HPLC METHODS FOR PHARMACEUTICAL ANALYSIS

Volumes 2-4

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A WILEY-INTERSCIENCE PUBLICATION

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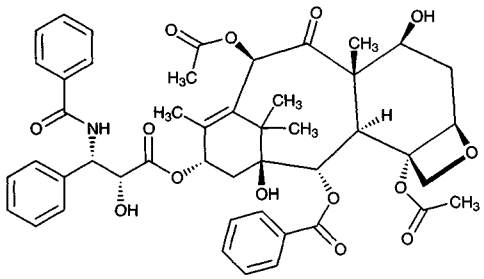
Paclitaxel

Molecular formula: $C_{47}H_{51}NO_{14}$
Molecular weight: 853.92
CAS Registry No.: 33069-62-4
Merck Index: 7117



SAMPLE

Matrix: blood
Sample preparation: Add 100 µL 10 µg/mL docetaxel in MeOH:water 50:50 and 5 mL MeCN: n-butyl chloride 20:80 to 1 mL plasma, vortex for 5 min, centrifuge at 4000 g for 5 min, evaporate the organic layer to dryness under a stream of nitrogen at 60°, reconstitute the residue with MeOH:water 50:50 with sonication for 1 min, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Inertsil ODS-80A (GL Science, Japan)
Mobile phase: MeOH:THF:water:ammonium hydroxide 60:2.5:37.5:0.1, pH adjusted to 6.0 with formic acid
Column temperature: 60
Flow rate: 1
Injection volume: 100
Detector: UV 230

CHROMATOGRAM

Retention time: 7.5
Internal standard: docetaxel (8.5)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, alizapride, codeine, dexamethasone, domperidone, lorazepam, metoclopramide, morphine, ranitidine
Interfering: paroxetine

KEY WORDS

pharmacokinetics; plasma

REFERENCE

Sparreboom,A.; de Bruijn,P.; Nooter,K.; Loos,W.J.; Stoter,G.; Verweij,J. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1998**, *705*, 159–164.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 1 mL Baker Bond cyanopropyl SPE cartridge with 1 mL MeCN, 1 mL mobile phase, and 1 mL 50 mM KH_2PO_4. Free paclitaxel. Add 500 µL plasma or urine to the SPE cartridge, wash with 1 mL water, 1 mL MeCN:water 30:70, and 1 mL 50 mM KH_2PO_4, elute with two 300 µL portions of mobile phase, inject a 200 µL aliquot of the eluate. Total paclitaxel. To 500 µL plasma or urine add 500 µL MeOH:100 mM KH_2PO_4 50:50 (pH 7.5), hydrolyze at 22° for 20 h, stop the hydrolysis by adding an equal volume of 500 mM KH_2PO_4. Add the hydrolyzed sample to the SPE cartridge, wash with 1 mL water, 1 mL MeCN: water 30:70, and 1 mL 50 mM KH_2PO_4, elute with two 300 µL portions of mobile phase, inject a 200 µL aliquot of the eluate.

HPLC VARIABLES

Guard column: 4 × 4 4 µm LiChrospher 100 RP-8e
Column: 250 × 4 4 µm Superspher 60 RP-8e
Mobile phase: MeCN:175 mM pH 4.6 KH_2PO_4 buffer 55:45
Flow rate: 0.45

Injection volume: 200
Detector: UV 229

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
dog; plasma; SPE

REFERENCE
Fraier,D.; Cenacchi,V.; Frigerio,E. Determination of a new polymer-bound paclitaxel derivative (PNU 166945), free paclitaxel and 7-epipaclitaxel in dog plasma and urine by reversed-phase high-performance liquid chromatography with UV detection, *J.Chromatogr.A*, **1998**, *797*, 295–303.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Dilute sample with MeOH containing 0.1% acetic acid. Injections. Dilute sample with MeCN:water:acetic acid 70:30:0.1. Method 1. Column A, gradient A. Method 2. Column B, gradient B. Method 3. Column A, gradient C. Method 4. Column B, gradient D. Methods 1 and 2 are for bulk drug potency determination. Methods 3 and 4 are for the determination of the degradation profile for bulk and injections, potency and content uniformity of the injections and chromatographic purity profile of the bulk drug.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Curosil PFP (Phenomenex) (A), 250 × 4.6 5 μm Whatman TAC-1 (PFP) (Whatman) (B)
Mobile phase: Gradient A. MeCN:water from 40:60 by linear gradient at 0.444%/min until the paclitaxel peak elutes, return rapidly to initial composition and equilibrate. Gradient B. MeCN:water 38:62 for 12 min, by linear gradient at 4%/min until paclitaxel peak elutes, return to initial composition and equilibrate. Gradient C. MeCN:water from 40:60 to 60:40 over 45 min, to 80:20 over 5 min, hold for 5 min, return to initial composition and equilibrate. Gradient D. MeCN:water 38:62 for 12 min, to 70:30 over 8 min, hold for 15 min, return to initial composition and equilibrate.
Flow rate: 1 (column A), 1.5 (column B)
Injection volume: 15
Detector: UV 230

CHROMATOGRAM
Retention time: 24 (Method 1), 22.5 (Method 3), 17 (Method 4)
Limit of detection: 370 ng/mL (method 1), 310 ng/mL (method 2)
Limit of quantitation: 1.11 μg/mL (method 1), 930 ng/mL (method 2)

OTHER SUBSTANCES
Simultaneous: 13-acetyl-9-dihydrobaccatin, baccatin III, cephalomannine, 10-deacetyl baccatin III, N-debenzoyl-N-phenylacetyl taxol, 10-deacetyl-7-epi-taxol, 10-deacetyl taxol, 10-deacetyl-7-xylosyl taxol, 10-deacetyl-7-xylosyl taxol B, 10-deacetyl-7-xylosyl taxol C, 7-epi-taxol, taxinine M, taxol C, 7-xylosyl taxol
Noninterfering: degradation products, Cremophor EL

KEY WORDS
injections

REFERENCE
Shao,L.K.; Locke,D.C. Determination of paclitaxel and related taxanes in bulk drug and injectable dosage forms by reversed phase liquid chromatography, *Anal.Chem.*, **1997**, *69*, 2008–2016.

SAMPLE
Matrix: cell culture

Sample preparation: Pulverize dry callus cell culture, pass through 40-mesh sieve. Extract ultrasonically with MeOH:dichloromethane 10:1 for 30 min. Evaporate extract to dryness, dissolve residue in MeOH. Add an aliquot to a Sep-Pak C18 SPE cartridge, wash with water, wash with MeOH:water 30:70, elute with MeOH:water 85:15. Evaporate the eluate to dryness and redissolve in a minimum amount of MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18
Mobile phase: MeCN:MeOH:water 35:25:45
Flow rate: 1.0
Injection volume: 10
Detector: UV 227

CHROMATOGRAM
Retention time: 27
Limit of quantitation: 11.5 ng

OTHER SUBSTANCES
Extracted: baccatin, cephalomannine, 10-deacetyltaxol, 10-deacetylcephalomannine, baccatin, 10-deacetylbaccatin

KEY WORDS
SPE

REFERENCE
Wu,Y.; Zhu,W. High performance liquid chromatographic determination of taxol and related taxanes from Taxus callus cultures, *J.Liq.Chromatogr.*, **1997**, *20*, 3147–3154.

SAMPLE
Matrix: formulations
Sample preparation: Add paclitaxel injection to 0.9% NaCl injection or 5% dextrose injection to make a paclitaxel concentration of 0.3 or 1.2 mg/mL, mix thoroughly. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere RP18
Mobile phase: MeOH:water 90:10
Column temperature: 28
Flow rate: 1.3
Injection volume: 500
Detector: UV 273

CHROMATOGRAM
Retention time: 2.1

OTHER SUBSTANCES
Simultaneous: polyoxyethylated castor oil, diethylhexyl phthalate

KEY WORDS
injections

REFERENCE
Mazzo,D.J.; Nguyen-Huu,J.-J.; Pagniez,S.; Denis,P. Compatibility of docetaxel and paclitaxel in intravenous solutions with polyvinyl chloride infusion materials, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 566–569.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18

Mobile phase: MeCN:water 40:60
Flow rate: 2.25
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.95

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: plant
Sample preparation: Bark. Condition a Supelclean LC-18 SPE cartridge with MeOH and water. Homogenize (Ystral) 20 g dried bark with 100 mL MeOH. Sonicate the homogenate in a bath for 10 min, shake at 110 rpm for 30 min, filter through a glass filter. Re-extract residue with 50 mL MeOH, combine two MeOH extracts, evaporate to dryness at 40-45°, reconstitute the residue with 5 mL MeOH. Add a 500 μL aliquot to the SPE cartridge. Needles, clippings. Add 3 g sample to 100 mL chloroform:EtOH 50:50 (Caution! Chloroform is a carcinogen!), sonicate, filter though a glass filter, evaporate the filtrate to dryness, reconstitute the residue in 5 mL MeOH. Add a 500 μL aliquot to the SPE cartridge. Wash the cartridge twice with 2 mL portions of water, with 2 mL MeOH:water 20:80, and with 2 mL MeOH:water 50:50. Elute with 2 mL MeOH, evaporate the fractions to dryness in a speed vac, reconstitute the residue with two 100 μL aliquots of MeCN, inject 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Lichrosorb RP-18
Column: 150 × 3.9 4 μm Novapak Phenyl
Mobile phase: Gradient. A was MeCN:50 mM ammonium acetate 30:70. B was MeCN:50 mM ammonium acetate 90:10. A:B 100:0, to 66:34 over 30 min, return to initial conditions over 2 min.
Flow rate: 0.8 (UV), 1 (MS)
Injection volume: 10
Detector: UV 227; MS, Finnigan MAT TSQ-70, electrospray, positive ion mode 150 V, sheath flow MeOH:water:acetic acid 80:20:1, mobile phase split 19:1 before MS

CHROMATOGRAM
Retention time: 22 (UV), 20 (MS)

OTHER SUBSTANCES
Extracted: baccatin III, cephalomannine, 10-DAB, 10-deacetyltaxol, 7-epi-10-deacetyltaxol, 7-epi-taxol, taxinine M, taxol C, 7-xylosyl-10-deacetyltaxol, 7-xylosyl-10-deacetyltaxol B, 7-xylosyl-10-deacetyltaxol C, 7-xylosyl-taxol

KEY WORDS
bark; needles; clippings; SPE

REFERENCE
Theodoridis,G.; Laskaris,G.; de Jong,C.F.; Hofte,A.J.P.; Verpoorte,R. Determination of paclitaxel and related diterpenoids in plant extracts by high-performance liquid chromatography with UV detection in high-performance liquid chromatography-mass spectrometry, *J.Chromatogr.A*, **1998**, *802*, 297–305.

SAMPLE
Matrix: plants
Sample preparation: Extract needles with MeOH, concentrate the extract under reduced pressure at <30°, partition concentrate with 0.8 volumes of water and 0.8 volumes of chloroform,

repeat the extraction with 0.6 volumes chloroform then 0.4 volumes chloroform, concentrate under reduced pressure, dry under vacuum at 35-40°, dissolve in MeCN/water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil C8
Mobile phase: MeCN:MeOH:water 50:10:40
Flow rate: 0.5
Detector: UV 254

KEY WORDS
needles; details of preparative HPLC in paper

REFERENCE
Rao,K.V.; Bhakuni,R.S.; Juchum,J.; Davies,R.M. A large scale process for paclitaxel and other taxanes from the needles of *Taxus* x *media Hicksii* and *Taxus floridana* using reverse phase column chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 427–447.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher diol
Mobile phase: Gradient. MeOH:carbon dioxide 8:92 for 3 min, to 28:72 over 25 min, to 35:65 over 5.7 min, maintain at 35:65 for 4 min.
Column temperature: 30
Flow rate: 2
Detector: UV 227

CHROMATOGRAM
Retention time: 15.77

OTHER SUBSTANCES
Simultaneous: impurities, degradation products

KEY WORDS
SFC; pressure 150 bar

REFERENCE
Jagota,N.K.; Nair,J.B.; Frazer,R.; Klee,M.; Wang,M.Z. Supercritical fluid chromatography of paclitaxel, *J.Chromatogr.A*, **1996**, *721*, 315–322.

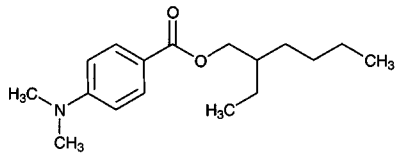
SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C-18 Guard Pak
Column: 100 × 8 10 μm Resolve C-18 radial compression (Waters)
Mobile phase: Gradient. MeCN:water from 45:55 to 100:0 over 20 min (exponential gradient), maintain at 100:0 for 5 min, re-equilibrate at initial conditions for 5 min.
Column temperature: 21
Flow rate: 2.5
Injection volume: 20
Detector: UV 227

CHROMATOGRAM
Retention time: 9.33

REFERENCE
Wenk,M.R.; Fahr,A.; Reszka,R.; Seelig,J. Paclitaxel partitioning into lipid bilayers, *J.Pharm.Sci.*, **1996**, *85*, 228–231.

Padimate O



Molecular formula: $C_{17}H_{27}NO_2$
Molecular weight: 277.41
CAS Registry No.: 21245-02-3
Merck Index: 3282

SAMPLE
Matrix: formulations
Sample preparation: Lotion. Weigh out lotion equivalent to about 90 mg oxybenzone, add 7 mL water, add MeOH slowly with vigorous shaking until total volume was 100 mL. Remove a 10 mL aliquot and make up to 100 mL with MeOH, filter (paper), discard first 5 mL of filtrate. Mix 4 mL filtrate and 1 mL 200 μg/mL sulfathiazole in MeOH, make up to 10 mL with MeOH, filter (0.45 μm), inject a 20 μL aliquot. Lipstick. Weigh out an amount equivalent to 25-90 mg oxybenzone, add 10 mL chloroform, add MeOH slowly with vigorous shaking until total volume was 100 mL. Remove a 10 mL aliquot and make up to 100 mL with MeOH, filter (paper), discard first 5 mL of filtrate. Mix 4 mL filtrate and 1 mL 200 μg/mL sulfathiazole in MeOH, make up to 10 mL with MeOH, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 25-37 μm Co:Pell ODS
Column: 250 × 4.6 10 μm Partisil PXS ODS-2
Mobile phase: MeCN:MeOH 10:90
Flow rate: 0.7
Injection volume: 20
Detector: UV 254

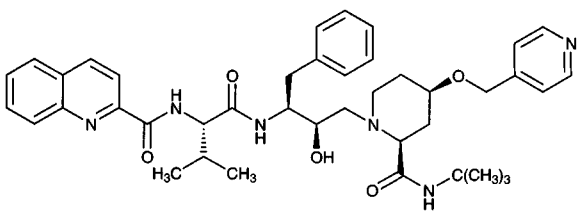
CHROMATOGRAM
Retention time: 7.4
Internal standard: sulfathiazole (3.9)
Limit of quantitation: 40 ng

OTHER SUBSTANCES
Simultaneous: oxybenzone, propyl paraben

KEY WORDS
lotion; lipstick; sun-screen

REFERENCE
Tan,H.S.I.; Sih,R.; Moseley,S.E.; Lichtin,J.L. Assay of mixtures of padimate-O and oxybenzone in sunscreen formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *291*, 275–282.

Palinavir



Molecular formula: $C_{41}H_{52}N_6O_5$
Molecular weight: 708.91
CAS Registry No.: 154612-39-2

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 1.5 M NaOH, extract three times with 2 mL portions of diethyl ether, vortex for 30 s, centrifuge at 3000 rpm for 10 min at 4°. Evaporate

the ether extract under a stream of nitrogen. Reconstitute the residue with 100 μL mobile phase. Inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 C-8 Nova-Pak
Mobile phase: MeCN:50 mM pH 3.0 potassium phosphate buffer 50:50 containing 0.1% dimethyloctylamine
Flow rate: 1.5
Injection volume: 80
Detector: UV 237

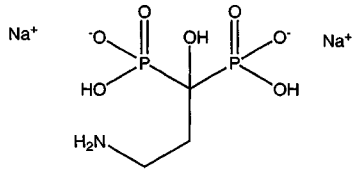
CHROMATOGRAM
Limit of detection: 2 nM

KEY WORDS
plasma; pharmacokinetics; rat

REFERENCE
Liard,F.; Jaramillo,J.; Paris,W.L.; Yoakim,C. Pharmacokinetic aspects of palinavir, an HIV protease inhibitor, in Sprague-Dawley rats, *J.Pharm.Sci.*, **1998**, *87*, 782–785.

Pamidronate

Molecular formula: $C_3H_9NNa_2O_7P_2$
Molecular weight: 279.01
CAS Registry No.: 57248-88-1, 109552-15-0 (pentahydrate), 40391-99-9 (free acid)
Merck Index: 7135



SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 500 mg Bakerbond quaternary amine SPE cartridge (J.T.Baker) with 2.5 mL water, 1 mL 1 mg/mL etidronate solution, 2.5 mL 100 mM nitric acid, and 5 mL water. Add 20 μL 5 mg/mL etidronate solution, 35 μL 100 mg/mL citric acid (to serum only), and 30 μL 2050 ng/mL neridronic acid solution to 1 mL serum or citrated plasma. Add 200 μL 20% trichloroacetic acid, vortex vigorously, centrifuge at 14000 g for 5 min. Add 30 μL 1 M calcium chloride, 40 μL 100 mM NaH_2PO_4, and 200 μL 1 M NaOH to the clear supernatant, vortex, centrifuge at 3900 g for 2 min, discard the liquid phase. Dissolve the residue in 25 μL 1 M HCl, dilute with 2.5 mL water, add to the SPE cartridge, wash with 1 mL water at 2 mL/min, wash with 4 mL water and 2.5 mL 10 mM nitric acid at 3 mL/min. Elute with 2.5 mL 100 mM nitric acid at 2.5 mL/min, evaporate the eluate to dryness under 0.6 bar nitrogen at 80° for 50-75 min, reconstitute the residue in 500 μL water, vortex. Add 50 μL 1 mg/mL etidronate, 75 μL triethylamine, and 500 μL 20 mg/mL 1-naphthylisothiocyanate in pyridine, mix by air bubbling to form a clear yellow solution. Heat the mixture at ca.80° for 15 min, extract twice with 2 mL 10 mg/mL tetrabutylammonium bromide in chloroform (Caution! Chloroform is a carcinogen!), mix the two phases by bubbling 3 mL air through the liquid, let the phases separate for 1 min, remove the lower organic layer. Mix 300 μL of the resulting sample and 90 μL 3% hydrogen peroxide, heat at ca.80° for 5 min, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 reversed-phase R2 (Chrompack)
Column: 100 × 4.6 3 μm Microspher C18 (Chrompack)
Mobile phase: MeCN:10 mM phosphate buffer containing 10 mM tetraoctylammonium bromide and 2 mM etidronate 62:38, pH 8.0
Flow rate: 0.8
Injection volume: 100
Detector: F ex 285 em 390

CHROMATOGRAM
Retention time: 12
Internal standard: neridronic acid (10.5)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS
derivatization; plasma; serum; SPE

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Beijnen,J.H.; Vermeij,P. Semi-automatic liquid chromatographic analysis of pamidronate in serum and citrate plasma after derivatization with 1-naphthylisothiocyanate, *J.Chromatogr.B*, **1998**, *705*, 331–339.

SAMPLE
Matrix: formulations
Sample preparation: Add 10 μL 300 μg/mL IS to 100 μL 30 μL aqueous solution prepared from injections or tablets, vortex with 50 μL EtOH, 40 μL pyridine, 10 μL triethylamine, and 2 μL phenylisothiocyanate to yield a clear solution. Heat at 80° for 5 min and evaporate under nitrogen at 80°. Reconstitute the dry residue in 1 mL water, wash twice by vortexing with 1 mL 2 g/L tetrabutylammonium bromide in chloroform (Caution! Chloroform is an carcinogen!), centrifuge at 3900 g for 2 min, discard the organic layer. Add 90 μL 0.06% hydrogen peroxide to 900 μL of the aqueous phase, heat at 80° for 2 min, evaporate to dryness at 80°, reconstitute the residue in 100 μL mobile phase. Inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 R2 (Chrompack, Netherlands)
Column: 100 × 4.6 3 μm Microspher C18 (Chrompack, Netherlands)
Mobile phase: MeCN:buffer 22.5:77.5 (Buffer was 30 mM pH 7.0 phosphate buffer containing 5 mM tetrabutylammonium hydroxide and 2 mM etidronate.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 6.8
Internal standard: neridronic acid (9.0)
Limit of quantitation: 100 ng/mL

KEY WORDS
tablets; injections; derivatization

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Ramp-Koopmanschap,W.M.; Langebroek,R.H.; Vermeij,P. The determination of pamidronate in pharmaceutical preparations by ion-pair liquid chromatography after derivatization with phenylisothiocyanate, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 491–497.

SAMPLE
Matrix: solutions
Sample preparation: Vortex 100 μL 0.5 mg/mL pamidronate disodium in water, 15 μL triethylamine, and 100 μL 20 mg/mL naphthylisothiocyanate in pyridine to yield a clear solution. Heat in a sealed tube at 80° for 15 min. Wash twice with 1 mL 10 mg/ml tetrabutyl ammonium bromide in chloroform, discard the organic solvent. Add 10 μL 0.2% hydrogen peroxide solution and heat at 80°. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.0 5μm Chromspher C18 (Chrompack)
Mobile phase: MeCN:buffer 60:40 (Buffer was 20 mM phosphate buffer containing 5 mM tetraoctylammonium bromide + 500 μM etidronate (adsorption supressor).)
Column temperature: 30

Flow rate: 0.4
Injection volume: 20
Detector: UV 285; F ex 285 em 390

CHROMATOGRAM
Retention time: 11.5
Limit of detection: 5 ng/mL

KEY WORDS
derivatization

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Beijnen,J.H.; Vermeij,P. Derivatization of pamidronate and other amino(bis)phosphonates with different isothiocyanates prior to ion-pair liquid chromatography, *J.Chromatogr.A*, **1997**, *782*, 211–217.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Plasma or urine + 100 μL 25 μg/mL IS in 100 mM NaOH, adjust pH to 3 with concentrated HCl, filter (0.45 μm fluoro-membrane, Skan Model Acro LC 13). Add 0.5 mL 1.5 M trichloroacetic acid to the filtrate, centrifuge at 1500 g for 15 min. Remove the supernatant, add 20 μL 2.5 M calcium chloride solution, add 40 μL 500 mM NaH_2PO_4, adjust pH to 12.0 with 6.25 M NaOH then with 0.5 M NaOH, centrifuge at 1500 g for 15 min. Wash the precipitate with water, dissolve the precipitate in 200 μL 130 mM disodium EDTA adjusted to pH 10 with 6.25 M NaOH, add 100 μL 3 mg/mL fluorescamine in MeCN while vortexing vigorously, add 200 μL dichloromethane, extract, centrifuge at 1000 g for 3 min, remove the aqueous phase, inject a 10 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:1 mM disodium EDTA adjusted to pH 6.5 with 1 M NaOH 3:97
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 395 em 480

CHROMATOGRAM
Retention time: 3.9
Internal standard: 6-amino-1-hydroxypentilidenebisphosphonate (CGP 33 637) (4.8)
Limit of detection: 10 nM (plasma, urine)
Limit of quantitation: 800 nM (plasma), 700 nM (urine)

KEY WORDS
derivatization; pharmacokinetics; plasma

REFERENCE
Flesch,G.; Tominaga,N.; Degen,P. Improved determination of the bisphosphonate pamidronate disodium in plasma and urine by pre-column derivatization with fluorescamine, high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.*, **1991**, *568*, 261–266.

SAMPLE
Matrix: bone
Sample preparation: Dissolve 25 mg ground (<20 μm) bone in 2 mL 200 mM HCl, add 5 μg IS, vortex, let stand overnight at room temperature, centrifuge. Remove a 500 μL aliquot and add it to 1 mL 10 mM NaOH and 50 μL 1 M NaOH, centrifuge at 1000 g for 10 min, wash the

pellet with 1 mL water, centrifuge, discard the supernatant. Dissolve the pellet in 200 μL 200 mM phosphoric acid, add 250 μL 200 mM pH 10.3 EDTA in 200 mM NaOH, add 200 μL resin, vortex, centrifuge, filter (0.2 μm) a 550 μL aliquot of the supernatant, add 10 μL 10 M NaOH to the filtrate. Remove a 50 μL aliquot and add it to 50 μL 1 M pH 10.7 carbonate buffer, add 10 μL 1 mg/mL 2,3-naphthalenedicarboxaldehyde, add 10 μL 1 mg/mL N-acetyl-D-penicillamine, mix, let stand for 2 min, inject a 20-50 μL aliquot. (The resin was AG 50W-X8 (K^+ form) resin. Prepare the resin by adding 3 volumes of 1 M KOH to 200-400 mesh AG 50W-X8 cation-exchange resin H^+ form (Bio-Rad), stir for 30 s, decant the supernatant, repeat the procedure twice, wash five times with 3 volumes of water, store at 4°, before use wash 2 or 3 times with three volumes of water (J. Chromatogr. 1992, 584, 213).)

HPLC VARIABLES

Guard column: present but not specified
Column: 150 × 4.6 PLRP-S (Phenomenex)
Mobile phase: MeCN:25 mM pH 6.5 citrate-phosphate buffer 16:94
Flow rate: 1
Injection volume: 20-50
Detector: F ex 436 em 508

CHROMATOGRAM

Retention time: 5.0
Internal standard: 1-hydroxypentanylidene-1,1-bisphosphonate (Ciba-Geigy) CGP 38146 (13.5)
Limit of quantitation: 7.5 μg/g

KEY WORDS

derivatization

REFERENCE

King,L.E.; Vieth,R. Extraction and measurement of pamidronate from bone samples using automated pre-column derivatization, high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.B*, **1996**, *678*, 325–330.

SAMPLE

Matrix: formulations
Sample preparation: Dilute injections 100-fold, inject a 20 μL aliquot. Disintegrate a 5 mg tablet in 100 mL water, sonicate for 5 min, centrifuge an aliquot at 3600 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 10 μm IC-PAK Anion HC (Waters)
Mobile phase: 1.5 mM Nitric acid containing 0.5 mM copper(II) nitrate (Prepare column by pumping ILC Regenerant A (Waters) and 100 mM nitric acid for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM

Retention time: 2
Limit of detection: 400 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: alendronate, clodronate, etidronate, neridronate, olpadronate

KEY WORDS

derivatization; complexation; injections; tablets

REFERENCE

Sparidans,R.W.; Den Hartigh,J.; Vermeij,P. High-performance ion-exchange chromatography with in-line complexation of bisphosphonates and their quality control in pharmaceutical preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1545–1550.

SAMPLE

Matrix: urine

Sample preparation: Filter dog urine through filter paper. 2 mL Urine + 100 μL IS in water, adjust pH to 3 with concentrated HCl, filter (0.45 μm fluoro-membrane, Skan Model Acro LC 13). Add 0.5 mL 1.5 M trichloroacetic acid to the filtrate, centrifuge at 1500 g for 15 min. Remove the supernatant, add 20 μL 2.5 M calcium chloride solution, add 40 μL 500 mM NaH_2PO_4, adjust pH to 12.0 with 6.25 M NaOH then with 0.5 M NaOH, centrifuge at 1500 g for 15 min. Wash the precipitate with water, dissolve the precipitate in 200 μL 130 mM disodium EDTA adjusted to pH 9 with 6.25 M NaOH, add 100 μL 1 mg/mL fluorescamine in MeCN while vortexing vigorously, add 200 μL dichloromethane, extract, centrifuge at 1000 g for 3 min, remove the aqueous phase, inject a 10 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:1 mM disodium EDTA adjusted to pH 6.5 with 1 M NaOH 3:97
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 395 em 480

CHROMATOGRAM

Retention time: 4
Internal standard: 6-amino-1-hydroxyhexylidenebisphosphonate (CGP 38 146) (8)
Limit of detection: 50 nM
Limit of quantitation: 1000 nM

KEY WORDS

derivatization; human; dog; pharmacokinetics

REFERENCE

Flesch,G.; Hauffe,S.A. Determination of the bisphosphonate pamidronate disodium in urine by pre-column derivatization with fluorescamine, high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.*, **1989**, *489*, 446–451.

SAMPLE

Matrix: urine

Sample preparation: Condition a 3 mL 500 mg Bakerbond quaternary amine SPE cartridge with 2.5 mL water, 1 mL 1 mg/mL etidronate, 2.5 mL 100 mM nitric acid, and two 2.5 mL portions of water. 2.5 mL Urine + 100 μL 1 mg/mL etidronate + 100 μL 2.16 μg/mL IS, mix, add 30 μL 1 M calcium chloride, vortex, add 25 μL portions of 1 M NaOH (vortexing after each addition) until a precipitate is clearly present, add 25 μL 1 M NaOH, vortex, centrifuge at 3900 g for 2 min. Remove the pellet and dissolve it in 50 μL 1 M HCl, add 2.5 mL water, add 50 μL 1 M NaOH, centrifuge. Remove the pellet and dissolve it in the minimum amount of 1 M HCl (30-50 μL), add 2.5 mL water, add 30 μL 1 M NaOH, centrifuge. Remove the pellet and dissolve it in the minimum amount of 1 M HCl (30-50 μL), add 2.5 mL water, add to the SPE cartridge, wash with two 2.5 mL portions of water, wash with 2.5 mL 10 mM nitric acid, elute with 2.5 mL 100 mM nitric acid. Evaporate the eluate to dryness under 0.8 bar nitrogen at 60° for 1.65-2 h, add 500 μL water, vortex. Remove a 250 μL aliquot and add it to 25 μL 1 mg/mL etidronate, add 40 μL triethylamine, add 250 μL 20 mg/mL 1-naphthylisothiocyanate in pyridine, vortex, heat at 80° for 15 min, cool, add 2 mL 10 mg/mL tetrabutylammonium bromide (?) in chloroform, vortex, centrifuge, discard the lower organic phase, repeat the procedure. Remove a 250 μL aliquot of the aqueous phase and add it to 75 μL 3% hydrogen peroxide (to convert the thiourea derivative to the corresponding urea), mix, heat at 80° for 5 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 R2 (Chrompack)
Column: 100 × 4.6 3 μm Microspher C18 (Chrompack)
Mobile phase: MeCN:buffer 65:35 (Buffer was 10 mM phosphate containing 10 mM tetraoctylammonium bromide and 2 mM etidronate (monosodium (1-hydroxyethylidene)bisphosphonate) (as adsorption suppressor), pH 7.6-7.9.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 100
Detector: F ex 285 em 390

CHROMATOGRAM
Retention time: 10
Internal standard: (3-amino-3-phenyl-1-hydroxypropylidene)bisphosphonic acid (17)
Limit of detection: 1 ng/mL
Limit of quantitation: 3 ng/mL

KEY WORDS
derivatization; SPE

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Beijnen,J.H.; Vermeij,P. Determination of pamidronate in urine by ion-pair liquid chromatography after derivatization with 1-naphthylisothiocyanate, *J.Chromatogr.B*, **1997**, *696*, 137–144.

Pancreatin

CAS Registry No.: 8049-47-6
Merck Index: 7137

SAMPLE
Matrix: solutions

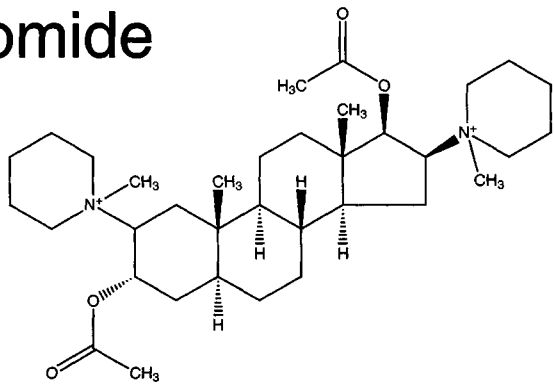
HPLC VARIABLES
Column: 250 × 4.6 Rexchrom 5 μm 300Å C8 (Regis)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B 100:0 to 0:100 over 40 min.
Flow rate: 1.5
Injection volume: 500
Detector: UV 214

CHROMATOGRAM
Retention time: 18-27 (multiple peaks)

REFERENCE
Baxter Scientific Products Catalog, 1990-1, p. 154.

Pancuronium bromide

Molecular formula: $C_{35}H_{60}Br_2N_2O_4$
Molecular weight: 732.68
CAS Registry No.: 15500-66-0
Merck Index: 7139
Lednicer No.: 2 163



SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 250 μL picric acid (1:10 dilution of saturated picric acid solution) + 250 μL vecuronium solution + 250 μL water + 5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 μL MeCN: water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 μPorasil
Mobile phase: MeCN:2 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100
Detector: conductivity 2500 nS full scale

CHROMATOGRAM

Retention time: 5.7
Internal standard: vecuronium (4.7)
Limit of detection: 20 ng/mL

KEY WORDS

plasma

REFERENCE

Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.027

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 0.5% solution in the mobile phase, inject a 20 μL aliquot.

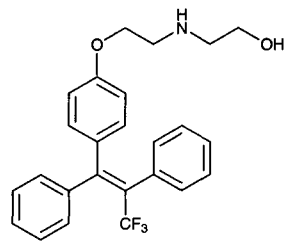
HPLC VARIABLES
Column: 250 × 4 5 μm SI 100 (Bio Separation Technologies)
Mobile phase: MeCN:100 mM sodium perchlorate 96:4
Flow rate: 1
Injection volume: 20
Detector: UV 213

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Simultaneous: pipecuronium
Interfering: vecuronium

REFERENCE
Gazdag,M.; Babják,M.; Kemenes-Bakos,P.; Görög,S. Analysis of steroids. XLI. Ion-pair high-performance liquid chromatographic separation of quaternary ammonium steroids on silica, *J.Chromatogr.*, **1991**, *550*, 639–644.

Panomifene



Molecular formula: $C_{25}H_{24}F_3NO_2$
Molecular weight: 427.47
CAS Registry No.: 77599-17-8

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL phenyl SPE cartridge with five 1 mL portions of MeCN, 1 mL water, and 1 mL 2.5 mL/L triethylamine in 50 mM pH 3.0 phosphate buffer. 980 μL Plasma + 20 μL MeOH:water 50:50 + 10 μL 2 μg/mL tamoxifen in MeOH:water 50:50 + 1 mL MeCN, vortex for 1 min, centrifuge at -10° at 2500 g for 1 h. Remove a 1.6 mL aliquot of the supernatant and add it to 400 μL 2% heptanesulfonic acid in 50 mM pH 3.0 KH_2PO_4/phosphoric acid buffer, add to the SPE cartridge, wash with two 100 μL portions of MeCN:buffer 80:20,

wash with 50 μL 25 mM sulfuric acid, elute with five 100 μL portions of MeCN:buffer 80:20. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL MeCN:buffer 70:30, inject a 10-30 μL aliquot. (Buffer was 5 mM heptanesulfonic acid in 50 mM pH 3.0 phosphate buffer.)

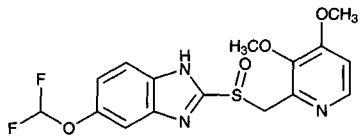
HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Si-100-S Phenyl (BST, Budapest)
Column: 250 × 4.6 10 μm Si-100-S Phenyl (BST, Budapest)
Mobile phase: MeCN:buffer 75:25 (Buffer was 50 mM pH 3.0 KH_2PO_4/phosphoric acid buffer containing 5 mM heptanesulfonic acid and 300 μL/L triethylamine. Temperature of MeCN was 60° and temperature of buffer was 80°.)
Flow rate: 1.2
Injection volume: 10-30
Detector: F ex 257 em 378 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID knitted PTFE coil irradiated by a mercury lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 5.73
Internal standard: tamoxifen (7.03)
Limit of detection: 1 ng/mL

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma; pharmacokinetics; SPE

REFERENCE
Erdélyi-Tóth,V.; Pap,E.; Kralovánszky,J.; Bojti,E.; Klebovich,I. Determination of panomifene in human plasma by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *668*, 419–425.

Pantoprazole



Molecular formula: $C_{16}H_{15}F_2N_3O_4S$
Molecular weight: 383.38
CAS Registry No.: 102625-70-7
Merck Index: 7146
Lednicer No.: 5 115

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 2000 g for 10 min, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 2 min backflush the contents of column A on to column B and start the gradient, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 25-40 μm LiChroprep RP-2; B 12 × 4.6 5 μm Hypersil RP-18 + 125 × 4.6 5 μm Hypersil RP-18
Mobile phase: A MeCN:50 mM pH 5 sodium acetate (Merck Extra Pure) buffer 10:90; B Gradient. MeOH:10 mM pH 6.5 ammonium phosphate buffer from 43:57 to 83:17 over 2 min, after 17 min flush at 100:0 for 2 min, re-equilibrate at initial conditions for 7 min.
Flow rate: A 1.5; B 1
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; column-switching

REFERENCE
Doyle,E.; McDowall,R.D.; Murkitt,G.S.; Picot,V.S.; Rogers,S.J. Two systems for the automated analysis of drugs in biological fluids using high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 67–77.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge serum or plasma at 2000 g for 10 min, inject a 200 μL aliquot on to column A and elute to waste with mobile phase A, after 2 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. Between runs flush column A with mobile phase A.

HPLC VARIABLES
Column: A 25-40 μm LiChroprep RP-2; B 12 × 4.6 5 μm Hypersil RP-18 + 125 × 4.6 5 μm Hypersil RP-18
Mobile phase: A 100 mM pH 5 sodium acetate buffer; B Gradient. MeOH:10 mM pH 6.5 $(NH_4)_2HPO_4$ 43:57 for 2 min, to 83:17 over 17 min, to 100:0 over 2 min, re-equilibrate for 7 min.
Flow rate: A 1.5; B 1
Injection volume: 200
Detector: UV 290

CHROMATOGRAM
Retention time: 16.5
Limit of detection: 4 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; plasma; dog; pharmacokinetics; column-switching

REFERENCE
Huber,R.; Muller,W.; Banks,M.C.; Rogers,S.J.; Norwood,P.C.; Doyle,E. High-performance liquid chromatographic determination of the H+/K+ ATPase inhibitor (BY 1023/SK&F 96,022) and its sulphone metabolite in serum or plasma by direct injection and fully automated pre-column sample clean-up, *J.Chromatogr.*, **1990**, *529*, 389–401.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in EtOH, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralpak AD
Mobile phase: Hexane:EtOH 80:20
Column temperature: 35
Flow rate: 1
Injection volume: 10-20
Detector: UV 302

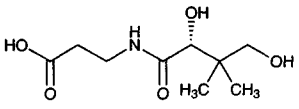
CHROMATOGRAM
Retention time: k' 6.10 (of first enantiomer)

OTHER SUBSTANCES
Simultaneous: lansoprazole, omeprazole, timoprazole

KEY WORDS
chiral; $\alpha = 1.19$

REFERENCE
Balmér,K.; Persson,B.-A.; Lagerström,P.-O. Stereoselective effects in the separation of enantiomers of omeprazole and other substituted benzimidazoles on different chiral stationary phases, *J.Chromatogr.A*, **1994**, *660*, 269–273.

Pantothenic acid



Molecular formula: $C_9H_{17}NO_5$
Molecular weight: 219.24
CAS Registry No.: 79-83-4 (D), 137-08-6 (Ca salt D), 6381-63-1 (Ca salt racemic), 599-54-2 (racemic)
Merck Index: 7147

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.772

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: food
Sample preparation: Completely dissolve 20 g of an elemental diet (Elental, Ajinomoto Co., Kawasaki) in 60 mL water at 50°, add 10 g sodium chloride, let stand at room temperature for 30 min. Dilute to 100 mL with water, wash with 10 mL hexane for 3 min to remove any oils. Inject a 20 µL aliquot of the aqueous layer onto column A and elute to waste with mobile phase A, after 4 min elute the contents of column A onto column B with mobile phase B, when the compounds of interest have moved onto column B remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 150 × 4.6 5 μm Capcellpak C18 (Shiseido, Tokyo); B 250 × 4.6 5 μm Capcellpak C18 (Shiseido, Tokyo)
Mobile phase: A MeCN:buffer 5:95; B MeCN:buffer 9:91 containing 1.5 mM sodium 1-heptanesulfonate (Buffer was water adjusted to pH 2.1 with phosphoric acid.)
Column temperature: 35
Flow rate: 1.2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Limit of detection: ca. 5 ng

KEY WORDS
elemental diet; column switching

REFERENCE
Iwase,H. Determination of pantothenic acid in an elemental diet by column-switching high-performance liquid chromatography with ultraviolet detection, *Anal.Sci.*, **1993**, *9*, 149–151.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate tablet in 40 mL mobile phase until it dissolves, make up to 50 mL with mobile phase, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Column: 200 mm long Nucleosil 7 C18
Mobile phase: Water:glacial acetic acid 95:5
Flow rate: 2
Injection volume: 100
Detector: RI

CHROMATOGRAM
Retention time: 4.50
Limit of detection: 500 ng/mL

KEY WORDS
tablets; detector temp 35

REFERENCE
Jonvel,P.; Andermann,G.; Barthelemy,J.F. Determination of calcium pantothenate in multivitamin preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *281*, 371–376.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets containing 50 mg calcium pantothenate to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: thiamine, riboflavin, niacinamide, pyridoxine

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, add 80 mL water, shake at 240 oscillations/min for 30 min, make up to 100 mL with water, mix well, centrifuge at 1500 rpm, dilute supernatant with water to a concentration of 45 μg/mL, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 3:97 (Buffer was 250 mM NaH_2PO_4 adjusted to pH 2.5 with phosphoric acid.) (Flush column with MeCN:water 5:95 at the end of each day.)
Flow rate: 2
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 5
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: saccharin, pantoyllactone, degradation products
Interfering: panthenol

KEY WORDS
tablets; stability-indicating

REFERENCE
Timmons,J.A.; Meyer,J.C.; Steible,D.J.; Assenza,S.P. Reverse phase liquid chromatographic assay for calcium pantothenate in multivitamin preparations and raw materials, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 510–513.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS,, **1993**.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 0.9

OTHER SUBSTANCES
Simultaneous: niacin, pyridoxine, riboflavin, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 780.

SAMPLE
Matrix: solutions

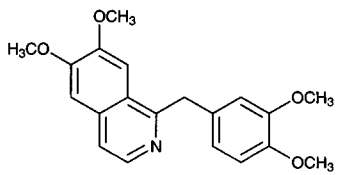
HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: biotin, folic acid, niacin, riboflavin, niacinamide

REFERENCE
MetaChem Catalog, **1995,** p. 21.

Papaverine



Molecular formula: $C_{20}H_{21}NO_4$
Molecular weight: 339.39
CAS Registry No.: 58-74-2, 61-25-6 (HCl)
Merck Index: 7151
Lednicer No.: 1 347

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 15 μL MeOH + 200 μL 4 M NaOH + 5 mL diethyl ether, vortex for 5 min, centrifuge at 2000 g for 10 min, remove the organic layer, extract the aqueous layer with 2 mL diethyl ether, centrifuge. Combine the organic layers and add them to 500 μL 1 M HCl, vortex for 1 min, centrifuge at 2000 g for 10 min. Remove the aqueous layer and add it to 500 μL 4 M NaOH, vortex for 1 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 25 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil 10 ODS
Mobile phase: MeOH:0.1% KH_2PO_4 65:35
Flow rate: 2
Detector: UV 238

CHROMATOGRAM
Retention time: 3.5
Internal standard: papaverine

OTHER SUBSTANCES
Extracted: ethaverine

KEY WORDS
plasma; papaverine is IS

REFERENCE
Brodie,R.R.; Chasseaud,L.F.; Walmsley,L.M.; Soegtrop,H.H.; Darragh,A.; O'Kelly,D.A. Determination of the antispasmodic agent ethaverine in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 379–386.

SAMPLE
Matrix: blood
Sample preparation: 4 mL Whole blood + 100 μL 8 μg/mL mepyramine maleate in water (prepare fresh daily), vortex, add 10 mL pH 10.0 phosphate buffer (μ = 0.4), vortex, add 5 mL chloroform:hexane 40:60, shake gently horizontally for 30 min, centrifuge. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micropak CN-10
Mobile phase: n-Hexane:dichloromethane:MeCN:propylamine 50:25:25:0.1
Column temperature: 30
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 3.4
Internal standard: pyrilamine maleate (mepyramine maleate) (4.0)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: carbetapentane, cocaine, diamorphine, dioxyline, ethaverine, fluphenazine, imipramine, papaveraldine, promethazine, strychnine, thonzylamine
Interfering: methapyrilene, procaine, yohimbine

KEY WORDS
whole blood; pharmacokinetics

REFERENCE
Hoogewijs,G.; Michotte,Y.; Lambrecht,J.; Massart,D.L. High-performance liquid chromatographic determination of papaverine in whole blood, *J.Chromatogr.*, **1981**, *226*, 423–430.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 100 μg/mL noscapine + 1 mL 100 mM HCl + 6 mL dichloromethane, extract, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: Lichrosorb Si-60
Mobile phase: Hexane:dichloromethane:MeOH:diethylamine 95:4:1:0.03
Injection volume: 40
Detector: F ex 238 no emission filter

CHROMATOGRAM
Internal standard: noscapine
Limit of detection: 5 ng/mL

KEY WORDS
plasma; normal phase; pharmacokinetics

REFERENCE
Berg,G.; Jonsson,K.-Å.; Hammar,M.; Norlander,B. Variable bioavailability of papaverine, *Pharmacol.Toxicol.*, **1988**, *62*, 308–310.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN: water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: Gradient A was MeCN. B was 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min.
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 14
Limit of detection: 0.05 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS
whole blood

REFERENCE
Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 300 μL 200 μg/mL laudanosine, mix, add 10 mL chloroform:isopropanol 95:5, vortex for 2 min, centrifuge for 30 min. Remove 8 mL of the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C8 (Brownlee)
Mobile phase: MeOH:15 mM pH 8.5 sodium borate buffer 58:42
Flow rate: 2.7
Injection volume: 20
Detector: UV 239

CHROMATOGRAM
Retention time: 5.0
Internal standard: laudanosine (9.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Noninterfering: caffeine, chlorothiazide, gentamicin, hydrochlorothiazide, oxytetracycline, tetracycline, theobromine, theophylline

KEY WORDS
plasma

REFERENCE
Gautam,S.R.; Nahum,A.; Baechler,J.; Bourne,D.W. Determination of papaverine in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 482–486.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a Toxiclean SPE cartridge (Alltech) with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μL Plasma or serum + 100 μL MeOH + 200 μL MeCN + 100 μL buffer, vortex for 1 min, centrifuge at 4000 rpm for 15 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2.5 μg/mL flufenamic acid in MeOH (?), inject an aliquot. Urine. Condition a Bond Elut C8 SPE cartridge with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μL Urine + 100 μL MeOH + 200 μL MeCN + 500 μL buffer, vortex for 1 min, centrifuge at 2000 rpm for 5 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2.5 μg/mL flufenamic acid in MeOH (?), inject an aliquot. (Buffer was 250 mL 25 mM sodium borate and 18 mL 100 mM NaOH, pH 9.2.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere HS C18
Mobile phase: MeCN:MeOH:1.2% ammonium acetate 15:40:45
Flow rate: 0.8
Detector: UV 239

CHROMATOGRAM
Retention time: 19.06
Internal standard: flufenamic acid (24.39)
Limit of quantitation: 60 ng/mL (urine), 20 ng/mL (plasma, serum)

OTHER SUBSTANCES
Extracted: codeine, monoacetylmorphine, morphine

KEY WORDS
SPE; plasma; serum

REFERENCE
Theodoridis,G.; Papadoyannis,I.; Tsoukali-Papadopoulou,H.; Vasilikiotis,G. A comparative study of different solid phase extraction procedures for the analysis of alkaloids of forensic interest in biological fluids by RP-HPLC/Diode array, *J.Liq.Chromatogr.*, **1995**, *18*, 1973–1975.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 251.1

CHROMATOGRAM
Retention time: 12.12

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 29.6

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, procaine

REFERENCE
Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE
Matrix: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:50 mM pH 4.7 $KH_2PO_4$40:60
Flow rate: 1
Injection volume: 75
Detector: UV 340

CHROMATOGRAM
Retention time: 9.5
Internal standard: rifampin
Limit of detection: 100-1000 ng/mL

REFERENCE
Darouiche,R.O.; Hamill,R.J. Antibiotic penetration of and bactericidal activity within endothelial cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1059–1064.

SAMPLE
Matrix: formulations
Sample preparation: Ampules. Dilute a 2 mL aliquot to 100 mL with buffer, dilute further with water to a papaverine concentration of 60 μg/mL, inject a 20 μL aliquot. Tablets. Crush a tablet, shake with 70 mL buffer for 10 min, make up to 100 mL with buffer, filter, dilute a 10 mL aliquot to 50 mL with water, inject a 20 μL aliquot. (The buffer was 1.248% NaH_2PO_4 adjusted to pH 3 with orthophosphoric acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: MeCN:buffer 70:30 (Buffer contained 2.88% sodium lauryl sulfate and 1.248% NaH_2PO_4 adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.16

OTHER SUBSTANCES
Simultaneous: moxaverine, drotaverine, ethaverine, codeine

KEY WORDS
ampules; tablets

REFERENCE
Girgis,E.H. Ion-pair reversed-phase liquid chromatographic identification and quantitation of papaverine congeners, *J.Pharm.Sci.*, **1993**, *82*, 503–505.

SAMPLE
Matrix: formulations
Sample preparation: Dilute syrup with mobile phase to a concentration of 5-100 μg/mL, shake, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 80 Å Ultrasphere CN
Mobile phase: MeCN:water:EtOH 60:38:2 containing 1 mM perchloric acid

Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: Conductivity, zero suppression 2, range 1 or 10

CHROMATOGRAM
Retention time: 12.1

OTHER SUBSTANCES
Simultaneous: bromhexine, chlorpheniramine, codeine, dextromethorphan, diphenhydramine, ephedrine, phenylephrine

KEY WORDS
syrup; indirect conductometric detection; presence of compound causes a decrease in mobile phase conductivity

REFERENCE
Lau,O.-W.; Mok,C.-S. High-performance liquid chromatographic determination of active ingredients in cough-cold syrups with indirect conductometric detection, *J.Chromatogr.A*, **1995**, *693*, 45–54.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.53

OTHER SUBSTANCES
Simultaneous: phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, naloxone, dextropropoxyphene, nalorphine, phenazocine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova Pak C18
Mobile phase: MeCN:50 mM pH 5.5 phosphate buffer 25:75
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 8.9

OTHER SUBSTANCES

Simultaneous: phentolamine

REFERENCE

Wang,D.-P.; Tu,Y.-H.; Allen,L.V.,Jr. Degradation kinetics of phentolamine hydrochloride in solution, *J.Pharm.Sci.*, **1988**, *77*, 972–976.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.16 μm PolyEncap ODS (n-octadecylacrylate copolymerized with vinyl silica in heptane, carrier Ultrasep ES 100; preparation described in paper)
Mobile phase: MeCN:pH 2.2 phosphate buffer 20:80
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: atropine, barbituric acid, codeine, diphenhydramine, noscapine

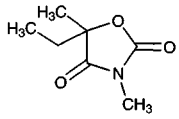
REFERENCE
Engelhardt,H.; Cuñat-Walter,M.A. Polymer encapsulated stationary phases with improved efficiency, *Chromatographia*, **1995**, *40*, 657–661.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge at 10000 rpm, dilute the supernatant with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil CPS
Mobile phase: MeCN:20 mM KH_2PO_4 50:50
Detector: UV 254

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

Paramethadione



Molecular formula: $C_7H_{11}NO_3$
Molecular weight: 157.17
CAS Registry No.: 115-67-3
Merck Index: 7161
Lednicer No.: 1 232

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 50 μL 7 μg/mL IS in water + 1 mL buffer, vortex for 10 s, add 5 mL n-hexane:ether:n-propanol 49:49:2, shake gently for 20 min, centrifuge at 1000 g for 5 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL mobile phase, inject a 50-100 μL aliquot. (Buffer was 10 mM sodium acetate:10 mM acetic acid 88.5:11.5, pH 5.5.) [Note: Extraction of paramethadione is implied but not explicitly stated.]

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:buffer 28:72 (Buffer was 300 μL 1 M KH_2PO_4 and 50 μL 900 mM phosphoric acid in 1.8 L water, pH 4.4.)
Column temperature: 50
Flow rate: 2.8
Injection volume: 50-100
Detector: UV 195

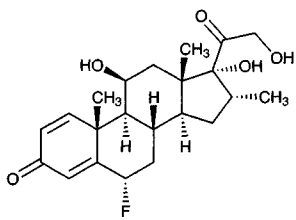
CHROMATOGRAM
Retention time: 3.8
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (11.5)

OTHER SUBSTANCES
Extracted: carbamazepine, ethosuximide, phenytoin, secobarbital
Simultaneous: mephobarbital, phenobarbital, primidone
Noninterfering: chlorazepate, clonazepam, diazepam, thioridazine, valproic acid

KEY WORDS
serum

REFERENCE
Levine,H.L.; Cohen,M.E.; Duffner,P.K.; Kustas,K.A.; Shen,D.D. An improved high-pressure liquid chromatographic assay for secobarbital in serum, *J.Pharm.Sci.*, **1982**, *71*, 1281–1283.

Paramethasone



Molecular formula: $C_{22}H_{29}FO_5$
Molecular weight: 392.47
CAS Registry No.: 53-33-8, 1597-82-6 (acetate)
Merck Index: 7162
Lednicer No.: 1 200

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 19.19
Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
Limit of detection: 1-10 ng/mL (245 nm)

OTHER SUBSTANCES
Extracted: betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluendrenolide, fluocinolone acetonide, fluorometholone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, prednisolone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone
Interfering: fluprednisolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J.Chromatogr.B*, **1995**, *666*, 347–353.

Parathyroid hormone

Molecular weight: ca. 9500
CAS Registry No.: 902-64-6
Merck Index: 7168

SAMPLE
Matrix: reaction mixtures
Sample preparation: Prepare a 40 μM solution in 100 mM pH 10.0 borate/NaOH/HCl buffer, add hydrogen peroxide to a concentration of 1 mM, inject an aliquot.

HPLC VARIABLES
Column: 150 × 6.4 YMC-Pack ODS-A A312 (YMC)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN:water 60:40. A:B from 60:40 to 50:50 over 10 min, to 40:60 over 40 min.
Flow rate: 1
Detector: UV 215

CHROMATOGRAM
Retention time: 41

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
recombinant

REFERENCE
Nabuchi,Y.; Fujiwara,E.; Ueno,K.; Kuboniwa,H.; Asoh,Y.; Ushio,H. Oxidation of recombinant human parathyroid hormone: Effect of oxidized position on the biological activity, *Pharm.Res.*, **1995**, *12*, 2049–2052.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-Hypersil
Mobile phase: Gradient. A was 155 mM NaCl containing 10 mM HCl, pH 2.1. B was MeCN. A:B 100:0 for 2.5 min, to 90:10 over 2.5 min, to 40:60 over 67 min.
Column temperature: 45
Flow rate: 1
Detector: UV 215 or bioassay

CHROMATOGRAM
Retention time: 40.5 (human), 43 (cow)

REFERENCE
Zanelli,J.M.; Kent,J.C.; Rafferty,B.; Nissenson,R.A.; Nice,E.C.; Capp,M.W.; O'Hare,M.J. High-performance liquid chromatographic methods for the analysis of human parathyroid hormone in reference standards, parathyroid tissue and biological fluids, *J.Chromatogr.*, **1983**, *276*, 55–68.

SAMPLE
Matrix: solutions

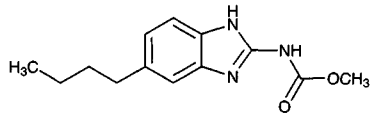
HPLC VARIABLES
Column: 250 × 4.6 RP C18 (Vydac)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was MeCN:0.1% aqueous trifluoroacetic acid 70:30. A: from 65:35 to 45:55 in 48 min, to 0:100 (step gradient), after 10 min return to initial conditions.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 32

KEY WORDS
human; recombinant

REFERENCE
Hogset,A.; Blingsmo,O.R.; Saether,O.; Gautvik,V.T.; Holmgren,E.; Hartmanis,M.; Josephson,S.; Gabrielsen,O.S.; Gordeladze,J.O.; Alestrom,P.; Gautvik,K.M. Expression and characterization of a recombinant human parathyroid hormone secreted by Escherichia coli employing the staphylococcal protein A promoter and signal sequence, *J.Biol.Chem.*, **1990**, *265*, 7338–7344.

Parbendazole



Molecular formula: $C_{13}H_{17}N_3O_2$
Molecular weight: 247.30
CAS Registry No.: 14255-87-9
Merck Index: 7169

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

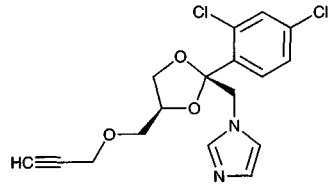
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM
Retention time: 18.15

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Parconazole



Molecular formula: $C_{17}H_{16}Cl_2N_2O_3$
Molecular weight: 367.23
CAS Registry No.: 61400-59-7, 62973-77-7 (HCl)
Lednicer No.: 3 133

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

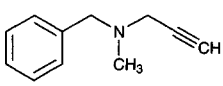
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 15.5

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pargyline



Molecular formula: $C_{11}H_{13}N$
Molecular weight: 159.23
CAS Registry No.: 555-57-7, 306-07-0 (HCl)
Merck Index: 7172

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

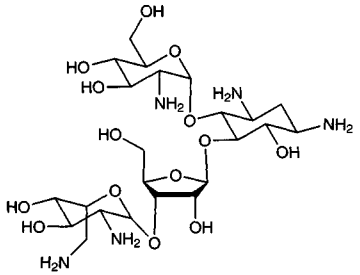
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Paromomycin



Molecular formula: $C_{23}H_{45}N_5O_{14}$
Molecular weight: 615.64
CAS Registry No.: 7542-37-2, 1263-89-4 (sulfate)
Merck Index: 7173

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 300 μL Plasma + 30 μL 101.4 μg/mL kanamycin B in water + 100 μL 2 M perchloric acid, vortex for 2-3 s, centrifuge at 1000 g for 5 min. Remove the supernatant and neutralize it with 1.5 M NaOH, add 300 μL buffer, add 400 μL DMSO, add 100 μL 2% 2,4-dinitrofluorobenzene in EtOH, vortex, heat at 64° for 30 min, add 3 mL toluene, vortex, centrifuge, discard the upper toluene layer, add 3 mL MeCN:toluene 50:50, vortex for 5-10 s. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 1 mL MeCN:water 50:50, inject a 20 μL aliquot. Urine. Dilute urine 100-fold with water. 300 μL Diluted urine + 30 μL 101.4 μg/mL kanamycin B in water + 300 μL buffer + 400 μL DMSO + 100 μL 2% 2,4-dinitrofluorobenzene in EtOH, vortex, heat at 64° for 30 min, add 3 mL toluene, vortex, centrifuge, discard the upper toluene layer, add 3 mL MeCN:toluene 50:50, vortex for 5-10 s. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 1 mL MeCN: water 50:50, inject a 20 μL aliquot. (Prepare buffer by mixing 80 mL 100 mM Na_2HPO_4 and 20 mL 100 mM NaH_2PO_4, adding 1 g Tris HCl, and adjusting the pH to 7.8 with 6 M HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax SB-C18
Mobile phase: MeOH:water 64:36, adjusted to pH 3.0 with phosphoric acid
Column temperature: 50
Flow rate: 2
Injection volume: 10-20
Detector: UV 350

CHROMATOGRAM
Retention time: 14.0
Internal standard: kanamycin B (24.0)
Limit of detection: 200 ng/mL (plasma), 500 ng/mL (urine)
Limit of quantitation: 500 ng/mL (plasma), 1 μg/mL (urine)

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Lu,J.; Cwik,M.; Kanyok,T. Determination of paromomycin in human plasma and urine by reversed-phase high-performance liquid chromatography using 2,4-dinitrofluorobenzene derivatization, *J.Chromatogr.B*, **1997**, *695*, 329–335.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 2 mg/mL solution in 20 mM pH 9.0 borate buffer, remove a 5 mL aliquot and add it to 15 mL 150 mM 2,4-dinitrofluorobenzene in MeOH (prepare fresh

daily), heat at 100° for 45 min, cool, make up to 250 mL with mobile phase, discard the upper aqueous phase, inject a 20 μL aliquot of the lower organic phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb SI-100
Mobile phase: Chloroform:THF:water 25:28.2:0.8
Flow rate: 1
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 12,25

KEY WORDS
normal phase; derivatization

REFERENCE
Tsuji,K.; Goetz,J.F.; VanMeter,W.; Gusciora,K.A. Normal-phase high-performance liquid chromatographic determination of neomycin sulfate derivatized with 1-fluoro-2,4-dinitrobenzene, *J.Chromatogr.*, **1979**, *175*, 141–152.

SAMPLE
Matrix: formulations
Sample preparation: Mix 2 g cream with 3 mL n-butanol, add 5 mL 2% sulfuric acid, mix thoroughly. Separate lower aqueous layer and re-extract the organic layer with another portion of sulfuric acid. Combine the aqueous layers and make up to 100 mL with water. Filter a portion of the extract through a 0.45 μm Nylon 66 syringe filter. Dilute filtrate with water, inject a 10 μL. aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Metachem Inertsil C8
Mobile phase: 200 mM Sodium sulfate containing 1.2 mM sodium 1-heptanesulfonate and 0.1% acetic acid
Flow rate: 1
Injection volume: 10
Detector: F ex 340 em 440 following post-column reaction. The column effluent mixed with reagent pumped at 1.0 mL/min and this mixture flowed through a 9 m × 0.25 mm I.D. stainless steel coil to the detector. (Reagent was 800 mg o-phthalaldehyde and 1 mL mercaptoethanol in 10 mL MeOH diluted to 1 L with 2.5% boric acid and adjusted to pH 10 with 2.5% KOH.)

CHROMATOGRAM
Retention time: 21.5
Limit of detection: 8 ng

OTHER SUBSTANCES
Also analyzed: gentamicin

KEY WORDS
cream; post-column reaction

REFERENCE
Pick,J.; Olson,L.L.; Ellis,W.Y.; Lim,P. Development and validation of a method to extract and quantitate paromomycin and gentamicin from an Aquaphilic cream formulation, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 131–137.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:water 17:83 containing 16 mM sodium 1-hexanesulfonate 20 mM Na_3PO_4, pH 3.5 (Connect a 250 × 4.6 column of Bondapak C18/Corasil or Co:Pell ODS between pump and injector. Flush column with MeOH:water 50:50 at the end of the day.)
Column temperature: 25
Flow rate: 1.5
Injection volume: 25
Detector: RI

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: neomycin

REFERENCE
Whall,T.J. Determination of streptomycin sulfate and dihydrostreptomycin sulfate by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *219*, 89–100.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 8 μm PLRP-S 1000 Å poly(styrene-divinylbenzene) (Polymer Laboratories)
Mobile phase: Water containing 70 g/L sodium sulfate, 1.4 g/L sodium 1-octanesulfonate, and 50 mL/L 200 mM pH 3.0 phosphate buffer
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: E, Dionex PED-1 pulsed electrochemical detector at 35°, 3 mm dia. gold working electrode, E_1 +0.05 V, E_2 +0.75 V, E_3 -0.15 V, t_1 0-0.40 s, t_2 0.41-0.60 s, t_3 0.61-1.00 s, measure signal between 0.2 and 0.4 s, stainless steel counter electrode, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.3 mL/min and the mixture flowed through a 1.2 m long 500 μL coil to the detector. (Prepare 500 mM NaOH solution by diluting 50% NaOH with helium-degassed water. Clean gold electrode after each 60 analyses.)

CHROMATOGRAM
Retention time: 9 (paromomycin II), 11 (paromomycin I)
Limit of detection: 5 ng
Limit of quantitation: 15 ng

OTHER SUBSTANCES
Simultaneous: neamine, neomycin B, neomycin C, neomycin LP-A, neomycin LP-B, paromamine

KEY WORDS
post-column reaction

REFERENCE
Adams,E.; Schepers,R.; Roets,E.; Hoogmartens,J. Determination of neomycin sulfate by liquid chromatography with pulsed electrochemical detection, *J.Chromatogr.A*, **1996**, *741*, 233–240.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Tissumizer) 1 g Ground tissue + 4 mL buffer at medium speed for 1 min, centrifuge at 3600 g for 20 min, remove the supernatant, re-homogenize pellet in 4 mL buffer for 10 min, centrifuge. Combine the supernatants, heat in a boiling water bath with occasional mixing for 5 min, centrifuge at 2000 g for 20 min, remove the supernatant, vortex the precipitate with 2 mL buffer for 30 s, centrifuge at 2000 g for 10 min. Combine the supernatants, acidify to pH 3.5-4 with 50-60 μL sulfuric acid, centrifuge at 2000 g for 10 min,

inject an aliquot of the supernatant. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water, pH 8.0.)

HPLC VARIABLES
Guard column: 10 μm RP-18
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 1.5:98.5 (Buffer was 10 mM sodium 1-pentanesulfonate, 56 mM sodium sulfate, and 7 mM acetic acid.)
Flow rate: 1.5
Detector: F ex 340 em 455 following post-column reaction with derivatization reagent pumped at 0.9 mL/min. (Derivatization reagent was commercially available (Pierce) or prepared by adding 2.5 mL 2-mercaptoethanol and 2.5 mL Brij-35 to 850 mg o-phthalaldehyde in 10 mL MeOH, mix until decolorization is complete, add 1 L buffer, filter (0.45 μm), and refrigerate until used. Buffer was prepared by adjusting pH of 250 mM boric acid to 9.5 with 5 M KOH.)

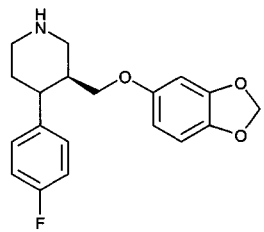
CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: neomycin
Simultaneous: dihydrostreptomycin, streptomycin

KEY WORDS
kidney; muscle; cow; pig; post-column reaction

REFERENCE
Shaikh,B.; Allen,E.H.; Gridley,J.C. Determination of neomycin in animal tissues, using ion-pair liquid chromatography with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 29–36.

Paroxetine



Molecular formula: $C_{19}H_{20}FNO_3$
Molecular weight: 329.37
CAS Registry No.: 61869-08-7
Merck Index: 7175
Lednicer No.: 5 87

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Carboxymethyl Isolute SPE cartridge with 1 mL MeOH and 1 mL 25 mM pH 6.8 phosphate buffer, dry under vacuum. Add 500 μL plasma to the SPE cartridge, wash with two 1 mL portions of 25 mM pH 6.8 phosphate buffer, dry under vacuum, elute with 1 mL 1% ammonia in MeOH, evaporate to dryness under vacuum at 40°, reconstitute the residue in 100 μL MeOH, vortex, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS/CN
Mobile phase: MeOH:50 mM pH 4.8 potassium phosphate buffer 70:30
Flow rate: 1
Injection volume: 25
Detector: E, ESA, Model 5100 A, Model 5010 analytical cell +650 mV on channel 1, +950 mV on channel 2, Model 5020 guard cell +980 mV

CHROMATOGRAM
Retention time: 9.6
Internal standard: paroxetine

inject an aliquot of the supernatant. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water, pH 8.0.)

HPLC VARIABLES
Guard column: 10 μm RP-18
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 1.5:98.5 (Buffer was 10 mM sodium 1-pentanesulfonate, 56 mM sodium sulfate, and 7 mM acetic acid.)
Flow rate: 1.5
Detector: F ex 340 em 455 following post-column reaction with derivatization reagent pumped at 0.9 mL/min. (Derivatization reagent was commercially available (Pierce) or prepared by adding 2.5 mL 2-mercaptoethanol and 2.5 mL Brij-35 to 850 mg o-phthalaldehyde in 10 mL MeOH, mix until decolorization is complete, add 1 L buffer, filter (0.45 μm), and refrigerate until used. Buffer was prepared by adjusting pH of 250 mM boric acid to 9.5 with 5 M KOH.)

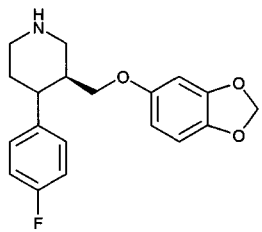
CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: neomycin
Simultaneous: dihydrostreptomycin, streptomycin

KEY WORDS
kidney; muscle; cow; pig; post-column reaction

REFERENCE
Shaikh,B.; Allen,E.H.; Gridley,J.C. Determination of neomycin in animal tissues, using ion-pair liquid chromatography with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 29–36.

Paroxetine



Molecular formula: $C_{19}H_{20}FNO_3$
Molecular weight: 329.37
CAS Registry No.: 61869-08-7
Merck Index: 7175
Lednicer No.: 5 87

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Carboxymethyl Isolute SPE cartridge with 1 mL MeOH and 1 mL 25 mM pH 6.8 phosphate buffer, dry under vacuum. Add 500 μL plasma to the SPE cartridge, wash with two 1 mL portions of 25 mM pH 6.8 phosphate buffer, dry under vacuum, elute with 1 mL 1% ammonia in MeOH, evaporate to dryness under vacuum at 40°, reconstitute the residue in 100 μL MeOH, vortex, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS/CN
Mobile phase: MeOH:50 mM pH 4.8 potassium phosphate buffer 70:30
Flow rate: 1
Injection volume: 25
Detector: E, ESA, Model 5100 A, Model 5010 analytical cell +650 mV on channel 1, +950 mV on channel 2, Model 5020 guard cell +980 mV

CHROMATOGRAM
Retention time: 9.6
Internal standard: paroxetine

OTHER SUBSTANCES
Extracted: desipramine, venlafaxine

KEY WORDS
plasma; SPE; paroxetine is IS

REFERENCE
Clement,E.M.; Odontiadis,J.; Franklin,M. Simultaneous measurement of venlafaxine and its major metabolite, oxydesmethylvenlafaxine, in human plasma by high-performance liquid chromatography with coulometric detection and utilisation of solid-phase extraction, *J.Chromatogr.B*, **1998**, *705*, 303–308.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 250 μL buffer + 200 μL water + 100 μL 25 ng/mL maprotiline in water + 4 mL toluene, extract on a tumble mixer for 15 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 50 μL acetone, add 25 μL 100 mM sodium bicarbonate, add 10 μL 1 mg/mL dansyl chloride in acetone (prepare fresh daily), vortex for 15 s, heat at 55° for 1 min, centrifuge for 1 min, let stand at room temperature for 30 min, add 25 μL 25 mg/mL L-proline in water (prepare fresh daily), vortex briefly, centrifuge for 1 min, let stand at room temperature for 5 min, add 500 μL water, add 2 mL toluene, agitate on a tumble mixer for 10 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject an aliquot. (Buffer was 8.6 g $Na_2HPO_4.12H_2O$ in 100 mL water, adjusted to pH 12.0 with 4 M NaOH, made up to 200 mL with water.)

HPLC VARIABLES
Guard column: 30 mm long 5 μm Spherisorb ODS
Column: 200 × 4 5 μm Spherisorb ODS
Mobile phase: MeOH:50 mM pH 4.5 sodium acetate buffer 84:16 (At the end of the day wash column with 95:5.)
Flow rate: 1.5
Injection volume: 100
Detector: F ex 340 em 520

CHROMATOGRAM
Retention time: 5.8
Internal standard: maprotiline (8.2)
Limit of detection: 0.2 ng/mL
Limit of quantitation: 0.5-1 ng/mL

OTHER SUBSTANCES
Noninterfering: cimetidine, digoxin, methyldopa, phenobarbital, phenytoin, procyclidine, tranylcypromine

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Brett,M.A.; Dierdorf,H.-D.; Zussman,B.D.; Coates,P.E. Determination of paroxetine in human plasma, using high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *419*, 438–444.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 75 μL 1.3 μg/mL norfemoxetine in water + 200 μL 1 M perchloric acid + 5 mL toluene, extract in a tumble mixer for 20 min, centrifuge at 1500 g for 10 min, let stand at -20° for 20 min. Remove the aqueous phase and add it to 750 μL 25 mM pH 12 phosphate buffer and 200 μL 1% lauryl sulfate, add 5 mL heptane:toluene 80:20, extract on a tumble mixer for 20 min, centrifuge at 2000 g for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 220-250 μL MeOH:pH 4.5 acetate buffer 30:70, mix thoroughly, inject a 180-200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 RP-Select B
Mobile phase: MeCN:EtOH:buffer 21:14:65 (Buffer was 50 mM acetic acid adjusted to pH 4.5 with 1 M NaOH containing 2 g/L (?) tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Injection volume: 180-200
Detector: UV 295

CHROMATOGRAM
Retention time: 11.5
Internal standard: norfemoxetine (9.5)
Limit of quantitation: 6 ng/mL

KEY WORDS
plasma

REFERENCE
Knoeller,J.; Vogt-Schenkel,R.; Brett,M.A. A simple and robust HPLC method for the determination of paroxetine in human plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 635–638.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.275

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: dialysate
Sample preparation: Inject dialysate onto column A and column B in series, elute with mobile phase, monitor the effluent from column B, after 15.3 min remove column B from the circuit, continue to elute column A with mobile phase, monitor the effluent from column A. (Serotonin elutes from column A and column B. The more highly retained paroxetine is eluted from the shorter column A.)

HPLC VARIABLES
Column: A 50 × 2.0 μm Nucleosil C18; B 250 × 2.1 5 μm Supelcosil LC-18-DB (Supelco, USA)
Mobile phase: MeCN:buffer 33:67 (Buffer was 0.23 mM 1-octanesulfonic acid sodium salt in 65 mM acetic acid, adjusted to pH 2.8 with glacial acetic acid.)
Flow rate: 0.127 for 13.7 min then 0.4
Injection volume: 10
Detector: F ex 280 em 340

CHROMATOGRAM
Retention time: 18.5
Limit of detection: 300 fmol
Limit of quantitation: 4.2 pmol

OTHER SUBSTANCES
Extracted: serotonin

KEY WORDS
brain; column switching; rat

REFERENCE
Ramaiya,A.; Karnes,H.T. Simultaneous measurement of serotonin and paroxetine in rat brain dialysate by a single-pump column-switching technique, *J.Chromatogr.B*, **1997**, *691*, 119–129.

SAMPLE
Matrix: formulations
Sample preparation: Extract tablets with mobile phase so as to give a paroxetine concentration of 400 μ g/mL.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM 1-decanesulfonic acid sodium salt containing 10 mM NaH_2PO_4, pH 3.0.)
Detector: UV 235

CHROMATOGRAM
Limit of quantitation: 800 ng/mL

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
tablets; stability-indicating

REFERENCE
Lambropoulos,J.; Spanos,G.A.; Lazaridis,N.V. Method development and validation for the HPLC assay in paroxetine 20 mg strength tablets (Abstract 3391), *Pharm.Res.*, **1997**, *14*, S591.

SAMPLE
Matrix: formulations
Sample preparation: Weight out powdered tablets equivalent to 20 mg paroxetine. Suspend the powder in three 5 mL portions of MeOH, stir for 30 min, filter, dry under a gentle stream of nitrogen. Reconstitute the residue in 20 mL 2-propanol. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralpak AD
Mobile phase: n-Hexane:EtOH:diethylamine 94:6:0.5 (At the end of each day wash column with ca. 100 mL n-hexane:2-propanol 90:10.)
Flow rate: 0.5
Injection volume: 20
Detector: UV 296

CHROMATOGRAM
Retention time: 39 (+), 45 (-)
Limit of detection: 2 ng
Limit of quantitation: 6 ng

KEY WORDS
chiral; tablets

REFERENCE
Ferretti,R.; Gallinella,B.; La Torre,F.; Turchetto,L. Validated chiral high-performance liquid chromatographic method for the determination of trans-(-)-paroxetine and its enantiomer in bulk and pharmaceutical formulations, *J.Chromatogr.B*, **1998**, *710*, 157–164.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

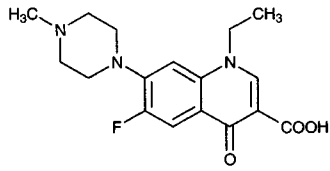
CHROMATOGRAM
Retention time: 11.06 (A), 5.77 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Pefloxacin



Molecular formula: $C_{17}H_{20}FN_3O_3$
Molecular weight: 333.36
CAS Registry No.: 70458-92-3, 70458-95-6 (mesylate)
Merck Index: 7197
Lednicer No.: 4 141

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 20 µL 20 µg/mL IS in MeOH. Vortex for 1 min with dichloromethane:diethyl ether 80:20, centrifuge at 1000 g for 10 min, separate the organic layer. Add 4 mL dichloromethane-diethyl ether 80:20, repeat the same extraction procedure twice, evaporate the organic phase to dryness under a stream of nitrogen, add 100 µL 10 mM NaOH to the residue, shake for 5 min, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 µm Vydac AXGU
Column: 250 × 4.6 5 µm Adsorbosphere SAX
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 9.2
Internal standard: 2-[4-(2-furoyl)phenyl]propionic acid (3.9)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: felbinac, fenbufen

KEY WORDS
plasma

REFERENCE
Carlucci,G.; Palumbo,G.; Mazzeo,P. Simple and rapid analysis of pefloxacin, fenbufen and felbinac in human plasma using high-performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1107–1115.

SAMPLE
Matrix: blood
Sample preparation: 200 µL Serum + 50 µL 400 µg/mL IS in water, vortex for 30 s. Add 500 µL MeCN, vortex for 1 min. Centrifuge at 6000 rpm for 10 min. Evaporate the supernatant to 200 µL at 40° under a stream of nitrogen, vortex for 30 s. Inject a 30-80 µL aliquot.

HPLC VARIABLES
Column: 100 × 8.0 4 µm Radial-pak Novapak C18
Mobile phase: MeCN:buffer 14:86 (Buffer was 2 g citric acid, 2 g sodium acetate, and 1 mL triethylamine in 1 L water.)
Flow rate: 2.5
Injection volume: 30-80
Detector: F ex 330 em 440

CHROMATOGRAM
Retention time: 5.2
Internal standard: acebutolol (7.4)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: norfloxacin
Simultaneous: ciprofloxacin, lomefloxacin, ofloxacin

KEY WORDS
serum; pharmacokinetics

REFERENCE
Abanmi,N.; Zaghloud,I.; El Sayed,N.; al-Khamis,K.I. Determination of pefloxacin and its main active metabolite in human serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1996**, *18*, 158–163.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 11:89 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1.2
Detector: UV 272

CHROMATOGRAM
Retention time: 9.97
Internal standard: pipemic acid (4.14)

OTHER SUBSTANCES
Simultaneous: norfloxacin

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL 10% trichloroacetic acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:18 mM KH_2PO_4 containing 0.13 mM heptanesulfonic acid:concentrated phosphoric acid 30:70:0.1
Injection volume: 20
Detector: F ex 277 em 475

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Griggs,D.J.; Wise,R. A simple isocratic high-pressure liquid chromatographic assay of quinolones in serum, *J.Antimicrob.Chemother.*, **1989**, *24*, 437–445.

SAMPLE
Matrix: blood
Sample preparation: Add two volumes of MeCN to plasma, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 5 μm Nucleosil C18
Mobile phase: MeOH:100 mM pH 4.9 phosphate buffer 50:50
Column temperature: 40
Flow rate: 1.2
Detector: F ex 275 em 415

CHROMATOGRAM
Limit of quantitation: 78 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jaehde,U.; Sörgel,F.; Stephan,U.; Schunack,W. Effect of an antacid containing magnesium and aluminum on absorption, metabolism, and mechanism of renal elimination of pefloxacin in humans, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1129–1133.

SAMPLE
Matrix: blood, dialysate
Sample preparation: 100 μL Plasma or dialysate + 400 μL MeOH, vortex, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: strong cation exchange
Mobile phase: MeCN:100 mM pH 3 citrate buffer 20:80
Detector: F ex 278 em 440

CHROMATOGRAM
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Rose,T.F.; Bremner,D.A.; Collins,J.; Ellis-Pegler,R.; Isaacs,R.; Richardson,R.; Small,M. Plasma and dialysate levels of pefloxacin and its metabolites in CAPD patients with peritonitis, *J.Antimicrob.Chemother.*, **1990**, *25*, 657–664.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 500 μL plasma with 5 μg IS, add 4 mL dichloromethane and 100 μL pH 7.4 phosphate buffer, agitate for 10 min, centrifuge at 5300 g for 10 min. Collect 3.5 mL organic phase. Add 4 mL dichloromethane to the aqueous phase again, agitate, centri-

fuge. Combine the organic phases, evaporate at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. Tissue. Mix 500 μL epiploic-fat and 4 mL dichloromethane and keep at 4°, add 5 μg IS, mix by using an automatic grinder (Ultra Turrax, Ika-Werk, Stauffen, Germany). Collect the mixture, centrifuge at 5300 g for 10 min. Add 4 mL 100 mM NaOH to the dichloromethane, agitate for 10 min, centrifuge at 5300 g for 5 min. Eliminate the organic phase, adjust the aqueous phase to pH 7.4 with concentrated trichloroacetic acid, add 4 mL dichloromethane, agitate for 10 min and centrifuge at 5300 g for 5 min. Evaporate the organic phase at 60°. Reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 4 5 μm 100 RP-18 Lichrosphere
Column: 125 × 4 5 μm 100 RP-18 endcapped Lichrosphere
Mobile phase: MeCN:pH 4.8 citrate buffer 85:15
Flow rate: 1
Injection volume: 20
Detector: F ex 330 em 418

CHROMATOGRAM
Internal standard: 4844P (pefloxacin analog)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: norfloxacin

KEY WORDS
epiploic-fat; plasma; pharmacokinetics; fat

REFERENCE
Jacoberger,B.; Ubeaud,G.; Freys,G.; Pottecher,T.; Jung,L.; Koffel,J.C. Concentrations of pefloxacin in plasma and tissue after administration as surgical prophylaxis, *Antimicrob.Agents Chemother.*, **1998**, *42*, 425–427.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Briefly vortex 500 μL plasma and 500 μL pH 7.4 phosphate buffer, add 5 mL dichloromethane:isopropanol 80:20, vortex for 30 s, shake gently for 15 min on an electric shaker, centrifuge at 1500 g for 10 min. Separate the lower layer using phase-separating filter paper (Whatman 1 PS), evaporate the organic phase under nitrogen at 25°, dissolve the residue in 100 μL mobile phase, inject a 20μL aliquot. Tissue. Pulverize prostatic tissue under liquid nitrogen, weigh a 200 mg aliquot, add 500 μL pH 7.4 phosphate buffer and 5 mL dichloromethane:isopropanol 80:20, vortex for 30 s, shake gently for 15 min on an electric shaker, centrifuge at 1500 g for 10 min. Separate the lower layer using phase-separating filter paper (Whatman 1 PS), evaporate the organic phase under nitrogen at 25°, dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (The pH 7.4 phosphate buffer was 28.2 g K_2HPO_4 and 5.17 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:pH 2.1 buffer 20.9:79.1 (Prepare the mobile phase by dissolving 18.1 g citric acid and 4.1 g ammonium perchlorate in about 300 mL distilled water, add 209 mL MeCN, dilute to 1 L with water, and add 3 mL tetrabutylammonium hydroxide. Filter through a 0.45 μm HV Millipore filter.)
Flow rate: 0.9
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 6.8
Internal standard: pefloxacin

OTHER SUBSTANCES
Extracted: enoxacin, 4-oxo-enoxacin

Noninterfering: amikacin, ciprofloxacin, fosfomycin, ofloxacin, rifampicin, roxithromycin, tobramycin, vancomycin

KEY WORDS
plasma; prostatic tissue; prostate; pefloxacin is IS

REFERENCE
Hamel,B.; Audran,M.; Costa,P.; Bressolle,F. Reversed-phase high-performance liquid chromatographic determination of enoxacin and 4-oxo-enoxacin in human plasma and prostatic tissue. Application to a pharmacokinetic study, *J.Chromatogr.A*, **1998**, *812*, 369–379.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize 100-250 mg tissue with 5 mL 500 mM pH 7.0 phosphate buffer, remove a 1 mL aliquot, add 100 µL 10 µg/mL IS, mix, add 10 mL chloroform: isopentanol 90:10, shake for 15 min, centrifuge , repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 100 µL 1% ammonia, inject a 25 µL aliquot. Plasma. 250-500 µL Plasma + 100 µL 10 µg/mL IS + 1 mL 500 mM pH 7.0 sodium phosphate buffer, mix, add 10 mL chloroform:isopentanol 90:10, shake for 15 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 100 µL 1% ammonia, inject a 25 µL aliquot.

HPLC VARIABLES
Column: 100 × 5 10 µm Nucleosil C18
Mobile phase: MeCN:water 15:85 containing 2 g/L sodium acetate trihydrate, 2 g/L citric acid monohydrate, and 1 mL/L triethylamine
Flow rate: 2
Injection volume: 25
Detector: F ex 330 em 440

CHROMATOGRAM
Retention time: 4.8
Internal standard: 1-allyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (Roger Bellon Laboratories) (6.6)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
prostate

REFERENCE
Montay,G.; Tassel,J.P. Improved high-performance liquid chromatographic determination of pefloxacin and its metabolite norfloxacin in human plasma and tissue, *J.Chromatogr.*, **1985**, *339*, 214–218.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 250 µL Plasma + 250 µL 10 µg/mL IS in water + 750 µL MeOH, stir, centrifuge at 2000 rpm for 5 min, inject a 50 µL aliquot of the supernatant. Urine. 500 µL Urine + 3.5 mL 8 µg/mL IS in water, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 Nucleosil C8
Mobile phase: MeCN:water:triethylamine:formic acid 11:87.5:0.1:1 containing 0.2% sodium acetate and 0.1% citric acid
Flow rate: 1
Injection volume: 50
Detector: F ex 280 em 450

CHROMATOGRAM
Internal standard: 1-ethyl-6-chloro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (RP 41983)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Humbert,G.; Brumpt,I.; Montay,G.; Le Liboux,A.; Frydman,A.; Borsa-Lebas,F.; Moore,N. Influence of rifampin on the pharmacokinetics of pefloxacin, *Clin.Pharmacol.Ther.*, **1991**, *50*, 682–687.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 278.3

CHROMATOGRAM
Retention time: 8.942

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve 40 mg freeze-dried nanoparticles in 25 mL acetone:MeOH 90:10 containing a few drops of 100 mM HCl, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil 10-ODS-1 C18
Mobile phase: MeCN:10 mM KH_2PO_4:triethylamine 14:86:0.2
Flow rate: 1.5
Injection volume: 20
Detector: UV 275

OTHER SUBSTANCES
Simultaneous: ofloxacin (UV 292)

KEY WORDS
nanoparticles

REFERENCE
Fresta,M.; Puglisi,G.; Giammona,G.; Cavallaro,G.; Micali,N.; Furneri,P.M. Pefloxacine mesilate and ofloxacin-loaded polyethylcyanoacrylate nanoparticles: Characterization of the colloidal drug carrier formulation, *J.Pharm.Sci.*, **1995**, *84*, 895–902.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) eye tissue with 3 mL 50 mM pH 5.8 sodium phosphate-citrate buffer and IS, centrifuge. Add the supernatant to 7 mL chloroform, agitate, centrifuge at 1000 g for 10 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Nucleosil C8
Mobile phase: MeCN:water 26:74 containing 2 g/L sodium acetate trihydrate, 2 g/L citric acid monohydrate, 4 mL/L triethylamine, and 2 mL/L formic acid, pH 4.8
Flow rate: 1
Injection volume: 5-20
Detector: UV 280

CHROMATOGRAM
Retention time: 1.89
Internal standard: 1-allyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (?) 4662 P (Roger-Bellon Laboratories) (2.95)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
rabbit; eye; pharmacokinetics

REFERENCE
Cochereau-Massin,I.; Bauchet,J.; Faurisson,F.; Vallois,J.M.; Lacombe,P.; Pocidalo,J.J. Ocular kinetics of pefloxacin after intramuscular administration in albino and pigmented rabbits, *Antimicrob.Agents Chemother.*, **1991**, *35*, 1112–1115.

SAMPLE
Matrix: urine
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:MeCN:100 mM pH 5.75 phosphate buffer 24.1:2.6:73.3
Flow rate: 1
Detector: F ex 275 em 415

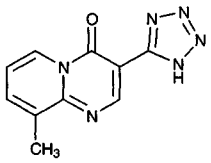
CHROMATOGRAM
Limit of quantitation: 780 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
pharmacokinetics

REFERENCE
Jaehde,U.; Sörgel,F.; Stephan,U.; Schunack,W. Effect of an antacid containing magnesium and aluminum on absorption, metabolism, and mechanism of renal elimination of pefloxacin in humans, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1129–1133.

Pemirolast



Molecular formula: $C_{10}H_8N_6O$
Molecular weight: 228.21
CAS Registry No.: 69372-19-6, 100299-08-9 (potassium salt)
Merck Index: 7205
Lednicer No.: 5 150

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 25 μL 2.5 μg/mL IS in water + 1 mL MeOH, mix, centrifuge at 13000 g for 5 min, inject a 50 μL aliquot of the supernatant.

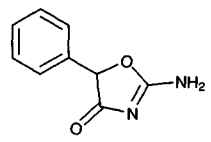
HPLC VARIABLES
Column: 100 × 9.4 5 μm ODS-3 RAC (Whatman)
Mobile phase: MeOH:water 45:55 containing 0.31% acetic acid
Flow rate: 1.5
Injection volume: 50
Detector: F ex 370 em 410 (360 nm cut-off filter)

CHROMATOGRAM
Retention time: 8.5
Internal standard: 7,9-dimethyl-3-(1H-tetrazol-5-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (BL 5609) (Mead Johnson) (13.3)
Limit of detection: 0.4 ng/mL
Limit of quantitation: 4.3 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Cheng,H.; Pittman,K.A.; Dandekar,K.A. Liquid chromatographic determination of 9-methyl-3-(1H-tetrazol-5-yl)-4H-pyrido[1,2-a]pyrimidin-4-one in human plasma with fluorescence detection, *J.Pharm.Sci.*, **1987**, *76*, 918–919.

Pemoline



Molecular formula: $C_9H_8N_2O_2$
Molecular weight: 176.17
CAS Registry No.: 2152-34-3
Merck Index: 7206

SAMPLE
Matrix: blood, tissue, urine

Sample preparation: Plasma. 100 μL Plasma + 500 μL 1 M pH 10 carbonate buffer + 4 mL dichloromethane, shake for 10 min, centrifuge at 1680 g for 5 min. Remove 3 mL of the organic layer and add it to 1 mL 1 μg/mL 5-methyl-5-phenylhydantoin in dichloromethane, evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot. Urine. 100 μL Urine + 500 μL 1 M pH 10 carbonate buffer + 4 mL dichloromethane, shake for 10 min, centrifuge at 1680 g for 5 min. Remove 1 mL of the organic layer and add it to 1 mL 4 μg/mL 5-methyl-5-phenylhydantoin in dichloromethane, evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 50 μL mobile phase, inject a 5 μL aliquot. Liver, kidney, lung, spleen, muscle. Homogenize on ice with 2 (liver, kidney, lung, spleen) or 3 (muscle) volumes ice-cold saline. 200 μL Homogenate + 500 μL 1 M pH 10 carbonate buffer + 4 mL dichloromethane, shake for 10 min, centrifuge at 1680 g for 5 min. Remove 3 mL of the organic layer and add it to 1 mL 1 μg/mL 5-methyl-5-phenylhydantoin in dichloromethane, evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot. Brain. Homogenize on ice with 2 volumes ice-cold saline. 200 μL Homogenate + 500 μL 1 M pH 10 carbonate buffer + 4 mL dichloromethane, shake for 10 min, centrifuge at 1680 g for 5 min. Remove 3 mL of the organic layer and add it to 1 mL 1 μg/mL 5-methyl-5-phenylhydantoin in dichloromethane, evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 50 μL mobile phase, centrifuge at 15300 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4 5 μm Kaseisorb LC C8-60-5 (Tokyo Kasei Kogo)
Mobile phase: MeCN:buffer 20:80 (Buffer was water adjusted to pH 5 with 15 mM phosphoric acid.)
Column temperature: 28
Flow rate: 0.7
Injection volume: 5-20
Detector: UV 215

CHROMATOGRAM

Retention time: 7
Internal standard: 5-methyl-5-phenylhydantoin (11)
Limit of detection: 5 ng (urine), 2 ng (tissue), 0.5 ng (plasma)

KEY WORDS

rat; plasma; brain; liver; kidney; lung; spleen; muscle; pharmacokinetics

REFERENCE

Aoyama,T.; Kotaki,H.; Saitoh,Y.; Nakagawa,F. Determination of pemoline in plasma, urine and tissues by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 351–360.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 1.50

OTHER SUBSTANCES

Simultaneous: phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenyl-

ephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

Interfering: benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recin-

namine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 4.91 (A), 3.84 (B)

OTHER SUBSTANCES

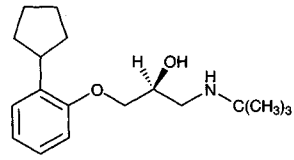
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluopromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Penbutolol



Molecular formula: $C_{18}H_{29}NO_2$
Molecular weight: 291.43
CAS Registry No.: 38363-40-5, 38363-32-5 (sulfate)
Merck Index: 7209

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 50 μL 100 ng/mL protriptyline hydrochloride in 2 M pH 10.6 aqueous Tris buffer + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject a 100 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 250 × 5 5 μm Spherisorb S5W
Mobile phase: Isooctane:MeOH:buffered MeOH:MTBE 55:15:10:20, apparent pH 5.7 (Buffered MeOH was 1 L 100 mM ammonium perchlorate in MeOH to which was added 10 mL 100 mM NaOH in MeOH, apparent pH 6.5.)
Flow rate: 2
Injection volume: 100
Detector: F ex 200 em no filter

CHROMATOGRAM
Retention time: 5.3
Internal standard: protriptyline (8)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: N-acetylprocainamide, ajmaline, atenolol, betaxolol, chlorpromazine, desipramine, dipyridamole, doxazosin, flecainide, gallopamil, imipramine, labetalol, metoprolol, mianserin, nadolol, norverapamil, orphenadrine, oxprenolol, pindolol, prajmaline, prazosin, procainamide, propranolol, quinidine, quinine, terazosin, trazodone, triamterene, verapamil
Noninterfering: acebutolol, amiodarone, desethylamiodarone, disopyramide, lidocaine, lorcainide, methyldopa, nifedipine, propafenone, sotalol, timolol, tocainide
Interfering: mexiletine, pyrimethamine

KEY WORDS
plasma; serum; normal phase

REFERENCE
Bhamra,R.K.; Flanagan,R.J.; Holt,D.W. Measurement of penbutolol and 4-hydroxypenbutolol in plasma or serum by HPLC, *Biomed.Chromatogr.*, **1986**, *1*, 140–142.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma or 0.5 mL plasma water + 1 mL 1 M NaOH + 12 mL n-heptane:isoamyl alcohol 98.5:1.5, shake mechanically for 10 min, centrifuge at 1680 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 50 μL EtOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hitachi Gel 3013 spherical styrene-divinylbenzene

Mobile phase: EtOH:buffer 65:35 (Buffer was 20 mM pH 2.0 perchloric acid/sodium perchlorate.)
Column temperature: 30
Flow rate: 0.2
Injection volume: 10
Detector: F ex 285 em 340

CHROMATOGRAM

Retention time: 18
Internal standard: penbutolol

OTHER SUBSTANCES

Extracted: propranolol
Simultaneous: quinidine, reserpine
Noninterfering: allopurinol, benzbromarone, diazepam, digoxin, diltiazem, dipyridamole, disopyramide, furosemide, isosorbide dinitrate, maprotiline, nifedipine, nitrazepam, trichlormethiazide, verapamil

KEY WORDS

penbutolol is IS; plasma; plasma water

REFERENCE

Yamamura,Y.; Uchino,K.; Kotaki,H.; Isozaki,S.; Saitoh,Y. Quantitative determination of propranolol in plasma and plasma water from normal subjects and patients with angina pectoris by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 311–319.

SAMPLE

Matrix: blood
Sample preparation: 100-500 μL Plasma + 5 ng bufarolol, mix, add 4 mL 500 mM pH 7.0 potassium phosphate buffer, add to a Sep-Pak C18 SPE cartridge, wash with 5 mL water, wash with 5 mL EtOH:water 30:70, elute with 5 mL EtOH:methylamine 99.9:0.1, evaporate to dryness, add 100 μL 2 mg/mL (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide in MeCN containing 0.1% quinuclidine, heat at 60° for 20 min, add 50 μL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL in EtOH:water 90:10, add to an 18 × 6 column packed with 100 mg carboxymethyl Sephadex LH-200, wash with EtOH:water 90:10 at 0.2 mL/min, elute with 5 mL 100 mM methylamine in EtOH:water 90:10. Evaporate the eluate to dryness, reconstitute with 50-100 μL mobile phase, inject an aliquot. (Derivatization occurs on the alcohol. Preparation of (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide is as follows. Treat 1-bromo-2-naphthol with sodium hydride in DMF, add iodomethane, stir at room temperature overnight to obtain 1-bromo-2-methoxynaphthalene (mp 85-86°). Add a solution of 37.7 g 1-bromo-2-methylnaphthalene in 200 mL ether over 1 h to a sonicated mixture of 7 g magnesium turnings in 50 mL ether, the mixture should reflux rapidly (Caution! There may be in an induction period!), sonicate for 2 h after addition is complete, add 200 mL benzene (Caution! Benzene is a carcinogen!), add this mixture dropwise to a stirred mixture of 100 mmoles 1-bromo-2-methoxynaphthalene and 655 mg bis(triphenylphosphine)nickel(II) chloride ($NiCl_2(PPh_3)_2$) in 150 mL benzene at room temperature over 1 h, stir at room temperature overnight, reflux for 3 h, remove the ether by distillation through a short Vigreux column, remove the solvent by evaporation under reduced pressure, remove excess 1-bromo-2-methylnaphthalene by heating at 150°/0.1 mm Hg, cool, dissolve the residue in hexane, pass through silica gel, evaporate to dryness, recrystallize from hexane to obtain 1-methoxy-2'-methylbinaphthalene (mp 118-121°). Reflux 10 mmoles 1-methoxy-2'-methyl-binaphthalene, 1.96 g N-bromosuccinimide, and 100 mg benzoyl peroxide in 70 mL carbon tetrachloride for 3 h, filter, evaporate the filtrate to obtain crude 1-bromomethyl-2'-methoxy-binaphthalene. Dissolve the crude 1-bromomethyl-2'-methoxy-binaphthalene in 60 mL DMSO under nitrogen, slowly add a sodium ethoxide/nitropropane mixture, stir at room temperature for 3 h, stir at 60° for 3 h, pour into 300 mL ice-water, extract with dichloromethane, wash with 2 M HCl, wash with 1 M sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to obtain crude 2'-methoxy-1,1'-binaphthalene-2-carboxaldehyde. (Prepare the sodium ethoxide/nitropropane mixture by dissolving 580 mg sodium in 35 mL EtOH, add 3.25 g 2-nitropropane.) Reflux the crude 2'-methoxy-1,1'-binaphthalene-2-carboxaldehyde in 60 mL acetone, add a solution of 2.36 g potassium permanganate in 60 mL hot water dropwise over 1 h, heat for an additional hour, pass sulfur dioxide through the solution until it becomes clear (sodium metabisulfite may work). Filter off the precipitate and dissolve it in 200 mL hot toluene, add a small amount of activated charcoal, filter while hot, concentrate to about a third of the volume, recrystallize

from EtOH:water 1:2 to obtain 2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (mp 258.5-260°) (Bull. Chem. Soc. Japan 1986, 59, 2044). Reflux 9.15 g racemic 2'-methoxy-1,1'-binaphthalene-2-carboxylic acid in 55 mL freshly distilled thionyl chloride for 5 h, evaporate under reduced pressure, add a little benzene, evaporate under reduced pressure, repeat the benzene evaporation twice more to obtain 2'-methoxy-1,1'-binaphthalene-2-carbonyl chloride as a brown solid. Dissolve the acid chloride in 70 mL benzene, add dropwise to 12.8 g (-)-menthol in 100 mL benzene containing 1 g 4-dimethylaminopyridine and 5 mL pyridine, stir overnight at room temperature, heat at 70° for 3 h, cool, dilute with benzene, wash with 2 M HCl, wash with 1 M sodium carbonate, wash with water, dry over anhydrous magnesium sulfate in the presence of activated charcoal, evaporate to dryness, remove as much menthol as possible by sublimation under vacuum, chromatograph twice on a column of silica gel with toluene to obtain the (aS,R) menthol ester (mp 145-146° from hexane) and the (aR,R) menthol ester (mp 126-129° from hexane) as well as a mixture of diastereomers. Reflux the (aS,R) menthol ester with KOH in aqueous EtOH for 8-10 h to obtain (aS)-2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (Bull. Chem. Soc. Japan 1989, 62, 1528). Add 1.5 mL oxalyl chloride to a solution of (aS)-2'-methoxy-1,1'-binaphthalene-2- carboxylic acid in 10 mL anhydrous benzene, reflux for 10 h, evaporate to dryness under reduced pressure. Take up the residue in 10 mL anhydrous benzene, add 1 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 5 h, evaporate to dryness, recrystallize from hexane/acetone to obtain (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide as orange-yellow needles (mp 143-146°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Kyoto)
Mobile phase: Hexane:ethyl acetate:triethylamine 83.3:16.7:0.005
Flow rate: 2
Detector: F ex 290 em 405

CHROMATOGRAM

Retention time: 10.5 (R), 13.5 (S)
Internal standard: bufarolol (8.5 (R), 12 (S))
Limit of detection: 30 pg

KEY WORDS

derivatization; plasma; chiral; normal phase; dog; SPE; pharmacokinetics

REFERENCE

Goto,J.; Shao,G.; Ito,M.; Kuriki,T.; Nambara,T. High-performance liquid chromatographic determination of penbutolol enantiomers in plasma with fluorescence detection, *Anal.Sci.*, **1991**, *7*, 723–726.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 270

CHROMATOGRAM

Retention time: 8.65
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Evaporate 0.5 mL 100 ng/mL propranolol in MeOH to dryness in a glass tube under a stream of nitrogen at 37°, add 1 mL plasma or urine, add 500 μL 1 M NaOH, add 8 mL freshly distilled diethyl ether, shake on a rotating shaker at 60 rpm for 15 min, centrifuge at 1200 g for 15 min. Remove 6.5 mL of the organic layer and pass it through a 20 × 4 column filled with glass wool, evaporate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. For conjugated compounds proceed as follows. Evaporate 0.5 mL 100 ng/mL propranolol in MeOH to dryness in a glass tube under a stream of nitrogen at 37°, add 1 mL plasma or urine, add 1 mL 100 mM pH 5 acetate buffer, add 100 μL solution containing 10000 U/mL β-glucuronidase and 0.6 U/mL sulfatase (Sigma), heat at 37° for 48 h, cool to room temperature, add 500 μL 1 M NaOH, add 8 mL freshly distilled diethyl ether, shake on a rotating shaker at 60 rpm for 15 min, centrifuge at 1200 g for 15 min. Remove 6.5 mL of the organic layer and add it to 6 mL 100 mM HCl, shake at 60 rpm on a rotating shaker for 15 min, centrifuge at 1200 g for 10 min. Remove 5.5 mL of the aqueous layer and add it to 700 μL 1 M NaOH, add 6 mL diethyl ether, shake on a rotating shaker at 60 rpm for 15 min, centrifuge at 1200 g for 10 min, remove 5 mL of the organic layer, evaporate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChrosorb RP-8

Mobile phase: MeCN:buffer 48:52 containing 1 g/L sodium heptanesulfonate (Buffer was 100 mM citric acid-sodium citrate buffer adjusted to pH 2.85 with 1 M HCl.)
Column temperature: 28
Flow rate: 1.7
Injection volume: 20
Detector: F ex 278 em 310

CHROMATOGRAM
Retention time: 7.75
Internal standard: propranolol (F ex 290 em 330) (4)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 4-hydroxypenbutolol (F ex 290 em 330)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Bernard,N.; Cuisinaud,G.; Sassard,J. Determination of penbutolol and its hydroxylated metabolite in biological fluids by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *228*, 355–361.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma + 100 µL 400 ng/mL propranolol in water + 20 µL 25 mg/mL ascorbic acid in water (prepare fresh daily) + 500 µL buffer, vortex, add 6 mL hexane:n-butanol 96:4, shake vigorously for 2 min, centrifuge briefly. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL MeOH:21 mM pH 5.5 ammonium acetate buffer 50:50, vortex, inject a 50 µL aliquot. To deconjugate samples proceed as follows. 300 µL Plasma or urine + 50 µL 200 mg/mL sodium bisulfite in water (prepare fresh daily) + 600 µL glucuronidase solution, vortex, flush tube with nitrogen, heat at 45 for 2 h, add 100 µL 400 ng/mL propranolol in water, add 20 µL 25 mg/mL ascorbic acid in water (prepare fresh daily), add 500 µL buffer, vortex, add 6 mL hexane:n-butanol 96:4, shake vigorously for 2 min, centrifuge briefly. Remove 5 mL of the organic layer and wash it with 2 mL buffer:25 mg/mL ascorbic acid 100:1. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL MeOH:21 mM pH 5.5 ammonium acetate buffer 50:50, vortex, inject a 50 µL aliquot. (Buffer was prepared by mixing saturated sodium carbonate and saturated sodium bicarbonate to pH 9.4. Glucuronidase solution was 50000 U type G-0258 (abalone entrails) and 40000 U type L-II (limpet) glucuronidases (Sigma) in 10 mL 50 mM pH 5 acetate buffer.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeOH:THF:buffer 60:6:34 (Buffer was 0.8 g ammonium acetate in 340 mL water, pH adjusted to 5.5 with acetic acid (if necessary).)
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: F ex 275 (slit width 6 nm) em 324 (slit width 10 nm)

CHROMATOGRAM
Retention time: 10
Internal standard: propranolol (8)
Limit of detection: 20 ng/mL (urine), 6 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: carazolol, metoprolol, physostigmine
Noninterfering: acetaminophen, aspirin, atenolol, bromocriptine, chloroquine, doxorubicin, hydrochlorothiazide, indomethacin, 17-methyltestosterone, nadolol, nandrolone, practolol, quinidine, salicyluric acid, sulfadiazine, sulfamerazine, sulfamethazine, timolol, triamterene, vinzolidine, warfarin

Interfering: pergolide

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Miner,D.J.; Binkley,D.A.; Bechtol,L.D. Liquid-chromatographic determination of penbutolol and its principal metabolites in plasma and urine, *Clin.Chem.*, **1984**, *30*, 717–723.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.928

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan,

benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve 100 ng penbutolol and 200 μg (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide in 200 μL 0.01% quinuclidine in MeCN, heat at 60° for 10 min, inject an aliquot. (Synthesis of (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide is as follows. Reflux 210 g 1-bromo-2-methylnaphthalene, 160 g N-bromosuccinimide, 1 g benzoyl peroxide, and 250 mL carbon tetrachloride for 2.5 h, add 250 mL carbon tetrachloride, filter while warm, wash the residue several times with solvent. Concentrate and cool the filtrate to give 1-bromo-2-bromomethylnaphthalene (mp 230-240°) (J. Org. Chem. 1949, 14, 375). Dissolve 90 g 1-bromo-2-bromomethylnaphthalene in 400 mL chloroform, reflux, add 46.5 g powdered hexamine in portions, remove the hexaminium salt by filtration. Reflux this salt in 650 mL 50% acetic acid for 1 h, add 105 mL concentrated HCl, reflux for 5 min, cool, obtain 1-bromo-2-naphthaldehyde (mp 119-120°) by filtration. Heat 11 g 1-bromo-2-naphthaldehyde in 275 mL acetone at 60-68°, add a hot solution of 14 g potassium permanganate in 330 mL water over 30 min, heat for another 30 min, pass in sulfur dioxide (sodium metabisulfite ?) until the solution is clear, pour into water to give 1-bromo-2-naphthoic acid, purify by forming the ammonium salt and reprecipitating. Reflux 1-bromo-2-naphthoic acid in MeOH in the presence of sulfuric acid to give methyl 1-bromo-2-naphthoate. Heat methyl-1-bromo-2-naphthoate with copper bronze at 270-280° for 20 min, while still hot extract with toluene, cool to obtain dimethyl 1,1'-binaphthalene-2,2'- dicarboxylate, obtain more crystals by evaporating some of the solvent, recrystallize from EtOH to give dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate (mp 158°) (J. Chem. Soc. 1955, 1242). Add 8 g lithium tri-tert-butoxyaluminohydride in portions to 2.8 g dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate in 150 mL anhydrous benzene:ether 50:50 (Caution! Benzene is a carcinogen!), heat at 80° for 2 h, acidify with 5% HCl. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness, chromatograph on 50 g silica gel with

hexane:ethyl acetate 80:20, recrystallize the product from hexane/acetone to give methyl 2-hydroxymethyl-1,1'-binaphthalene-2'-dicarboxylate (mp 117.5-118.5°). Add 5 mL 30% hydrogen bromide in acetic acid to 2 g methyl 2-hydroxymethyl-1,1'- binaphthalene-2'-dicarboxylate in 10 mL acetic acid, stir at 50° for 10 min, pour into ice-water, filter, chromatograph the solid on 40 g silica gel with hexane:ethyl acetate 30:1 to give methyl 2-bromomethyl-1,1'-binaphthalene-2'-dicarboxylate as pale yellow needles (mp 137-138°). Add 400 mg sodium borohydride to 1.9 g methyl 2-bromomethyl-1,1'-binaphthalene-2'-dicarboxylate in 10 mL DMSO, stir at 60° for 15 min, pour into ice-water, acidify with concentrated HCl, chromatograph the crude product on 40 g silica gel with hexane:ethyl acetate 10:1, recrystallize from MeOH to give methyl 2-methyl-1,1'-binaphthalene-2'-carboxylate as colorless needles mp 97-98°. Add 30 mL 10% KOH to 1.2 g methyl 2-methyl-1,1'-binaphthalene-2'-carboxylate in 50 mL MeOH, reflux for 3 h, pour into ice-water, filter, recrystallize from hexane/ethyl acetate to give 2-methyl-1,1'-binaphthalene-2'-carboxylic acid as colorless needles (mp 232-233°). Add 4.1 g (-)-brucine in 20 mL EtOH to 3.3 g 2-methyl-1,1'-binaphthalene-2'-carboxylic acid dissolved in 60 mL EtOH, allow to stand overnight, filter, recrystallize the precipitate several times from EtOH. Add 5% HCl to the salt and extract with ethyl acetate, wash the organic layer with water, dry over anhydrous sodium sulfate, evaporate to dryness, recrystallize from hexane/acetone to give (-)-2-methyl-1,1'-binaphthalene-2'-carboxylic acid as colorless needles (mp 229-229.5°; $[\alpha]_D^{20}$ -41.3° (c = 0.58 in chloroform). Add 3 mL oxalyl chloride to 500 mg (-)-2-methyl-1,1'-binaphthalene-2'-carboxylic acid in 30 mL anhydrous dichloromethane, stir at room temperature for 2 h, evaporate to give an oily residue, take up in 10 mL dichloromethane, add 2 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, chromatograph on 5 g silica gel with hexane to give (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide as a yellow oil ($[\alpha]_D^{20}$ -42.8° (c = 1.05 in chloroform).)

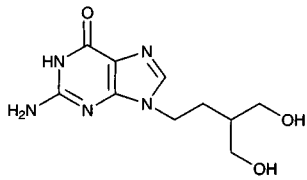
HPLC VARIABLES
Column: 150 × 4.6 5 μm spherical silica (Waters)
Mobile phase: Hexane:chloroform:MeOH 100:5:0.3
Detector: F ex 342 em 420

CHROMATOGRAM
Retention time: 11 (+), 12 (-)
Limit of detection: 200 pg

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Goto,J.; Goto,N.; Shao,G.; Ito,M.; Hongo,A.; Nakamura,S.; Nambara,T. Fluorescence chiral derivatization reagents for high performance liquid chromatographic resolution of enantiomeric hydroxyl compounds, *Anal.Sci.*, **1990**, *6*, 261–264.

Penciclovir



Molecular formula: $C_{10}H_{15}N_5O_3$
Molecular weight: 253.26
CAS Registry No.: 39809-25-1
Merck Index: 7210

SAMPLE
Matrix: reaction mixtures
Sample preparation: Filter (0.45 μm) a reaction mixture containing activated sludge, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-AQ C18 (YMC)
Mobile phase: MeOH:23 mM pH 7.0 potassium phosphate buffer 5:95
Column temperature: 30
Flow rate: 1

Injection volume: 20
Detector: UV 260

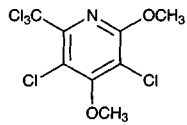
CHROMATOGRAM
Retention time: 17
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hsu,L.C.; Constable,D.J.C.; Orvos,D.R.; Hannah,R.E. Comparison of high-performance liquid chromatography and capillary zone electrophoresis in penciclovir biodegradation kinetic studies, *J.Chromatogr.B*, **1995**, *669*, 85–92.

Penclomedine



Molecular formula: $C_8H_6Cl_5NO_2$
Molecular weight: 325.41
CAS Registry No.: 108030-77-9

SAMPLE
Matrix: bile, blood, tissue
Sample preparation: Plasma, red cells. Add 1 mL plasma or red cells to 2.8 mL ethyl acetate and 200 μL 700 mM pH 2.7 ammonium phosphate buffer, homogenize, centrifuge. Draw off the organic layer, add 50 μL dimethyl sulfoxide, evaporate to approximately 100 μL under nitrogen, add 50 μL MeCN, inject an aliquot. Liver slices. Add 1 mL incubation to 500 μL Krebs-Henseleit buffer containing 2.25% bovine serum albumin, homogenize, centrifuge. Draw off the organic layer, add 50 μL dimethyl sulfoxide, evaporate to approximately 100 μL under nitrogen, add 50 μL MeCN, inject an aliquot. Bile. Dilute bile with an equal volume mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere HS C18 (Alltech)
Mobile phase: Gradient. A was MeCN. B was 10 mM pH 2.7 ammonium phosphate buffer. A:B from 0:100 to 100:0 in 25 min, maintain at 100:0 for 15 min.
Flow rate: 1
Detector: UV 240; Radioactivity, Radiomatic Flo-One/Beta A140, with 500 μL cell, using Flo-Scint VI scintillant at ratio 2:1

CHROMATOGRAM
Retention time: 32

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver; mouse; ^{14}C labeled; plasma; red cells; erythrocytes

REFERENCE
Hartman,N.R.; Leo,K.U.; Brewer,T.G.; Strong,J.M. The in vitro metabolism of penclomedine in mouse, rat, and human systems, *Drug Metab.Dispos.*, **1998**, *26*, 513–519.

SAMPLE
Matrix: microsomal incubations

Sample preparation: Microsomes. Add 1 mL microsomal incubation to 2.8 mL ethyl acetate and 200 μL 700 mM pH 2.7 ammonium phosphate buffer, vortex, centrifuge. Draw off the organic layer, add 50 μL dimethyl sulfoxide, evaporate to approximately 100 μL under nitrogen, add 50 μL MeCN to the residue, inject an aliquot. Liver slices. Mix 1 mL microsomal incubation with 500 μL Krebs-Henseleit buffer containing 2.25% bovine serum albumin, homogenize, centrifuge. Draw off the organic layer, add 50 μL dimethyl sulfoxide, evaporate to approximately 100 μL under nitrogen. Reconstitute the residue with 50 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Adsorbosphere HS C18 (Alltech)
Mobile phase: Gradient. A was MeCN. B was 10 mM pH 2.7 ammonium phosphate buffer. A:B from 0:100 to 100:0 over 25 min, maintain at 100:0.
Flow rate: 1
Detector: UV 240; Radioactivity, Radiomatic Flo-One/Beta A140, with 500 μL cell, using Flo-Scint VI scintillant at ratio 2:1

CHROMATOGRAM

Retention time: 32

OTHER SUBSTANCES

Extracted: metabolites

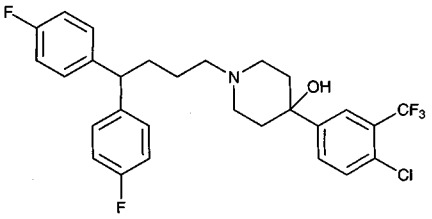
KEY WORDS

human; liver; mouse; ^{14}C labeled

REFERENCE

Hartman,N.R.; Leo,K.U.; Brewer,T.G.; Strong,J.M. The in vitro metabolism of penclomedine in mouse, rat, and human systems, *Drug Metab.Dispos.*, **1998**, *26*, 513–519.

Penfluridol



Molecular formula: $C_{28}H_{27}ClF_5NO$
Molecular weight: 523.97
CAS Registry No.: 26864-56-2
Merck Index: 7213
Lednicer No.: 2 334

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 266

CHROMATOGRAM

Retention time: 18.25

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

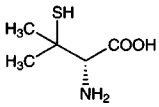
Detector: UV 200.5

CHROMATOGRAM
Retention time: 20.183

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Penicillamine



Molecular formula: $C_5H_{11}NO_2S$
Molecular weight: 149.21
CAS Registry No.: 52-67-5
Merck Index: 7214

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 150 μL 25% trichloroacetic acid, vortex, cool on ice for 10 min, centrifuge at 6500 g for 2 min. Remove a 500 μL aliquot of the supernatant and add it to 200 μL 1% NaOH in water, add 250 μL buffer, add 1 mL 1 mM N-[p-(2-benzoxazolyl)phenyl] maleimide (Eastman) in EtOH, heat at 37° overnight, inject a 50 μL aliquot. (Buffer was 500 mM sodium citrate adjusted to pH 5.0 with perchloric acid.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:100 μM sodium acetate 48:52
Flow rate: 2
Injection volume: 50
Detector: F ex 319 em 360 (cutoff filter)

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 250 nM
Limit of quantitation: 1 μM

KEY WORDS
derivatization; plasma

REFERENCE
Miners,J.O.; Fearnley,I.; Smith,K.J.; Birkett,D.J.; Brooks,P.M.; Whitehouse,M.W. Analysis of D-penicillamine in plasma by fluorescence derivatisation with N-[*p*-(2-benzoxazolyl)-phenyl] maleimide and high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *275*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma and 400 μL 18% (w/v) trichloroacetic acid in a 100 × 15 polypropylene tube, let stand at 0° for 5 min, centrifuge at 4° at 1700 g for 10 min, remove the supernatant as completely as possible. Suspend the precipitate in 1 mL 5% (w/v) trichloroacetic acid by stirring magnetically, centrifuge at room temperature at 2000 g for 5 min, discard the supernatant, repeat this washing step. Air-dry the precipitate then blanket it with nitrogen, add 2 mL 200 mM pH 8.0 Tris buffer, pass nitrogen over the mixture for 1 h, add 100 μL 250 mM EDTA, add 50 μL octanol, add 100 mg solid sodium borohydride, remove the

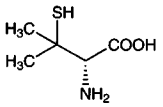
Detector: UV 200.5

CHROMATOGRAM
Retention time: 20.183

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Penicillamine



Molecular formula: $C_5H_{11}NO_2S$
Molecular weight: 149.21
CAS Registry No.: 52-67-5
Merck Index: 7214

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 150 µL 25% trichloroacetic acid, vortex, cool on ice for 10 min, centrifuge at 6500 g for 2 min. Remove a 500 µL aliquot of the supernatant and add it to 200 µL 1% NaOH in water, add 250 µL buffer, add 1 mL 1 mM N-[p-(2-benzoxazolyl)phenyl] maleimide (Eastman) in EtOH, heat at 37° overnight, inject a 50 µL aliquot. (Buffer was 500 mM sodium citrate adjusted to pH 5.0 with perchloric acid.)

HPLC VARIABLES
Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: MeOH:100 µM sodium acetate 48:52
Flow rate: 2
Injection volume: 50
Detector: F ex 319 em 360 (cutoff filter)

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 250 nM
Limit of quantitation: 1 µM

KEY WORDS
derivatization; plasma

REFERENCE
Miners,J.O.; Fearnley,I.; Smith,K.J.; Birkett,D.J.; Brooks,P.M.; Whitehouse,M.W. Analysis of D-penicillamine in plasma by fluorescence derivatisation with N-[*p*-(2-benzoxazolyl)-phenyl] maleimide and high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *275*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma and 400 µL 18% (w/v) trichloroacetic acid in a 100 × 15 polypropylene tube, let stand at 0° for 5 min, centrifuge at 4° at 1700 g for 10 min, remove the supernatant as completely as possible. Suspend the precipitate in 1 mL 5% (w/v) trichloroacetic acid by stirring magnetically, centrifuge at room temperature at 2000 g for 5 min, discard the supernatant, repeat this washing step. Air-dry the precipitate then blanket it with nitrogen, add 2 mL 200 mM pH 8.0 Tris buffer, pass nitrogen over the mixture for 1 h, add 100 µL 250 mM EDTA, add 50 µL octanol, add 100 mg solid sodium borohydride, remove the

hydrogen line, allow hydrogen to vent through a pin-hole in the cap, stir slowly, after 10 min cool in ice and slowly add 1 mL ice-cold 2 M perchloric acid, stir briefly, centrifuge an aliquot at room temperature at 8000 g for 30 s, remove 200 μL of the supernatant and add it to 10 μL 1 mM L-cysteine in water, inject an aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm C18 "Short One" (Rainin)
Mobile phase: MeCN:buffer 6:94 (Buffer was 100 mM pH 3.0 monochloroacetic acid/NaOH containing 1 g/L heptanesulfonic acid.) (Rigorously degas mobile phase by refluxing, negative-pressure filtration, and passing helium through it for 3 h before starting the assay.)
Flow rate: 0.6
Injection volume: 20-50
Detector: E, Bioanalytical Systems BAS LC-4B/19, BAS TL-6A Au/Hg working electrode + 150 mV, glassy carbon auxiliary electrode, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 4.66
Limit of detection: 1.2 μM

KEY WORDS

plasma

REFERENCE

Joyce,D.A.; Wade,D.N. Assay for D-penicillamine-protein conjugate in human plasma utilising chemical reduction followed by high-performance liquid chromatography with gold/mercury electrochemical detection, *J.Chromatogr.*, **1988**, *430*, 319–327.

SAMPLE

Matrix: blood
Sample preparation: 1 Volume plasma + 0.4 volume 10 mM monobromobimane (Calbiochem; Molecular Probes, Eugene OR) + 0.01 volume 1 M acetic acid + 2.5 volume MeCN, mix, centrifuge at 4° at 1000 g for 10 min, filter (5 μm) the supernatant, inject a 100 μL aliquot.

HPLC VARIABLES

Column: μBondapak C18 radial compression
Mobile phase: MeOH:water:glacial acetic acid 22:77.75:0.25, pH adjusted to 3.9 with NaOH. (After each injection wash column with MeOH at 4 mL/min for 10 min.)
Flow rate: 1.5
Injection volume: 100
Detector: F ex 365 em 418

CHROMATOGRAM

Retention time: 13.60
Internal standard: d-penicillamine
Limit of detection: 10 nM

OTHER SUBSTANCES

Extracted: cysteine, glutathione, homocysteine

KEY WORDS

derivatization; plasma; penicillamine is IS

REFERENCE

Velury,S.; Howell,S.B. Measurement of plasma thiols after derivatization with monobromobimane, *J.Chromatogr.*, **1988**, *424*, 141–146.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Plasma + 10 μL 25 mM monobromobimane in MeCN, let stand for 5 min at room temperature, add 20 μL 20% perchloric acid.

HPLC VARIABLES

Column: 150 × 4.6 7 μm Nucleosil RP-18
Mobile phase: Gradient. A was MeCN. B was 1% aqueous acetic acid containing 1 g/L octanesulfonic acid. A:B from 5:95 to 8:92 over 2 min, to 10:90 over 13 min (Waters convex), to 30:70 over 20 min (Waters convex), maintain at 30:70 for 6 min, re-equilibrate at initial conditions for 9 min.
Flow rate: 1.4
Detector: F (wavelengths not given)

CHROMATOGRAM

Internal standard: penicillamine

OTHER SUBSTANCES

Extracted: mesna

KEY WORDS

plasma; derivatization; penicillamine is IS

REFERENCE

Stofer-Vogel,B.; Cerny,T.; Borner,M.; Lauterburg,B.H. Oral bioavailability of mesna tablets, *Cancer Chemother.Pharmacol.*, **1993**, *32*, 78–81.

SAMPLE

Matrix: blood, cells
Sample preparation: Blood. Mix 9 mL whole blood with 1 mL 3.8% sodium citrate, centrifuge at 4° at 150 g for 15 min, wash the erythrocytes three times with isotonic saline. Suspend 100 μL erythrocytes in 700 μL 6 mM EDTA, mix gently for 1 min, add 200 μL 25% metaphosphoric acid, mix for 10 min, centrifuge at 5000 g for 15 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot of the filtrate. Cells. Wash 500 mg (wet weight) E. coli cells with water, add 2 mL 5% metaphosphoric acid, sonicate, centrifuge at 4° at 15000 g for 15 min, filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 Fine Sil C18-10 (Japan Spectroscopic)
Mobile phase: 33 mM KH_2PO_4 adjusted to pH 2.2 with phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 344 following post-column reaction. The column effluent mixed with the 1.5 mM 6,6'-dithiodinicotinic acid in 200 mM pH 7.0 sodium phosphate buffer pumped at 1 mL/min and the mixture flowed through a 60 cm × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM

Retention time: 8.2
Limit of detection: 0.1 nmole

OTHER SUBSTANCES

Extracted: cysteamine, cysteine, glutathione, homocysteine

KEY WORDS

post-column reaction; whole blood; erythrocytes

REFERENCE

Nishiyama,J.; Kuninori,T. Assay of biological thiols by a combination of high-performance liquid chromatography and postcolumn reaction with 6,6'-dithiodinicotinic acid, *Anal.Biochem.*, **1984**, *138*, 95–98.

SAMPLE

Matrix: blood, sea water, urine
Sample preparation: Blood. Centrifuge blood at 550 g for 30 min. Mix the clear solution with MeOH and centrifuge at 500 g for 30 min. Filter (0.22 mm) and centrifuge at 2500 g. Dilute the clear liquid with an equal amount of mobile phase. Centrifuge 2500 g for 5 min inject an

aliquot of the supernatant. Sea water, urine. Filter (0.22 mm), dilute with an equal amount of mobile phase, store at -5°, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:50 mM pH 2.2 trichloroacetic acid 1:99
Column temperature: 25
Flow rate: 0.3
Injection volume: 50
Detector: E, PAR-174, 1.0 mm tungsten wire electrode at -0.3 V, Ag/AgCl reference electrode, 1.0 mm dia. Pt wire auxiliary electrode (construction details for cell in paper) following post-column reaction. The column effluent mixed with 100 mM pH 3.1 phosphate buffer containing 25 μM or 25 mM Hg^{2+} (both quantities in paper) pumped at 1.0 mL/min and the mixture flowed to the detector.

CHROMATOGRAM

Retention time: 16.0
Limit of detection: 1 ng

OTHER SUBSTANCES

Extracted: cysteine, glutathione, homocysteine, mercaptopropionic acid, thiourea

KEY WORDS

plasma

REFERENCE

Hidayat,A.; Hibbert,D.B.; Alexander,P.W. Amperometric detection of organic thiols at a tungsten wire electrode following their separation by liquid chromatography, *J.Chromatogr.B*, **1997**, *693*, 139–146.

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 1 mL ice-cold 2 M perchloric acid containing 4 mM EDTA, vortex, centrifuge at 4° at 4000 g for 10 min. Neutralize an aliquot of the supernatant with cold 2 M LiOH solution, adjust to 10% with pH 8.4 borate buffer. Remove a 100 μL aliquot and add it to 100 μL 50 mM N-acetylcysteine solution, 100 μL reagent solution, and 700 μL pH 8.4 borate buffer, vortex for a few s, let stand at room temperature for 1 h, add an equal volume of the mobile phase, inject a 10 μL aliquot. Tissue. Powder tissue at low temperature. Add 4 volumes ice-cold 2 M perchloric acid containing 25 mM EDTA to 0.1-0.5 g powdered tissue, vortex quickly, homogenize (Brinkman). Neutralize an aliquot with cold 2 M LiOH solution, adjust to 1% (brain, lung, liver, kidney, testes, small intestine) or 2.5% (aorta, heart, spleen) with pH 8.4 borate buffer. Remove a 100 μL aliquot and add it to 100 μL 50 mM N-acetylcysteine solution, 100 μL reagent solution, and 700 μL pH 8.4 borate buffer, vortex for a few s, let stand at room temperature for 1 h, add an equal volume of the mobile phase, inject a 10 μL aliquot. (Prepare reagent, 2-(4-N-maleimidephenyl)-6-methylbenzothiazole, as follows. Recrystallize 2-(4-aminophenyl)-6-methylbenzothiazole from chloroform before use. Add 500 mg maleic anhydride in 2 mL chloroform dropwise to 1.2 g 2-(4-aminophenyl)-6-methylbenzothiazole in 10 mL DMF, stir at room temperature for 2 h, filter, wash with 30 mL chloroform, recrystallize from DMF to give 2-(4-N-phenylmaleamic acid)-6-methylbenzothiazole as yellow crystals (mp 242°). Reflux 2 g 2-(4-N-phenylmaleamic acid)-6-methylbenzothiazole, 100 mg anhydrous sodium acetate, and 25 mL acetic anhydride for 2 h, cool on ice, filter, wash the solid with water. Neutralize the filtrate with cold 10% NaOH, extract with chloroform. Dry the organic layer over anhydrous magnesium sulfate and evaporate it to dryness under reduced pressure. Combine this product with the solid obtained earlier and recrystallize from isopropanol to give 2-(4-N-maleimidephenyl)-6-methylbenzothiazole as yellow needles (mp 254-6°). Prepare the reagent solution by dissolving 50 μmoles of this compound in 10 mL DMF and diluting 25-fold with MeCN.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:buffer 35:65, pH 4.5 (Buffer was 10 mM KH_2PO_4 containing 0.1% sodium hexanesulfonate.)
Flow rate: 1.5
Injection volume: 10

Detector: F ex 320 em 405

CHROMATOGRAM
Retention time: 17
Internal standard: N-acetylcysteine (7)
Limit of detection: 20 fmole

OTHER SUBSTANCES
Extracted: N-acetylpenicillamine, coenzyme A, cysteine, glutathione, homocysteine

KEY WORDS
derivatization; plasma; rat; aorta; heart; lung; liver; kidney; testes; spleen; brain; small intestine

REFERENCE
Haj-Yehia,A.I.; Benet,L.Z. Determination of aliphatic thiols by fluorometric high-performance liquid chromatography after precolumn derivatization with 2-(4-N-maleimidophenyl)-6-methylbenzothiazole,*Pharm.Res.*, **1995**, *12*, 155–160.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water:67 mM pH 7.0 phosphate buffer 70:25:5
Flow rate: 0.45
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: k' 7.37 (L), 10.05 (D)

OTHER SUBSTANCES
Simultaneous: amino acids

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve about 1 mg of the contents of a capsule in 10 mL water and dilute with MeCN to a penicillamine concentration of 8 mM. 50 μL Solution + 200 μL buffer + 150 μL reagent, heat at 60° for 30 min, cool, inject a 10 μL aliquot. (Buffer was prepared by adjusting the pH of a solution containing 100 mM boric acid and 100 mM KCl to 8.5 with 100 mM sodium carbonate. Reagent was 200 μM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide (DBPM) in MeCN. Synthesis of N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide is as follows. Add 8.8 g aluminum trichloride to 12.50 g 3-dimethylaminophenol in 185 mL chloroform and 84 g triethyl orthoformate, mix at room temperature for 10 min, when the exothermic reaction ceases add 50 mL 10% HCl, stir to hydrolyze the acetal, neutralize with 10% NaOH, filter through a short column of Celite, wash through with chloroform, wash the filtrate with saturated aqueous NaCl, dry over magnesium sulfate, concentrate under reduced pressure, recrystallize from chloroform to give 4-(dimethylam-

ino)salicylaldehyde (mp 78-79°). Add 400 mg KOH in 3 mL EtOH to a solution of 1 g 4-(dimethylamino)salicylaldehyde and 1.3 g (?) 4-nitrobenzylbromide in 12 mL EtOH, reflux for 7 h, cool, filter to recover the crystals, wash with water, dry under vacuum, recrystallize from EtOH to give 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde (mp 179-180°). Add a solution of 900 mg 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde in 6 mL DMF to a sodium methoxide solution (prepared from 69 mg sodium in 1 mL MeOH), reflux for 20 min, add 1 mL MeOH, filter the crystals, recrystallize from EtOH to give 6-dimethylamino-2-(4-nitrophenyl)benzofuran as red needles (mp 209.5-210.5°). Reflux 1 g 6-dimethylamino-2-(4-nitrophenyl)benzofuran in 20 mL benzene (Caution! Benzene is a carcinogen!) and 18 mL MeOH containing 80 mg active carbon and a catalytic amount of ferric chloride hexahydrate for 10 min, add 2.30 g 98% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) dropwise, reflux for 7 h, filter, concentrate the filtrate, recrystallize from cyclohexane to give 6-dimethylamino-2-(4-aminophenyl)benzofuran as orange needles (mp 198.5-200°). Stir 605 mg 6-dimethylamino-2-(4-aminophenyl)benzofuran and 230 mg maleic anhydride in 5 mL chloroform at room temperature for 3 h, filter the crystals, wash with a small amount of chloroform, recrystallize from EtOH to obtain N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid (mp 219.5-221°). Reflux a mixture of 1.17 g N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid and 30 mg sodium acetate in 18 mL acetic anhydride, cool in an ice bath, collect the crystals of product, wash with water. Neutralize the filtrate with 20% NaOH, extract twice with 30 mL portions of chloroform, wash the organic layers with saturated aqueous NaCl, dry over anhydrous magnesium sulfate, evaporate to give more product. Combine the products and recrystallize them from acetone to give N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide as reddish purple crystals (mp 203-204°) (Bull.Chem.Soc.Jpn. 1985, 58, 2192).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Sumichiral OA-2500S Pirkle-type (Sumika Chemical Analysis Service)
Mobile phase: MeOH:water 75:25 containing 150 mM ammonium acetate and 50 mM tetra-n-butylammonium bromide
Flow rate: 1
Injection volume: 10
Detector: F ex 360 em 455

CHROMATOGRAM

Retention time: 27 (D), 31 (L) (Each enantiomer gives 2 peaks, the later peaks are used for quantitation.)
Limit of detection: 350 fmole (L), 290 fmole (D)

KEY WORDS

capsules; chiral; derivatization

REFERENCE

Nakashima,K.; Ishimaru,T.; Kuroda,N.; Akiyama,S. High-performance liquid chromatographic separation of penicillamine enantiomers labelled with *N*-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide on a chiral stationary phase, *Biomed.Chromatogr.*, **1995**, *9*, 90–93.

SAMPLE

Matrix: formulations
Sample preparation: Dissolve about 1 mg of the contents of a capsule in 10 mL water and dilute with MeCN to a penicillamine concentration of 8 mM. 50 μL Solution + 200 μL buffer + 150 μL reagent, heat at 60° for 30 min, cool, inject a 10 μL aliquot. (Buffer was prepared by adjusting the pH of a solution containing 100 mM boric acid and 100 mM KCl to 8.5 with 100 mM sodium carbonate. Reagent was 200 μM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide (DBPM) in MeCN. Synthesis of N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide is as follows. Add 8.8 g aluminum trichloride to 12.50 g 3-dimethylaminophenol in 185 mL chloroform and 84 g triethyl orthoformate, mix at room temperature for 10 min, when the exothermic reaction ceases add 50 mL 10% HCl, stir to hydrolyze the acetal, neutralize with 10% NaOH, filter through a short column of Celite, wash through with chloroform, wash the filtrate with saturated aqueous NaCl, dry over magnesium sulfate, concentrate under reduced pressure, recrystallize from chloroform to give 4-(dimethylamino)salicylaldehyde (mp 78-79°). Add 400 mg KOH in 3 mL EtOH to a solution of 1 g 4-(dimethylamino)salicylaldehyde and 1.3 g (?) 4-nitrobenzylbromide in 12 mL EtOH, reflux for 7 h, cool, filter to recover the crystals, wash with water, dry under vacuum, recrystallize from

EtOH to give 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde (mp 179-180°). Add a solution of 900 mg 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde in 6 mL DMF to a sodium methoxide solution (prepared from 69 mg sodium in 1 mL MeOH), reflux for 20 min, add 1 mL MeOH, filter the crystals, recrystallize from EtOH to give 6-dimethylamino-2-(4-nitrophenyl)benzofuran as red needles (mp 209.5-210.5°). Reflux 1 g 6-dimethylamino-2-(4-nitrophenyl)benzofuran in 20 mL benzene (Caution! Benzene is a carcinogen!) and 18 mL MeOH containing 80 mg active carbon and a catalytic amount of ferric chloride hexahydrate for 10 min, add 2.30 g 98% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) dropwise, reflux for 7 h, filter, concentrate the filtrate, recrystallize from cyclohexane to give 6-dimethylamino-2-(4-aminophenyl)benzofuran as orange needles (mp 198.5-200°). Stir 605 mg 6-dimethylamino-2-(4-aminophenyl)benzofuran and 230 mg maleic anhydride in 5 mL chloroform at room temperature for 3 h, filter the crystals, wash with a small amount of chloroform, recrystallize from EtOH to obtain N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid (mp 219.5-221°). Reflux a mixture of 1.17 g N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid and 30 mg sodium acetate in 18 mL acetic anhydride, cool in an ice bath, collect the crystals of product, wash with water. Neutralize the filtrate with 20% NaOH, extract twice with 30 mL portions of chloroform, wash the organic layers with saturated aqueous NaCl, dry over anhydrous magnesium sulfate, evaporate to give more product. Combine the products and recrystallize them from acetone to give N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide as reddish purple crystals (mp 203-204°) (Bull.Chem.Soc.Jpn. 1985, 58, 2192).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Sumichiral OA-2500S Pirkle-type (Sumika Chemical Analysis Service)
Mobile phase: MeOH:water 75:25 containing 150 mM ammonium acetate and 50 mM tetra-n-butylammonium bromide
Flow rate: 1
Injection volume: 10
Detector: F ex 360 em 455

CHROMATOGRAM

Retention time: 27 (D), 31 (L) (Each enantiomer gives 2 peaks, the later peaks are used for quantitation.)
Limit of detection: 350 fmole (L), 290 fmole (D)

KEY WORDS

capsules; chiral; derivatization

REFERENCE

Reichelova,V.; Liliemark,J.; Albertioni,F. Structure-activity relationships of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine and related analogues: Protein binding, lipophilicity, and retention in reversed-phase LC, *J.Liq.Chromatogr.*, **1995**, *18*, 1123–1135.

SAMPLE

Matrix: solutions
Sample preparation: Add 1 mL 50 μg/mL N-(4-anilinophenyl)maleimide in 33 mM pH 6.85 phosphate buffer to 0.1-4 μg thiol, let stand at 0° for 90 min, wash twice with 2 mL portions of ether, heat the aqueous phase to 50° for 20 min, inject an aliquot. (Prepare N-(4-anilinophenyl)maleimide as follows. Add dropwise 1.1 g maleic anhydride in 10 mL chloroform to 1 g N-phenylphenylenediamine (4-aminodiphenylamine) stirred in 10 mL chloroform at 0°, filter, dry to give N-(4-anilinophenyl)maleamic acid. Heat 100 mg N-(4-anilinophenyl)maleamic acid and 25 mg sodium acetate in 400 μL acetic anhydride on a water bath for 2 h, cool, pour into ice-water, filter, recrystallize from ethyl acetate/hexane to give N-(4-anilinophenyl)maleimide as yellow needles (mp 135-6°).)

HPLC VARIABLES

Column: 305 × 6.3 μBondapak C18
Mobile phase: MeCN:0.5% pH 3.0 $(NH_4)H_2PO_4$ 4:7
Flow rate: 1
Injection volume: 10
Detector: E, Yanagimoto model VMD-101, glassy carbon electrode +1.0 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: N-acetyl-L-cysteine, L-cysteine, glutathione

KEY WORDS
derivatization

REFERENCE
Shimada,K.; Tanaka,M.; Nambara,T. Sensitive derivatization reagents for thiol compounds in high-performance liquid chromatography with electrochemical detection, *Anal.Chim.Acta*, **1983**, *147*, 375–380.

SAMPLE
Matrix: solutions
Sample preparation: Add 1.05-3 equivalents 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate to 10 mL of a 100 μM solution of the thiol in MeCN:water 50:50 containing 1-3 equivalents triethylamine, vortex briefly, let stand at room temperature for 30 min, dilute with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel ODS-80TM (Tosoh)
Mobile phase: MeCN:10 mM pH 2.8 potassium phosphate buffer 60:40
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 3.99 (L), 5.24 (D)

OTHER SUBSTANCES
Simultaneous: cysteine, homocysteine

KEY WORDS
derivatization; chiral

REFERENCE
Ito,S.; Ota,A.; Yamamoto,K.; Kawashima,Y. Resolution of the enantiomers of thiol compounds by reversed-phase liquid chromatography using chiral derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, *J.Chromatogr.*, **1992**, *626*, 187–196.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of a 30 μM solution in 2 mM disodium EDTA containing 3% triethylamine with 10 μL 12 mM R-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in MeCN, let stand for 40 min, inject a 10 μL aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole, (R)-(-)-NBD-PyNCS, is as follows. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the

minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL water, extract 4 times with 80 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as dark red crystals (mp 178-181°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as red crystals (mp 165-170°) (Analyst 1995, 120, 385).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultron VX-ODS (Shinwa, Kyoto)
Mobile phase: MeCN:water:trifluoroacetic acid 35:65:0.1
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 455 em 568

CHROMATOGRAM
Retention time: 20, 23 (enantiomers)

KEY WORDS
derivatization; chiral

REFERENCE
Jin,D.; Takehana,K.; Toyo'oka,T. Chiral separation of racemic thiols based on diastereomer formation with a fluorescent chiral tagging reagent by reversed-phase liquid chromatography, *Anal.Sci.*, **1997**, *13*, 113–115.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 200 μM solution in buffer with three volumes of a 400 μM solution of 5,5'-dithio-(bis-2-nitrobenzoic acid) in buffer, let stand at room temperature for 30 min, inject a 75 μL aliquot. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS1
Mobile phase: Gradient. MeCN:buffer 0:100 for 20 min, to 17.5:82.5 over 40 min. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)
Flow rate: 0.25 for 20 min, to 1 over 40 min
Injection volume: 75
Detector: UV 357

CHROMATOGRAM
Retention time: 45

OTHER SUBSTANCES
Simultaneous: N-acetylcysteine, N-acetylpenicillamine, captopril, cysteine, glutathione, thiomalic acid

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KEY WORDS
derivatization

REFERENCE

Russell,J.; McKeown,J.A.; Hensman,C.; Smith,W.E.; Reglinski,J. HPLC determination of biologically active thiols using pre-column derivatization with 5,5'-dithio-(bis-2-nitrobenzoic acid), *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1757–1763.

SAMPLE

Matrix: tissue

Sample preparation: Freeze tissue in liquid nitrogen and pulverize. Homogenize 50-100 mg tissue in 1 mL MeCN:20 mM EDTA 30:70, centrifuge at 4° at 4000 g for 5 min, adjust to 1-2.5% w/v with pH 8.4 borate buffer, keep on ice. 100 μL Sample + 100 μL 0.05 mM N-acetylcysteine + 100 μL 0.25 mM reagent in MeCN:DMF 95:5 + 700 μL pH 8.4 borate buffer, vortex for a few s, let stand for 1 h at room temperature, dilute with an equal volume of mobile phase, inject a 10 μL aliquot. (Reagent was 2-(4-maleimidophenyl)-6-methoxybenzofuran, a partial synthesis is given in the paper.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere-ODS

Mobile phase: MeCN:buffer 35:65 adjusted to pH 4.5 (Buffer was 10 mM KH_2PO_4 containing 0.1% sodium hexanesulfonate.)

Flow rate: 1.5

Injection volume: 10

Detector: F ex 310 em 390

CHROMATOGRAM

Retention time: 10

Internal standard: N-acetylcysteine (8)

Limit of detection: 75 fmole

OTHER SUBSTANCES

Extracted: glutathione, homomocysteine, acetylpenicillamine

KEY WORDS

rat; heart; lung; liver; kidney; testes; spleen; derivatization

REFERENCE

Haj-Yehia,A.I.; Benet,L.Z. 2-(4-N-Maleimidophenyl)-6-methoxybenzofuran: a superior derivatizing agent for fluorimetric determination of aliphatic thiols by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *666*, 45–53.

SAMPLE

Matrix: urine

Sample preparation: For each 1 mL urine add 1-2 mg EDTA and 5 μg homocysteine, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 45 × 4.6 5 μm ODS Hypersil

Column: 100 × 4.6 5 μm ODS Hypersil

Mobile phase: 1 g/L pH 4 Heptanesulfonic acid in water containing 150 mg/L sodium EDTA

Flow rate: 1

Injection volume: 20

Detector: UV 412 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 150 × 2 column filled with 40 μm glass beads to the detector. (Prepare reagent by dissolving 200 mg 5,5'-dithiobis(2-nitrobenzoic acid) and 10 g tripotassium citrate in 100 mL 250 mM pH 7.4 phosphate buffer, dilute 10-fold with water immediately before use.)

CHROMATOGRAM

Retention time: 1.3

Internal standard: homocysteine (0.8)

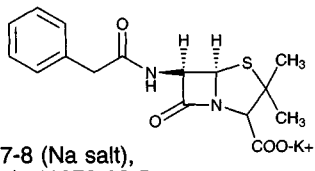
Limit of quantitation: 10 ng

OTHER SUBSTANCES
Extracted: cysteine

KEY WORDS
post-column reaction

REFERENCE
Beales,D.; Finch,R.; McLean,A.E.M.; Smith,M.; Wilson,I.D. Determination of penicillamine and other thiols by combined high-performance liquid chromatography and post-column reaction with Ellman's reagent: application to human urine, *J.Chromatogr.*, **1981**, *226*, 498–503.

Penicillin G



Molecular formula: $C_{16}H_{18}N_2O_4S$
Molecular weight: 334.40
CAS Registry No.: 113-98-4 (potasium salt), 61-33-6 (free acid), 69-57-8 (Na salt), 751-84-8 (penicillin G benethamine), 1538-09-6 (penicillin G benzathine), 41372-02-5 (penicillin G benzathine tetrahydrate), 1538-11-0 (penicillin G benzhydrylamine), 973-53-5 (Ca salt), 3344-16-9 (penicillin G hydrabamine), 6130-64-9 (penicillin G procaine monohydrate), 54-35-3 (penicillin G procaine)
Merck Index: 7225

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 24:76 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 4.8
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, piperacillin, ticarcillin

KEY WORDS
serum

REFERENCE
Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE
Matrix: blood

Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 3 mL MeOH and 3 mL buffer. 1 mL Plasma + 2 mL buffer, vortex, add to SPE cartridge, wash with buffer, elute with 500 μL MeOH:10 mM pH 5.2 potassium phosphate buffer 90:10, inject a 25 μL aliquot. (Buffer was 121 g Trizma base in 1 L water, adjust pH to 7.0 with concentrated HCl. Dilute 1:100 to obtain the 10 mM buffer.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeOH:10 mM pH 5.2 potassium phosphate buffer 20:80 (Buffer was 1 M KH_2PO_4 adjusted to pH 5.2 with 5 M KOH, dilute 1:100 with water to give working buffer.)
Column temperature: 50
Flow rate: 0.9
Injection volume: 25
Detector: UV 225

CHROMATOGRAM
Retention time: 412
Internal standard: penicillin G

OTHER SUBSTANCES
Extracted: cefteran (ceftetrane)

KEY WORDS
plasma; SPE; method stated to be applicable to urine (no details); penicillin G is IS

REFERENCE
Hicks,C.M.; Powell,M.L. Rapid analysis of ceftetrame in human plasma using sorbent extraction and high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *497*, 349–354.

SAMPLE
Matrix: blood
Sample preparation: Condition a 55 × 5 100-200 mesh AG 50W-X8 (H^+) column (Bio-Rad) with 10 mL MeCN:water 50:50. 600 μL Serum + 600 μL MeCN, vortex for 1 min, centrifuge at 2000 g for 5 min, add a 1 mL aliquot of the supernatant to the column, discard the first 200 μL effluent, collect the rest of the effluent. Remove a 450 μL aliquot and add it to 50 μL 10% sodium carbonate solution, heat at 60° for 1 h (to hydrolyse the β-lactam ring), cool in an ice bath. Remove a 100 μL aliquot and add it to 15 μL 200 mM pH 6.0 phosphate buffer, add 35 μL 80 mM 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole in MeCN, heat at 60° for 10 min, cool in an ice bath, add 30 μL 1 M HCl, inject a 5-10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS-80TM (Tosoh)
Mobile phase: MeOH:100 mM pH 3.0 phosphate buffer 40:60
Flow rate: 1
Injection volume: 5-10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 10
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: methicillin, piperacillin

KEY WORDS
derivatization; serum; SPE

REFERENCE
Iwaki,K.; Okumura,N.; Yamazaki,M.; Nimura,N.; Kinoshita,T. Precolumn derivatization technique for high-performance liquid chromatographic determination of penicillins with fluorescence detection, *J.Chromatogr.*, **1990**, *504*, 359–367.

SAMPLE

Matrix: blood

Sample preparation: Condition a Baker C18 SPE cartridge with 5 mL water and 5 mL 2% NaCl, do not allow to run dry. 2 mL Plasma + 120 μL 5 μg/mL penicillin V + 30 mL water + 2 mL 170 mM sulfuric acid +2 mL 5% sodium tungstate solution, vortex for 30 s, centrifuge at 2200 g for 10 min, filter supernatant (GF/B glass fiber filter), add 10 mL 20% NaCL, mix, add to SPE cartridge at 3 mL/min, wash with 5 mL 2% NaCl, wash with 5 mL water, draw air through cartridge for 5 min, elute with 500 μL elution solution. Add 500 μL derivatization reagent to the eluate, vortex for 20 s, allow to react at 65° for 30 min, cool to room temperature, vortex, filter (0.45 μm), inject 50-100 μL aliquots. (Prepare derivatization reagent by dissolving 34.45 g 1,2,4-triazole in 150 mL water, add 25 mL 10 mM mercuric chloride solution, mix, adjust the pH to 9.0 ± 0.5 with 5 M NaOH, dilute to 250 mL with water. Prepare elution solution by mixing 60 mL MeCN and 5 mL buffer and making up to 100 mL with water. The buffer was 0.994 g Na_2HPO_4 + 1.794 g $NaH_2PO_4.H_2O$ in 100 mL water, pH 6.5.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: MeCN:buffer 25:75 (Buffer contained 4.969 g Na_2HPO_4 + 8.969 g $NaH_2PO_4.H_2O$ + 2.482 g anhydrous sodium thiosulfate per liter.)

Flow rate: 1

Injection volume: 50-100

Detector: UV 325

CHROMATOGRAM

Retention time: 4.5

Internal standard: penicillin V (5.8)

Limit of detection: 5 ng/mL

KEY WORDS

plasma; cow; SPE; derivatization

REFERENCE

Boison,J.O.; Korsrud,G.O.; MacNeil,J.D.; Keng,L.; Papich,M. Determination of penicillin G in bovine plasma by high-performance liquid chromatography after pre-column derivatization, *J.Chromatogr.*, **1992**, *576*, 315–320.

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 10.28

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS
plasma; serum; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 8.5
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Plasma. Add 500 μL MeCN to 500 μL plasma while mixing on a Whirlmixer, rotate for 10 min, centrifuge at 1000 g for 5 min. Remove a 700 μL aliquot of the supernatant and ad it to 3.5 mL dichloromethane, mix for 30 s, centrifuge at 1000 g for 1 min, inject a 20 μL aliquot of the aqueous layer. Urine. Dilute with Sörensen buffer, inject an aliquot. CSF. Inject an aliquot directly.

HPLC VARIABLES
Column: 100 × 3 5 μm MOS-Hypersil C8
Mobile phase: MeCN:MeOH:buffer 12:26:62 containing 3 mM tetrabutylammonium bromide (Buffer was 5 mM pH 5.0 sodium acetate.)
Column temperature: 22
Flow rate: 1
Injection volume: 20

Detector: UV 231

CHROMATOGRAM
Retention time: 3
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: probenecid

KEY WORDS
plasma

REFERENCE
van Gulpen,C.; Brokerhof,A.W.; van der Kaay,M.; Tjaden,U.R.; Mattie,H. Determination of benzylpenicillin and probenecid in human body fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *381*, 365–372.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Plasma. 50 μL Plasma + 50 μL IS solution + 50 μL MeCN, mix for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot of the supernatant. Urine. Mix 100 μL IS solution with 200 μL MeCN and 100 μL urine for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot. Tissue. Weight out finely chopped tissue and suspend it in 200 μL water. Add 100 μL 100 μg/mL IS, sonicate for 60 s. Add 200 μL MeCN, vortex for 30 s, centrifuge at 10000 g for 15 min. Inject an aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard C18 (Alltech)
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeCN:50 mM pH 5.0 sodium dihydrogen phosphate 30:70
Flow rate: 1.0
Detector: UV 214

CHROMATOGRAM
Retention time: 6.4
Internal standard: dicloxacillin (13.9)
Limit of quantitation: 800 ng/mL (plasma), 1 μg/mL (urine), 5 μg/g (tissue)

OTHER SUBSTANCES
Extracted: flucloxacillin

KEY WORDS
plasma; muscle; rat; pharmacokinetics

REFERENCE
Cross,S.E.; Thompson,M.J.; Roberts,M.S. Distribution of systemically administered ampicillin, benzylpenicillin, and flucloxacillin in excisional wounds in diabetic and normal rats and effects of local topical vasodilator treatment, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1703–1710.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma, serum. 200 μL Plasma or serum + 200 μL 50 mM pH 6.0 sodium phosphate buffer + 800 μL MeCN, vortex for 30 s, centrifuge at 3000 g for 10 min. Remove the clear supernatant and add it to 2 mL dichloromethane, mix for 30 s, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot of the upper aqueous layer. Urine. 100 μL Urine + 9.9 mL 50 mM pH 6.0 sodium phosphate buffer, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Brownlee C 18 guard column
Column: 250 × 4.6 5 μm Hypersil ODS (Keystone)

Mobile phase: Gradient. A was MeCN:10 mM sodium phosphate adjusted to pH 2.7 with concentrated phosphoric acid 3:97. B was MeCN:10 mM sodium phosphate adjusted to pH 2.7 with concentrated phosphoric acid 90:10. A:B from 95:5 to 50:50 over 9 min and then to 95:5 over 1 min.
Flow rate: 1.5
Injection volume: 25
Detector: UV 220

CHROMATOGRAM
Retention time: 12.5
Internal standard: penicillin G

OTHER SUBSTANCES
Extracted: piperacillin, tazobactam
Simultaneous: amoxicillin, ampicillin, cefoperazine, cefometazole, cefotaxime, cefotetan, cefuroxime, mezlocillin

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KEY WORDS
plasma; serum; penicillin G is IS

REFERENCE
Ocampo,A.P.; Hoyt,K.D.; Wadgaonkar,N.; Carver,A.H.; Puglisi,C.V. Determination of tazobactam and piperacillin in human plasma, serum, bile and urine by gradient elution reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *496*, 167–179.

SAMPLE
Matrix: cell suspensions
Sample preparation: 300 μL Cell suspension + 300 μL MeCN, vortex, centrifuge, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 80 × 4 Nucleosil 120 3 C18
Mobile phase: MeCN:20 mM phosphoric acid 25:75
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 3.1

REFERENCE
Kersten,A.; Poitschek,C.; Rauch,S.; Aberer,E. Effects of penicillin, ceftriaxone, and doxycycline on morphology of *Borrelia burgdorferi*, *Antimicrob.Agents Chemother.*, **1995**, *39*, 1127–1133.

SAMPLE
Matrix: cheese, milk, yogurt
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl, do not allow to go dry. 5 mL Milk (or 5 g yogurt or cottage cheese + 4 mL 1 M pH 6 phosphate buffer) + 20 μL 20 μg/mL penicillin V in water + 25 mL water + 4 mL 170 mM sulfuric acid + 40 mL 5% sodium tungstate, vortex for 30 s, centrifuge at 1500 g for 10 min, remove the supernatant, add 10 mL 20% NaCl to the residue, vortex for 10 s, centrifuge. Combine the supernatants and add them to the SPE cartridge, wash with 10 mL 2% NaCl, wash with 10 mL water, elute with 750 μL MeCN:200 mM ammonium acetate:water 60:5:35, filter (Acro 0.45 μm), inject a 50-100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeCN. B was MeCN:150 mM ammonium acetate 10:90. A:B 0:100 for 10 min, to 30:70 over 10 min, return to initial conditions over 10 min.
Flow rate: 0.9
Injection volume: 50-100

Detector: MS, VG Trio II, probe tip 255°, source 180°, thermospray/plasmaspray, m/z 335, m/z 160

CHROMATOGRAM
Internal standard: penicillin V
Limit of detection: 5 ng/mL

KEY WORDS
cow; SPE

REFERENCE
Boison,J.O.K.; Keng,L.J.-Y.; MacNeil,J.D. Analysis of penicillin G in milk by liquid chromatography, *J.AOAC Int.*, **1994**, *77*, 565–570.

SAMPLE
Matrix: fermentation solutions
Sample preparation: Adjust pH of fermentation broth to 7, centrifuge at 8000 g for 10 min, add MeCN, centrifuge, add dichloromethane to the supernatant, vortex for 10 s, shake for 15 min, centrifuge at 8000 g for 15 min. Add 1 mL of the aqueous layer to 100 μL reagent, heat at 50° for 50 min, cool in an ice bath, inject a 20 μL aliquot. (Prepare reagent by dissolving 4.125 g imidazole in 2.5 mL water, add 1 mL HCl, add 500 μL 110 mM mercury(II) chloride, add 1.5 mL HCl. Recrystallize imidazole twice from isopropanol.)

HPLC VARIABLES
Guard column: 10 × 4 5 μm Spherisorb C18
Column: 20 × 4.6 5 μm Spherisorb C18 S5ODS2
Mobile phase: Gradient. MeCN:buffer from 16.5:83.5 to 31.5:68.5 over 17 min (Buffer was 10 mM NaH_2PO_4 containing 10 mM EDTA, adjusted to pH 6.5 with 2 M NaOH.)
Flow rate: 2
Injection volume: 20
Detector: UV 325

CHROMATOGRAM
Retention time: 13
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: methicillin, penicillin V, penicillin X

KEY WORDS
derivatization

REFERENCE
Rogers,M.E.; Adlard,M.W.; Saunders,G.; Holt,G. High-performance liquid chromatographic determination of penicillins following derivatization to mercury-stabilized penicillenic acids, *J.Liq.Chromatogr.*, **1983**, *6*, 2019–2031.

SAMPLE
Matrix: fermentation solutions
Sample preparation: Filter (0.22 μm) fermentation broth. Mix 980 μL filtrate with 20 μL 200 mM benzoic anhydride in MeCN at room temperature for 3 min, add 100 μL reagent, mix, heat at 45° for 1 h, cool to room temperature, inject a 40 μL aliquot. (Prepare reagent by dissolving 6.76 g 1-hydroxybenzotriazole hydrate in 10 mL water, add 2.5 mL mercury(II) chloride solution (no concentration given), adjust pH to 9.2 with 4 M NaOH, make up to 25 mL.)

HPLC VARIABLES
Guard column: 10 × 4 5 μm Spherisorb S5ODS2
Column: 259 × 4.9 5 μm Spherisorb S5ODS2
Mobile phase: Gradient. MeCN:20 mM pH 6.5 potassium phosphate buffer:20 mM sodium thiosulfate from 10:45:45 to 25:37.5:37.5 over 25 min, maintain at 25:37.5:37.5 for 10 min (The mobile phase flowed through a 50 × 4 column of 5 μm Spherisorb S5ODS2 before the injector.)

Flow rate: 1 for 25 min then 1.2
Injection volume: 40
Detector: UV 328

CHROMATOGRAM
Retention time: 24
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: 6-aminopenicillanic acid, ampicillin, penicillin K, penicillin V, penicillin X

KEY WORDS
derivatization

REFERENCE
Shah,A.J.; Adlard,M.W.; Holt,G. Determination of natural penicillins in fermentation media by high-performance liquid chromatography using precolumn derivatization with 1-hydroxybenzotriazole, *Analyst*, **1988**, *113*, 1197–1200.

SAMPLE
Matrix: fermentation solutions
Sample preparation: Centrifuge fermentation broth at 4° at 5000 g for 20 min, filter (0.45 μm) a 1 mL aliquot of the supernatant, add 50 μL 1 M NaOH to the filtrate. Remove a 50 μL aliquot and add it to 50 μL 3 mM N-dansylaziridine (Sigma) in dioxane (Caution! Dioxane is a carcinogen!), heat at 100° for 30 min, cool to room temperature, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Spherisorb C18
Column: 250 × 4.6 5 μm Spherisorb C18
Mobile phase: Gradient. MeCN:20 mM pH 4.4 acetate buffer containing 0.5 mM EDTA from 19:81 to 23:77 over 15 min, to 40:60 over 10 min, to 65:35 over 2.5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 339 em 540

CHROMATOGRAM
Retention time: 24
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine, 6-aminopenicillanc acid, isopenicillin N

KEY WORDS
derivatization

REFERENCE
Orford,C.D.; Perry,D.; Adlard,M.W. The determination of naturally produced penicillins and their biosynthetic precursors using pre-column derivatisation with dansylaziridine, *J.Liq.Chromatogr.*, **1991**, *14*, 2665–2684.

SAMPLE
Matrix: formulations
Sample preparation: Blend tablets and capsules with water in a high-speed blender for 5 min, filter, dilute with mobile phase, inject a 20 μL aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 70 mm long Co:Pell ODS
Column: 300 × 4.6 10 μm Chromegabond C18 (E.S. Industries)
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 19:11:70
Flow rate: 1

Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 1700 ng/mL

OTHER SUBSTANCES
Simultaneous: amoxicillin, ampicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin V

KEY WORDS
tablets; capsules; oral suspensions; injections

REFERENCE
Briguglio,G.T.; Lau-Cam,C.A. Separation and identification of nine penicillins by reverse phase liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 228–231.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova Pak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 5 mM pH 7.5 containing 1.3 mM tetrabutylammonium hydroxide.)
Flow rate: 0.5
Detector: UV 254

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; water; stability-indicating

REFERENCE
Stiles,M.L.; Tu,Y.H.; Allen,L.V.,Jr. Stability of cefazolin sodium, cefoxitin sodium, ceftazidime, and penicillin G sodium in portable pump reservoirs, *Am.J.Hosp.Pharm.*, **1989**, *46*, 1408–1412.

SAMPLE
Matrix: milk
Sample preparation: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 mL MeCN, shake vigorously for 1 min, centrifuge at 9000 g for 10 min, decant, add 5 g NaCl, swirl to dissolve, add 100 mL dichloromethane, shake for 1 min, centrifuge at 1000 g for 10 min. Remove top aqueous layer and extract organic layer with 25 mL 10% NaCl by shaking and centrifuging as before. Combine aqueous layers, add 1 mL 0.3% mercuric chloride in water, let stand 30 min, add 1 mL 2 M HCl, extract with three 50 mL portions of dichloromethane by shaking each portion for 1 min and centrifuging at 1000 g for 10 min, filter dichloromethane extracts through 30 g anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 35°, if water remains add 5-10 mL MeOH to flask and complete evaporation. Dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 2 mL IS solution, inject a 20 μL aliquot. (Prepare IS solution by dissolving 10 μL benzaldehyde in 100 mL dichloromethane, evaporate 1 mL to dryness under reduced pressure, dissolve res-

idue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 100 mL MeCN then dilute an aliquot 1:4 with MeCN.)

HPLC VARIABLES
Column: 250 × 4 10 μm Lichrosorb RP-18
Mobile phase: MeCN:water 58:42
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 500 filter

CHROMATOGRAM
Retention time: 5.74
Internal standard: benzaldehyde (derivatized) (12.18)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: penicillin V, phenethicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin
Interfering: methicillin

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Shimoda,W.; Roybal,J.E.; Vieira,C. Multiresidue method for determination of eight neutral β-lactam penicillins in milk by fluorescence-liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 968–971.

SAMPLE
Matrix: milk
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 2 mL 2% NaCl. Pass through 30 g filtered (glass-wool plug) milk at 2 mL/min, wash with 5 mL water, wash with 10 mL MeOH:water:20% NaCl 10:80:10 containing 20 mM 18-crown-6, elute with 10 mL 15% (v/v) MeOH, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 Permaphase ETH (Du Pont)
Column: 150 × 4.3 LiChrosorb RP-18
Mobile phase: MeOH:water:0.2 M pH 4.0 phosphate buffer 25:65:10 containing 11 mM sodium 1-heptanesulfonate
Column temperature: 45
Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 9
Limit of detection: 30 ng/g

OTHER SUBSTANCES
Extracted: ampicillin, penicillin V

KEY WORDS
cow; SPE

REFERENCE

Terada,H.; Sakabe,Y. Studies on residual antibacterials in foods. IV. Simultaneous determination of penicillin G, penicillin V and ampicillin in milk by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *348*, 379–387.

SAMPLE

Matrix: milk

Sample preparation: 500 μL Milk + 500 μL MeCN:MeOH:water 40:20:40, vortex for 10-15 s, filter (Amicon Centricon-10, 10000 dalton cut-off) while centrifuging at 2677 g for 30 min, inject a 10-60 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 220 × 2.1 5 μm Spheri-5 phenyl

Mobile phase: MeCN:buffer 25:75 (Buffer was 2.5 mM octanesulfonate, 2.5 mM dodecanesulfonate, 0.5% 85% phosphoric acid, and 0.5% triethylamine.)

Column temperature: 40

Flow rate: 0.3-0.5

Injection volume: 10-60

Detector: UV 210

CHROMATOGRAM

Retention time: 6.3

Limit of detection: 10 ppb

KEY WORDS

cow; ultrafiltrate

REFERENCE

Tyczkowska,K.; Voyksner,R.D.; Aronson,A.L. Development of an analytical method for penicillin G in bovine milk by liquid chromatography with ultraviolet-visible detection and confirmation by mass spectrometric detection, *J.Chromatogr.*, **1989**, *490*, 101–113.

SAMPLE

Matrix: milk

Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl, do not allow to go dry. 5 mL Milk (or 5 g yogurt or cottage cheese + 4 mL 1 M pH 6 phosphate buffer) + 20 μL 20 μg/mL penicillin V in water + 25 mL water + 4 mL 170 mM sulfuric acid + 40 mL 5% sodium tungstate, vortex for 30 s, centrifuge at 1500 g for 10 min, remove the supernatant, add 10 mL 20% NaCl to the residue, vortex for 10 s, centrifuge. Combine the supernatants and add them to the SPE cartridge, wash with 10 mL 2% NaCl, wash with 10 mL water, elute with 1 mL MeCN:200 mM pH 6.5 sodium phosphate buffer:water 60:5:35. Add 1 mL reagent to the eluate, vortex for 10 s, heat at 65° for 30 min, cool to room temperature, vortex, filter (Acro 0.45 μm), inject a 50-100 μL aliquot of the filtrate. (Prepare reagent by dissolving 34.45 g 1,2,4-triazole in 150 mL water, add 25 mL 10 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 5 M NaOH, make up to 250 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: MeCN:buffer 25:75 (Buffer was 4.696 g Na_2HPO_4, 8.969 g $NaH_2PO_4.H_2O$, and 2.482 g anhydrous sodium thiosulfate in 1 L water.)

Flow rate: 0.8

Injection volume: 50-100

Detector: UV 325

CHROMATOGRAM

Retention time: 5.5

Internal standard: penicillin V (7)

Limit of detection: 3 ng/mL

KEY WORDS

derivatization; cow; SPE

REFERENCE

Boison,J.O.K.; Keng,L.J.-Y.; MacNeil,J.D. Analysis of penicillin G in milk by liquid chromatography, *J.AOAC Int.*, **1994**, *77*, 565–570.

SAMPLE

Matrix: milk

Sample preparation: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of 1:1 methylene chloride:hexane, centrifuge aqueous phase at 3000 rpm for 10 min. Dilute 3:1 with 20 mM sodium acetate buffer and filter (0.2 μm nylon). Inject 50 μL onto column with mobile phase A, run mobile phase A for 30 min and elute to waste. After 30 min switch to mobile phase B and elute through detector.

HPLC VARIABLES

Column: 100 × 8 Radial-Pak 10 μm μBondapak C18

Mobile phase: A 20 mM sodium acetate buffer; B Gradient. MeCN:MeOH:20 mM sodium acetate buffer from 15:10:75 to 30:0:70 over 15 min and hold at 30:0:70

Flow rate: A 3; B 2

Injection volume: 50

Detector: E, Waters 464 pulsed electrochemical detector using a thin layer cell with a Ag/AgCl reference electrode. E1 = 1300 mV for 0.166 s, E2 = 1500 mV for 0.166 s, E3 = -200 mV for 0.333 s.

CHROMATOGRAM

Retention time: 9

Limit of detection: 0.2 ppm

OTHER SUBSTANCES

Simultaneous: penicillin V, ampicillin, methicillin, oxacillin, cloxacillin, nafcillin, dicloxacillin.

REFERENCE

Kirchmann,E.; Earley,R.L.; Welch,L.E. The electrochemical detection of penicillins in milk, *J.Liq.Chromatogr.*, **1994**, *17*, 1755–1772.

SAMPLE

Matrix: milk

Sample preparation: Condition a 1 mL 100 mg Bond Elut C2 SPE cartridge with 1 mL MeCN and two 1 mL portions of water, suck dry for 5 s. 1 mL Milk + 200 μL water + 10 mL acetone, mix for 10 s, centrifuge at 3000 rpm for 3 min. Remove the organic layer and evaporate it to 600 μL under a stream of nitrogen at 45°, add 1 mL hexane, shake vigorously for 5 s, centrifuge for 2 min, discard the hexane layer, repeat the hexane wash, evaporate the aqueous layer to dryness, reconstitute the residue in 350 μL MeCN:water 20:80, add slowly to the SPE cartridge, suck dry for 5 s, wash with two 50 μL portions of MeCN:water 10:90, suck dry for 5 s, elute with four 100 μL portions of MeCN:water 20:80. Centrifuge the eluate at 3000 rpm for 3 min, inject a 125 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-C18 DB

Column: 250 × 4.6 5 μm Supelcosil LC-C18 DB

Mobile phase: MeCN:buffer 34:66 (Prepare buffer by dissolving 1.78 g $Na_2HPO_4.2H_2O$ and 4.45 g sodium 1-heptanesulfonate in 750 mL water, adjust pH to 2.15 with 5 M phosphoric acid, make up to 1 L with water.)

Column temperature: 35

Flow rate: 0.9 for 3 min, 0.6 for 10 min, 2 for 2 min

Injection volume: 125

Detector: UV 200

CHROMATOGRAM

Retention time: 12

Limit of detection: 2 ng/mL

Limit of quantitation: 4 ng/mL

KEY WORDS
cow; SPE

REFERENCE
Hormazal,V.; Yndestad,M. Detection of benzylpenicillin in milk by HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 2469–2474.

SAMPLE
Matrix: milk
Sample preparation: 10 mL Milk + 2 mL 200 mM tetraethylammonium chloride, stir, slowly add 38 mL MeCN over 30 s, let stand for 5 min, decant the supernatant through a plug of glass wool. 40 mL Filtrate + 1 mL water, evaporate under reduced pressure to 1-2 mL, make up to 4 mL with water, filter (0.45 μm polyvinylidene difluoride). Inject 2 mL into an LC system (150 × 4.6 5 μm Supelcosil LC-18; MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 40:60 over 27 min, to 0:100 over 1 min; 1 mL/min; UV 210 and 295), collect a 1.5 mL fraction at retention time for penicillin G (23 min), evaporate to 1 mL, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 29:71 (Buffer was 3.3 mM phosphoric acid and 6.7 mM KH_2PO_4.)
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Limit of quantitation: 2-5 ppb

OTHER SUBSTANCES
Also analyzed: ampicillin, amoxicillin, cephapirin, ceftiofur, penicillin V, cloxacillin

KEY WORDS
cow

REFERENCE
Moats,W.A.; Harik-Khan,R. Liquid chromatographic determination of β-lactam antibiotics in milk: A multiresidue approach, *J.AOAC Int.*, **1995**, *78*, 49–54.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM
Retention time: 15.33

OTHER SUBSTANCES
Extracted: ampicillin, ceftiofur, cephapirin, cloxacillin, dicloxacillin, nafcillin, oxacillin
Simultaneous: amoxicillin

KEY WORDS
SPE

REFERENCE
Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM
Retention time: 27
Internal standard: penicillin V (28.5)
Limit of detection: 1.3 ng/mL
Limit of quantitation: 1.9 ng/mL

OTHER SUBSTANCES
Extracted: amoxicillin, ampicillin, cloxacillin, dicloxacillin, oxacillin

KEY WORDS
derivatization; cow; SPE

REFERENCE
Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE

Matrix: milk, tissue

Sample preparation: Milk. Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound in a tube containing 100 μL Na_2HPO_4 (ca. 24.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. Tissue. Blend 5 g tissue, 5 mL water, 2 mL 100 mM tetraethylammonium chloride (for liver and kidney 1 mL 200 mM tetraethylammonium chloride and 1 mL 5 mM KH_2PO_4), and 40 mL MeCN at half power for 1 min, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate (20 mL for liver and kidney), add 2 mL buffer, add 5 mL water, add 5 mL t-butanol, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Proceed as above. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18-DB (milk) or Inertsil ODS-2 (tissue)

Mobile phase: MeCN:buffer 28:72 (milk) or 30:70 (tissue) (Buffer was 3.3 mM phosphoric acid containing 6.7 mM potassium dihydrogen phosphate.)

Flow rate: 1

Injection volume: 200

Detector: UV 215

KEY WORDS

muscle; liver; kidney

REFERENCE

Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm ODS-Hypersil

Mobile phase: MeCN:10 mM ammonium acetate 20:80

Flow rate: 2

Injection volume: 25

Detector: UV 227

OTHER SUBSTANCES

Also analyzed: imipenem (UV 300)

REFERENCE

Eley,A.; Greenwood,D. Beta-lactamases of type culture strains of the *Bacteroides fragilis* group and of strains that hydrolse cefoxitin, latamoxef and imipenem, *J.Med.Microbiol.*, **1986**, *21*, 49–57.

SAMPLE

Matrix: solutions

Sample preparation: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: present but not specified

Column: 150 × 4.6 4 μm Micropak SPC-18 C18

Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid from 15:85 to 60:40 over 20 min

Flow rate: 1

Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Simultaneous: dicloxacillin, methicillin, penicillin V, cloxacillin, nafcillin, carbenicillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 12.8

OTHER SUBSTANCES
Extracted: carbenicillin, cephapirin, cloxacillin, dicloxacillin, hetacillin, methicillin, nafcillin, oxacillin

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 200 μL aliquot of an aqueous solution with 30 μL reagent, heat at 60° for 20 min, inject an aliquot. (Reagent was imidazole:water:mercury(II) chloride 40:59.9: 0.1, adjusted to pH 6.8 with phosphoric acid.)

HPLC VARIABLES
Guard column: 40 × 4 5 μm LiChrosorb RP-18

Column: 150 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeOH:water 45:55 containing 2% imidazole and 50 μM mercury(II) chloride, pH adjusted to 6.6 with phosphoric acid (At the end of each day wash the column with 30 mL MeOH:pH 3.0 phosphate buffer (μ = 0.1) 45:55.)
Column temperature: 50
Detector: UV 325

CHROMATOGRAM
Retention time: 10
Limit of detection: 2 ng

KEY WORDS
derivatization

REFERENCE
Wiese,B.; Martin,K. Basic extraction studies of benzylpenicillin and its determination by liquid chromatography with pre-column derivatisation, *J.Pharm.Biomed.Anal.*, **1989**, 7, 67–78.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 22:78
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 220

OTHER SUBSTANCES
Also analyzed: methicillin, cefoperazone, cephalothin

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 mg/mL solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:water:500 mM pH 3.5 phosphate buffer 36:54:10
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
comparison with capillary electrophoresis

REFERENCE

Yongxin,Z.; Dalle,J.; Van Schepdael,A.; Roets,E.; Hoogmartens,J. Analysis of benzylpenicillin by capillary electrophoresis, *J.Chromatogr.A*, **1997**, *792*, 83–88.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl, do not allow to go dry. 5 g Tissue + 75 μL 20 μg/mL penicillin V in water + 20 mL water, homogenize (Polytron, 20 mm probe), rinse probe with water so that total volume is 35 mL, shake mechanically for 5 min, add 5 mL 170 mM sulfuric acid, add 5 mL 5% sodium tungstate, vortex for 20 s, centrifuge at 2200 g for 10 min, remove the supernatant, add 15 mL water to the residue, shake for 5 min, centrifuge at 2200 g for 10 min. Combine the supernatants and filter (Whatman GF/B) them, add 10 mL 20% NaCl to the filtrate, mix thoroughly, add to the SPE cartridge at 3 mL/min, wash with 10 mL 2% NaCl, wash with 10 mL water, draw air through the cartridge for 5 min, immediately elute with 1 mL MeCN:200 mM pH 6.5 sodium phosphate buffer:water 60:5:35. Add 1 mL reagent to the eluate, vortex, heat at 65° for 30 min, cool rapidly to room temperature, vortex, filter (Acro 0.45 μm), inject a 80-100 μL aliquot of the filtrate. (Prepare reagent by dissolving 34.45 g 1,2,4-triazole in 150 mL water, add 25 mL 10 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 5 M NaOH, make up to 250 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: MeCN:buffer 25:75 (Buffer was 4.969 g Na_2HPO_4, 8.969 g $NaH_2PO_4.H_2O$, and 2.482 g anhydrous sodium thiosulfate in 1 L water.)

Flow rate: 0.8

Injection volume: 80-100

Detector: UV 325

CHROMATOGRAM

Retention time: 5.8

Internal standard: penicillin V (7.6)

Limit of detection: 5 ng/g

OTHER SUBSTANCES

Simultaneous: ampicillin, chloramphenicol

KEY WORDS

muscle; liver; kidney; derivatization; cow; SPE

REFERENCE

Boison,J.O.; Salisbury,C.D.C.; Chan,W.; MacNeil,J.D. Determination of penicillin G residues in edible animal tissues by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 497–501.

SAMPLE

Matrix: tissue

Sample preparation: Blend 15 g tissue with 45 mL (60 mL for liver and kidney) water in a 300 or 500 mL blender jar at half power (or less to control foaming) for 2 min. Add a 20 mL aliquot of homogenate to 40 mL MeCN, mix, after 5 min decant supernatant through a plug of glass wool, collect 30 mL. Shake vigorously 30 mL filtrate, 10 mL 200 mM phosphoric acid, and 20 mL dichloromethane, remove organic layer and extract aqueous layer with 10 mL dichloromethane (and 10 mL MeCN for liver and kidneys). Combine dichloromethane layers, add 15 mL MeCN, add 40 mL hexane, wash the mixture twice with 4 mL portions of water, extract the organic layer four times with 1 mL 10 mM pH 7 buffer. Combine extracts and add 0.1-0.2 mL tert-butanol, place in a rotary evaporator without heating at first. When the flask becomes cold warm to 50°, concentrate to less than 1 mL, adjust to a final volume of 1 mL, filter (Gelman Acrodisc LCPVDF), inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: Polymer Labs guard cartridge

Column: 150 × 4.6 5 μm 100 Å PLRP-S polystyrene-divinylbenzene (Polymer Labs)

Mobile phase: MeCN:buffer 15:85, after run was over flush at 35:65 for 5 min then re-equilibrate with 15:85 for 9 min. (Buffer was 10 mM pH 7 phosphate buffer prepared from 1.36 KH_2PO_4 and 2.84 g Na_2HPO_4 in 3 L water.)
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 10 ng/g

KEY WORDS
cow; pig

REFERENCE
Moats,W.A. High-performance liquid chromatographic determination of penicillin G, penicillin V and cloxacillin in beef and pork tissues, *J.Chromatogr.*, **1992**, *593*, 15–20.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 20 mL 2% NaCl. Shake 10 g tissue and 20 mL MeCN on a mechanical shaker for 30 min, centrifuge, remove the supernatant, repeat the extraction with 20 and 10 mL portions of MeCN. Combine the extracts and add them to 30 mL 4% NaCl, remove the MeCN under reduced pressure at 40°, filter (Whatman GF/C and Gelman 0.45 μm membrane) the remaining aqueous mixture, add the filtrate to the SPE cartridge at <2 mL/min, wash with 15 mL 2% NaCl, elute with 5 mL MeCN. Add 100 μL 20 μg/mL penicillin V in MeCN to the eluate, evaporate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 1 mL water, vortex, add 1 mL 2 M pH 9 1,2,4-triazole containing 1 mM mercuric chloride, vortex, heat at 65° for 30 min, cool, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:buffer 22.5:77.5 (Prepare buffer by dissolving 4.96 g Na_2HPO_4, 10.14 g $NaH_2PO_4.2H_2O$, and 3.90 g sodium thiosulfate in 1 L water.)
Flow rate: 1.2
Injection volume: 50
Detector: UV 325

CHROMATOGRAM
Retention time: 5
Internal standard: penicillin V (6.5)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: cloxacillin

KEY WORDS
derivatization; cow; sheep; kidney; liver; muscle; SPE

REFERENCE
Gee,H.-E.; Ho,K.-B.; Toothill,J. Liquid chromatographic determination of benzylpenicillin and cloxacillin in animal tissues and its application to a study of the stability at -20°C of spiked and incurred residues of benzylpenicillin in ovine liver, *J.AOAC Int.*, **1996**, *79*, 640–644.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 25 g tissue with 25 mL MeCN for 1 min, add 5 mL 500 mM pH 2.2 phosphate buffer while the homogenizer is still running, add 65 mL MeCN, homogenize for 1 min, centrifuge at 4000 g for 10 min. Remove the supernatant and add it to 7 g NaCl and 50 mL dichloromethane, shake for 2 min, allow to stand for 30 min.

Remove the upper organic layer and add it to 5 g anhydrous sodium sulfate, shake for 30 s, filter through a cotton-wool plug, evaporate to about 4 mL under reduced pressure at 30°, add 3 mL dichloromethane, evaporate to about 4 mL, add 3 mL light petroleum, evaporate to about 0.5 mL, Suspend this residue with sonication in three 3 mL portions of light petroleum and place these fractions in a separate tube, rinse the original tube with 2 mL pH 7 phosphate buffer. Add the phosphate buffer rinse to the light petroleum extracts, vortex for 30 s, centrifuge, remove the aqueous layer. Extract the light petroleum layer with 2 mL pH 7 phosphate buffer and with two 1.5 mL portions of pH 7 phosphate buffer, combine all the aqueous phase, centrifuge, inject a 200 μL aliquot on to column A and elute to waste with mobile phase B, after 15 min elute to waste with mobile phase C at 2 mL/min, after 10 min elute the contents of column A on to column B with mobile phase D, after 2 min remove column A from the circuit, elute column B with mobile phase D, monitor the effluent from column B. (Wash column A with mobile phase A at 2 mL/min for 7 min, with mobile phase A at 1 mL/min for 5 min, with mobile phase B at 2 mL/min for 8 min, and with mobile phase B at 1 mL/min for 6 min.)

HPLC VARIABLES

Column: A 4 × 4 5 μm LiChrospher 100 RP-18e; B 250 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: A MeCN:water 50:50; B 20 mM pH 7 phosphate buffer; C MeCN:20 mM pH 3 phosphate buffer 10:90; D MeCN:200 mM pH 3.0 phosphate buffer 35:65 containing 2 mM disodium EDTA
Column temperature: 35
Flow rate: 1 (except where indicated)
Injection volume: 200
Detector: E, Merck Model L3500, glassy carbon working electrode +0.65 V, stainless-steel auxiliary electrode, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID woven PTFE coil illuminated by a UV 254 low-pressure mercury lamp to the detector.

CHROMATOGRAM

Retention time: 5.2
Limit of detection: 1.2 ng

OTHER SUBSTANCES

Extracted: cloxacillin, dicloxacillin, oxacillin, penicillin V

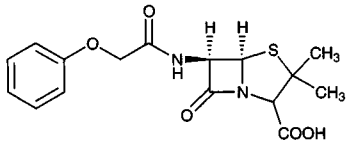
KEY WORDS

post-column reaction; post-column photochemical derivatization; cow; muscle; column-switching

REFERENCE

Lihl,S.; Rehorek,A.; Petz,M. High-performance liquid chromatographic determination of penicillins by means of automated solid-phase extraction and photochemical degradation with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 229–235.

Penicillin V



Molecular formula: $C_{16}H_{18}N_2O_5S$
Molecular weight: 350.40
CAS Registry No.: 87-08-1, 132-98-9 (potassium salt), 5928-84-7 (benzathine), 63690-57-3 (benzathine tetrahydrate), 6591-72-6 (hydrabamine)
Merck Index: 7230
Lednicer No.: 7230

SAMPLE

Matrix: bulk
Sample preparation: Dissolve a 50 mg sample in 50 mL 50 mM pH 6.5 potassium dihydrogen phosphate buffer. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18

Mobile phase: MeOH:water:500 mM pH 3.5 phosphate buffer 39:51.2:9.8
Column temperature: 50
Flow rate: 1.0
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13.8

OTHER SUBSTANCES
Simultaneous: 4-hydroxyphenoxymethylpenicillin

KEY WORDS
use gradient to determined impurities; details for HPLC

REFERENCE
Yongxin,Z.; Roets,E.; Trippen,B.; Christiansen,C.-P.; Arevalo,M.P.; Porqueras,E.; Maichel,B.; Inama,P.; Söderholm,S.; Miller,J.H.MB.; Spieser,J.M.; Hoogmartens,J. Interlaboratory study of analysis of phenoxymethylpenicillin by liquid chromatography, *Chromatographia*, **1998**, *47*, 152–156.

SAMPLE
Matrix: bulk
Sample preparation: Inject an aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.65 μm Hypersil C18
Mobile phase: MeOH:water:500 mM pH 3.5 phosphate buffer 40:50:10
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Limit of detection: 11.8 pg
Limit of quantitation: 23.6 pg

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Zhu,Y.; Van Schepdael,A.; Roets,E.; Hoogmartens,J. Micellar electrokinetic capillary chromatography for the separation of phenoxymethylpenicillin and related substances, *J.Chromatogr.A*, **1997**, *781*, 417–422.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound, evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 33:67 (Buffer was 5 mM phosphoric acid containing 5 mM potassium dihydrogen phosphate.)

Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE

Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE

Matrix: milk

Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)

Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM

Retention time: 28.5
Internal standard: penicillin V

OTHER SUBSTANCES

Extracted: amoxicillin, ampicillin, cloxacillin, dicloxacillin, oxacillin, penicillin G

KEY WORDS

derivatization; cow; SPE; penicillin V is IS

REFERENCE

Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 25 g tissue with 25 mL MeCN for 1 min, add 5 mL 500 mM pH 2.2 phosphate buffer while the homogenizer is still running, add 65 mL MeCN, homogenize for 1 min, centrifuge at 4000 g for 10 min. Remove the supernatant and add it to 7 g NaCl and 50 mL dichloromethane, shake for 2 min, allow to stand for 30 min. Remove the upper organic layer and add it to 5 g anhydrous sodium sulfate, shake for 30 s, filter through a cotton-wool plug, evaporate to about 4 mL under reduced pressure at 30°, add 3 mL dichloromethane, evaporate to about 4 mL, add 3 mL light petroleum, evaporate to about 0.5 mL, Suspend this residue with sonication in three 3 mL portions of light petroleum and place these fractions in a separate tube, rinse the original tube with 2 mL pH 7 phosphate buffer. Add the phosphate buffer rinse to the light petroleum extracts, vortex for 30 s, centrifuge, remove the aqueous layer. Extract the light petroleum layer with 2 mL pH 7 phosphate buffer and with two 1.5 mL portions of pH 7 phosphate buffer, combine all the aqueous phase, centrifuge, inject a 200 µL aliquot on to column A and elute to waste with mobile phase B, after 15 min elute to waste with mobile phase C at 2 mL/min, after 10 min elute the contents of column A on to column B with mobile phase D, after 2 min remove column A from the circuit, elute column B with mobile phase D, monitor the effluent from column B. (Wash column A with mobile phase A at 2 mL/min for 7 min, with mobile phase A at 1 mL/min for 5 min, with mobile phase B at 2 mL/min for 8 min, and with mobile phase B at 1 mL/min for 6 min.)

HPLC VARIABLES
Column: A 4 × 4 5 µm LiChrospher 100 RP-18e; B 250 × 4 5 µm LiChrospher 100 RP-18e
Mobile phase: A MeCN:water 50:50; B 20 mM pH 7 phosphate buffer; C MeCN:20 mM pH 3 phosphate buffer 10:90; D MeCN:200 mM pH 3.0 phosphate buffer 35:65 containing 2 mM disodium EDTA
Column temperature: 35
Flow rate: 1 (except where indicated)
Injection volume: 200
Detector: E, Merck Model L3500, glassy carbon working electrode +0.65 V, stainless-steel auxiliary electrode, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID woven PTFE coil illuminated by a UV 254 low-pressure mercury lamp to the detector.

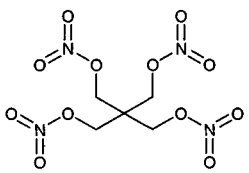
CHROMATOGRAM
Retention time: 6.1
Limit of detection: 1.4 ng

OTHER SUBSTANCES
Extracted: cloxacillin, dicloxacillin, oxacillin, penicillin G

KEY WORDS
post-column reaction; post-column photochemical derivatization; cow; muscle; column-switching

REFERENCE
Lihl,S.; Rehorek,A.; Petz,M. High-performance liquid chromatographic determination of penicillins by means of automated solid-phase extraction and photochemical degradation with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 229–235.

Pentaerythritol tetranitrate



Molecular formula: $C_5H_8N_4O_{12}$
Molecular weight: 316.14
CAS Registry No.: 78-11-5
Merck Index: 7249

SAMPLE
Matrix: bulk, formulations

Sample preparation: Weigh out amount of bulk drug or powdered tablets or capsules equivalent to about 25 mg pentaerythritol tetranitrate, add 125 mL mobile phase, if clumping occurs sonicate for 5 min, shake for 30 min, add 5 mL IS solution, dilute to 250 mL with mobile phase, filter (0.45 μm), inject a 20 μL aliquot. (Prepare IS solution by adding 10 g 10% nitroglycerin solution in lactose to 125 mL MeOH, sonicate for 5 min, shake mechanically for 30 min, dilute to 200 mL with MeOH, let undissolved lactose settle, and filter through paper.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:water 65:35
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 7.8
Internal standard: nitroglycerin (6)

KEY WORDS
tablets; capsules; collaborative study

REFERENCE
Carlson,M. Liquid chromatographic determination of pentaerythritol tetranitrate in pharmaceuticals: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 693–697.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out a portion equivalent to 2 mg pentaerythritol tetranitrate, add to 10 mL 75 μg/mL nitroglycerin in MeOH, sonicate for 2 min, shake mechanically for 30 min, filter, inject an aliquot

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HPLC VARIABLES
Guard column: 40 × 4.6 μBondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 25.5
Internal standard: nitroglycerin (14)

OTHER SUBSTANCES
Simultaneous: isosorbide dinitrate, erythrityl tetranitrate

KEY WORDS
tablets

REFERENCE
Olsen,C.S.; Scroggins,H.S. High-performance liquid chromatographic determination of the nitrate esters isosorbide dinitrate, pentaerythritol tetranitrate, and erythrityl tetranitrate in various tablet forms, *J.Pharm.Sci.*, **1984**, *73*, 1303–1304.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out an amount of finely powdered tablets or capsules equivalent to about 25 mg of drug. Add 50 mL buffer, shake for 30 min, add 10 mL 5 mg/mL nitroglycerin in MeOH, make up to 100 mL with buffer, filter (0.45 μm), inject a 20 μL aliquot. If the sample clumps when the buffer is added, agitate with a stirring rod and sonicate. (Buffer was MeOH: 200 mM ammonium acetate buffer:water 55:10:35.)

HPLC VARIABLES
Guard column: 50 × 6.4 25-37 μm Whatman Co-Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:200 mM ammonium acetate buffer:water 55:10:35
Flow rate: 1
Injection volume: 20
Detector: UV 254

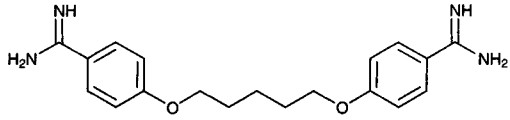
CHROMATOGRAM
Retention time: 15.2
Internal standard: nitroglycerin (8)

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate, saccharin, isosorbide dinitrate

KEY WORDS
tablets; capsules

REFERENCE
Carlson,M.; Thompson,R.D.; Snell,R.P. Determination of isosorbide dinitrate in pharmaceutical products by HPLC, *J.Chromatogr.Sci.*, **1988**, *26*, 574–578.

Pentamidine



Molecular formula: $C_{19}H_{24}N_4O_2$
Molecular weight: 340.43
CAS Registry No.: 100-33-4, 6823-79-6 (dimethanesulfonate), 140-64-7 (isethionate)
Merck Index: 7254

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 4.92 μg/mL hexamidine, vortex briefly, add 500 μL 2 M NaOH, vortex, add 500 μL 2 M HCl, vortex, add 1 mL pH 10 carbonate buffer, vortex, add 4 mL 20 mM di(2-ethylhexyl)phosphoric acid in chloroform, vortex for 1 min, centrifuge at 700 g for 15 min. Remove the chloroform layer and add it to 1 mL 20 mM HCl, vortex for 1 min. Remove the aqueous layer and adjust the pH to 12 with 4 drops 2 M NaOH, add 2 mL dichloromethane, vortex for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL solvent, vortex for 30 s, inject a 20 μL aliquot. (Solvent was MeOH:buffer 50:50. Buffer was 50 mM sodium heptanesulfonate containing 0.4% triethylamine, pH adjusted to 3.0 with phosphoric acid.)

HPLC VARIABLES
Column: 150 × 2.1 5 μm solvent miser C18 (Alltech)
Mobile phase: MeOH:buffer 60:40 (Buffer was 50 mM sodium heptanesulfonate containing 14 mM triethylamine, pH adjusted to 3.0 with phosphoric acid.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.7
Internal standard: hexamidine (8.2)
Limit of detection: 5 ng/mL

KEY WORDS
serum; dog; human; pharmacokinetics

HPLC VARIABLES
Guard column: 50 × 6.4 25-37 μm Whatman Co-Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:200 mM ammonium acetate buffer:water 55:10:35
Flow rate: 1
Injection volume: 20
Detector: UV 254

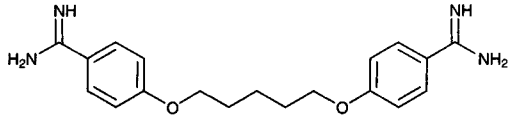
CHROMATOGRAM
Retention time: 15.2
Internal standard: nitroglycerin (8)

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate, saccharin, isosorbide dinitrate

KEY WORDS
tablets; capsules

REFERENCE
Carlson,M.; Thompson,R.D.; Snell,R.P. Determination of isosorbide dinitrate in pharmaceutical products by HPLC, *J.Chromatogr.Sci.*, **1988**, *26*, 574–578.

Pentamidine



Molecular formula: $C_{19}H_{24}N_4O_2$
Molecular weight: 340.43
CAS Registry No.: 100-33-4, 6823-79-6 (dimethanesulfonate), 140-64-7 (isethionate)
Merck Index: 7254

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 4.92 μg/mL hexamidine, vortex briefly, add 500 μL 2 M NaOH, vortex, add 500 μL 2 M HCl, vortex, add 1 mL pH 10 carbonate buffer, vortex, add 4 mL 20 mM di(2-ethylhexyl)phosphoric acid in chloroform, vortex for 1 min, centrifuge at 700 g for 15 min. Remove the chloroform layer and add it to 1 mL 20 mM HCl, vortex for 1 min. Remove the aqueous layer and adjust the pH to 12 with 4 drops 2 M NaOH, add 2 mL dichloromethane, vortex for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL solvent, vortex for 30 s, inject a 20 μL aliquot. (Solvent was MeOH:buffer 50:50. Buffer was 50 mM sodium heptanesulfonate containing 0.4% triethylamine, pH adjusted to 3.0 with phosphoric acid.)

HPLC VARIABLES
Column: 150 × 2.1 5 μm solvent miser C18 (Alltech)
Mobile phase: MeOH:buffer 60:40 (Buffer was 50 mM sodium heptanesulfonate containing 14 mM triethylamine, pH adjusted to 3.0 with phosphoric acid.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.7
Internal standard: hexamidine (8.2)
Limit of detection: 5 ng/mL

KEY WORDS
serum; dog; human; pharmacokinetics

REFERENCE
Dickinson,C.M.; Navin,T.R.; Churchill,F.C. High-performance liquid chromatographic method for quantitation of pentamidine in blood serum, *J.Chromatogr.*, **1985**, *345*, 91–97.

SAMPLE
Matrix: blood
Sample preparation: Condition an SPE-C8 SPE cartridge (Jones Chromatography) with two 1 mL aliquots of MeOH, two 1 mL aliquots of MeCN:1 M pH 3 ammonium acetate 75:25, and five 1 mL aliquots of water, do not allow to dry. 100 μL Plasma + 100 μL water, mix, add to the SPE cartridge, wash with three 1 mL aliquots of MeOH, add 80 μL 500 ng/mL melphalan in MeOH to the SPE cartridge, elute with 1 mL MeCN:1 M pH 3 ammonium acetate 75:25. Evaporate the eluate to dryness under reduced pressure, reconstitute in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:MeOH:triethylamine:200 mM ammonium acetate 18:2:0.5:79.5, pH adjusted to 3.8 with acetic acid
Flow rate: 1.4
Injection volume: 50
Detector: F ex 265 em 345

CHROMATOGRAM
Retention time: 5.5
Internal standard: melphalan (10)
Limit of detection: 8.6 ng/mL

KEY WORDS
plasma; rat; SPE; pharmacokinetics

REFERENCE
Yeh,T.-K.; Dalton,J.T.; Au,J.L.-S. High-performance liquid chromatographic determination of pentamidine in plasma, *J.Chromatogr.*, **1993**, *622*, 255–261.

SAMPLE
Matrix: blood
Sample preparation: Let blood stand at 4° for 4 h, centrifuge at 15000 g for 10 min, add sodium azide to a concentration of 0.01%, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax RX-C8
Mobile phase: Gradient. A was 4.2 mM phosphoric acid containing 10 mM sodium heptanesulfonate and 10 mM tetramethylammonium chloride. B was MeCN:water 75:25 containing 4.2 mM phosphoric acid, 10 mM sodium heptanesulfonate, and 10 mM tetramethylammonium chloride. A:B from 90:10 to 10:90 over 30 min, return to initial conditions over 7 min, re-equilibrate for 3 min.
Flow rate: 1.5
Injection volume: 25
Detector: UV 265

CHROMATOGRAM
Retention time: 18
Internal standard: pentamidine

OTHER SUBSTANCES
Extracted: guanylhydrazones

KEY WORDS
serum; mouse; pentamidine is IS; rat

REFERENCE
Cerami,C.; Zhang,X.; Ulrich,P.; Bianchi,M.; Tracey,K.J.; Berger,B.J. High-performance liquid chromatographic method for guanylhydrazone compounds, *J.Chromatogr.B*, **1996**, *675*, 71–75.

SAMPLE
Matrix: blood, broncho-alveolar lavage fluid, cells, urine
Sample preparation: Plasma. 0.5 mL Plasma + 1 mL 30 ng/mL hexamidine in MeCN, vortex, centrifuge at 1000 g for 10 min, add the supernatant to a Bond Elut C8 SPE cartridge, wash with 1 mL water, wash with 1 mL MeOH:water 50:50, wash with 1 mL MeOH, elute with MeOH:water:sodium heptanesulfonate:tetramethylammonium chloride:phosphoric acid 97.2:2.2:0.5:0.02:0.1, evaporate the eluate to 200 μL under a stream of nitrogen, inject a 50 μL aliquot. Bronchoalveolar lavage fluid. Centrifuge bronchoalveolar lavage fluid, add 1 mL supernatant to 1 mL Sorensen's buffer and 1 mL 30 ng/mL hexamidine in MeCN, vortex, centrifuge, add the supernatant to a Bond Elut C8 SPE cartridge, wash with water, wash with MeOH:water 50:50, wash with MeOH, elute with MeOH:water:sodium heptanesulfonate:tetramethylammonium chloride:phosphoric acid 97.2:2.2:0.5:0.02:0.1, evaporate the eluate to 200 μL under a stream of nitrogen, inject a 50 μL aliquot. Alveolar cells. Wash alveolar cells (from 10 mL bronchoalveolar lavage fluid) twice with phosphate buffered saline, resuspend in 1 L Sorensen's buffer, vortex for 1 min. 250 μL Suspension + 250 μL Sorensen's buffer + 1 mL 30 ng/mL hexamidine in MeCN, vortex, centrifuge, add the supernatant to a Bond Elut C8 SPE cartridge, wash with water, wash with MeOH:water 50:50, wash with MeOH, elute with MeOH:water:sodium heptanesulfonate:tetramethylammonium chloride:phosphoric acid 97.2:2.2:0.5:0.02:0.1, evaporate the eluate to 200 μL under a stream of nitrogen, inject a 50 μL aliquot. Urine. 200 μL Urine + 1 mL 750 ng/mL hexamidine in MeCN, vortex for 1 min, centrifuge at 1000 g for 5 min, inject a 20 μL aliquot (J.Infect.Dis. 1986, 154, 823).

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Octyl 5
Mobile phase: MeCN:water 21:79 containing 0.02% tetramethylammonium chloride and 0.1% phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: F ex 275 em 340

CHROMATOGRAM
Internal standard: hexamidine
Limit of detection: 2.29 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Conte,J.E.,Jr.; Golden,J.A. Intrapulmonary and systemic pharmacokinetics of aerosolized pentamidine used for prophylaxis of *Pneumocystis carinii* pneumonia in patients infected with the human immunodeficiency virus, *J.Clin.Pharmacol.*, **1995**, *35*, 1166–1173.

SAMPLE
Matrix: cells
Sample preparation: 400 μL Cells in phosphate saline glucose (99:1) buffer + 10 μL 10% orthophosphoric acid, vortex, filter (Ultrafree-MC polysulfone membrane, 100000 molecular mass cut-off) while centrifuging at 5000 g for 5 min, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm pellicular Spherisorb RP-18
Column: 200 × 2 5 μm Nucleosil C18
Mobile phase: MeOH:5 mM citric acid 50:50 containing 5 mM sodium pentanesulfonate, pH adjusted to 4.0 with NaOH
Flow rate: 0.5
Injection volume: 20
Detector: UV 261

CHROMATOGRAM
Retention time: 6
Limit of detection: 5.7 ng/mL

REFERENCE
Rabanal,B.; De Arriba,R.G.; Garzón,M.J.; Reguera,R.M.; Balaña-Fouce,R.; Negro,A. Determination of pentamidine in *Leishmania* infantum promastigotes by ion-paired liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2017–2029.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 6 mL microsomal incubation + 500 μL MeCN + hexamidine, add to a Prep-Sep C18 SPE cartridge (Fisher), wash with water, wash with MeCN, elute with 1 mL MeCN: buffer 95:5. Evaporate the eluate to near dryness under a stream of air, reconstitute the residue in 200 μL mobile phase, inject a 5 μL aliquot. (Buffer was 4.2 mM phosphoric acid containing 10 mM heptanesulfonate and 10 mM tetramethylammonium chloride.)

HPLC VARIABLES
Column: 250 X 4.6 Zorbax 5 μm RX diisopropyl C8
Mobile phase: Gradient. MeCN:buffer from 22.5:77.5 to 45:55 over 25 min (Buffer was 4.2 mM phosphoric acid containing 10 mM heptanesulfonate and 10 mM tetramethylammonium chloride.)
Column temperature: 40
Injection volume: 5
Detector: UV 265

CHROMATOGRAM
Retention time: 16
Internal standard: hexamidine

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver; SPE

REFERENCE
Berger,B.J.; Reddy,V.V.; Le,S.T.; Lombardy,R.J.; Hall,J.E.; Tidwell,R.R. Hydroxylation of pentamidine by rat liver microsomes, *J.Pharmacol.Exp.Ther.*, **1991**, *256*, 883–889.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Lyophilize microsomal incubation. Dissolve lyophilizate in 150 μL mobile phase, mix thoroughly for 5 min, centrifuge at 6000 g for 5 min, inject a 15 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 60 RP-select B
Column: 125 × 4 5 μm Lichrospher 60 RP-select B
Mobile phase: Gradient. A was 10 mM sodium octylsulfonate, 10 mM tetramethylammonium chloride, and 20 mM phosphoric acid adjusted to pH 3.0 with ammonia. B was MeOH. A:B from 60:40 to 30:70 over 30 min.
Flow rate: 1
Injection volume: 15
Detector: UV 260

CHROMATOGRAM
Retention time: 22
Limit of detection: 0.02 nmole

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rabbit; liver

REFERENCE
Clement,B.; Jung,F. *N*-Hydroxylation of the antiprotozoal drug pentamidine catalyzed by rabbit liver cytochrome P-450 2C3 or human liver microsomes, microsomal retroreduction, and further oxidative transformation of the formed amidoximes Possible relationship to the biological oxidation of arginine to NG-hydroxyarginine, citrulline, and nitric oxide, *Drug Metab.Dispos.*, **1994**, *22*, 486–497.

SAMPLE
Matrix: urine
Sample preparation: Directly inject an aliquot of urine. Alternatively add urine to a Sep-Pak SPE cartridge, elute with 2 mL MeCN:water 50:50, inject an aliquot of the eluate.

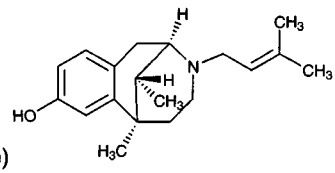
HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:50 mM pH 6 ammonium acetate buffer:triethylamine 3:96.8:0.2. B was MeCN:50 mM pH 6 ammonium acetate buffer:triethylamine 50:49.8:0.2. A:B from 100:0 to 0:100 in 60 min (?)
Injection volume: 100-150
Detector: MS, Finnigan TSQ 700, chemical ionization APCI interface, vaporizer 400°, heated capillary 200°, current 5 μA, m/z 357 (The effluent from the column was directed to waste for 2 min, then to the MS.)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rat; SPE

REFERENCE
Nordin,J.; Wilkström,I.; Bronner,U.; Gustafsson,L.L.; Ericsson,. Liquid chromatography-tandem mass spectrometry applied to a study of the metabolism of pentamidine. Discussion of possibilities and problems, *J.Chromatogr.A*, **1997**, *777*, 73–79.

Pentazocine



Molecular formula: $C_{19}H_{27}NO$
Molecular weight: 285.45
CAS Registry No.: 359-83-1, 64024-15-3 (HCl), 17146-95-1 (lactate)
Merck Index: 7261
Lednicer No.: 1 297; 2 325

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 ng doxepin or desipramine + 100 μL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 μL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8

Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 7.4
Internal standard: doxepin (12.2), desipramine (14.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: cocaine, dextromoramide, meperidine, methadone, normeperidine, norpropoxyphene, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

KEY WORDS
serum

REFERENCE
Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 1 M NaOH + 6 mL 20 ng/mL levallorphan tartrate in diethyl ether, agitate at 4° for 15 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, dissolve the residue in 250-500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Nucleosil RP 18
Mobile phase: MeCN:5 mM phosphoric acid 33:67
Flow rate: 1
Injection volume: 50
Detector: F ex 278 em 324

CHROMATOGRAM
Retention time: 6.2
Internal standard: levallorphan tartrate (4.9)
Limit of detection: 1 ng/mL
Limit of quantitation: 4 ng/mL

KEY WORDS
plasma

REFERENCE
Moeller,N.; Dietzel,K.; Nuernberg,B.; Geisslinger,G.; Brune,K. High-performance liquid chromatographic determination of pentazocine in plasma, *J.Chromatogr.*, **1990**, *530*, 200–205.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN: water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: Gradient A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 20
Limit of detection: 0.50 ppm

OTHER SUBSTANCES

Extracted: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS

whole blood

REFERENCE

Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE

Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 1.9
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, promazine, propafenone, propoxyphene, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol,

MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: encainide, lidocaine, propranolol

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 4.33
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine;

bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents

Sample preparation: 1 mL Whole blood or gastric contents + 50 μL 400 μg/mL IS in MeOH + 1 mL EtOH + 5 drops 1 M pH 9 potassium carbonate + 2 mL water + 8 mL n-hexane:MTBE 25:75, rotate for 15 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil BDS C18

Mobile phase: Gradient. A was MeCN:MeOH:1.5 M ammonium acetate:water 10:10:3:77. B was MeCN:MeOH:1.5 M ammonium acetate:water 40:40:3:17. A:B from 95:5 to 50:50 over 20 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 15

Internal standard: N-allylnormetazocine (Sigma) (9)

OTHER SUBSTANCES

Extracted: zopiclone

KEY WORDS

whole blood

REFERENCE

Van Bocxlaer,J.; Meyer,E.; Clauwaert,K.; Lambert,W.; Piette,M.; De Leenheer,A. Analysis of zopiclone (Imovane) in postmortem specimens by GC-MS and HPLC with diode-array detection, *J.Anal.Toxicol.*, **1996**, *20*, 52–54.

SAMPLE

Matrix: blood, saliva, tissue, urine

Sample preparation: Homogenize (Polytron) tissue with 4 (whole brain) or 8 (brain striata) volumes of 100 mM pH 4.5 NaH_2PO_4 containing 0.5% NaF. Add 500 μL brain homogenate or 500 μL plasma, saliva, or urine containing 15 μL saturated NaF solution to 75 μL 150 μg/mL IS, add 50 μL 50% perchloric acid, mix vigorously for 10 s, let stand at room temperature for 10 min, add 1 mL water, mix briefly, centrifuge at 10° at 2500 (?) for 30 min. Remove the supernatant and add it to 750 μL saturated sodium carbonate solution, mix briefly, add 7.5 mL pentane:chloroform 95:5, rock gently for 10 min, centrifuge in a desk-top centrifuge for 2 min, freeze in dry ice/acetone for 2 min. Remove the organic layer and add it to 250 μL 100 mM HCl, mix vigorously for 10 s, centrifuge in a desk-top centrifuge for 1-2 min, freeze in dry ice/acetone for 3-5 min, discard the organic layer. Allow the aqueous layer to thaw, remove any trace of organic solvent with a stream of nitrogen, inject a 75 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Brownlee RP-8

Column: 250 × 4.6 5 μm Zorbax RX-C18
Mobile phase: MeCN:buffer 18:82 (Buffer was 100 mM K_2HPO_4 containing 0.5% triethylamine, adjusted to pH 2.7 with phosphoric acid.)
Flow rate: 2
Injection volume: 75
Detector: UV 235

CHROMATOGRAM
Retention time: 11.5
Internal standard: 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (RTI-31) (Research Biochemical International, Natick MA) (11.4)

OTHER SUBSTANCES
Extracted: chlordiazepoxide, clozapine, cocaine, gepirone, methylphenidate, pseudococaine
Simultaneous: acetaminophen, acetophenazine, amoxapine, amphetamine, atropine, benperidol, buspirone, caffeine, carbamazepine, chlorpheniramine, codeine, dextromethorphan, diazepam, diphenhydramine, flupenthixol, flurazepam, haloperidol, hydergine, hydrocodone, hydromorphone, lidocaine, loxapine, mepazine, meperidine, mesoridazine, methaquaalude, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, 3,4-methylenedioxymethamphetamine, morphine, norcocaine, oxazepam, pentobarbital, phenylpropanolamine, procainamide, procaine, propyl benzoylecgonine, quinidine, quinine, salicylic acid, secobarbital, theophylline, trazodone, 3-tropanyl-3,5-dichlorobenzoate, vancomycin, WIN 35428
Noninterfering: amitriptyline, benztropine methanesulfonate, butaperazine, butriptyline, carphenazine, chlorpromazine, clomipramine, cyclobenzaprine, dextropropoxyphene, dronabinol, ephedrine, ethchlorvynol, fluoxetine, fluphenazine, imipramine, meprobamate, methadone, methamphetamine, nicotine, norfluoxetine, nortriptyline, PCP, phenothiazine, pseudoephedrine

KEY WORDS
rat; cow; plasma; brain

REFERENCE
Bonate,P.L.; Davis,C.M.; Silverman,P.B.; Swann,A. Determination of cocaine in biological matrices using reversed phase HPLC: Application to plasma and brain tissue, *J.Liq.Chromatogr.*, **1995**, *18*, 3473–3494.

SAMPLE
Matrix: blood, tissue, vitreous humor
Sample preparation: Blood, vitreous humor. Mix 1 mL sample with 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 200 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer. Tissue. Mix 500 μL liver homogenate with 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 400 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 3.9 5 μm NovaPak-Phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 55:45, adjusted to pH 3.0
Flow rate: 1.5
Injection volume: 30
Detector: UV 214

OTHER SUBSTANCES
Extracted: pimozide, sertraline

KEY WORDS
liver; pentazocine is IS

REFERENCE
McIntyre,I.M.; King,C.V.; Staikos,V.; Gall,J.; Drummer,O.H. A fatality involving moclobemide, sertraline, and pimozide, *J.Forensic Sci.*, **1997**, *42*, 951–953.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 12.522

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak CN

Mobile phase: MeOH:3 mM ammonium acetate 90:10

Flow rate: 1.3

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 5.3

OTHER SUBSTANCES

Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, promethazine, protriptyline, pyrilamine, pyrimethamine, tripelennamine

KEY WORDS

tablets; syrups; elixirs; injections

REFERENCE

Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.21

OTHER SUBSTANCES
Simultaneous: thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, normethadone, meperidine, dipipanone, diamorphine, acetylcodeine, monoacetylmorphine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipa-

none, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, imino-

stilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.91 (A), 5.47 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, met-

ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 2.8

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

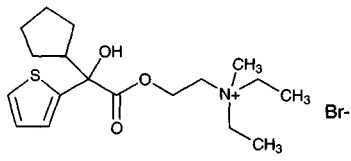
Extracted: nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine

Interfering: amphetamine, phenmetrazine, lidocaine, ephedrine, methamphetamine, desipramine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

Penthienate bromide



Molecular formula: $C_{18}H_{30}BrNO_3S$
Molecular weight: 420.41
CAS Registry No.: 60-44-6
Merck Index: 7268

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.9

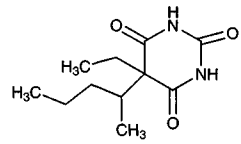
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, pri-

maquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Pentobarbital



Molecular formula: $C_{11}H_{18}N_2O_3$
Molecular weight: 226.28
CAS Registry No.: 76-74-4, 57-33-0 (Na salt)
Merck Index: 7272
Lednicer No.: 1 269

SAMPLE

Matrix: blood
Sample preparation: 200 µL Serum + 200 µL 50 µg/mL hexobarbital in MeCN + 25 µL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 µL aliquot of the supernatant.

HPLC VARIABLES

Column: µBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM

Retention time: 22.4
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 1 mL buffer, vortex, add 10 mL n-butyl chloride containing 10 μg/mL barbital and 4 μg/mL thiamylal, extract vigorously for 3 min, centrifuge at 3000 g for 5 min. Remove the upper organic layer and add it to 100 μL 450 mM NaOH, extract vigorously for 3 min, centrifuge for 10 min or until lower aqueous phase is clear, inject a 15 μL aliquot of the lower aqueous phase. (Soak glassware in 1 M HCl overnight, rinse with water, dry. Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)

HPLC VARIABLES
Column: 125 × 4.6 5 μm C-18 (Perkin-Elmer)
Mobile phase: MeOH:THF:buffer 50:7:43 (Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)
Flow rate: 2
Injection volume: 6
Detector: UV 240

CHROMATOGRAM
Retention time: 2.4
Internal standard: barbital (0.8), thiamylal (5.2)
Limit of detection: 2 μg/mL

OTHER SUBSTANCES
Extracted: thiopental
Simultaneous: acetaminophen, acetazolamide, aspirin, butabarbital, cefazolin, chlorothiazide, chlorzoxazone, dicloxacillin, ethosuximide, furosemide, hydrochlorothiazide, ibuprofen, oxacillin, phenobarbital, phenylbutazone, phenytoin, salicylic acid, secobarbital, sulfamethoxazole, theophylline, ascorbic acid
Noninterfering: ampicillin, penicillin G, valproic acid
Interfering: amobarbital

KEY WORDS
serum

REFERENCE
Kelner,M.; Bailey,D.N. Reversed-phase liquid-chromatographic simultaneous analysis for thiopental and pentobarbital in serum, *Clin.Chem.*, **1983**, *29*, 1097–1100.

SAMPLE
Matrix: blood
Sample preparation: 50 μL 25 μg/mL 5-Ethyl-5-p-tolylbarbituric acid in MeOH added to 150 × 10 mm glass centrifuge tube and blow dry under a stream of nitrogen, add 500 μL plasma, add 5 mL dichloromethane, mix on rotary mixer for 5 min, centrifuge. Remove organic layer, evaporate to dryness under nitrogen, take up in 500 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 μBondapak C18 Corasil B
Column: 300 × 4 5 μm μBondapak C18
Mobile phase: MeOH:10 mM potassium phosphate adjusted to pH 4.40 ± 0.05 with 150 mM phosphoric acid 50:50
Flow rate: 1.7
Injection volume: 40
Detector: UV 212

CHROMATOGRAM
Retention time: 8.90
Internal standard: 5-ethyl-5-p-tolylbarbituric acid (4.62)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: thiopental (UV 284)

Simultaneous: acetaminophen, amobarbital, barbital, butalbital, butabarbital, caffeine, carbamazepine, phenacetin, phenobarbital, phenytoin, secobarbital, theobromine, theophylline, vinbarbital

KEY WORDS
plasma

REFERENCE
Houdret,N.; Lhermitte,M.; Lalau,G.; Izydorczak,J.; Roussel,P. Determination of thiopental and pentobarbital in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 437–442.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C8 SPE cartridge with 2 volumes of MeOH, 2 volumes of water, and 1 volume of 100 mM pH 5.59 Sörensen's phosphate buffer. 500 μL Plasma + 10 μL 1 mg/mL sodium secobarbital in EtOH, add to the SPE cartridge, wash with 2 volumes of 100 mM pH 5.59 Sörensen's phosphate buffer, wash with 1 volume of water, elute with 500 μL MeOH. Evaporate the eluate to dryness under vacuum, reconstitute in 50 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak C18 (Waters)
Column: 100 × 8 10 μm Radial-Pak C8 (Waters)
Mobile phase: MeOH:THF:100 mM pH 7.72 Sörensen's phosphate buffer 28:16:52
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.21
Internal standard: secobarbital (6.39)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: methohexital, thiopental
Noninterfering: ketamine

KEY WORDS
plasma; dog; pharmacokinetics; SPE

REFERENCE
Avram,M.J.; Krejcie,T.C. Determination of sodium pentobarbital and either sodium methohexital or sodium thiopental in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1987**, *414*, 484–491.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 5.86
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental
Simultaneous: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

KEY WORDS
serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: Vigorously shake equal volumes of plasma and MeCN, centrifuge at 10000 g for 3 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 110 × 4.6 PartiSphere C8 (Whatman)
Mobile phase: MeCN:120 mM pH 6.2 phosphate buffer 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted PTFE coil irradiated with a Sylvania G8-T5 lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 3.28
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: thiopental

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma

REFERENCE
Schmid,R.W.; Wolf,C. Simultaneous determination of thiopental and its metabolite, pentobarbital, in blood by high-performance liquid chromatography and post-column photochemical reaction, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1749–1755.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 9.18
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood
Sample preparation: 200-500 μL Whole blood + 1 mL 100 mM pH 7.5 phosphate buffer, vortex for 1 min, add 7 mL n-hexane:diethyl ether 50:50, add 50 μL 100 μg/mL secobarbital in EtOH:water 75:25, shake for 15 min, centrifuge at 4° at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, vortex, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Nucleosil C18
Mobile phase: MeCN:water 32:68
Flow rate: 0.3
Injection volume: 5-20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: secobarbital (9)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: thiopental

KEY WORDS
whole blood

REFERENCE
Celardo,A.; Bonati,M. Determination of thiopental measured in human blood by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 220–225.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, centrifuge at 10000 g, dilute supernatant with an equal volume of water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 110 × 4.7 5 μm PartiSphere C18 (Whatman)
Mobile phase: MeCN:15 mM pH 7.0 phosphate buffer 30:70
Flow rate: 0.8
Injection volume: 50
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted coil of PTFE tubing irradiated by an 8 W low-pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Extracted: aprobarbital, butethal, secobarbital

KEY WORDS
plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Wolf,C.; Schmid,R.W. Enhanced UV-detection of barbiturates in HPLC analysis by on-line photochemical reaction, *J.Liq.Chromatogr.*, **1990**, *13*, 2207–2216.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 20 μL 500 μg/mL phenylbutazone in 2 mM NaOH + 1.1 mL ether:n-hexane 20:80 + 20 μL 3 M phosphoric acid, vortex at 1200 rpm for 1 min, centrifuge at 2000 g for 5 min, freeze in dry ice for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure (16 mbar) at 40° for 10 min, reconstitute the residue in 50 μL 400 μM NaOH, inject a 2-40 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 AGP (ChromTech)
Column: 100 × 4 AGP-CSP (ChromTech)
Mobile phase: Isopropanol:100 mM pH 6.2 phosphate buffer 4.5:95.5
Flow rate: 0.9
Injection volume: 2-40
Detector: UV 220

CHROMATOGRAM
Retention time: 3.1 (R(+)), 4.1 (S(-))
Internal standard: phenylbutazone (15.7)

OTHER SUBSTANCES
Extracted: thiopental (UV 287)

KEY WORDS
sheep; plasma; chiral

REFERENCE
Huang,J.L.; Mather,L.E.; Duke,C.C. High-performance liquid chromatographic determination of thiopentone enantiomers in sheep plasma, *J.Chromatogr.B*, **1995**, *673*, 245–250.

SAMPLE
Matrix: blood

Sample preparation: Mix serum with an equal volume of 1 M pH 5.0 phosphate buffer, add IS, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3500 rpm for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:10 mM pH 5.0 sodium phosphate buffer 55:45
Flow rate: 1
Injection volume: 10-20
Detector: UV

CHROMATOGRAM

Internal standard: 5-(p-methylphenyl)-5-phenylhydantoin
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES

Also analyzed: thiopental

KEY WORDS

serum; rat; pharmacokinetics

REFERENCE

Nakashima,E.; Matsushita,R.; Ohshima,T.; Tsuji,A.; Ichimura,F. Quantitative relationship between structure and peritoneal membrane transport based on physiological pharmacokinetic concepts for acidic drugs, *Drug Metab.Dispos.*, **1995**, *23*, 1220–1224.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 12.12
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 1.50
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, mianserin, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: moclobemide, tranylcypromine, metoclopramide

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.437

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve injection in mobile phase to give a pentobarbital sodium concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Partisil ODS-3 or 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:buffer:propylene glycol 55:45:4 (Buffer was 4.1 g anhydrous sodium acetate and 15 mL acetic acid in 1 L water.)
Flow rate: 2
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rugged; injections

REFERENCE
Reif,V.D.; Kaufmann,K.L.; DeAngelis,N.J.; Frankhouser,M.C. Liquid chromatographic assays for barbiturate injections, *J.Pharm.Sci.*, **1986**, *75*, 714–716.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 20-200 μg/mL solution in acetone with 50 μL of a 0.4-1.6 mg/mL solution of 2-bromo-2'-acetonaphthone in acetone, add 5-10 mg cesium carbonate, heat at 30° for 30 min, add 50 μL glacial acetic acid, mix, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 2
Detector: UV 249

CHROMATOGRAM
Retention time: 8.75
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: amobarbital, barbital, butobarbital, heptobarbital, hexobarbital, mephobarbital, phenobarbital, secobarbital

KEY WORDS
derivatization

REFERENCE
Hulshoff,A.; Roseboom,H.; Renema,J. Improved detectability of barbiturates in high-performance liquid chromatography by pre-column labelling and ultraviolet detection, *J.Chromatogr.*, **1979**, *186*, 535–541.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mm KH_2PO_4 + 5 mM 1-decanesulfonic acid 30:70 adjusted to pH 3.2 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 11.5
Internal standard: methyl paraben (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, butalbital, aprobarbital, mephobarbital, phenobarbital, secobarbital, talbutal, vinbarbital

KEY WORDS
stability-indicating

REFERENCE
Ibrahim,F.B. Simultaneous determination and separation of several barbiturates and analgesic products by ion-pair high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2835–2851.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 2.09

OTHER SUBSTANCES
Simultaneous: aprobarbital, amobarbital, butabarbital, butalbital, secobarbital, thiopental, phenobarbital

REFERENCE

Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 2 μBondapak C18
Mobile phase: MeCN:water 30:70 adjusted to pH 3.0 with formic acid
Flow rate: 0.27
Injection volume: 5
Detector: MS, VG TRIO 2000 single quadrupole MS with EI or CI or UV 270

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: butethal, butabarbital, talbutal, butalbital, amobarbital

KEY WORDS
mass spectra given

REFERENCE
Ryan,T.W. Identification of barbiturates using high performance liquid chromatography-particle beam EI/CI mass spectrometry, *J.Liq.Chromatogr.*, **1994**, *17*, 867–881.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.92 (A), 5.58 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine,

methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine +1 mL 500 mM pH 5.5 phosphate buffer, add to an Extrelut 3 SPE cartridge, let stand for 10 min, elute with 15 mL dichloromethane:isopropanol 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm Lichrospher 100 RP8
Column: 250 × 4 5 µm Lichrospher 100 RP8
Mobile phase: Gradient. MeCN:10 mM pH 4.4 phosphate buffer from 30:70 to 40:60 over 8 min, maintain at 40:60 for 6 min, to 30:70 over 1 min
Flow rate: 1
Injection volume: 20
Detector: UV 212

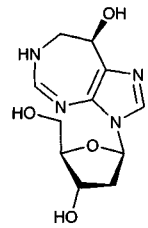
CHROMATOGRAM
Retention time: 11.1
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: barbital, allobarbital, butabarbital, phenobarbital, secobarbital
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, triflupromazine, trimethoprim, trimipramine

KEY WORDS
SPE

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

Pentostatin



Molecular formula: $C_{11}H_{16}N_4O_4$
Molecular weight: 268.27
CAS Registry No.: 53910-25-1
Merck Index: 7277

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 20 μL isoamyl alcohol + 50 μL chloroform, vortex for 30 s, centrifuge at 20931 g for 10 min. Remove the aqueous layer and add it to 1 mL cold acetone (0°), vortex for 10 s, centrifuge for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb RP-8
Mobile phase: MeCN:buffer 4:96 (Buffer was 5 mM sodium pentanesulfonate, pH 7.2.)
Column temperature: 40
Flow rate: 1
Detector: UV 250

CHROMATOGRAM

Retention time: 5.82

OTHER SUBSTANCES

Extracted: vidarabine
Noninterfering: chlorothiazide, cytosine arabinoside, guanine arabinoside, hydroxyzine, kanamycin, metaproterenol, nystatin, penicillin G, phenobarbital, prednisone, sulfamethoxazole, theophylline, trimethoprim, uracil arabinoside

REFERENCE

Bowman,D.B.; Kauffman,R.E. Reversed-phase high-performance liquid chromatographic method to determine vidarabine and hypoxanthine arabinoside in biological fluids, *J.Chromatogr.*, **1982**, *229*, 487–491.

SAMPLE

Matrix: fermentation solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Phenomenex C8
Mobile phase: MeCN:MeOH:50 mM $(HN_4)_2HPO_4$ 2.5:2.5:95 adjusted to pH 7.4 with phosphoric acid
Flow rate: 1.5
Detector: UV 258

CHROMATOGRAM

Retention time: 6.9

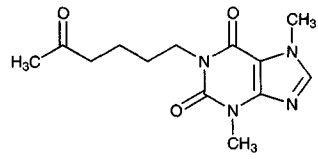
OTHER SUBSTANCES

Extracted: cytosine, coformycin, (8S)-pentostatin, 2'-deoxyguanosine, Ara-A

REFERENCE

Showalter,H.D.H.; Bunge,R.H.; French,J.C.; Hurley,T.R.; Leeds,R.L.; Leja,B.; McDonnell,P.D.; Edmunds,C.R. Improved production of pentostatin and identification of fermentation cometabolites, *J.Antibiot.(Tokyo)*, **1992**, *45*, 1914–1918.

Pentoxifylline



Molecular formula: $C_{13}H_{18}N_4O_3$
Molecular weight: 278.31
CAS Registry No.: 6493-05-6
Merck Index: 7278
Lednicer No.: 2 466

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM

Retention time: 11.477

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Add 6 mL ice-cold dichloromethane to 500 μL microsomal incubation, add 100 μL 10 μg/mL CT-2410 R, shake on a reciprocal shaker for 10 min, centrifuge at 3000 g for 10 min, evaporate the organic layer to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject 30-60 μL aliquot.

HPLC VARIABLES

Guard column: Opti-Guard (Optimize Technologies, Inc.)
Column: 100 × 4.6 3 μm Microsorb-MV C18
Mobile phase: MeOH:buffer 35:65 (Buffer was 25 mM ammonium phosphate containing 0.25% acetic acid, pH adjusted to 4.5 with ammonium hydroxide.)
Flow rate: 0.7
Injection volume: 30-60
Detector: UV 273

CHROMATOGRAM

Retention time: 9.3

Internal standard: CT-2410 R (20.0)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: lisofylline

KEY WORDS
human; liver

REFERENCE
Lee,S.H.; Slattery,J.T. Cytochrome P450 isozymes involved in lisofylline metabolism to pentoxifylline in human liver microsomes, *Drug Metab.Dispos.*, **1997**, *25*, 1354–1358.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

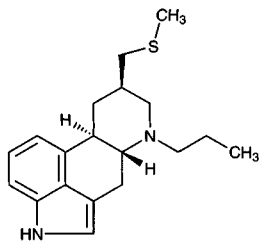
HPLC VARIABLES
Column: 150 × 6 5 μm 3-aminopropylsilyl silica gel with the amino group derivatized with 1,8-naphthalic anhydride (Bunseki Kagaku 1993, 42, 817)
Mobile phase: MeOH:buffer 50:50 (Prepare buffer by dissolving 6.183 g boric acid and 1.461 g NaCl in 500 mL water, adjust pH to 6.4 with sodium borate solution.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 20
Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)

OTHER SUBSTANCES
Simultaneous: caffeine, hypoxanthine, propentofylline, theobromine, theophylline, uric acid, xanthine

REFERENCE
Nakashima,K.; Inoue,K.; Mayahara,K.; Kuroda,N.; Hamachi,Y.; Akiyama,S. Use of 3-(1,8-naphthalimido)propyl-modified silyl silica gel as a stationary phase for the high-performance liquid chromatographic separation of purine derivatives, *J.Chromatogr.A*, **1996**, *722*, 107–113.

Pergolide



Molecular formula: $C_{19}H_{26}N_2S$
Molecular weight: 314.49
CAS Registry No.: 66104-22-1, 66104-23-2 (mesylate)
Merck Index: 7304
Lednicer No.: 3 249

SAMPLE
Matrix: cell cultures
Sample preparation: Evaporate 100 μL 1 mg/mL lergotrile in MeOH in the bottom of a glass tube under a stream of nitrogen, add 2 mL culture homogenate (Polytron), add 2 mL 100 mM pH 8.5 sodium carbonate/sodium bicarbonate buffer, add 4 mL isoamyl alcohol, shake at 30 oscillations/min for 30 min, centrifuge at 1230 g for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250-500 μL mobile phase, filter, inject an aliquot.

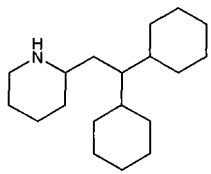
HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM pH 8.4 ammonium carbonate buffer 65.2:34.8
Flow rate: 2
Injection volume: 50
Detector: UV 290

CHROMATOGRAM
Retention time: 6.74
Internal standard: lergotrile (2.42)
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Kerr,K.M.; Smith,R.V.; Davis,P.J. High-performance liquid chromatographic determination of pergolide and its metabolite, pergolide sulfoxide, in microbial extracts, *J.Chromatogr.*, **1981**, *219*, 317–320.

Perhexiline



Molecular formula: $C_{19}H_{35}N$
Molecular weight: 277.49
CAS Registry No.: 6621-47-2, 6724-53-4 (maleate)
Merck Index: 7305

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 1.4 μg/mL hexadiline hydrochloride in 100 mM HCl + 50 μL 2 M pH 8.75 Tris-HCl buffer + 4 mL n-hexane, shake horizontally at 100 oscillations/min for 15 min, centrifuge at 2500 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL 100 mM pH 10 sodium bicarbonate buffer, add 100 μL 5 mM dansyl chloride in acetone (freshly prepared), vortex, heat at 37° for 20 min, add 1.5 mL n-hexane, vortex, centrifuge at 10° at 2500 rpm for 3 min, freeze in dry ice/EtOH. Remove the organic layer and evaporate it to dryness at 60°, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.2 3 μm Velosep (Brownlee)
Mobile phase: MeOH:water 86:14
Column temperature: 45
Flow rate: 0.5
Injection volume: 25
Detector: F ex 366 em 470

CHROMATOGRAM
Retention time: 15.9, 16.8 (isomers)
Internal standard: hexadiline (19.5)
Limit of quantitation: 150 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE

Morris,R.G.; Sallustio,B.C.; Saccoia,N.C.; Mangas,S.; Fergusson,L.K.; Kassapidis,C. Application of an improved HPLC perhexiline assay to human plasma specimens, *J.Liq.Chromatogr.*, **1992**, *15*, 3219–3232.

SAMPLE

Matrix: blood

Sample preparation: 400 µL Plasma + 400 µL 1 M diammonium hydrogen phosphate + 50 µL 2.5 µg/mL di-n-hexylamine in water + 100 µL 1 mg/mL diisopropylethylamine + 5 mL isopentane:dichloromethane 60:40, vortex for 1.5 min, centrifuge at 1500 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 100 µL 10 mg/mL trans-4-nitrocinnamoyl chloride in dry MeCN, vortex for 15 s, let stand at room temperature for 30 min, add 100 µL 25 mM sodium carbonate, vortex for 15 s, let stand at room temperature for 5 min, add 100 µL MeCN:50 mM ammonium acetate 50:50, vortex for 30 s, centrifuge at 1500 g for 5 min, inject a 130 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherisorb phenyl
Mobile phase: MeCN:water:glacial acetic acid 46:53.5:0.5
Flow rate: 2
Injection volume: 130
Detector: UV 340

CHROMATOGRAM

Retention time: 18.4
Internal standard: di-n-hexylamine (10.2)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: dealkyldisopyramide, desethylamiodarone, desipramine, flecainide, mexiletine, nortriptyline, protriptyline, sotalol

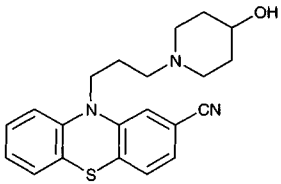
KEY WORDS

derivatization; plasma

REFERENCE

Grgurinovich,N. Method for the analysis of perhexiline and its hydroxy metabolite in plasma using high-performance liquid chromatography with precolumn derivatization, *J.Chromatogr.B*, **1997**, *696*, 75–80.

Pericyazine



Molecular formula: $C_{21}H_{23}N_3OS$
Molecular weight: 368.47
CAS Registry No.: 2622-26-6
Merck Index: 7306

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20

REFERENCE
Morris,R.G.; Sallustio,B.C.; Saccoia,N.C.; Mangas,S.; Fergusson,L.K.; Kassapidis,C. Application of an improved HPLC perhexiline assay to human plasma specimens, *J.Liq.Chromatogr.*, **1992**, *15*, 3219–3232.

SAMPLE
Matrix: blood
Sample preparation: 400 µL Plasma + 400 µL 1 M diammonium hydrogen phosphate + 50 µL 2.5 µg/mL di-n-hexylamine in water + 100 µL 1 mg/mL diisopropylethylamine + 5 mL isopentane:dichloromethane 60:40, vortex for 1.5 min, centrifuge at 1500 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 100 µL 10 mg/mL trans-4-nitrocinnamoyl chloride in dry MeCN, vortex for 15 s, let stand at room temperature for 30 min, add 100 µL 25 mM sodium carbonate, vortex for 15 s, let stand at room temperature for 5 min, add 100 µL MeCN:50 mM ammonium acetate 50:50, vortex for 30 s, centrifuge at 1500 g for 5 min, inject a 130 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Spherisorb phenyl
Mobile phase: MeCN:water:glacial acetic acid 46:53.5:0.5
Flow rate: 2
Injection volume: 130
Detector: UV 340

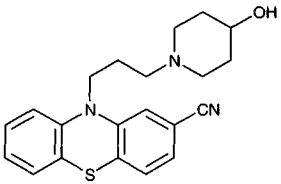
CHROMATOGRAM
Retention time: 18.4
Internal standard: di-n-hexylamine (10.2)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: dealkyldisopyramide, desethylamiodarone, desipramine, flecainide, mexiletine, nortriptyline, protriptyline, sotalol

KEY WORDS
derivatization; plasma

REFERENCE
Grgurinovich,N. Method for the analysis of perhexiline and its hydroxy metabolite in plasma using high-performance liquid chromatography with precolumn derivatization, *J.Chromatogr.B*, **1997**, *696*, 75–80.

Pericyazine



Molecular formula: $C_{21}H_{23}N_3OS$
Molecular weight: 368.47
CAS Registry No.: 2622-26-6
Merck Index: 7306

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20

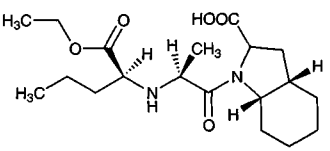
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Perindopril



Molecular formula: $C_{19}H_{32}N_2O_5$
Molecular weight: 368.47
CAS Registry No.: 82834-16-0, 107133-36-8 (erbumine salt)
Merck Index: 7311
Lednicer No.: 5 111

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM

Retention time: 13.698

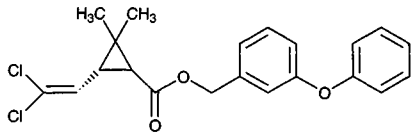
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Permethrin



Molecular formula: $C_{21}H_{20}Cl_2O_3$
Molecular weight: 391.29
CAS Registry No.: 52645-53-1
Merck Index: 7321

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize (Labsonic 2000 U sonicator with titanium probe) sciatic nerve or brain with 2 mL water. Homogenize (Stomacher Lab Blender) liver samples. Shake plasma or tissue homogenate with 8 mL n-pentane, centrifuge, repeat twice more. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30°, reconstitute the residue in 500 µL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 120 × 4 5 µm Nucleosil 120 C18
Mobile phase: MeOH:water:chloroform:acetic acid 54.5:39.5:5:1
Flow rate: 1.1
Detector: UV 254

CHROMATOGRAM

Limit of quantitation: 200 ng/mL (tissue), 100 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
rat; plasma; brain; sciatic nerve; pharmacokinetics

REFERENCE
Anadón,A.; Martinez-Larrañaga,M.R.; Diaz,M.J.; Bringas,P. Toxicokinetics of permethrin in the rat, *Toxicol.Appl.Pharmacol.*, **1991**, *110*, 1–8.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve the compound in benzene. Inject an aliquot. (Protect the sample from light! Caution! Benzene is a carcinogen!)

HPLC VARIABLES
Column: 250 × 4 10 μm Lichrospher Si60
Mobile phase: Hexane:benzene 50:50
Flow rate: 1
Detector: UV 280; Polarimeter, diode-laser polarimetric Chiral Monitor 2000 (Applied Chromatography System, Great Britain) collimated laser diode 30 mW at 830 nm, flow cell 4.8 cm, volume 73 μL

CHROMATOGRAM
Retention time: 3.63 (cis 1RS, 3SR), 4.39 (trans 1RS, 3RS) (UV detection); 3.76 (cis 1R, 3S [+]), 4.03 (cis 1S, 3R [-]), 4.52 (trans 1R, 3R [+]), 4.85 (trans 1S, 3S [-]) (polarimetric detection)
Limit of detection: 91 μg

OTHER SUBSTANCES
Simultaneous: cypermethrin, deltamethrin

KEY WORDS
chiral; normal phase

REFERENCE
Díaz,A.N.; Sánchez,F.G.; Pareja,A.G. Resolution of deltamethrin, permethrin, and cypermethrin enantiomers by high-performance liquid chromatography with diode-laser polarimetric detection, *J.Chromatogr.Sci.*, **1998**, *36*, 210–216.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 229

CHROMATOGRAM
Limit of detection: 400 pg

OTHER SUBSTANCES
Also analyzed: amitraz (UV 313), chlorpyrifos (UV 313), coumaphos (UV 313), crotoxyphos (UV 229), phosmet (UV 229)

REFERENCE
Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.

SAMPLE
Matrix: fruit, vegetables
Sample preparation: Prepare a cleanup column by placing 4 g Florisil, 1 g activated charcoal, and a 20 mm layer of anhydrous sodium sulfate in a 400 × 10 glass column, wash with 40 mL toluene, wash with 40 mL toluene:MeCN 99:1. Homogenize 25 g chopped fruit or vegetable with 70 mL MeOH at high speed for 3 min, filter, homogenize solid with 30 mL MeOH, filter. Combine the filtrates and add them to 60 mL toluene and 300 mL 10% NaCl in water, shake well for 3 min, let layers separate. Dry the organic layer by passing it through 20 g anhydrous sodium sulfate in a 20 mm diameter column, concentrate to about 5 mL under reduced pressure at 80°, add to the cleanup column, elute with 40 mL toluene:MeCN 99:1. Evaporate the eluate just to dryness under reduced pressure at 80°, reconstitute with 1 mL MeOH, inject an aliquot. (Reflux activated charcoal (20-40 mesh) with 1 M HCl for 4 h, wash with water until the washings are neutral, dry at 95-100° (J.Assoc.Off.Anal.Chem. 1983, 66, 1013). Heat 60-100 mesh Florisil at 200° for 24 h, cool, add 4% water, mix thoroughly, store in a sealed jar (J.Assoc.Off.Anal.Chem. 1983, 66, 1003).)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:water from 62:38 to 78:22 over 32 min (Waters curve 6).
Column temperature: 50
Flow rate: 1.5
Detector: UV 206

CHROMATOGRAM
Retention time: 24.60, 27.01
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Simultaneous: allethrin, biphenthrin, cypermethrin, fenpropathrin, fenvalerate, flucythrinate, methothrin, Py-115, tetramethrin

KEY WORDS
cucumber; tomato; cabbage; apple; pear; peach; SPE

REFERENCE
Pang,G.-F.; Chao,Y.-Z.; Liu,X.-S.; Fan,C.-L. Multiresidue liquid chromatographic method for simultaneous determination of pyrethroid insecticides in fruits and vegetables, *J.AOAC Int.*, **1995**, *78*, 1474–1480.

SAMPLE
Matrix: rice
Sample preparation: 30 g Rice + 50 mL acetone, let stand with occasional shaking for 48 h, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: Guard Pak
Column: 150 × 3.9 Novapak C18
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 10
Detector: UV 225

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 1 μg/g

OTHER SUBSTANCES
Extracted: bioremethrin, deltamethrin, fenvalerate, phenothrin, piperonyl butoxide

REFERENCE
Haddad,P.R.; Brayan,J.G.; Sharp,G.J.; Dilli,S.; Desmarchelier,J.M. Determination of pyrethroid residues on paddy rice by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *461*, 337–346.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm silica (IBM)
Mobile phase: Hexane:dichloromethane:isopropanol 99:1:0.07
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.86 (cis), k' 6.68 (trans)

OTHER SUBSTANCES
Also analyzed: allethrin, chrysanthemol, dimethrin, ethyl chrysanthemate, cyfluthrin (baythroid), phenothrin, resmethrin, RU-11679, tetramethrin

KEY WORDS
normal phase

REFERENCE
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 PartiSphere Silica (Whatman)
Mobile phase: Hexane:dichloromethane 99:1
Flow rate: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 1.57 (cis), 2.17 (trans)

KEY WORDS
normal phase

REFERENCE
Baxter Scientific Products Catalog, 1990-1, p. 173.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

HPLC VARIABLES
Guard column: 5 μm Spherisorb NH2
Column: 250 × 4.6 Pirkle ionic type 1-A column (Technicol)
Mobile phase: Hexane:isopropanol 99.95:0.05
Flow rate: 0.8
Detector: UV 230

CHROMATOGRAM
Retention time: 18.2, 18.8, 26.5, 27.1 (enantiomers)

OTHER SUBSTANCES
Also analyzed: phenothrin, resmethrin

KEY WORDS
chiral

REFERENCE
Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

SAMPLE
Matrix: solutions
Sample preparation: Inject 100 mL river water on to column A at 5 mL/min and let the effluent flow to waste, backflush the contents of column A on to column B and start the gradient, monitor the effluent from column B. At the end of each run backflush column A with 5 mL water, with 30 mL 100 mM pH 2 sodium citrate buffer, with 10 mL water, with 5 mL MeCN, and with 10 mL hexane:dichloromethane 50:50. Wash new column A with 10 mL water.

HPLC VARIABLES
Column: A 20 × 3 10 μm PRP-1 (Hamilton); B 30 × 4.6 10 μm RP-18 (Brownlee) + 250 × 4.6 5 μm LiChrospher C18
Mobile phase: Gradient. MeCN:water from 5:95 to 90:10 over 1 h, maintain at 90:10 for 4 min, return to initial conditions (?) over 1 min, re-equilibrate for 10 min.
Flow rate: 1 for 64 min, to 1.5 over 1 min, maintain at 1.5 for 5 min, to 1 over 5 min
Injection volume: 100000
Detector: UV 210

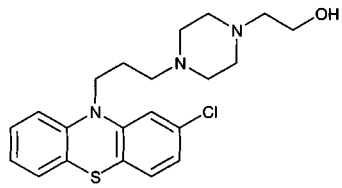
CHROMATOGRAM
Retention time: 62.2 (trans), 63.6 (cis)
Limit of detection: 100 ng/L

OTHER SUBSTANCES
Simultaneous: alachlor, aldicarb, aldicarb oxime, atrazine, carbofuran, chlorobenzilate, chlorothalonil, chlorpyrifos methyl, chlortoluron, cypermethrin, DDT, deltamethrin, diazinon, diclofop methyl, dimethoate, diuron, ethofumesate, fenitrothion, fenvalerate, fluazifop butyl, fluometuron, linuron, metalaxyl, metamitron, methomyl, metobromuron, metolachlor, molinate, oxamyl, paraoxon, paraoxon methyl, parathion, parathion methyl, pendimethalin, phenmedipham, pirimphos, pirimphos methyl, prometryne, propanil, propiconazole, simazine, terbuthylazine, trifluraline
Interfering: p,p'-DDE

KEY WORDS
river water; column-switching

REFERENCE
Papadopoulou-Mourkidou,E.; Patsias,J. Development of a semi-automated high-performance liquid chromatographic-diode array detection system for screening pesticides at trace levels in aquatic systems of the Axios River basin, *J.Chromatogr.A*, **1996**, *726*, 99–113.

Perphenazine



Molecular formula: $C_{21}H_{26}ClN_3OS$
Molecular weight: 403.98
CAS Registry No.: 58-39-9
Merck Index: 7323

SAMPLE
Matrix: blood
Sample preparation: 3 mL Serum + 50 ng thiethylperazine maleate + 3 mL diluted Titrisol (pH 10 borate buffer, Merck) + 4 mL heptane:isoamyl alcohol 97:3, shake thoroughly for 15 s,

centrifuge at 2500 g for 5 min. Remove the organic phase and add it to 1.5 mL 50 mM sulfuric acid containing 0.1% $Na_2S_2O_2$, mix for 15 s, centrifuge at 2500 g for 10 min. Remove the aqueous phase. Repeat the extraction and back extraction. Combine the aqueous phases and add them to 1.5 mL 2 M pH 9.1 glycine buffer, add 200 μL n-hexane:isoamyl alcohol 97:3, vortex for 25 s. Remove the organic phase and evaporate it to dryness in a desiccator, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 65:35:3 containing 10 mM dodecyl hydrogen sulfate
Flow rate: 2
Detector: UV 257

CHROMATOGRAM
Retention time: 8.5
Internal standard: thiethylperazine (13)
Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: alimemazine, biperidine, carbamazepine, chlorpromazine, clomipramine, diazepam, dihydroergotamine, disulfiram, dixyrazine, haloperidol, levomepromazine, nitrazepam, orphenadrine, promethazine, propiomazine, thioridazine, trimipramine, vitamins

KEY WORDS
serum

REFERENCE
Larsson,M.; Forsman,A. A high-performance liquid chromatographic method for the assay of perphenazine and its dealkylated metabolite in serum after therapeutic doses, *Ther.Drug Monit.*, **1983**, *5*, 225–228.

SAMPLE
Matrix: blood
Sample preparation: 2.5 mL Plasma + 7.5 ng IS + 100 μL 1 M NaOH + 6 mL ethyl acetate: hexane 2:1, shake vigorously for 30 s, centrifuge for 3 min. Remove the organic phase and add it to 2 mL 100 mM HCl, shake vigorously for 30 s, centrifuge for 3 min. Remove the aqueous phase and add it to 100 μL 6 M NaOH, add 3 mL hexane, shake vigorously for 30 s. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 75 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 20 × 4.6 3 μm C18 (Perkin-Elmer)
Mobile phase: MeOH:water:dichloromethane:ammonia 200:40:10:3
Flow rate: 1
Injection volume: 20
Detector: UV 257

CHROMATOGRAM
Retention time: 2.1
Internal standard: 4-[3-(2,8-dichlorophenothiazin-10-yl)propyl]-1-piperazinethanol(2.5)
Limit of quantitation: 0.5 nM

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: amitriptyline, biperidine, clomipramine, disulfiram, imipramine, levomepromazine, nitrazepam, nortriptyline, orphenadrine
Interfering: diazepam

KEY WORDS
plasma

REFERENCE

Larsen,N.-E.; Hansen,L.B.; Knudsen,P. Quantitative determination of perphenazine and its dealkylated metabolite using high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *341*, 244–250.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 10 μL 1 mg/mL prochlorperazine in 25 mM pH 2.4 KH_2PO_4, adjust pH to 9.0 with 1 M NaOH, add 6 mL n-hexane:ethyl acetate 1:2, shake for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and add it to 100 μL pH 2.4 KH_2PO_4, shake for 30 s, centrifuge at 3000 g for 3 min, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES

Column: 120 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:MeOH:buffer 26:4:70 (Buffer was 10 mM KH_2PO_4 containing 5 mM tetramethylammonium chloride adjusted to pH 2.4 with 85% phosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: E, ESA Coulochem Model 5100A, detector 1 +0.20 V, detector 2 +0.73 V, guard cell 0.75 V

CHROMATOGRAM

Retention time: 15.01

Internal standard: prochlorperazine dimaleate (17.80)

Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: alprazolam, chlorpheniramine, diltiazem, lorazepam, mesoridazine, nifedipine, ranitidine

Noninterfering: doxepin, metoprolol, nordoxepin, nortriptyline, propranolol, theophylline, trifluoperazine, trihexyphenidyl, verapamil

KEY WORDS

plasma; protect from light

REFERENCE

Foglia,J.P.; Sorisio,D.; Kirshner,M.A.; Mulsant,B.H.; Perel,J.M. Quantitative determination of perphenazine and its metabolites in plasma by high-performance liquid chromatography and coulometric detection, *J.Chromatogr.B*, **1995**, *668*, 291–297.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 255.8

CHROMATOGRAM
Retention time: 15.96

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Add 5 mL water to powdered tablets containing 4 mg perphenazine, heat on a water-bath for 3 min. Cool, add 50 mL water, shake for 15 min and make up to 100 mL with MeOH. Filter, remove a 5 mL aliquot, add IS to a concentration of 3 μg/mL, make up to 50 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Novapak-phenyl-4
Mobile phase: MeOH:15 mM pH 6.5 sodium acetate buffer 81:19
Flow rate: 1.0
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4.4
Internal standard: pindolol (2.5)
Limit of detection: 100 pg

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
tablets

REFERENCE
Al-Obaid,A.M.; Hagga,M.E.M.; El-Khawad,I.E.; El-Mahi,O.H.M. Simultaneous quantitation of some phenothiazine drug substances and their monosulphoxide degrades by high performance liquid chromatography (HPLC), *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1369–1389.

SAMPLE
Matrix: formulations
Sample preparation: Syrup, injections. Measure amount of syrup equivalent to about 5 mg perphenazine, add 5 mL 0.5 mg/mL trifluoperazine hydrochloride in MeOH, make up to 50 mL with MeOH, mix, inject a 10 μL aliquot. Tablets. Finely powder tablets, weigh out amount equivalent to 10 mg perphenazine, add 20 mL 1% HCl, shake for 20 min, centrifuge. Remove a 10 mL aliquot and add it to 5 mL 0.5 mg/mL trifluoperazine hydrochloride in MeOH, make up to 50 mL with MeOH, mix, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:MeOH:25 mM pH 4.5 acetate buffer 40:30:30
Flow rate: 2.5
Injection volume: 10
Detector: UV 229

CHROMATOGRAM
Retention time: 4.6
Internal standard: trifluoperazine (6)

OTHER SUBSTANCES
Simultaneous: perphenazine sulfoxide, degradation products

KEY WORDS
tablets; syrup; injections

REFERENCE
Beaulieu,N.; Lovering,E.G. Liquid chromatographic method for perphenazine and its sulfoxide in pharmaceutical dosage forms for determination of stability, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 167–169.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 1.59

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl

Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05

Column temperature: 50

Flow rate: 3

Injection volume: 5

Detector: UV 220

CHROMATOGRAM

Retention time: 3.75

OTHER SUBSTANCES

Simultaneous: benactyzine, buclizine, hydroxyzine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

KEY WORDS

SFC; pressure 200 bar

REFERENCE

Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl

Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03

Column temperature: 50

Flow rate: 3

Injection volume: 5

Detector: UV 254

CHROMATOGRAM
Retention time: 6.0

OTHER SUBSTANCES
Simultaneous: trifluprομazine, carphenazine, methotrimeprazine, promazine, chlorprothixene, deserpidine, thiothixene, reserpine
Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 5 μm Diol (Keystone)
Mobile phase: Carbon dioxide:MeOH containing 1 mM t-butylammonium hydroxide
Flow rate: 0.5 (carbon dioxide), 0.1 (MeOH/t-butylammonium hydroxide)
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
outlet pressure 3500 psi; SFC; back-pressure regulator heated to 60°

REFERENCE
Ashraf-Khorassani,M.; Levy,J.M. Addition of modifier in supercritical fluid chromatography using a microbore reciprocating pump, *Chromatographia*, **1995**, *40*, 78–84.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 13.16 (A), 6.33 (B)

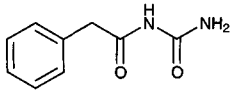
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide,

chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Phenacemide



Molecular formula: $C_9H_{10}N_2O_2$
Molecular weight: 178.19
CAS Registry No.: 63-98-9
Merck Index: 7343
Lednicer No.: 1 95

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 2.36
Internal standard: tolylphenobarbital (7.57)

Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine,desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin
Interfering: methyprylon

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: solutions

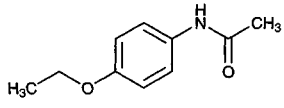
HPLC VARIABLES
Column: 100 × 4.6 3 μm 208HS3410 (Vydac)
Mobile phase: Gradient. MeCN:water from 15:85 to 60:40 over 10 min.
Flow rate: 1.5
Detector: UV 210 (?)

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: barbital, carbamazepine, diazepam, ethotoin, mephenytoin, methsuximide, phenobarbital, phensuximide

REFERENCE
Vydac HPLC Catalog, 1994-5, **1994**, p. 26.

Phenacetin



Molecular formula: $C_{10}H_{13}NO_2$
Molecular weight: 179.22
CAS Registry No.: 62-44-2
Merck Index: 7344
Lednicer No.: 1 111

SAMPLE
Matrix: solution

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18 (Alltech)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:THF:100 mM ammonium acetate 22.5:5.5:72
Flow rate: 1.0

Injection volume: 50
Detector: UV 283

CHROMATOGRAM
Retention time: 10.9

OTHER SUBSTANCES
Simultaneous: chlorzoxazone

REFERENCE
Frye,R.F.; Stiff,D.D. Determination of chlorzoxazone and 6-hydroxychlorzoxazone in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *686*, 291–296.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer 22.5:37.5:40
Flow rate: 0.8
Detector: UV 220

CHROMATOGRAM
Retention time: 5.4

OTHER SUBSTANCES
Simultaneous: 4-hydroxy-midazolam, α-hydroxy-midazolam, midazolam

REFERENCE
von Moltke,L.L.; Greenblatt,D.J.; Schmider,J.; Duan,S.X.; Wright,C.E.; Harmatz,J.S.; Shader,R.I. Midazolam hydroxylation by human liver microsomes in vitro: Inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents, *J.Clin.Pharmacol.*, **1996**, *36*, 783–791.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

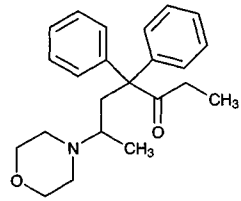
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fen-

camfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phenadoxone



Molecular formula: $C_{23}H_{29}NO_2$
Molecular weight: 351.49
CAS Registry No.: 467-84-5, 545-91-5 (HCl)
Merck Index: 7348
Lednicer No.: 1 80

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.35

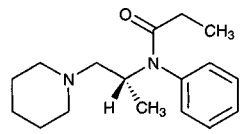
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Phenampromide



Molecular formula: $C_{17}H_{26}N_2O$
Molecular weight: 274.41
CAS Registry No.: 129-83-9
Merck Index: 7353

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2

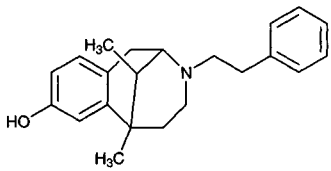
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Phenazocine



Molecular formula: $C_{22}H_{27}NO$
Molecular weight: 321.46
CAS Registry No.: 127-35-5, 1239-04-9 (±-hydrobromide)
Merck Index: 7360
Lednicer No.: 1 298

SAMPLE
Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupentixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

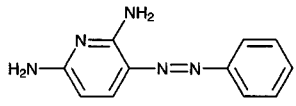
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phenazopyridine



Molecular formula: $C_{11}H_{11}N_5$
Molecular weight: 213.24
CAS Registry No.: 94-78-0, 136-40-3 (HCl)
Merck Index: 7361
Lednicer No.: 1 255

SAMPLE
Matrix: formulations
Sample preparation: Dissolve a capsule in 1 L 100 mM HCl, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax TMS
Mobile phase: MeCN:isopropanol:acetic acid:water 30:30:1:39
Flow rate: 1.8
Injection volume: 20
Detector: UV 405

CHROMATOGRAM
Retention time: 5.25

OTHER SUBSTANCES
Noninterfering: sulfamethizole, tetracycline

KEY WORDS
capsules

REFERENCE
Du Preez,J.L.; Botha,S.A.; Lötter,A.P. High-performance liquid chromatographic determination of phenazopyridine hydrochloride, tetracycline hydrochloride and sulphamethizole in combination, *J.Chromatogr.*, **1985**, *333*, 249–252.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, add 40 mg trimethoprim, dissolve in 70 mL MeOH, filter (paper), wash filter with MeOH, make up filtrate to 100 mL with MeOH. Dilute a 1 mL aliquot to 10 mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:MeCN:water:triethylamine 20:20:60:0.15, pH adjusted to 3.0 with phosphoric acid
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 7
Internal standard: trimethoprim (3)
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: nalidixic acid

KEY WORDS
tablets

REFERENCE

Sane,R.T.; Ghadge,J.K.; Jani,A.B.; Vaidya,A.J.; Kotwal,S.S. Simultaneous high-performance liquid chromatographic determination of haloperidol with propantheline bromide, nalidixic acid with phenazopyridine hydrochloride, and dipyridamole with aspirin in combined dosage (forms), *Indian Drugs*, **1992**, *29*, 240–244.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Filtered (Millex-GS 0.22 μm) urine + 500 μL 200 mM pH 5 sodium acetate buffer + 3.75 μL Glusulase + 12.5 μg 2,6-diamino-3-(2-chlorophenylazo)pyridine + 20 μg 3-acetaminophenol, heat at 37° for 16 h, freeze dry, reconstitute in 100 μL water, add 900 μL MeOH, sonicate, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 500 μL MeOH:MeCN:10 mM pH 6.5 ammonium formate buffer 37.5:37.5:25, sonicate, filter (Xydex LID/X ORG.2ns, 0.2 μm PTFE membrane), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 Supelcosil LC18 DB
Mobile phase: Gradient. A was MeCN:MeOH 50:50. B was 10 mM pH 6.5 ammonium formate buffer. A:B from 15:85 to 25:75 over 15 min, to 80:20 over 10 min.
Injection volume: 20
Detector: UV 430

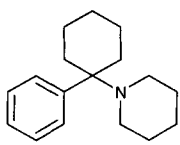
CHROMATOGRAM
Retention time: 28.5
Internal standard: 2,6-diamino-3-(2-chlorophenylazo)pyridine (30.2), 3-acetaminophenol (10.4)
Limit of quantitation: 2 μM

OTHER SUBSTANCES
Extracted: metabolites (UV 248 and UV 430)

KEY WORDS
rat; guinea pig; mouse; human

REFERENCE
Thomas,B.H.; Whitehouse,L.W.; Solomonraj,G.; Paul,C.J. Excretion of phenazopyridine and its metabolites in the urine of humans, rats, mice, and guinea pigs, *J.Pharm.Sci.*, **1990**, *79*, 321–325.

Phencyclidine



Molecular formula: $C_{17}H_{25}N$
Molecular weight: 243.39
CAS Registry No.: 77-10-1, 956-90-1 (HCl)
Merck Index: 7364
Lednicer No.: 1 56

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 262

CHROMATOGRAM

Retention time: 5.22
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

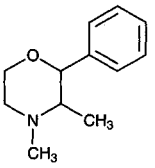
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phendimetrazine



Molecular formula: $C_{12}H_{17}NO$
Molecular weight: 191.27
CAS Registry No.: 634-03-7, 50-58-8 (tartrate)
Merck Index: 7365
Lednicer No.: 1 260

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 1.74

OTHER SUBSTANCES

Simultaneous: hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: mazindol, tranylcypromine, caffeine, fenethyline, methylphenidate, phenelzine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

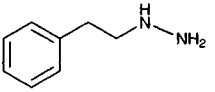
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine,

chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phenelzine



Molecular formula: $C_8H_{12}N_2$
Molecular weight: 136.20
CAS Registry No.: 51-71-8, 156-51-4 (sulfate)
Merck Index: 7366
Lednicer No.: 1 74

SAMPLE

Matrix: blood

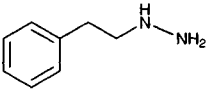
Sample preparation: 2 mL Plasma + 400 μL 10% acetic acid + 7 mL diethyl ether:dichloromethane 2:1, shake, centrifuge at 2059 g for 10 min. Remove the aqueous layer and add it to 600 μL 10% acetic acid, add 300 μL 0.1% salicaldehyde in EtOH, heat at 60° for 30 min, cool to room temperature, add 1 mL 1 M K_2PO_4 (sic), extract with 7 mL diethyl ether, centrifuge at 2059 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL buffer, inject a 20 μL aliquot. (Buffer was 5 mM heptanesulfonic acid in MeCN:water:triethylamine 70:30:0.4.)

chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phenelzine



Molecular formula: $C_8H_{12}N_2$
Molecular weight: 136.20
CAS Registry No.: 51-71-8, 156-51-4 (sulfate)
Merck Index: 7366
Lednicer No.: 1 74

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 400 μL 10% acetic acid + 7 mL diethyl ether:dichloromethane 2:1, shake, centrifuge at 2059 g for 10 min. Remove the aqueous layer and add it to 600 μL 10% acetic acid, add 300 μL 0.1% salicaldehyde in EtOH, heat at 60° for 30 min, cool to room temperature, add 1 mL 1 M K_2PO_4 (sic), extract with 7 mL diethyl ether, centrifuge at 2059 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL buffer, inject a 20 μL aliquot. (Buffer was 5 mM heptanesulfonic acid in MeCN:water:triethylamine 70:30:0.4.)

HPLC VARIABLES
Guard column: 50 × 4.6 30 μm C8
Column: 250 × 4.6 Spherisorb S5 ODS2 C18
Mobile phase: Gradient. MeCN:buffer:water 0:75:25 for 5 min, 15:85:0 for 12 min (step gradient). (Buffer was 5 mM heptanesulfonic acid in MeCN:water:triethylamine 70:30:0.4.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 11.09
Internal standard: phenelzine

OTHER SUBSTANCES
Extracted: isoniazid, hydrazine, monoacetylhydrazine

KEY WORDS
derivatization; phenelzine is IS

REFERENCE
Walubo,A.; Smith,P.; Folb,P.I. Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 391–396.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200-500 μL Plasma or CSF + 100 μL 10% (?) aqueous acetic acid + 5 mL n-hexane, shake for 30 min, centrifuge at 1870 g for 10 min. Discard the organic layer. Add 300 μL 0.1% salicaldehyde in EtOH and 400 μL 10% aqueous acetic acid to the aqueous layer, heat at 60° for 30 min, cool, add 1 mL 1 M pH 6.5 K_2HPO_4, shake for 10 s, add 5 mL diethyl ether, shake for 10 min, centrifuge at 1870 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 30 μm C8 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:triethylamine 70:30:0.4 containing 5 mM heptanesulfonic acid, pH adjusted to 6.0 with acetic acid
Flow rate: 1
Injection volume: 25
Detector: UV 320

CHROMATOGRAM
Retention time: 3
Internal standard: phenelzine sulfate

OTHER SUBSTANCES
Extracted: hydrazine, isoniazid
Noninterfering: p-aminosalicylic acid, pyrazinamide, rifampin

KEY WORDS
plasma; rabbit; derivatization; phenelzine is IS

REFERENCE
Walubo,A.; Chan,K.; Wong,C.L. Simultaneous assay for isoniazid and hydrazine metabolite in plasma and cerebrospinal fluid in the rabbit, *J.Chromatogr.*, **1991**, *567*, 261–266.

SAMPLE
Matrix: formulations
Sample preparation: Mix powdered tablet with 5 mL 200 mM pH 6 sodium acetate buffer, rotate at 30 rpm for 30 min, centrifuge. Remove a 1 mL aliquot of the supernatant and add it

to 1 mL 15 mg/mL benzaldehyde in MeOH:water 50:50, rotate at 30 rpm for 10 min, add 20 mL 3 μg/mL IS in mobile phase, rotate at 30 rpm for 30 min, inject a 50 μL aliquot of the upper layer.

HPLC VARIABLES
Column: 150 × 4.6 Ultrasphere Si
Mobile phase: n-Hexane:chloroform 95:5
Flow rate: 2
Injection volume: 50
Detector: UV 313

CHROMATOGRAM
Retention time: 17
Internal standard: iminodibenzyl (2)

OTHER SUBSTANCES
Simultaneous: hydrazine

KEY WORDS
derivatization; tablets; normal phase

REFERENCE
Matsui,F.F.; Butterfield,A.G.; Curran,N.M.; Lovering,E.G.; Sears,R.W.; Robertson,D.L. Determination of hydrazine in pharmaceuticals. Part 2. Phenelzine sulfate, *Can.J.Pharm.Sci.*, **1981**, *16*, 20–22.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.81

OTHER SUBSTANCES
Simultaneous: normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.8

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

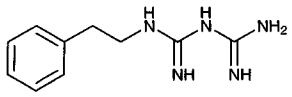
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phenformin



Molecular formula: $C_{10}H_{15}N_5$
Molecular weight: 205.26
CAS Registry No.: 114-86-3, 834-28-6 (HCl)
Merck Index: 7376
Lednicer No.: 1 75

SAMPLE
Matrix: microsomal incubations
Sample preparation: Mix 2 mL microsomal incubation with 4 mL MeOH:DMSO 80:20, centrifuge at 300 g for 20 min, dilute 1:2 with water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil-BDS C18
Mobile phase: MeOH:100 mM pH 6.5 ammonium acetate 20:80
Flow rate: 1
Injection volume: 200-500
Detector: UV 236; MS, VG Quattro BQ tandem quadrupole, API, ESI, positive ion mode, source 150°, cone voltage and lens 2 40 and 50 V, m/z 206

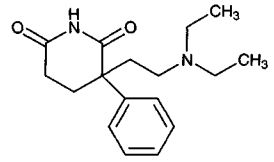
CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
comparison with capillary electrophoresis; rat; liver

REFERENCE
Llambias,E.B.; Luo,J. Study of phenformin metabolism in rat liver microsomes by HPLC, CE and on-line HPLC-electrospray ionization mass spectrometry, *Biomed.Chromatogr.*, **1996**, *10*, 155–160.

Phenglutarimide



Molecular formula: $C_{17}H_{24}N_2O_2$
Molecular weight: 288.39
CAS Registry No.: 1156-05-4, 1674-96-0 (HCl)
Merck Index: 7377

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.6

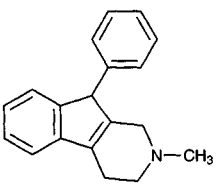
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Phenindamine



Molecular formula: $C_{19}H_{19}N$
Molecular weight: 261.37
CAS Registry No.: 82-88-2, 569-59-5 (tartrate)
Merck Index: 7380

SAMPLE

Matrix: bulk
Sample preparation: Disperse 100 mg phenindamine tartrate in 5 mL 1 mM nitric acid with gentle shaking, make up to 10 mL with MeOH, shake until dissolution is complete, inject a 5 μL aliquot.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeOH:buffer 50:50 (Protect mobile phase from light, prepare fresh daily.) (Buffer was 40 g/L silver nitrate in 1 mM nitric acid.)

Flow rate: 0.8
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: isophenindamine

KEY WORDS
protect from light

REFERENCE
Tscherne,R.J.; Umagat,H. Determination of isophenindamine in phenindamine tartrate using an argentated high-performance liquid chromatographic mobile phase, *J.Pharm.Sci.*, **1980**, *69*, 342–344.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine,

prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 10

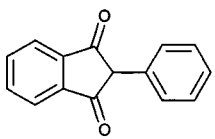
OTHER SUBSTANCES

Simultaneous: thonzylamine, pheniramine, tripelennamine, chlorpheniramine, brompheniramine, phenyltoxamine, clemizole

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Phenindione



Molecular formula: $C_{15}H_{10}O_2$
Molecular weight: 222.24
CAS Registry No.: 83-12-5
Merck Index: 7381

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

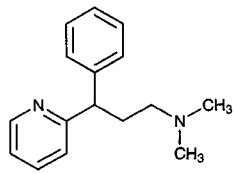
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 226.3

CHROMATOGRAM
Retention time: 18.062

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pheniramine



Molecular formula: $C_{16}H_{20}N_2$
Molecular weight: 240.35
CAS Registry No.: 86-21-5, 132-20-7 (maleate)
Merck Index: 7383
Lednicer No.: 1 77

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 30 μL 10 μg/mL amitriptyline in water + 500 μL 1 M sodium carbonate, vortex for 30 s, add 5 mL diethyl ether, vortex for 2 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, vortex for 30 s, centrifuge at 12000 rpm for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 62:38 adjusted to pH 3.5 with phosphoric acid
Column temperature: 40
Flow rate: 1.2
Detector: UV 262

CHROMATOGRAM
Retention time: 4.5
Internal standard: amitriptyline (6.1)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: diazepam, diltiazem, flurbiprofen, ibuprofen, itraconazole, ketoprofen, mebeverine, metoclopramide, phenylbutazone
Interfering: chlorpheniramine

KEY WORDS
plasma; pharmacokinetics; dog

REFERENCE
El-Sayed,Y.M.; Niazy,E.M.; Khidir,S.H. High-performance liquid chromatographic method for the quantitative determination of pheniramine in plasma, *J.Liq.Chromatogr.*, **1995**, *18*, 763–777.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 4.07
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: cell incubations
Sample preparation: 40 mL Cell incubation + 50 mL MeOH, shake vigorously for 1 min, centrifuge at 2000 rpm for 10 min, wash the pellet twice with 50 mL portions of MeOH. Combine the supernatants and add 100 mL water, extract three times with 150 mL portions of dichloromethane. Filter the extracts through anhydrous sodium sulfate, evaporate the filtrate to dryness under reduced pressure at 40°, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano-propyl (Beckman)
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 containing 20 mM triethylamine, pH 7.0.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.9

OTHER SUBSTANCES
Extracted: metabolites
Interfering: brompheniramine, chlorpheniramine

KEY WORDS
also semipreparative details

REFERENCE
Hansen,E.B.,Jr.; Cho,B.P.; Korfmacher,W.A.; Cerniglia,C.E. Fungal transformations of antihistamines: metabolism of brompheniramine, chlorpheniramine, and pheniramine to *N*-oxide and *N*-demethylated metabolites by the fungus *Cunninghamella elegans*, *Xenobiotica*, **1995**, *25*, 1081–1092.

SAMPLE
Matrix: formulations
Sample preparation: Crush 10 tablets, add 250 mL 50 mM HCl in EtOH:water 50:50, heat for 15 min on a steam bath, shake mechanically for 2 h, filter (glass fiber GF/A, Whatman), inject a 30 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil-10-ODS
Mobile phase: MeCN:buffer 50:50 (Buffer was 2.85 mM ethylenediamine sulfate adjusted to pH 7.44 ± 0.02 with 1 M ammonium hydroxide.)
Flow rate: 3.8
Injection volume: 30
Detector: UV 216.5

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: aposcopolamine, methscopolamine, phenylpropanolamine, pyrilamine, tropic acid

KEY WORDS
tablets

REFERENCE
Heidemann,D.R. High-pressure liquid chromatographic determination of methscopolamine nitrate, phenylpropanolamine hydrochloride, pyrilamine maleate, and pheniramine maleate in tablets, *J.Pharm.Sci.*, **1981**, *70*, 820–822.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. One tablet + 50 mL MeOH, sonicate, make up to 100 mL with MeOH, centrifuge for 15 min. Remove 1 mL supernatant, make up to 10 mL with mobile phase, inject a 50 μL aliquot. Drops. Dilute drops with the mobile phase so that the concentration of pheniramine maleate is 25 μg/mL, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Cyclobond I (Advanced Separation Technologies)
Mobile phase: MeOH:50 mM NaH_2PO_4 adjusted to pH 7.0 with 0.1 M NaOH 30:70
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.2

OTHER SUBSTANCES
Simultaneous: pyrilamine (mepyramine), phenylpropanolamine

KEY WORDS
tablets; drops

REFERENCE
el-Gizawy,S.M.; Ahmed,A. High-performance liquid chromatographic determination of mepyramine maleate, pheniramine maleate and phenylpropanolamine hydrochloride in tablets and drops, *Analyst*, **1987**, *112*, 867–869.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Crush tablets, add 100 mL water and 30-40 mL MeCN, dissolve, add N,N-dimethylbenzylamine, make up to 250 or 500 mL with water, centrifuge an aliquot, inject a 20 μL aliquot of the supernatant. Urine. Inject a 100 μL aliquot of urine directly.

HPLC VARIABLES
Column: 150 × 4.6 Asahipak ODP-50 C18
Mobile phase: MeCN:200 mM pH 7.0 phosphate buffer 27:73
Flow rate: 0.8
Injection volume: 20-100
Detector: Chemiluminescence following post-column reaction. Oxidize a 1 mM tris(2,2'-bipyridine) ruthenium(II) hexachloride solution in 50 mM pH 5.5 acetate buffer to Ru(III) using a Princeton Applied Research polarographic analyzer with a platinum gauze working electrode, platinum wire auxiliary electrode, and a silver wire reference electrode, +950 mV. Pump the reagent solution at 0.28 mL/min and mix with the column effluent, allow to flow through detector. The chemiluminescence detector was a fluorescence detector with the light source removed.

CHROMATOGRAM
Retention time: 3.5
Internal standard: N,N-dimethylbenzylamine
Limit of detection: 90 ng/mL

OTHER SUBSTANCES
Simultaneous: brompheniramine, chlorpheniramine, pyrilamine, diphenhydramine

KEY WORDS
tablets

REFERENCE
Holeman,J.A.; Danielson,N.D. Liquid chromatography of antihistamines using post-column tris(2, 2'-bipyridine) ruthenium(III) chemiluminescence detection, *J.Chromatogr.A*, **1994**, *679*, 277–284.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 305 × 7 PRP-1 (Hamilton)
Mobile phase: Gradient. A was water:triethylamine 99.9:0.1. B was MeCN:triethylamine 99.9:0.1. A:B 60:40 for 7 min, to 20:80 over 5 min, maintain at 20:80 for 5 min, to 60:40 over 6 min, re-equilibrate at 60:40 for 2 min.
Column temperature: 40
Flow rate: 3.5
Injection volume: 500
Detector: UV 254

CHROMATOGRAM

Retention time: 12.0

OTHER SUBSTANCES

Simultaneous: diphenylpyraline, doxylamine, guaifenesin, hydrocodone, phenylephrine, phenylpropanolamine, pyrilamine
Interfering: etafedrine

REFERENCE

Black,D.B.; By,A.W.; Lodge,B.A. Isolation and identification of hydrocodone in narcotic cough syrups by high-performance liquid chromatography with infrared spectrometric identification, *J.Chromatogr.*, **1986**, *358*, 438–443.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: thonzylamine, tripelennamine, chlorpheniramine, brompheniramine, pheninda-mine, phenyltoxamine, clemizole

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 3.4

OTHER SUBSTANCES

Simultaneous: amitriptyline, buprenorphine, chlorpromazine, cocaine, desipramine, desmethyl-doxepin, dextromoramide, diphenhydramine, doxepin, imipramine, meperidine, methadone, normeperidine, norpropoxyphene, nortriptyline, oxazepam, pentazocine, pericyazine, propoxy-phene, propranolol, quinine, thiopropazate, thioridazine
Interfering: codeine, ephedrine, oxycodone

REFERENCE

Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:10 mM pH 2.5 KH_2PO_4 60:40
Flow rate: 2.5

Injection volume: 20-40
Detector: E, Environmental Science Associates Coulochem Model 5100A, Model 5100 guard cell +0.85 V (between pump and injector), Model 5010 analytical cell +0.8 V, pre-analytical cell +0.3 V

CHROMATOGRAM
Retention time: 4.9

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, chlorpromazine, desmethyldoxepin, fluphenazine, mesoridazine, perphenazine, phenylephrine, prochlorperazine, reduced haloperidol, thioridazine, thiothixene, trazodone, triflupromazine, trimeprazine, tripelennamine
Noninterfering: diazepam, diphenhydramine, ethopropazine, fluoxetine, nordiazepam, oxazepam, phenylpropanolamine, pseudoephedrine, trifluoperazine
Interfering: desipramine, doxepin, haloperidol, imipramine, loxapine, nortriptyline, promazine, promethazine

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REFERENCE
Hariharan,M.; VanNoord,T.; Kindt,E.K.; Tandon,R. A simple, sensitive liquid chromatographic assay of cis-thiothixene in plasma with coulometric detection, *Ther.Drug Monit.*, **1991**, *13*, 79–85.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, ox-

ymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 4.00

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.49 (A), 4.49 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

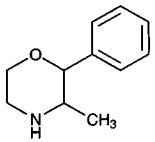
KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Phenmetrazine



Molecular formula: $C_{11}H_{15}NO$
Molecular weight: 177.25
CAS Registry No.: 134-49-6, 1707-14-8 (HCl)
Merck Index: 7385
Lednicer No.: 1 260

SAMPLE

Matrix: blood
Sample preparation: Buffer 1 mL plasma to 9.1 with 50 mM Tris buffer, extract with diethyl ether. Extract the diethyl ether layer with 10 mM phosphoric acid, wash the aqueous layer with n-hexane, inject an aliquot.

HPLC VARIABLES

Guard column: CO:PELL:ODS
Column: 110 × 4.6 5 μm Partisphere C8 (Whatman)
Mobile phase: MeCN:10 mM pH 2.3 phosphate buffer 64:36
Flow rate: 0.45
Detector: UV 210

CHROMATOGRAM
Internal standard: phenmetrazine

OTHER SUBSTANCES
Extracted: reboxetine

KEY WORDS
plasma; phenmetrazine is IS

REFERENCE
Edwards,D.M.F.; Pellizzoni,C.; Breuel,H.P.; Berardi,A.; Castelli,M.G.; Frigerio,E.; Poggesi,I.; Rocchetti,M.; Dubini,A.; Strolin Benedetti,M. Pharmacokinetics of reboxetine in healthy volunteers. Single oral doses, linearity and plasma protein binding, *Biopharm.Drug Dispos.*, **1995**, *16*, 443–460.

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Simultaneous: amphetamine, ephedrine, methamphetamine, phentermine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.);

D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

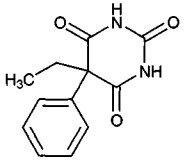
CHROMATOGRAM
Retention time: k' 2.7
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine
Interfering: phentermine, amphetamine, lidocaine, ephedrine, pentazocine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

Phenobarbital



Molecular formula: $C_{12}H_{12}N_2O_3$
Molecular weight: 232.24
CAS Registry No.: 50-06-6, 57-30-7 (sodium salt)
Merck Index: 7386
Lednicer No.: 1 268

SAMPLE
Matrix: blood
Sample preparation: Mix 500 µL plasma with 500 µL MeCN and 2 µg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, clonazepam, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenytoin, primidone

D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

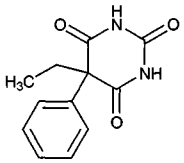
CHROMATOGRAM
Retention time: k' 2.7
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine
Interfering: phentermine, amphetamine, lidocaine, ephedrine, pentazocine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

Phenobarbital



Molecular formula: $C_{12}H_{12}N_2O_3$
Molecular weight: 232.24
CAS Registry No.: 50-06-6, 57-30-7 (sodium salt)
Merck Index: 7386
Lednicer No.: 1 268

SAMPLE
Matrix: blood
Sample preparation: Mix 500 µL plasma with 500 µL MeCN and 2 µg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, clonazepam, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenytoin, primidone

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood
Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 4.0
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: ethosuximide, primidone, phenytoin, carbamazepine, valproic acid

KEY WORDS
human; plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: Dilute 20 μL serum with 100 μL pH 3.7 phosphate buffer, shake vigorously for 10 s, add to a 45 μL PTFE column packed with 50 μm ODS-silica (Asahi Chemicals, Tokyo) (Extrashot-ODS device), wash with 100 μL water, elute with 130 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:MeOH:pH 4.4 potassium phosphate buffer 14:21:65
Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Extracted: carbamazepine, phenytoin

KEY WORDS
SPE

REFERENCE

Kouno,Y.; Ishikura,C.; Homma,M.; Oka,K. Extrashot-ODS, a syringe-type minicolumn sample injector for a reversed-phase high-performance liquid chromatographic column. Application to antiepileptics in human sera, *J.Chromatogr.B*, **1997**, *695*, 349–353.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL heptabarbital in MeOH + 500 μL 400 mM pH 7.0 sodium phosphate buffer + 10 mL ethyl acetate, extract. Evaporate the extract to dryness at 50°, reconstitute the residue in 20 μL MeOH, inject a 3 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 2.1 Whatman Co:Pell ODS
Column: 125 × 4.5 5 μm SAS Hypersil
Mobile phase: MeCN:buffer 20:80 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 7.5 with phosphoric acid.)
Flow rate: 1.6
Injection volume: 3
Detector: UV 200

CHROMATOGRAM

Retention time: 6.6
Internal standard: heptabarbital (9.8)
Limit of quantitation: 2.5 μM

OTHER SUBSTANCES

Extracted: ethosuximide, primidone, pheneturide, carbamazepine, phenytoin
Simultaneous: phenylethylmalonamide, sulthiame, sulfamethoxazole, butabarbital, pentobarbital, methsuximide, cyclobarbital, ethylphenacemide, amobarbital, glutethimide, secobarbital, barbital
Interfering: ethotoin

KEY WORDS

plasma; horse

REFERENCE

Christofides,J.A.; Fry,D.E. Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography, *Clin.Chem.*, **1980**, *26*, 499–501.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 20 μg/mL IS in MeOH:water 10:90 + 75 μL glacial acetic acid, vortex for 30 s, add 5 mL chloroform, shake for 5 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 2.1 Permaphase ETH (DuPont)
Column: 250 × 4.6 CLC 1 C8 (DuPont)
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM KH_2PO_4 and 1 mM K_2HPO_4 adjusted to pH 5.6.)
Column temperature: 25
Flow rate: 2
Injection volume: 40
Detector: UV 220

CHROMATOGRAM

Retention time: 3.5
Internal standard: alphenal (5-allyl-5-phenylbarbituric acid) (4.4)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: ethosuximide, phenytoin, carbamazepine, primidone
Simultaneous: amobarbital, barbital, chlordiazepoxide, codeine, cortisol, glutethimide, hexobarbital, mephenytoin, mephobarbital, metharbital, methsuximide, nitrazepam, pentobarbital, phenacetin, phensuximide, secobarbital
Noninterfering: acetaminophen, acetazolamide, amphetamine, bilirubin, caffeine, diazepam, dimenhydrinate, meperidine, meprobamate, methamphetamine, methaqualone, methylphenidate, nicotine, propoxyphene, theophylline, valproate
Interfering: ethotoin

KEY WORDS
plasma; serum

REFERENCE
Rydzewski,R.S.; Gadsden,R.H.; Phelps,C.A. Simultaneous rapid HPLC determination of anticonvulsant drugs in plasma and correlation with EMIT, *Ann.Clin.Lab.Sci.*, **1980**, *10*, 89–94.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 13.8
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS
serum

REFERENCE
Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Serum or plasma + 400 μL 10 μg/mL IS in acetone, vortex for 10 s, centrifuge at 4500-5000 g for 1 min, remove the supernatant to another tube, centrifuge for 30 s, inject a 5-7.5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:MeOH:buffer 17:28:55, final pH 6.8-7.0 (Buffer was 400 μL 1 M KH_2PO_4 in 1 L water, pH adjusted to 6.0 with 900 mM phosphoric acid.)
Column temperature: 30
Flow rate: 0.7
Injection volume: 5-7.5
Detector: UV 195

CHROMATOGRAM
Retention time: 9.4
Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (13.8)

OTHER SUBSTANCES
Extracted: carbamazepine, N-desmethylmethsuximide, ethosuximide, phenytoin, primidone
Simultaneous: acetaminophen, butalbital, caffeine, hexobarbital, methsuximide, phenacetin, phenylethylmalonamide, salicylic acid

KEY WORDS
plasma; serum

REFERENCE
Szabo,G.K.; Browne,T.R. Improved isocratic liquid-chromatographic simultaneous measurement of phenytoin, phenobarbital, primidone, carbamazepine, ethosuximide, and N-desmethylmethsuximide in serum, *Clin.Chem.*, **1982**, *28*, 100–104.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 10 μg/mL IS in MeCN, vortex for 10 s, centrifuge at 3000 g for 1 min, remove the supernatant and place it in another tube, centrifuge for 1 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 5 μm Nova Pak C18 Radial pak
Mobile phase: MeCN:MeOH:acetone:buffer 8:21:10:61 adjusted to pH 7.95 ± 0.02 with NaOH (Buffer was 1.36 g/L KH_2PO_4.)
Flow rate: 2.8
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 2.68
Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (4.89)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: ethosuximide, primidone, carbamazepine, phenytoin, metabolites
Simultaneous: acetaminophen, N-acetylprocainamide, aspirin, ampicillin, caffeine, cephapirin, chloramphenicol, digoxin, disopyramide, hexobarbital, indomethacin, lidocaine, mephobarbital, methsuximide, nafcillin, pentobarbital, phenylethylmalonamide, procainamide, quinidine, salicylic acid, secobarbital, sulfamerazine, sulfamethazine, terbutaline, tetracycline, theobromine, theophylline
Noninterfering: acetazolamide, amikacin, cephalosporin C, gentamicin, propranolol, sulfadiazine, sulfamethoxazole, sulfisoxazole, tobramycin, valproic acid, verapamil

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Rognerud,C.L. Simultaneous measurement of ethosuximide, primidone, phenobarbital, phenytoin, carbamazepine, and their bioactive metabolites by liquid chromatography, *Clin.Chem.*, **1984**, *30*, 1667–1670.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 200 μL 1 M HCl saturated with ammonium sulfate, vortex for 20 s, add 60 μL 10 μg/mL 4-methylprimidone in MeCN, vortex for 20 s, centrifuge at 2700 g for 5 min, inject a 5-10 μL aliquot of the MeCN layer.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeOH:THF:50 mM pH 5.9 phosphate buffer 44:1:55
Column temperature: 50
Flow rate: 1.1
Injection volume: 5-10
Detector: UV 210

CHROMATOGRAM
Retention time: 4
Internal standard: 4-methylprimidone (5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, phenytoin, primidone, valproic acid
Simultaneous: acetaminophen, salicylic acid, ethylphenylmalonamide, theophylline, caffeine, ethosuximide, chloramphenicol, methylphenobarbital, glutethimide, pentobarbital, lidocaine, diazepam

KEY WORDS
plasma

REFERENCE
Kushida,K.; Ishizaki,T. Concurrent determination of valproic acid with other antiepileptic drugs by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 131–139.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 1 mL saturated solution of $NaH_2PO_4.H_2O$ + 5 μg mephobarbital, vortex for 15 s, add 6 mL dichloromethane, rotate for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 5 5 μm Waters Radial-Pak C18
Mobile phase: MeOH:200 mM NaCl 40:60
Flow rate: 1.6
Injection volume: 50
Detector: E, Bioanalytical Systems LC4B, glassy carbon working electrode operated in parallel mode, stainless steel auxiliary electrode, W1 +0.85 V, W2 +1.00, V Ag/AgCl reference electrode following a 9.144 m × 0.5 mm i.d. figure eight Teflon tubing UV irradiation unit maintained at 0-5° with an ice bath

CHROMATOGRAM
Retention time: 4.5
Internal standard: mephobarbital 8.5
Limit of detection: 10 ng/mL

KEY WORDS
serum; post-column photochemical derivatization

REFERENCE
Selavka,C.M.; Krull,I.S.; Lurie,I.S. Photolytic derivatization for improved LCEC determinations of pharmaceuticals in biological fluids, *J.Chromatogr.Sci.*, **1985**, *23*, 499–508.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 1 mL 5 μg/mL 5-tolyl-5-phenylhydantoin in MeCN, agitate for 3 min. Remove the supernatant and evaporate it to dryness, dissolve the residue in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: pellicular reversed phase (Chrompack 28653)
Column: 100 × 3 octyl CP-tm-Spher C8 glass column (Chrompack)
Mobile phase: MeCN:50 mM NaH_2PO_4 30:70 adjusted to pH 2.2 with phosphoric acid
Flow rate: 0.9
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 3
Internal standard: 5-tolyl-5-phenylhydantoin (11)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: phenytoin

KEY WORDS
serum

REFERENCE
Van Damme,M.; Molle,L.; Abi Khalil,F. Useful sample handlings for reversed phase high performance liquid chromatography in emergency toxicology, *J.Toxicol.Clin.Toxicol.*, **1985**, *23*, 589–614.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 3.45
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental
Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin

Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: diflunisal, diazoxide, nirvanol

KEY WORDS
serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 3.59
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 2 μg 10-methoxycarbamazepine + 25 μL 1 M NaOH + 1.2 mL dichloromethane, mix for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 20 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.9 10 μm LiChrosorb RP8
Mobile phase: MeCN:water 32:68
Flow rate: 1.8
Injection volume: 10

Detector: UV 215

CHROMATOGRAM
Retention time: 2.3
Internal standard: 10-methoxycarbamazepine (9.3)

OTHER SUBSTANCES
Extracted: carbamazepine, oxcarbazepine, primidone
Noninterfering: clobazam, clonazepam, diazepam, ethosuximide, phenytoin, valproic acid

KEY WORDS
plasma

REFERENCE
Elyas,A.A.; Goldberg,V.D.; Patsalos,P.N. Simple and rapid micro-analytical high-performance liquid chromatographic technique for the assay of oxcarbazepine and its primary active metabolite 10-hydroxycarbazepine, *J.Chromatogr.*, **1990**, *528*, 473–479.

SAMPLE
Matrix: blood
Sample preparation: Inject 20 μL serum onto column A with mobile phase A and elute to waste, after 1.5 min backflush the contents of column A onto column B with mobile phase B, after 1 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 30 × 4.6 IRSP silica (for preparation see Anal. Chem. 1989, 61, 2445); B 150 × 4.6 5 μm Nucleosil C18
Mobile phase: A 14 mM NaH_2PO_4 containing 6 mM Na_2HPO_4; B MeCN:MeOH:14 mM NaH_2PO_4 containing 6 mM Na_2HPO_4 15:20:65
Flow rate: 0.8
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 11
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, phenytoin, primidone

KEY WORDS
serum; column-switching

REFERENCE
Haginaka,J.; Wakai,J.; Yasuda,H.; Kimura,Y. Determination of anticonvulsant drugs and methyl xanthine derivatives in serum by liquid chromatography with direct injection: column-switching method using a new internal-surface reversed-phase silica support as a precolumn, *J.Chromatogr.*, **1990**, *529*, 455–461.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 200 μL 20 μg/mL butalbital in MeCN, vortex, centrifuge 5 min, inject supernatant.

HPLC VARIABLES
Column: 125 × 4 LiChroSpher RP-8 5 μm
Mobile phase: MeCN:water:100 mM pH 7.0 phosphate buffer 20:75:5
Column temperature: 45
Flow rate: 2
Injection volume: 50

Detector: UV 212

CHROMATOGRAM
Retention time: 2
Internal standard: butalbital (3.8)

OTHER SUBSTANCES
Simultaneous: carbamazepine, phenytoin

KEY WORDS
serum

REFERENCE
Hannak,D.; Haux,P.; Scharbert,F.; Kattermann,R. Liquid chromatographic analysis of phenobarbital, phenytoin, and theophylline, *Wien.Klin.Wochenschr.Suppl.*, **1992**, *191*, 27–31.

SAMPLE
Matrix: blood
Sample preparation: Condition an Extrashot-Silica (diatomaceous earth) SPE cartridge (Kusano Scientific) with 200 μL EtOH and 200 μL dichloromethane, force out the remaining solvent with 500 μL air. Add 5 μL serum to the surface of the cartridge and pass 130 μL dichloromethane gently through the cartridge into the 100 μL sample loop.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrosorb Si60
Mobile phase: n-Hexane:dichloromethane:EtOH:acetic acid 82.8:15:2:0.2
Flow rate: 1
Injection volume: 100
Detector: UV 240

CHROMATOGRAM
Retention time: 7.2
Limit of quantitation: 5000 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, phenytoin

KEY WORDS
serum; normal phase; SPE

REFERENCE
Kouno,Y.; Ishikura,C.; Homma,M.; Oka,K. Simple and accurate high-performance liquid chromatographic method for the measurement of three antiepileptics in therapeutic drug monitoring, *J.Chromatogr.*, **1993**, *622*, 47–52.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 600 μL allobarbital in 75 mM pH 6.8 buffer, add 200 units β-glucuronidase (Type VII-A from E. coli), incubate at 37° for 30 min, add 1 mL of the sample to an Extrelut-1 SPE cartridge, after 10 min elute with 2.5 mL MTBE, dry the eluate under a stream of nitrogen, dissolve the residue in 50 μL MeOH:water 1:1, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 4 μm Superspher RP-18e (Merck)
Mobile phase: MeOH:11.2 mM β-cyclodextrin in 20 mM KH_2PO_4 5:95
Flow rate: 0.8
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 5
Internal standard: allobarbital (16)
Limit of detection: 1.9 ng/mL

OTHER SUBSTANCES
Simultaneous: mephobarbital, zonisamide

KEY WORDS
serum; SPE

REFERENCE
Eto,S.; Noda,H.; Noda,A. Simultaneous determination of antiepileptic drugs and their metabolites, including chiral compounds, via β-cyclodextrin inclusion complexes by a column-switching chromatographic technique, *J.Chromatogr.B*, **1994**, *658*, 385–390.

SAMPLE
Matrix: blood
Sample preparation: Add two volumes of MeCN to the mouse serum, mix, centrifuge at 1500 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Sentry (Waters)
Column: 150 × 4.6 Nova-Pak C18
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110
Column temperature: 40
Flow rate: 0.5
Injection volume: 5
Detector: UV 214

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: phenylethyl malonamide, primidone, carbamazepine, phenytoin, carbamazepine-10,11-epoxide

KEY WORDS
serum; mouse

REFERENCE
Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of 1 M pH 5.0 phosphate buffer, add IS, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3500 rpm for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:10 mM pH 5.0 sodium phosphate buffer 35:65
Flow rate: 1
Injection volume: 10-20
Detector: UV

CHROMATOGRAM
Internal standard: primidone
Limit of quantitation: 1 μg/mL

KEY WORDS
serum; rat; pharmacokinetics

REFERENCE
Nakashima,E.; Matsushita,R.; Ohshima,T.; Tsuji,A.; Ichimura,F. Quantitative relationship between structure and peritoneal membrane transport based on physiological pharmacokinetic concepts for acidic drugs, *Drug Metab.Dispos.*, **1995**, *23*, 1220–1224.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.22 μm), inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: MeCN:50 mM pH 6.9 phosphate buffer 12:88
Flow rate: 0.6
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: phenytoin, carbamazepine

KEY WORDS
serum; direct injection

REFERENCE
Nimura,N.; Itoh,H.; Kinoshita,T. Diol-bonded silica gel as a restricted access packing forming a binary-layered phase for direct injection of serum for the determination of drugs, *J.Chromatogr.A*, **1995**, *689*, 203–210.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 100 μL MeCN, centrifuge, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 3.5 μm Zorbax SB
Mobile phase: MeCN:MeOH:10 mM pH 7.1 phosphate buffer 7:34:59
Flow rate: 1.5
Injection volume: 5
Detector: UV 220

CHROMATOGRAM
Limit of detection: <1 μM

OTHER SUBSTANCES
Extracted: carbamazepine, carbamazepine epoxide, hydroxycarbamazepine, lamotrigine, oxcarbazepine, phenytoin
Also analyzed: ibuprofen, naproxen, trimethoprim

KEY WORDS
plasma

REFERENCE
Lessing,U.; Vielemeyer,O.; Heilmann,P.; Schöneshöfer,M. Routine determination of serum primidone levels with a fully automated liquid chromatographic method: Comparison with an immuno-assay-technique (Abstract 100), *Ther.Drug Monit.*, **1995**, *17*, 408.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM
Retention time: 9.75
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES
Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS
plasma; serum; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, dialysate
Sample preparation: Dialyze 400 μL plasma against 175 μL acceptor solution through a Cuprophane membrane (15 kDa cut-off) at 37° for 10 min, inject 500 μL acceptor solution (including the portion used for dialysis) onto column A at 0.71 mL/min, elute the contents of column A onto column B with mobile phase, remove column A from circuit and condition it with 1 mL acceptor solution, elute column B with mobile phase and monitor the effluent. Flush acceptor channel with 5 mL acceptor solution and plasma channel with 8 mL acceptor solution containing 25 μg/mL Triton X-100. (Acceptor solution contained 5.9 g NaCl, 4.1 g sodium acetate, 0.3 g KCl, and 1.65 g sodium citrate in 1 L water, adjusted to pH 7.4 with citric acid.)

HPLC VARIABLES
Column: A 5 × 1.6 Hypersil ODS-2; B 100 × 3 5 μm Spherisorb ODS-2
Mobile phase: MeCN:THF:20 mM pH 6.0 phosphate buffer 22:6.5:71.5
Column temperature: 37
Flow rate: 0.6
Detector: UV 240

CHROMATOGRAM
Retention time: 3
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, phenytoin

KEY WORDS
plasma; column-switching; dialysis

REFERENCE

Johansen,K.; Krogh,M.; Andresen,A.T.; Christophersen,A.S.; Lehne,G.; Rasmussen,K.E. Automated analysis of free and total concentrations of three antiepileptic drugs in plasma with on-line dialysis and high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *669*, 281–288.

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Serum. 100 μL Serum + 200 μL MeCN, vortex for 10 s, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Saliva. 250 μL Saliva + 50 μL MeCN, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Urine. Condition a Sep-Pak SPE cartridge with 5 mL MeCN then 20 mL water. Add 2 mL urine to the cartridge, wash with 20 mL water, elute with 500 μL MeCN, inject 2 μL of the eluent.

HPLC VARIABLES

Guard column: 20 × 2 3 μm ODS-Hypersil
Column: 250 × 2 3 μm ODS-Hypersil
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110
Column temperature: 40
Flow rate: 0.2
Injection volume: 2
Detector: UV 200

CHROMATOGRAM

Retention time: 6.2
Limit of quantitation: 1560 ng/mL

OTHER SUBSTANCES

Simultaneous: p-hydroxyphenobarbital, phenylethylmaleimide, primidone, dihydrodihydroxycarbamazepine, 5-(p-hydroxyphenyl)-5-phenylhydantoin, carbamazepine, 5-(m-hydroxyphenyl)-5-phenylhydantoin, phenytoin, carbamazepine-10,11-epoxide, hexobarbital, nitrazepam, clonazepam

Noninterfering: oxazepam, nordiazepam, cyheptamide, diazepam, prezepam, temazepam, lorazepam, chlordiazepoxide

KEY WORDS

serum; SPE

REFERENCE

Liu,H.; Delgado,M.; Forman,L.J.; Eggers,C.M.; Montoya,J.L. Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1993**, *616*, 105–115.

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Homogenize 20-200 mg brain tissue with 1 mL 1.5 μg/mL IS in 1% ammonium acetate + 1% sodium azide buffer:MeCN 99:1, flush apparatus with 1 mL extraction buffer, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 μL MeOH, add 50 μL water, inject a 10-25 μL aliquot. Serum. 100 μL Serum + 1 mL 1.5 μg/mL IS in 1% ammonium acetate + 1% sodium azide buffer:MeCN 99:1,mix, add 1 mL extraction buffer, mix, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 μL MeOH, add 50 μL water, inject a 10-25 μL aliquot. (Extraction buffer was 20 g $NaH_2PO_4.2H_2O$ + 4.5 g $Na_2HPO_4.2H_2O$ + 1.5 NaN_3 in 1 L water, pH 6. Extraction solvent was dichloromethane:isopropanol 97:3.)

HPLC VARIABLES

Column: 200 × 2.1 5 μm Hypersil ODS

Mobile phase: Gradient. A was MeCN:50 mM $(NH_4)H_2PO_4$ (pH 4.4) 10:90. B was MeCN:50 mM $(NH_4)H_2PO_4$ (pH 4.4) 60:40. A:B from 85:15 to 55:45 over 9.5 min, keep at 55:45 for 0.5 min, return to 85:15 over 0.5 min.

Column temperature: 65
Flow rate: 0.3
Injection volume: 10-25
Detector: UV 207

CHROMATOGRAM
Retention time: 6.22
Internal standard: 5-ethyl-5-(p-tolyl)barbituric acid (9.07)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: primidone, N-desmethylmethsuximide, carbamazepine-10,11-epoxide, phenytoin, carbamazepine

KEY WORDS
serum; SPE; brain

REFERENCE
Juergens,U.; Rambeck,B. Sensitive analysis of antiepileptic drugs in very small portions of human brain by microbore HPLC, *J.Liq.Chromatogr.*, **1987**, *10*, 1847–1863.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Inject a 5-20 μL aliquot directly onto the column with mobile phase A or B. Urine. Inject a 5 μL aliquot directly onto the column with mobile phase C.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 13:87 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer 14:86 for 5 min, to 25:75 over 1 min, to 30:70 over 2 min, to 50:50 over 3 min, maintain at 50:50 for 6 min (C)
Flow rate: 1
Injection volume: 5 (A, C), 20 (B)
Detector: UV 254 (serum);, UV 230 (urine)

CHROMATOGRAM
Retention time: 3.58 (serum, A), 10.5 (serum, B), 8.2 (urine, C)

OTHER SUBSTANCES
Simultaneous: acetaminophen (B), barbital (B), carbamazepine (B,C), phenobarbital (B), phenytoin (B,C), primidone (B,C), sulfapyridine (B)
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.993

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve injection in mobile phase to give a phenobarbital sodium concentration of 1.2 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Partisil ODS-3 or 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:buffer:propylene glycol 35:65:4 (Buffer was 4.1 g anhydrous sodium acetate and 15 mL acetic acid in 1 L water.)
Flow rate: 2
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rugged; injections

REFERENCE
Reif,V.D.; Kaufmann,K.L.; DeAngelis,N.J.; Frankhouser,M.C. Liquid chromatographic assays for barbiturate injections, *J.Pharm.Sci.*, **1986**, *75*, 714–716.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 20-200 μg/mL solution in acetone with 50 μL of a 0.4-1.6 mg/mL solution of 2-bromo-2'-acetonaphthone in acetone, add 5-10 mg cesium carbonate, heat at 30° for 30 min, add 50 μL glacial acetic acid, mix, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18

Mobile phase: MeOH:water 80:20
Flow rate: 2
Detector: UV 249

CHROMATOGRAM
Retention time: 4.95
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: amobarbital, butobarbital, heptobarbital, hexobarbital, mephobarbital, pentobarbital, secobarbital
Interfering: barbital

KEY WORDS
derivatization

REFERENCE
Hulshoff,A.; Roseboom,H.; Renema,J. Improved detectability of barbiturates in high-performance liquid chromatography by pre-column labelling and ultraviolet detection, *J.Chromatogr.*, **1979**, *186*, 535–541.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate a solution in water, MeOH, or diethyl ether to dryness, add a 3-fold molar excess of triethylamine, add 0.5-3 mL MeCN, add a 3-fold molar excess of N-chloromethyl-4-nitrophthalimide, heat at 60° for 1 h, inject an aliquot. (Preparation of N-chloromethyl-4-nitrophthalimide is as follows. Suspend 130 g 4-nitrophthalimide in 80 mL 40% formaldehyde solution, add 200 mL water, reflux for 4 h, filter while hot, N-(hydroxymethyl-4-nitrophthalimide crystallizes on cooling (cf. J. Am. Chem. Soc. 1922, 44, 817). Mix a suspension of 2.26 g N-hydroxymethyl-4-nitrophthalimide in 10-15 mL ether with a suspension of 2.1 g phosphorus pentachloride in 10-15 mL ether, after 10 min heat on a water bath, cool in an ice-salt mixture, add ice-water dropwise with shaking, filter to obtain N-chloromethyl-4-nitrophthalimide, dry under vacuum (cf. Chem. Ber. 1959, 9, 1258).)

HPLC VARIABLES
Column: 7 μm LiChrosorb RP8
Mobile phase: MeCN:water 60:40
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 4 ng

OTHER SUBSTANCES
Simultaneous: amobarbital, cyclobarbital, methylphenobarbital, secobarbital

KEY WORDS
derivatization

REFERENCE
Lindner,W.; Santi,W. N-chloromethylphthalimides as derivatization reagents for high-performance liquid chromatography, *J.Chromatogr.*, **1979**, *176*, 55–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.

Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: allobarbital, amobarbital, barbital, barbituric acid, butabarbital, mephobarbital, methabarbital, methohexital, phenytoin, secobarbital, thiamylal

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mm KH_2PO_4 + 5 mM 1-decanesulfonic acid 30:70, adjusted to pH 3.2 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 6.6
Internal standard: methyl paraben (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, butalbital, aprobarbital, mephobarbital, pentobarbital, secobarbital, talbutal, vinbarbital

KEY WORDS
stability-indicating

REFERENCE
Ibrahim,F.B. Simultaneous determination and separation of several barbiturates and analgesic products by ion-pair high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2835–2851.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 3 μm 208HS3410 (Vydac)
Mobile phase: Gradient. MeCN:water from 15:85 to 60:40 over 10 min.
Flow rate: 1.5
Detector: UV 210 (?)

CHROMATOGRAM
Retention time: 4.2

OTHER SUBSTANCES
Simultaneous: barbital, carbamazepine, diazepam, ethotoin, mephenytoin, methsuximide, phenacemide, phensuximide

REFERENCE
Vydac HPLC Catalog, 1994-5, **1994**, p. 26.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 2.95

OTHER SUBSTANCES
Simultaneous: aprobarbital, pentobarbital, amobarbital, butabarbital, butalbital, thiopental, secobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone,

naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.61 (A), 4.74 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, ser-

traline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.77

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.76

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil

Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: Filter urine (0.45 μm), directly inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 2 × 20 37-53 μm ODS (Whatman, USA)
Column: 4.6 × 250 5 μm Econosphere
Mobile phase: MeCN:25 mM pH 2.2-2.3 H_3PO_4 12:88 (After each analysis change the mobile phase to MeCN:water 40:60 over 1 min and wash the column for 20 min, then re-equilibrate at initial conditions for 20 min before the next injection.)
Injection volume: 25
Detector: UV 240 following post-column reaction. The column effluent mixed with 50 mM pH 12.7-12.9 borate buffer pumped at 0.3 mL/min and the mixture flowed through a 50 × 4.6 mm i.d. Supelco reaction coil filled with acid washed silanized glass beads to the detector.

CHROMATOGRAM
Retention time: 44.2
Limit of detection: 1 μM
Limit of quantitation: 3.2 μM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
post-column reaction

REFERENCE
Paibir,S.G.; Soine,W.H. High-performance liquid chromatographic analysis of phenobarbital and phenobarbital metabolites in human urine, *J.Chromatogr.B*, **1997**, *691*, 111–117.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 3 mL 5 M NaOH, vortex 30 s, add 12 mL diethyl ether, rotate for 5 min, centrifuge at 2500 rpm for 5 min. Remove the ether layer and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech C18
Mobile phase: MeOH:water 50:50 containing 7 mL/L butylamine, adjusted to pH 3.2 with sulfuric acid
Flow rate: 1.8
Injection volume: 50
Detector: E, Bioanalytical Systems Model LC4B, dual glassy carbon working electrode cell half operated in the parallel mode + 1.0 V and +0.9 V, stainless steel auxiliary electrode cell half, Ag/AgCl reference electrode. The detector was preceded by a Photronix Model 816 UV irradiator which irradiated the mobile phase in a 9.144 m length of 0.5 mm i.d. × 1.6 mm o.d. Teflon tubing in a three-dimensional figure eight configuration. The irradiation apparatus was maintained at 0-5° using an ice bath.

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 10 ppb

OTHER SUBSTANCES
Simultaneous: methylphenidate, chlordiazepoxide, nitrazepam, cocaine

KEY WORDS
post-column photochemical derivatization

REFERENCE
Selavka,C.M.; Krull,I.S.; Lurie,I.S. Photolytic derivatization for improved LCEC determinations of pharmaceuticals in biological fluids, *J.Chromatogr.Sci.*, **1985**, *23*, 499–508.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.5 μm) urine, inject 10 μL directly onto column A with mobile phase A, run with mobile phase A for 2 min, backflush column A onto column B with mobile phase B for 10 min then switch column B out of circuit, elute column B with mobile phase B and monitor the eluant, re-equilibrate column A with mobile phase A for at least 5 min.

HPLC VARIABLES
Column: A 15 × 3.2 5 μm Brownlee ODS; B 250 × 1 5 μm Adsorbosphere ODS
Mobile phase: A 25 mM pH 7.5 phosphate buffer; B MeCN:25 mM pH 7.5 phosphate buffer 15:85
Flow rate: A 1; B 0.045
Injection volume: 10
Detector: UV 208

CHROMATOGRAM
Retention time: 18
Limit of detection: 2000 ng/mL

KEY WORDS
column-switching; microbore

REFERENCE
Koenigbauer,M.J.; Curtis,M.A. Use of micellar mobile phases and microbore column switching for the assay of drugs in physiological fluids, *J.Chromatogr.*, **1988**, *427*, 277–285.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES
Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.);

D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN: buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM
Retention time: k' 1.1
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine
Interfering: cotinine, benzoylecgonine, secobarbital, oxazepam, nordiazepam

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KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine +1 mL 500 mM pH 5.5 phosphate buffer, add to an Extrelut 3 SPE cartridge, let stand for 10 min, elute with 15 mL dichloromethane:isopropanol 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm Lichrospher 100 RP8
Column: 250 × 4 5 µm Lichrospher 100 RP8
Mobile phase: Gradient. MeCN:10 mM pH 4.4 phosphate buffer from 30:70 to 40:60 over 8 min, maintain at 40:60 for 6 min, to 30:70 over 1 min
Flow rate: 1
Injection volume: 20
Detector: UV 212

CHROMATOGRAM
Retention time: 7.8
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: barbital, allobarbital, butabarbital, pentobarbital, secobarbital
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, trifluprom azine, trimethoprim, trimipramine

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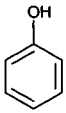
KEY WORDS
SPE

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

Phenol



Molecular formula: C_6H_6O
Molecular weight: 94.11
CAS Registry No.: 108-95-2
Merck Index: 7390

SAMPLE
Matrix: air
Sample preparation: Pull 5-150 L air through 10 mL 0.06% NaOH in water at 1-2 L/min. Add 1 mL buffer and 3 mL 0.1% 4-nitrobenzenediazonium tetrafluoroborate in water, make up to 20 mL with water, let stand for 15 min, inject a 2-40 μL aliquot. (Buffer was 0.51% sodium bicarbonate containing 0.21% sodium carbonate, pH 11.5.)

HPLC VARIABLES
Column: 200 × 4.6 Polygosil 60-5 C18 (Macherey-Nagel)
Mobile phase: MeOH:water 85:15
Flow rate: 1.1
Injection volume: 2-40
Detector: UV 365

CHROMATOGRAM
Retention time: 4
Limit of detection: 50 pg

OTHER SUBSTANCES
Also analyzed: o-cresol, m-cresol, p-cresol, 1-naphthol, 2-naphthol, 2,3-xylenol, 2,4-xylenol, 2,5-xylenol, 2,6-xylenol, 3,4-xylenol, 3,5-xylenol

KEY WORDS
derivatization

REFERENCE
Kuwata,K.; Uebori,M.; Yamazaki,Y. Determination of phenol in polluted air as *p*-nitrobenzeneazophenol derivative by reversed phase high performance liquid chromatography, *Anal.Chem.*, **1980**, *52*, 857–860.

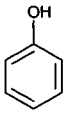
SAMPLE
Matrix: air
Sample preparation: Pull 5-150 L air through 10 mL 0.12% NaOH in water at 1-2 L/min. Remove a 5 mL aliquot, add 1 mL buffer, add 3 mL 0.1% 4-nitrobenzenediazonium tetrafluoroborate in water, make up to 20 mL with water, let stand for 30 min, add 1 mL 1% NaOH, add 1 mL carbon tetrachloride, shake, centrifuge, inject a 2-40 μL aliquot of the aqueous (p-unsubstituted phenols) layer or a 2-10 μL aliquot of the organic (p-substituted phenols) layer. (Buffer was 0.51% sodium bicarbonate containing 0.21% sodium carbonate, pH 11.5.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm LiChrosorb RP-18
Mobile phase: MeOH:water 85:15
Flow rate: 1.1
Injection volume: 2-40
Detector: UV 365

CHROMATOGRAM
Retention time: 3.89

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

Phenol



Molecular formula: C_6H_6O
Molecular weight: 94.11
CAS Registry No.: 108-95-2
Merck Index: 7390

SAMPLE
Matrix: air
Sample preparation: Pull 5-150 L air through 10 mL 0.06% NaOH in water at 1-2 L/min. Add 1 mL buffer and 3 mL 0.1% 4-nitrobenzenediazonium tetrafluoroborate in water, make up to 20 mL with water, let stand for 15 min, inject a 2-40 μL aliquot. (Buffer was 0.51% sodium bicarbonate containing 0.21% sodium carbonate, pH 11.5.)

HPLC VARIABLES
Column: 200 × 4.6 Polygosil 60-5 C18 (Macherey-Nagel)
Mobile phase: MeOH:water 85:15
Flow rate: 1.1
Injection volume: 2-40
Detector: UV 365

CHROMATOGRAM
Retention time: 4
Limit of detection: 50 pg

OTHER SUBSTANCES
Also analyzed: o-cresol, m-cresol, p-cresol, 1-naphthol, 2-naphthol, 2,3-xylenol, 2,4-xylenol, 2,5-xylenol, 2,6-xylenol, 3,4-xylenol, 3,5-xylenol

KEY WORDS
derivatization

REFERENCE
Kuwata,K.; Uebori,M.; Yamazaki,Y. Determination of phenol in polluted air as *p*-nitrobenzeneazophenol derivative by reversed phase high performance liquid chromatography, *Anal.Chem.*, **1980**, *52*, 857–860.

SAMPLE
Matrix: air
Sample preparation: Pull 5-150 L air through 10 mL 0.12% NaOH in water at 1-2 L/min. Remove a 5 mL aliquot, add 1 mL buffer, add 3 mL 0.1% 4-nitrobenzenediazonium tetrafluoroborate in water, make up to 20 mL with water, let stand for 30 min, add 1 mL 1% NaOH, add 1 mL carbon tetrachloride, shake, centrifuge, inject a 2-40 μL aliquot of the aqueous (p-unsubstituted phenols) layer or a 2-10 μL aliquot of the organic (p-substituted phenols) layer. (Buffer was 0.51% sodium bicarbonate containing 0.21% sodium carbonate, pH 11.5.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm LiChrosorb RP-18
Mobile phase: MeOH:water 85:15
Flow rate: 1.1
Injection volume: 2-40
Detector: UV 365

CHROMATOGRAM
Retention time: 3.89

Limit of detection: 0.05 ng

OTHER SUBSTANCES
Simultaneous: o-chlorophenol, m-chlorophenol, p-chlorophenol, o-cresol, m-cresol, p-cresol, o-ethylphenol, m-ethylphenol, p-ethylphenol, α-naphthol, β-naphthol, 2,3-xylenol, 2,4-xylenol, 2,5-xylenol, 2,6-xylenol, 3,4-xylenol, 3,5-xylenol

KEY WORDS
derivatization

REFERENCE
Kuwata,K.; Uebori,M.; Yamazaki,Y. Reversed-phase liquid chromatographic determination of phenols in auto exhaust and tobacco smoke as *p*-nitrobenzeneazophenol derivatives, *Anal.Chem.*, **1981**, *53*, 1531–1534.

SAMPLE
Matrix: air
Sample preparation: Condition a Sep Pak silica SPE cartridge with 10 mL dichloromethane and dry with helium at 5 L/min. Pull air through a 0.80 μm cellulose ester membrane filter and the SPE cartridge at 2 L/min for 1 h, desorb the filter with 5 mL 1% acetic acid with sonication for 10 min, elute the SPE cartridge with 5 mL 1% acetic acid, inject aliquots of the eluates.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 1% acetic acid. B was MeCN:acetic acid 99:1. A:B from 0:100 to 90:10 over 10.5 min, to 78:22 to 24.5 min, to 0:100 (step gradient), maintain at 0:100 for 5 min, re-equilibrate for 12 min.
Flow rate: 2
Injection volume: 200
Detector: F ex 304 em 338 for 6.3 min, ex 280 em 325 for 7.7 min, ex 257 em 330 for 5.3 min, ex 342 em 464 for 4.7 min, ex 285 em 310 for 11 min

CHROMATOGRAM
Retention time: 15.5
Limit of detection: 0.29 μg/cu.m.

OTHER SUBSTANCES
Simultaneous: catechol, cresol, hydroquinone, 3-methylcatechol, scopoletin

KEY WORDS
SPE

REFERENCE
Risner,C.H. The quantification of hydroquinone, catechol, phenol, 3-methylcatechol, scopoletin, m+p-cresol and o-cresol in indoor air samples by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4117–4140.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 100 μg/mL p-ethylphenol in water, acidify to pH 1.0 with 1 M HCl, saturate with 100 mg NaCl, add 300 μL ethyl acetate, shake for 10 min, centrifuge at 1300 g for 5 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Finepak Sil C18S (Jasco)
Mobile phase: MeCN:water 30:70
Flow rate: 1
Injection volume: 10
Detector: F ex 260 em 305 or MS, Hitachi M-1000S quadrupole, APCI, desolvation 399°, vaporization 300°, drift voltage 40 V, negative-ion mode, m/z = 93

CHROMATOGRAM
Retention time: 5.8
Internal standard: p-ethylphenol (m/z = 121) (16)
Limit of detection: <1 μM

OTHER SUBSTANCES
Extracted: p-cresol

KEY WORDS
serum

REFERENCE
Niwa,T. Phenol and p-cresol accumulated in uremic serum measured by HPLC with fluorescence detection, *Clin.Chem.*, **1993**, *39*, 108–111.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 3.40
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclam-

ide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.422

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk, formulations

Sample preparation: Bulk. Weigh out 100 mg morphine, dissolve in 25 mL MeOH:water:acetic acid 24:72:1, dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL morphine, filter (0.45 μm), inject a 20 μL aliquot. Injections. Dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL morphine, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:5 mM sodium 1-heptanesulfonate:acetic acid 24:72:1

Flow rate: 1.5
Injection volume: 20
Detector: UV 284

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: morphine, 2-mercaptobenzothiazole (UV 230), pseudomorphine (UV 230)

KEY WORDS
injections

REFERENCE
Bello,A.C.; Jhangiani,R.K. Liquid chromatographic determination of morphine sulfate and some contaminants in injections and bulk drug material: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1046–1048.

SAMPLE
Matrix: feces, urine
Sample preparation: Urine. 5 mL Urine + 2 mL concentrated HCl + 1 mL 250 μg/mL p-chlorophenol in water, boil for 1 h, cool, add 4 mL diethyl ether, extract by repeated inversion for 1 min, centrifuge at 500 g for 10 min. Remove the organic layer and add it to 3 mL 50 mM NaOH in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL water, filter (0.45 μm), inject a 50 μL aliquot of the filtrate. Feces. 500 mg Feces + 5 mL 100 mM pH 5.5 phosphate buffer + 50 μL 250 μg/mL p-chlorophenol in water, vortex, centrifuge at 500 g for 10 min. Remove the top layer and add it to 2 mL concentrated HCl, boil for 1 h, cool, add 4 mL diethyl ether, extract by repeated inversion for 1 min, centrifuge at 500 g for 10 min. Remove the organic layer and add it to 3 mL 50 mM NaOH in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL water, filter (0.45 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Econosil RP-18
Mobile phase: MeOH:20 mM pH 4.0 phosphate buffer 48:52
Flow rate: 0.7
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 14
Internal standard: p-chlorophenol (37)
Limit of detection: 200 ng

OTHER SUBSTANCES
Extracted: p-cresol

REFERENCE
Birkett,A.M.; Jones,G.P.; Muir,J.G. Simple high-performance liquid chromatographic analysis of phenol and p-cresol in urine and feces, *J.Chromatogr.B*, **1995**, *674*, 187–191.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL aliquot to a theoretical concentration of 30 μg/mL with MeCN:water 2:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 mm long 5 μm Waters Resolve C18
Mobile phase: MeOH:100 mM ammonium acetate:diethylamine 1280:2720:4, adjusted to a pH of 7.5
Column temperature: 30
Flow rate: 1

Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: nizatidine sulfoxide, nizatidine amide, nizatidine

KEY WORDS
injections

REFERENCE
Raineri,D.L.; Cwik,M.J.; Rodvold,K.A.; Deyo,K.L.; Scaros,L.P.; Fischer,J.H. Stability of nizatidine in commonly used intravenous fluids and containers, *Am.J.Hosp.Pharm.*, **1988**, *45*, 1523–1529.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:THF:isopropanol:water 30:3.5:1.5:65
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 245

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: methylparaben, propylparaben, flumazenil
Noninterfering: aminophylline, cimetidine, dobutamine, dopamine, famotidine, lidocaine, procainamide, ranitidine

KEY WORDS
injections; 5% dextrose

REFERENCE
Olsen,K.M.; Gurley,B.J.; Davis,G.A.; Christensen,R.; Monaghan,M.S. Stability of flumazenil with selected drugs in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1993**, *50*, 1907–1912.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4 5 μm Zorbax RX-C18
Column: 250 × 4.6 5 μm Zorbax RX-C18
Mobile phase: MeCN:buffer 20:80 (Buffer was 50 mM NaH_2PO_4 + 1 mM tetramethylammonium chloride + 0.5 mM 1-octanesulfonic acid adjusted to pH 3.5 with concentrated orthophosphoric acid.)
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 203

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: atropine, tropic acid, obidoxime, HI-6
Also analyzed: pralidoxime chloride

KEY WORDS
nerve agent antidote mixtures

REFERENCE
Paddle,B.M.; Dowling,M.H. Simple high-performance liquid chromatographic method for assessing the deterioration of atropine-oxime mixtures employed as antidotes in the treatment of nerve agent poisoning, *J.Chromatogr.*, **1993**, *648*, 373–380.

SAMPLE
Matrix: formulations
Sample preparation: Injections and ophthalmic solutions. Dilute with water to an atropine concentration of 80 μg/mL, inject a 20 μL aliquot. Ointment. Weigh out ointment equivalent to about 4 mg atropine sulfate, add 10 mL THF:water 80:20, sonicate and swirl until the ointment is completely dispersed, make up to 50 mL with water, filter (0.45 μm), inject a 20 μL aliquot

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:50 mM NaH_2PO_4 10:90, pH adjusted to 4.0 with 10% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: atropine, tropic acid
Noninterfering: benzyl alcohol, methylparaben, benzalkonium chloride, chlorobutanol

KEY WORDS
injections; ophthalmic solutions; ointments

REFERENCE
Lehr,G.J.; Yuen,S.M.; Lawrence,G.D. Liquid chromatographic determination of atropine in nerve gas antidotes and other dosage forms, *J.AOAC Int.*, **1995**, *78*, 339–343.

SAMPLE
Matrix: honey
Sample preparation: Condition a 3 mL Baker-10 C18 SPE cartridge with 3 mL MeOH and 3 mL water. Dissolve 10 g honey in 90 mL water, add 30 g NaCl, add 2 mL 10% phosphoric acid, distil about 30 mL at about 5 mL/min. Add a 15 mL aliquot of the distillate to 6 g NaCl and 5 mL 5% sodium bicarbonate, extract with 5 mL benzene (Caution! Benzene is a carcinogen!). Wash the organic layer with 3 mL 1% sodium bicarbonate, extract with 3 mL 100 mM NaOH, extract with 2 mL 100 mM NaOH. Combine the aqueous extracts and adjust the pH to 3.0 with 0.33 mM phosphoric acid, add 4.5 g NaCl, add to the SPE cartridge, let cartridge dry under vacuum for 3 min, elute with 1 mL MeOH, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 70 × 2 30 μm Co-Pell ODS
Column: 150 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:buffer 20:80 (Buffer was 10 mM NaH_2PO_4 containing 2 mM EDTA adjusted to pH 5.0.)
Flow rate: 1
Injection volume: 10
Detector: E, Irica Model E-520, glassy carbon electrode 0.7 V, Ag/AgCl reference electrode or UV 270

CHROMATOGRAM
Retention time: 11
Limit of detection: 2 ppb

KEY WORDS
SPE

REFERENCE
Takeba,K.; Matsumoto,M.; Shida,Y.; Nakazawa,H. Determination of phenol in honey by liquid chromatography with amperometric detection, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 602–604.

SAMPLE
Matrix: perfusate
Sample preparation: 100 μL Perfusate (Kreb's Henselit buffer containing 1% bovine serum albumin) + 200 μL 5 μg/mL p-cresol in MeOH, vortex for 20 s, centrifuge at 10000 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:water:orthophosphoric acid 40:60:0.1, pH 2.7 ± 0.02
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: p-cresol (10.3)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: phenyl-β-D-glucuronide

REFERENCE
Thompson,M.J.; Ballinger,L.N.; Cross,S.E.; Roberts,M.S. High-performance liquid chromatographic determination of phenol, 4-nitrophenol, β-naphthol and a number of their glucuronide and sulphate conjugates in organ perfusate, *J.Chromatogr.B*, **1996**, *677*, 117–122.

SAMPLE
Matrix: solutions
Sample preparation: Mix solution with 5 mL 200 g/L sodium acetate trihydrate and 650 μL reagent, let stand for 1 min, add 5 mL 160 g/L sodium carbonate monohydrate, add 150 mg tetrabutylammonium bromide, extract with 2 mL n-butanol, centrifuge, inject an aliquot of the organic layer. (Prepare reagent by mixing 2.566 g p-aminobenzonitrile and 108 mL concentrated HCl in 1 L water. Cool a 25 mL aliquot in an ice bath, slowly add with stirring 3 mL of a 25 g/L sodium nitrite solution. Use reagent within 1 h.)

HPLC VARIABLES
Column: 250 × 2.6 HC ODS/SIL-X C18 (Perkin-Elmer)
Mobile phase: MeOH:water 64:36
Flow rate: 1
Injection volume: 10
Detector: UV 370

CHROMATOGRAM
Retention time: 3.20
Limit of detection: 10 ppb

OTHER SUBSTANCES
Simultaneous: 2-sec-butylphenol, 2-tert-butylphenol, 3-tert-butylphenol, catechol, o-cresol, m-cresol, p-cresol, 2,3-dimethylphenol, 2-5-dimethylphenol, 2,6-dimethylphenol, 3,5-dimethylphenol, 3-hydroxybenzoic acid, 3-nitrophenol, salicylic acid, 2,3,5,6-tetramethylphenol, 3-trifluoromethylphenol

KEY WORDS
derivatization

REFERENCE
Baiocchi,C.; Campi,E.; Gennaro,M.; Mentasti,E.; Mirti,P. Reversed phase liquid chromatographic separation of phenolic compounds with a new derivatizing reaction, *Chromatographia*, **1982**, *15*, 660–664.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 mL of a 50 μg/mL solution with 2 mL 100 mg/mL NaOH and 5 mL reagent, mix, let stand for 15 min, add 3.4 mL 15 mg/mL tetrabutylammonium bromide in butanol (saturated with water), extract, inject a 10 μL aliquot of the organic layer. (Prepare reagent by mixing 5 volumes 7.6 mg/mL sulfanilic acid with 1 volume 470 mg/mL sulfuric acid, cool in an ice bath, slowly add 5 volumes 3.4 mg/mL sodium nitrite. Discard the reagent after 10 min.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak RP phenyl
Mobile phase: MeOH:water 48:52 containing 3 mM tetrabutylammonium bromide
Flow rate: 2
Injection volume: 10
Detector: UV 370

CHROMATOGRAM
Retention time: 5.12
Limit of quantitation: 100 ppb

OTHER SUBSTANCES
Simultaneous: 2-sec-butylphenol, 3-t-butylphenol, 3-chlorophenol, 2-methylphenol, 3-methylphenol, 4-nitrophenol, 2,3-xylenol, 2,5-xylenol, 2,6-xylenol, 3,5-xylenol
Noninterfering: 2-t-butylphenol, 4-methylphenol

KEY WORDS
derivatization

REFERENCE
Baiocchi,C.; Gennaro,M.C.; Campi,E.; Mentasti,E.; Aruga,R. HPLC identification and separation of phenolic compounds derivatized with diazotized sulfanilic acid. Structural effects on retention times, *Anal.Lett.*, **1982**, *15*, 1539–1548.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeOH:10 mM pH 5.5 potassium phosphate buffer 3.5:96.5
Flow rate: 2-3
Detector: UV 254

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Simultaneous: catechol, hydroquinone (quinol), phenyl glucuronide, phenyl glucoside, phenyl galactopyranoside, phenyl sulfate, resorcinol

REFERENCE
Beyer,J.; Frank,G. Hydroxylation and conjugation of phenol by the frog *Rana temporaria*, *Xenobiotica*, **1985**, *15*, 277–280.

SAMPLE

Matrix: solutions

Sample preparation: Mix 3.5 mL of a 40 nM-50 μM solution in benzene with 500 μL 3 mM reagent in benzene and 100 μL 3.7 mM pyridine in benzene (Caution! Benzene is a carcinogen!), heat in the dark at 100° for 40 min (primary and secondary alcohols) or at 140° for 50 min (tertiary alcohols), cool, dilute 100-fold with mobile phase, inject a 20 μL aliquot. (The reagent is 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (Dojindo Laboratories, Kumamoto, Japan). Synthesis is as follows. Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Add 2 mL phosphorus oxychloride dropwise to a solution of 2 g sesamol in 3 mL DMF at 0°, heat on a steam bath with frequent shaking for 1 h, cool in ice, add 50 mL saturated sodium acetate solution, heat on a steam bath for 30 min, cool, filter, recrystallize the solid from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as colorless needles (mp 125-126°) (Bull. Chem. Soc. Jpn. 1962, 35, 1321). Stir 1.4 g ethyl 2-chloromethyl-5-oxazolecarboxylate, 1.5 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 2 g potassium carbonate, and 50 mL anhydrous DMF at 120° overnight, cool, filter. Evaporate the filtrate to dryness under reduced pressure to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 186°) (yield 39%). Reflux 260 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran, 100 mg KOH, 20 mL EtOH, and 30 mL water for 2 h, concentrate under reduced pressure, dissolve the residue in 100 mL water, wash with ethyl acetate, treat the aqueous layer with activated carbon, acidify the aqueous layer to pH 2 with 2 M HCl. Filter the precipitate and recrystallize it from EtOH to give 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 294-295°). Reflux 150 mg 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran and 5 mL thionyl chloride for 2 h, pour the reaction mixture into 300 mL petroleum ether. Filter the precipitate and dry it over KOH to give 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (mp 290°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeCN:water 70:30
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 460

CHROMATOGRAM

Retention time: 4.3
Limit of detection: 750 fmole

OTHER SUBSTANCES

Simultaneous: benzyl alcohol, cyclohexanol, 1-butanol, 1-hexanol, 3-methyl-1-butanol, 2-methyl-2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 1-propanol
Noninterfering: aldehydes, amino acids, aromatic amines, carboxylic acids, ketones, sulfhydryl compounds
Interfering: 2-propanol

KEY WORDS

derivatization

REFERENCE

Nagaoka,H.; Nohta,H.; Kaetsu,Y.; Saito,M.; Ohkura,Y. 2-(5-Chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as fluorescence derivatization reagent for alcohols in high performance liquid chromatography, *Anal.Sci.*, **1989**, *5*, 525–530.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot of a solution in 1% acetic acid.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere-ODS C18
Mobile phase: Gradient. A was MeCN:acetic acid 99:1. B was 1% acetic acid in water. A:B from 0:100 to 10:90 over 10 min, to 20:80 over 25 min, wash with A for 6 min, re-equilibrate for 14 min.
Flow rate: 2
Injection volume: 20
Detector: F ex 274 em 298

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: catechol (F ex 280 em 325), hydroquinone (F ex 304 em 338), resorcinol (F ex 284 em 313)

REFERENCE
Risner,C.H.; Cash,S.L. A high-performance liquid chromatographic determination of major phenolic compounds in tobacco smoke, *J.Chromatogr.Sci.*, **1990**, *28*, 239–244.

SAMPLE
Matrix: solutions
Sample preparation: Inject 50 μL onto column A in series with column B, after 1.1 min switch so that column A comes after column B, continue to elute.

HPLC VARIABLES
Column: A 15 × 3.2 7 μm New Guard RP-18 (Applied Biosystems); B 100 × 4.6 3 μm Econosphere C18 (Alltech)
Mobile phase: MeCN:water:phosphoric acid 25:75:0.2
Flow rate: 1
Injection volume: 50
Detector: UV 200

CHROMATOGRAM
Retention time: 5
Limit of detection: 0.05 ppm

OTHER SUBSTANCES
Extracted: toluene, cresol, benzoic acid

KEY WORDS
groundwater; water; column-switching

REFERENCE
Chamkasem,N.; Hill,K.D.; Sewell,G.W. High-performance liquid chromatographic column-switching technique for the determination of intermediates of anaerobic degradation of toluene in ground water microcosm, *J.Chromatogr.*, **1991**, *587*, 185–191.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6

Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.85 (A), 5.29 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.2 μm) water, remove a 100 μL aliquot and add it to 500 μL 2.59 μM 2-(9-anthryl)ethyl chloroformate in MeCN and 200 μL 25 mM pH 9.6 borate buffer, heat at 43° for 35 min, inject a 10 μL aliquot. (Prepare 2-(9-anthryl)ethyl chloroformate as follows. Stir a solution of 3 g of 9-bromoanthracene in 100 mL ether at 0° under argon or nitrogen, add 9 mL 1.6 M n-butyllithium over 5 min, stir for 30 min, add an ice-cold solution of 3 g ethylene oxide (Caution! Ethylene oxide is a carcinogen!) in 16 mL ether, stir for 1 h, add 70 mL water, add 50 mL ether, remove the organic layer, extract the aqueous layer with 100 mL dichloromethane. Combine the organic layers and wash them with water, dry over anhydrous sodium sulfate, evaporate to dryness, chromatograph on silica gel with dichloromethane to give 2-(9-anthryl)ethanol as pale yellow crystals (mp 106-8°) (J.Org.Chem. 1986, 51, 2956). Stir a solution of 2-(9-anthryl)ethanol in ether in the presence of pyridine (as an HCl scavenger) at 0°, add a solution of phosgene in toluene. 2-(9-anthryl)ethyl chloroformate is obtained as colorless crystals (mp 86-87° from pentane). Protect stock solutions from light and store them in the refrigerator (Anal.Chem. 1991, 63, 292).)

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18

Mobile phase: Gradient. MeCN:water from 70:30 to 100:0 over 10 min, maintain at 100:0 for 10 min
Flow rate: 0.75
Injection volume: 10
Detector: F ex 256 em 418 (cut-off filter)

CHROMATOGRAM
Retention time: 8.57
Limit of detection: 7 nM

OTHER SUBSTANCES
Simultaneous: 4-tert-butylphenol, 3,4-dimethylphenol, 4-methylphenol

KEY WORDS
derivatization; wastewater

REFERENCE
Landzettel,W.J.; Hargis,K.J.; Caboot,J.B.; Adkins,K.L.; Strein,T.G.; Veening,H.; Becker,H.-D. High-performance liquid chromatographic separation and detection of phenols using 2-(9-anthrylethyl) chloroformate as a fluorophoric derivatizing agent, *J.Chromatogr.A*, **1995**, *718*, 45–51.

SAMPLE
Matrix: solutions
Sample preparation: Wash column A with MeOH at 2 mL/min for 1 min, wash column A with 5 mM tetrabutylammonium bromide at 2 mL/min for 1 min, pump sample through column A at 2 mL/min for 2.5 min and elute to waste, backflush the contents of column A on to column B with MeOH for 1 min, remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 2 15-25 μm PLRP-S (Spark Holland); B 250 × 4 ODS-2
Mobile phase: Gradient. MeOH:1% acetic acid from 25:75 to 60:40 over 25 min, to 100:0 over 5 min, maintain at 100:0 for 2 min, return to initial conditions over 2 min.
Column temperature: 65
Flow rate: 1
Injection volume: 5000
Detector: UV 280

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.1-2 ng/mL

OTHER SUBSTANCES
Simultaneous: 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2,6-dimethylphenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol (UV 302), 2,4,6-trichlorophenol, 2,4,6-trimethylphenol

KEY WORDS
waste water; river water; column-switching

REFERENCE
Pocurull,E.; Marcé,R.M.; Borrull,F. Improvement of on-line solid-phase extraction for determining phenolic compounds in water, *Chromatographia*, **1995**, *41*, 521–526.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18

Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.79

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.15

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: Add 1 mL 12 M HCl to 5 mL urine. Heat at 100° for 30 min. Centrifuge at 2500 rpm for 5 min and dilute 40 fold with water. Extract a 1 mL aliquot of this solution with 1 mL isoamyl alcohol saturated with 6 M HCl. Mix for 2 min. Remove a 500 μL aliquot of the organic layer and add it to 500 μL 500 mM NaOH, vortex for 2 min. Inject a 10 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 4.6 10 μm MicroPak RP18
Mobile phase: MeCN:10 mM HCl 20:80
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 11.93
Limit of detection: 50 ng/mL

REFERENCE
Menezes,M.L.; Demarchi,A.C.C.O. Off line extraction of phenol from human urine sample with isoamyl alcohol and determination by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2355–2363.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH to 5, hydrolyze with β-glucuronidase/arylsulfatase at 37° for 12 h, add p-chlorophenol at a final concentration of 50 μg/mL, adjust pH to 2 with HCl. 2 mL Sample + 4 mL dichloromethane, vortex, centrifuge at 2400 g for 15 min. Remove 2.5 mL of the organic layer and add it to 500 μL 200 mM NaOH, vortex. Remove 300 μL of the aqueous layer, adjust pH to 7.0 with HCl, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 33 × 4.6 3 μm Pecosphere 3 × 3 C18
Mobile phase: MeOH:water:orthophosphoric acid 30:70:0.1
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 2.02
Internal standard: p-chlorophenol (8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: p-nitrophenol, cresols, p-methoxyphenol

REFERENCE
Brega,A.; Prandini,P.; Amaglio,C.; Pafumi,E. Determination of phenol, m-, o- and p-cresol, p-aminophenol and p-nitrophenol in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *535*, 311–316.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.22 μm) urine. Dilute a 100 μL aliquot with 900 μL mobile phase, inject a 200 μL aliquot. Hydrolyze conjugates as follows. 1 mL Urine + 1 mL 200 mM pH 4.8 acetate buffer + 200 μL Helix pomatia juice (containing 100000 Fishman Units of β-glucuronidase and 1000000 Roy Units of sulfatase, IBF, France), heat at 37° overnight, dilute 10-fold with mobile phase, centrifuge at 10000 g for 5 min, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4.6 10 μm ODS 2
Column: 250 × 4.6 5 μm Sup-Rs Classic ODS 2 (Prolabo)
Mobile phase: MeCN:1% phosphoric acid 10:90
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 20.1
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: hippuric acid, mandelic acid, 3-methylhippuric acid, phenylglyoxylic acid

REFERENCE
Astier,A. Simultaneous high-performance liquid chromatographic determination of urinary metabolites of benzene, nitrobenzene, toluene, xylene and styrene, *J.Chromatogr.*, **1992**, *573*, 318–322.

SAMPLE
Matrix: urine
Sample preparation: Condition a 500 mg Bond Elut SAX SPE cartridge with 3 mL MeOH and 3 mL water. Dilute 125 μL urine to 4 mL with water, adjust to pH 4.5 with ascorbic acid, add 12.5 μL enzyme solution, heat at 37° for 48 h, add to the SPE cartridge, wash with 3 mL 5 mM pH 7 phosphate buffer. Acidify the eluate to pH <3 with concentrated HCl, add 5 mL ether, vortex, repeat the extraction twice. Combine the organic layers and evaporate them to dryness under reduced pressure at 30°, reconstitute the residue in 1 mL 1% aqueous phosphoric acid, inject a 20 μL aliquot. (The enzyme solutions used to deconjugate glucuronides and sulfate esters were β-glucuronidase/arylsulfatase (Merck, 4114), arylsulfatase (Sigma, S 1629), and β-glucuronidase diluted 1:6 with water (Boehringer, 127051).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeOH:5 mM pH 3.4 phosphate buffer 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 9.3
Limit of detection: 36 μg/mL

OTHER SUBSTANCES
Extracted: catechol, hydroquinone

KEY WORDS
mouse; SPE

REFERENCE
Schad,H.; Schäfer,F.; Weber,L.; Seidel,H.J. Determination of benzene metabolites in urine of mice by solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *593*, 147–151.

SAMPLE
Matrix: urine
Sample preparation: Freeze urine, thaw, centrifuge at 6000 g for 10 min, filter (0.45 μm PVDF), dilute with 3 volumes of 40 mM pH 6.8, inject an aliquot onto column A with mobile phase A (phenol conjugates are hydrolyzed to phenol on this column) and elute onto column B, after 10 min remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 4.6 immobilized enzyme reactor (Prepare as follows. Stir 1 g Supelclean LC-NH2 (Supelco) in 25 mL 1% glutaraldehyde for 1 h, rinse solid with water and 40 mM pH 6.8 phosphate buffer, stir solid in 25 mL 1 mg/mL β-glucuronidase/sulfatase (type H-2 from Helix pomatia, Sigma) in 40 mM pH 6.8 buffer at 4° for 24 h, wash the solid with water, wash with 100 mM NaCl. Make a slurry of 0.5 g of the immobilized enzyme in 40 mM pH 6.8 buffer and pack in a 50 × 4.6 column using high pressure nitrogen, rinse with 40 mM pH 6.8 phosphate buffer. When not in use store in 100 mM NaCl at 4°.); B 150 × 4.6 5 μm ODS (J & W)
Mobile phase: A MeOH:40 mM pH 6.8 phosphate buffer 30:70; B MeOH:40 mM pH 6.8 phosphate buffer 30:70
Flow rate: 1
Injection volume: 1000
Detector: F ex 270 em 300

CHROMATOGRAM
Retention time: 20
Limit of detection: 10 ppb
Limit of quantitation: 50 ppb

KEY WORDS
column-switching; immobilized enzyme reactor

REFERENCE
Jen,J.-F.; Tsai,M.-Y. Determination of phenol in urine by high-performance liquid chromatography with on-line precolumn enzymatic hydrolysis of the conjugates, *J.Chromatogr.B*, **1994**, *658*, 87–92.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.2 μm), inject an aliquot directly. Hydrolyze conjugates by heating with 6 M HCl at 37° for 18 h, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:1.5% trifluoroacetic acid on water 10:90
Flow rate: 0.5
Detector: UV or radioactivity

CHROMATOGRAM
Retention time: 5.9

OTHER SUBSTANCES
Extracted: metabolites, hydroquinone, phenyl glucuronide, phenyl sulfate

KEY WORDS
rat

REFERENCE
Hughes,M.F.; Hall,L.L. Disposition of phenol in rat after oral, dermal, intravenous, and intratracheal administration, *Xenobiotica*, **1995**, *25*, 873–883.

SAMPLE
Matrix: water
Sample preparation: Add disodium EDTA to sample. 100 mL Water + 10 mL pH 8-9 Britton-Robinson buffer (μ = 0.09) + 1 mL 1.5% 4-aminoantipyrine in water + 5 mL 2% potassium ferricyanide in water + 10 mL chloroform, stir for 10 min, inject a 10 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 480

CHROMATOGRAM
Retention time: k' 1.36
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: 4-chloro-3-methylphenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 2,3-dichlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,3-dimethylphenol, 2,5-dimethylphenol, 2,6-dimethylphenol, 3,5-dimethylphenol, 2-ethylphenol, 3-ethylphenol, 2-methylphenol, 3-methylphenol, 2-nitrophenol, 3-nitrophenol, 2,3,5,6-tetramethylphenol, 2,4,6-trichlorophenol, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol

KEY WORDS
derivatization

REFERENCE
Blo,G.; Dondi,F.; Betti,A.; Bighi,C. Determination of phenols in water samples as 4-aminoantipyrine derivatives by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *257*, 69–79.

SAMPLE
Matrix: water
Sample preparation: 100 mL Water + 2 mL 50 mM KCl in water + 1 mL 0.6% dextrin in water + 1 mL 80 mM silver nitrate in water + 2 mL pH 9 borax buffer + 500 μL 2% 4-aminoantipyrine in water + 10 mL chloroform, stir at 40° for 40 min, inject an aliquot of the organic layer.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 0.5 ppm

KEY WORDS
derivatization

REFERENCE
Blo,G.; Dondi,F.; Bighi,C. High-performance liquid chromatographic determination of phenols as 4-aminoantipyrine derivatives; silver chloride as oxidizing agent in the derivatization reaction, *J.Chromatogr.*, **1984**, *295*, 231–235.

SAMPLE
Matrix: water
Sample preparation: Condition a Baker amino SPE cartridge with dichloromethane, dry with nitrogen. Adjust pH to 12 with 1 M NaOH. Remove a 500 μL aliquot and add it to 100 μL 30 mg/mL pH 12 tetrabutylammonium bromide in water, add 500 μL pH 12 water, add 600 μL 100 μg/mL dansyl chloride in dichloromethane, vortex vigorously for 2 min, add a 500 μL aliquot of the dichloromethane layer to the amino SPE cartridge, let stand for 10 min, elute with 3 mL dichloromethane. Evaporate the eluate to dryness, reconstitute the residue in 500 μL MeOH:water 50:50, inject a 100 μL aliquot. (Excess dansyl chloride reacts with the amino groups in the SPE cartridge.)

HPLC VARIABLES
Column: 200 × 3.1 3 μm LiChrosorb RP-18
Mobile phase: Gradient. A was MeOH:100 mM pH 7.0 imidazole buffer 97.5:2.5. B was MeOH: 2.5 mM pH 7.0 imidazole buffer 2.5:97.5. A:B 75:25 for 9.5 min, to 85:15 over 0.5 min, maintain at 85:15 for 4.5 min, to 95:5 over 0.5 min, maintain at 95:5 for 4.5 min, to 100:0 over 0.5 min, maintain at 100:0 for 20 min, return to initial conditions over 1 min, re-equilibrate for 20 min.
Flow rate: 0.5
Injection volume: 100
Detector: Chemiluminescence (470 nm cut-off filter) following post-column reaction. The column effluent flowed through a 130 × 0.3 mm ID PTFE coil irradiated with a fan-cooled 90 w Philips Model 93110E mercury lamp. The effluent from this coil mixed with 50 mM hydrogen peroxide in MeCN containing 5 mM bis(2-nitrophenyl) oxalate pumped at 0.3 mL/min and this mixture flowed to the detector.

CHROMATOGRAM
Retention time: 14
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Simultaneous: 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, 2,4,6-trichlorophenol

KEY WORDS
derivatization; post-column reaction; SPE; post-column photochemical derivatization

REFERENCE
Kwakman,P.J.M.; Kamminga,D.A.; Brinkman,U.A.T.; de Jong,G.J. Sensitive liquid chromatographic determination of alkyl-, nitro- and chlorophenols by precolumn derivatization with dansyl chloride, postcolumn photolysis and peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1991**, *553*, 345–356.

SAMPLE
Matrix: water
Sample preparation: Prepare an SPE cartridge by adding 500 mg 120-400 mesh Carbograph 4 graphitized carbon black (210 m^2/g, Carbochimica Romana, Rome) to a 65 × 13 polypropylene tube using polyethylene frits. Condition with 10 mL 10 mM tetrabutylammonium chloride in dichloromethane:MeOH 80:20, 2 mL MeOH, and 14 mL water acidified to pH 2 with HCl. Filter (Whatman GF/C 1.5 μm glass fiber) river water, pass 4 L through the SPE cartridge at 100 mL/min, wash with 7 mL water at 5-7 mL/min, pull air through the SPE cartridge for 1 min, wash with 800 μL MeOH, dry under vacuum for 1 min, elute in a reverse fashion with 6 mL 10 mM tetrabutylammonium chloride in dichloromethane:MeOH 80:20 at 6 mL/min. Remove a 3 mL aliquot of the eluate and evaporate it to dryness under a stream of nitrogen at 27°, reconstitute the residue in 150 μL 100 mM sodium carbonate in MeCN:water 20:80, add 40 μL acetic anhydride, heat at 50° for 6 min, inject a 50 μL aliquot. (Phenols can also be determined without derivatization. Derivatization provides confirmation of peak identity.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltima LC-18 (Alltech)
Mobile phase: Gradient. A was 0.025% trifluoroacetic acid in water. B was 0.0125% trifluoroacetic acid in MeCN. A:B from 78:22 to 10:90 over 27 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280 for 18 min then UV 220

CHROMATOGRAM
Retention time: 12.4
Limit of detection: <50 ng/mL

OTHER SUBSTANCES
Simultaneous: 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 4,6-dinitro-2-methylphenol, 2,4-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, 2,4,6-trichlorophenol

KEY WORDS
derivatization; SPE; river water

REFERENCE
Di Corcia,A.; Bellioni,A.; Madbouly,M.D.; Marchese,S. Trace determination of phenols in natural waters. Extraction by a new graphitized carbon black cartridge followed by liquid chromatography and re-analysis after phenol derivatization, *J.Chromatogr.A*, **1996**, *733*, 383–393.

SAMPLE
Matrix: water
Sample preparation: Condition a 500 mg ENVI Chrom P highly cross-linked styrene-divinylbenzene SPE cartridge (Supelco) with 10 mL MeOH, 10 mL water, and 2 mL 5 mM tetrabutylammonium bromide, dry. Filter (0.45 μm) sample, add 3 mL 10% sodium sulfite solution to each 1 L of water, adjust pH to 9 with 1 M NaOH, add tetrabutylammonium bromide to a final concentration of 5 mM, pass a 500 mL aliquot through the SPE cartridge, elute with 5 mL MeOH, acidify with 1% acetic acid, evaporate to 1 mL under reduced pressure, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS-2
Mobile phase: Gradient. MeOH:1% pH 2.8 acetic acid from 25:75 to 40:60 over 25 min, to 100:0 over 5 min, maintain at 200:0 for 2 min, return to initial conditions over 2 min
Column temperature: 65
Flow rate: 1
Injection volume: 20
Detector: UV 280

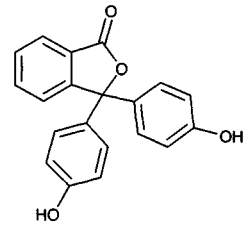
CHROMATOGRAM
Retention time: 6
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Simultaneous: other phenolic compounds

KEY WORDS
river water; SPE

REFERENCE
Pocurull,E.; Calull,M.; Marcé,R.M.; Borrull,F. Determination of phenolic compounds at low μg l^{-1} levels by various solid-phase extractions followed by liquid chromatography and diode-array detection, *J.Chromatogr.A*, **1996**, *719*, 105–112.

Phenolphthalein



Molecular formula: $C_{20}H_{14}O_4$
Molecular weight: 318.33
CAS Registry No.: 77-09-8
Merck Index: 7392

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of water, do not allow to go dry. Serum. 1 mL Serum + 25 μL 400 μg/mL bromocresol purple in water + 3 mL acidified acetone, vortex for 5 min, centrifuge at 1200 g for 10 min. Remove 3 mL of the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 100 mM pH 7.4 sodium phosphate buffer containing 0.9% NaCl, sonicate for 10 min, add to the SPE cartridge, wash with 2 column volumes of water, allow to dry. Elute with two 800 μL aliquots of MeOH, evaporate the eluate to dryness under reduced pressure, reconstitute in 1 mL MeCN:50 mM NaH_2PO_4 10:90, centrifuge at 1200 g for 10 min, inject a 100 μL aliquot. Urine. 100 μL Urine + 25 μL 400 μg/mL bromocresol purple in water + 875 μL 100 mM pH 7.4 sodium phosphate buffer containing 0.9% NaCl, mix, add 3 mL acetone:water 88:12, vortex for 5 min, centrifuge at 1200 g for 10 min. Remove 3 mL of the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 100 mM pH 7.4 sodium phosphate buffer containing 0.9% NaCl, sonicate for 10 min, add to the SPE cartridge, wash with 2 column volumes of water, allow to dry. Elute with two 800 μL aliquots of MeOH, evaporate the eluate to dryness under reduced pressure, reconstitute in 1 mL MeCN:50 mM NaH_2PO_4 10:90, centrifuge at 1200 g for 10 min, inject a 100 μL aliquot. Bile. 50 μL Bile + 950 μL MeCN:50 mM NaH_2PO_4 10:90, centrifuge at 1200 g for 10 min, inject a 50 μL aliquot of the supernatant. (Acidified acetone was 0.5 mL glacial acetic acid in 880 mL acetone, made up to 1 L with water.)

HPLC VARIABLES
Guard column: 5.3 × 4 10 μm Bondapak C18 guard-pak
Column: 150 × 4.6 5 μm LC-18DB (Supelco)

Mobile phase: Gradient. A was MeCN:50 mM NaH_2PO_4 10:90. B was MeCN:50 mM NaH_2PO_4. A:B 65:35 for 5 min, to 40:60 over 0.1 min, maintain at 40:60 for 8 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 11.4
Internal standard: bromocresol purple (9.5)
Limit of detection: 10 μg/mL (bile), 500 ng/mL (urine), 100 ng/mL (serum)

OTHER SUBSTANCES
Extracted: metabolites, glucuronides

KEY WORDS
serum; dog; SPE; pharmacokinetics

REFERENCE
Wilhelm,J.A.; Bailey,L.C.; Shepard,T.A.; Venturella,V.S. Simultaneous determination of phenolphthalein and phenolphthalein glucuronide from dog serum, urine and bile by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *578*, 231–238.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize tissue with 2 volumes of water. 1 mL Homogenate + 300 μL MeOH + 1 mL buffer + 10 mL dichloromethane, rotate, centrifuge. Remove the organic layer and add it to 3 mL 100 mM NaOH, rotate, centrifuge. Remove the aqueous layer and acidify it with 1 mL 1 M HCl, extract with 10 mL dichloromethane. Remove the organic layer and evaporate it to dryness at 40°, reconstitute the residue in 300 μL MeOH, inject a 20 μL aliquot. Blood. 500 μL Whole blood + 500 μL buffer + 200 μL MeOH + 5 mL dichloromethane, rotate, centrifuge. Remove the organic layer and evaporate it to dryness at 40°, reconstitute the residue in 300 μL MeOH, inject a 20 μL aliquot. (Buffer was prepared by mixing 500 mM Na_2HPO_4 with 500 mM KH_2PO_4 to pH 5.5.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm RP-8 (Hewlett-Packard) or 260 × 4.6 10 μm Spherisorb C18
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 2.96
Internal standard: phenolphthalein

OTHER SUBSTANCES
Extracted: thiopental
Simultaneous: carbamazepine, pentobarbital (UV 210)
Noninterfering: amobarbital, butabarbital, glutethimide, meprobamate, methaqualone, methyprylon, phenobarbital, phenytoin, secobarbital

KEY WORDS
phenolphthalein is IS; whole blood

REFERENCE
Levine,B.; Blanke,R.; Valentour,J. Liquid chromatographic analysis of thiopental in blood and tissues, *J.Anal.Toxicol.*, **1983**, *7*, 207–208.

SAMPLE
Matrix: formulations

Sample preparation: Weigh out formulation containing 500 mg phenolphthalein, make up to 100 mL with MeOH, sonicate for 5 min, filter (0.45 μm). Dilute 5 mL of the filtrate to 100 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 7 μm Nucleosil C18
Mobile phase: MeOH:water:acetic acid 50:50:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3.9
Limit of detection: 4 μg/mL

OTHER SUBSTANCES
Simultaneous: aloin

KEY WORDS
granules; tablets; dragees; emulsions; comparison with spectrophotometric method

REFERENCE
Torrado,S.; Fraile,S.; Torrado,J.J.; Selles,V.E. Comparison of reversed-phase liquid chromatography with colorimetry for analysis of phenolphthalein preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1167–1172.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher CH-18
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 75:25 containing 2.5 mM cethexonium bromide (Rinse with 100 mL MeOH:EtOH:water 50:25:25 at the end of the day.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.3

OTHER SUBSTANCES
Extracted: glucuronides

REFERENCE
Liu,H.-F.; Leroy,P.; Nicolas,A.; Magdalou,J.; Siest,G. Evaluation of a versatile reversed-phase high-performance liquid chromatographic system using cethexonium bromide as ion-pairing reagent for the analysis of glucuronic acid conjugates, *J.Chromatogr.*, **1989**, *493*, 137–147.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS (Altex)
Mobile phase: MeOH:water 5:1
Flow rate: 2
Injection volume: 20
Detector: UV 290

OTHER SUBSTANCES
Simultaneous: thiamylal

REFERENCE
Costantino,A.G.; Caplan,Y.H.; Levine,B.S.; Dixon,A.M.; Smialek,J.E. Thiamylal: review of the literature and report of a suicide, *J.Forensic Sci.*, **1990**, *35*, 89–96.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 6.05 (A), 5.66 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

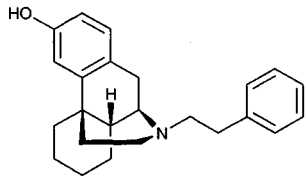
CHROMATOGRAM
Retention time: k' 0.96

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

Phenomorphan



Molecular formula: $C_{24}H_{29}NO$
Molecular weight: 347.50
CAS Registry No.: 468-07-5
Merck Index: 7399
Lednicer No.: 1 294

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.3

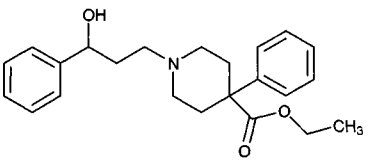
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, er-

gosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Phenoperidine



Molecular formula: $C_{23}H_{29}NO_3$
Molecular weight: 367.49
CAS Registry No.: 562-26-5, 3627-49-4 (HCl)
Merck Index: 7400
Lednicer No.: 1 302

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.7

OTHER SUBSTANCES

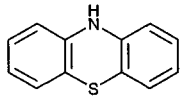
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine,

chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Phenothiazine



Molecular formula: $C_{12}H_9NS$
Molecular weight: 199.28
CAS Registry No.: 92-84-2
Merck Index: 7404

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 14.122, 14.265

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Evaporate reaction mixture (if necessary, chromatograph residue on deactivated alumina with MeOH), dissolve residue in MeOH with IS, inject an aliquot.

HPLC VARIABLES
Column: 150 × 5 Spherisorb A5Y alumina
Mobile phase: Hexane:ethyl acetate containing 0.6% water 95:5
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.32
Internal standard: 4-methoxy-2-nitroaniline (k' 8.60)

KEY WORDS
normal phase

REFERENCE
Lunn,G. *Nitrogen-containing Reactive Intermediates in Heterocyclic Synthesis*, Ph.D. Thesis, University of Edinburgh, **1975**.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.0

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquin-

amide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 1.56

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Mix 100 µL of a 0.05-1 µg/mL solution in MeOH with 20 µL 5% sodium carbonate and about 35 mg of a suspension of W-2 Raney nickel in EtOH (80 µL), stir at 70° for 15 min, cool in ice-water, add 50 µL 400 ng/mL 1-naphthol in MeOH, mix well, filter (0.45 µm), inject a 5 µL aliquot of the filtrate. (Raney nickel can be purchased from Aldrich or

prepared from aluminum-nickel alloy (Org. Syn. 1955, Coll. Vol. 3, 181). Phenothiazine is desulfurized to diphenylamine.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:water 75:25 containing 50 mM sodium perchlorate and 5 mL/L glacial acetic acid
Column temperature: 35
Flow rate: 0.6
Injection volume: 5
Detector: E, Showa Denko Shodex EC-1, glassy carbon working electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 12.5
Internal standard: 1-naphthol (8.5)
Limit of detection: 10 pg

KEY WORDS
derivatization; desulfurization

REFERENCE
Shimada,K.; Mino,T.; Nakajima,M.; Wakabayashi,H.; Yamato,S. Application of the desulfurization of phenothiazines for a sensitive detection method by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *661*, 85–91.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 1 g tissue + 5 mL MeOH, centrifuge at 1600 g for 5 min. Remove 1 mL of the supernatant and evaporate it to dryness, reconstitute the residue in 1 mL cyclohexane, centrifuge, inject a 50 μL aliquot of the supernatant.

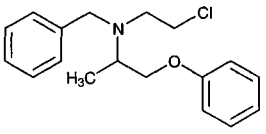
HPLC VARIABLES
Guard column: 30 × 2.8 30 μm pellicular beads
Column: 250 × 4.6 Partisil-10
Mobile phase: Cyclohexane:n-propanol 99.7:0.3
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Limit of detection: 50 ppb

KEY WORDS
sheep; kidney; muscle; liver; fat

REFERENCE
Blackman,G.L.; Ho,A.C.; Jozsa,A.; Kelly,J.D. High performance liquid chromatographic determination of phenothiazine residues in sheep tissues, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 988–991.

Phenoxybenzamine



Molecular formula: $C_{18}H_{22}ClNO$
Molecular weight: 303.83
CAS Registry No.: 59-96-1, 63-92-3 (HCl)
Merck Index: 7409
Lednicer No.: 1 55

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize tissue with 3 parts of 50 mM pH 7.4 phosphate buffer, sonicate adipose tissue homogenates for 30 s. 1 mL Plasma or tissue homogenate + 500 μL 500 mM sodium carbonate + 6 mL ethyl acetate, extract for 1 h, centrifuge. Remove the organic layer and dry it by mixing with anhydrous sodium sulfate for 20 s, centrifuge, evaporate 5 mL to dryness under a stream of nitrogen at 50°, reconstitute the residue in 500 μL n-heptane, add 200 μL MeOH:1 M HCl 90:10, mix for 4 min, centrifuge, discard the heptane layer, inject a 10 μL aliquot of the MeOH/HCl layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2
Mobile phase: MeCN:MeOH:10 mM pH 8.0 phosphate buffer 20:66:14
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM

Limit of detection: 10 ng

KEY WORDS

rat; plasma; liver; kidney; lung; brain; heart; muscle; adipose tissue

REFERENCE

Moor,M.J.; Bickel,M.H. Tissue distribution of phenoxybenzamine in the rat. Lack of adipose tissue storage, *Life Sci.*, **1987**, *41*, 2041–2046.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextro-

propoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 1.78

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

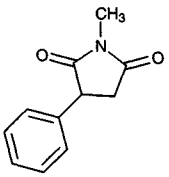
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 0.70 (of first (-) enantiomer)

KEY WORDS
chiral; α 1.13

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

Phensuximide



Molecular formula: $C_{11}H_{11}NO_2$
Molecular weight: 189.21
CAS Registry No.: 86-34-0
Merck Index: 7414
Lednicer No.: 1 226

SAMPLE
Matrix: solutions
Sample preparation: Inject a 6-10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 4.22
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetanilide, N-acetylcysteine, N-acetylprocainamide, amobarbital, ampicillin, aspirin, barbital, butalbital, caffeine, carbamazepine, chloramphenicol, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, ethosuximide, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, mephobarbital, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, pentobarbital, phenacetin, phenobarbital, phenylbutazone, phenytoin, primidone, procainamide, salicylamide, salicylic acid, secobarbital, sulfamethoxazole, sulindac, theophylline, thiopental, tolmetin, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: butabarbital, trimethoprim

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 3 μm 208HS3410 (Vydac)
Mobile phase: Gradient. MeCN:water from 15:85 to 60:40 over 10 min.
Flow rate: 1.5
Detector: UV 210 (?)

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: barbital, carbamazepine, diazepam, ethotoin, mephenytoin, methsuximide, phenacemide, phenobarbital

REFERENCE
Vydac HPLC Catalog, 1994-5, **1994**, p. 26.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

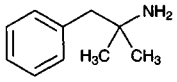
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyril-

amine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phentermine



Molecular formula: $C_{10}H_{15}N$
Molecular weight: 149.24
CAS Registry No.: 122-09-8, 1197-21-3 (HCl)
Merck Index: 7415
Lednicer No.: 1 72

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Simultaneous: amphetamine, ephedrine, methamphetamine, phenmetrazine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE

Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

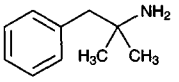
HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco

amine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phentermine



Molecular formula: $C_{10}H_{15}N$
Molecular weight: 149.24
CAS Registry No.: 122-09-8, 1197-21-3 (HCl)
Merck Index: 7415
Lednicer No.: 1 72

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Simultaneous: amphetamine, ephedrine, methamphetamine, phenmetrazine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE

Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco

Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 2.9
Internal standard: promazine (5.2)

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, amoxapine, amphetamine, buprion, chlordiazepoxide, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, disopyramide, doxepin, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, iprindole, maprotiline, meperidine, mianserin, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, procainamide, prochlorperazine, prolixin, promethazine, propoxyphene, protriptyline, pyrilamine, quinidine, thioridazine, trifluoperazine, trimeprazine, trimipramine, zimeldine
Noninterfering: thiopropazine
Interfering: amitriptyline, chlorimipramine, fluphenazine, loxepin, methadone, perphenazine, triflupromazine

KEY WORDS
normal phase

REFERENCE
Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.45

OTHER SUBSTANCES
Simultaneous: norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, fenfluramine, methylenedioxyamphetamine, amphetamine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 15:1.5:0.5:83
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Simultaneous: phenylpropanolamine, ephedrine, hydroxyamphetamine, amphetamine, methamphetamine, mephentermine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 9.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: β-hydroxyethyltheophylline

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM

Retention time: 7.5

OTHER SUBSTANCES

Simultaneous: diethylpropion, phenylpropanolamine, ephedrine, amphetamine, methamphetamine, fenfluramine
Also analyzed: amitriptyline, chlordiazepoxide, chlorpromazine, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, doxepin, flurazepam, imipramine, mesoridazine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE

Rainin Catalog, C1-94, **1994,** p. 7.24.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, meth-

yltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 2.4

Internal standard: chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam

Interfering: phenylpropanolamine, amphetamine, phenmetrazine, lidocaine, ephedrine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Adsorbex SCX cation-exchange SPE cartridge (Merck) with 2 mL MeOH, 1 mL water, and 1 mL 17 mM KH_2PO_4, do not allow to dry. Centrifuge urine at 2000 g for 5 min. 1 mL Urine + 500 μL 50 mM KH_2PO_4, sonicate for 1 min, add to the SPE cartridge, rinse vial with 50 μL 50 mM KH_2PO_4 and add to cartridge, dry cartridge for 1 min, wash with three 500 μL portions of 17 mM KH_2PO_4, wash with 1 mL MeOH, dry under vacuum for 1 min, elute with four 500 μL portions of MeOH:7.3% HCl (97.5:2.5) at a flow rate of 0.5 mL/min, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL (8.5 g) 85% orthophosphoric acid and 280 μL (0.22 g) hexylamine per liter. B was MeCN containing 100 mL water, 5 mL (8.5 g) 85% orthophosphoric acid, and 280 μL (0.22 g) hexylamine per liter. A:B 94.5:5.5 for 10.6 min, then to 61:39 over 11 min.
Column temperature: 40
Flow rate: 0.8
Injection volume: 10
Detector: UV 198

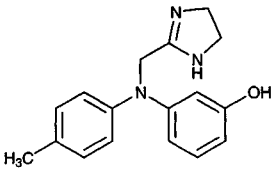
CHROMATOGRAM
Retention time: 10
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: 3,4-methylenedioxyamphetamine, amphetamine, 4-methoxyamphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, 5-methoxy-3,4-methylenedioxyamphetamine, 3,4,5-trimethoxyamphetamine, 3,4-methylenedioxyethylamphetamine, 2,5-dimethoxyamphetamine, 4-bromo-2,5-dimethoxyphenylethylamine, 2,5-dimethoxy-4-methylamphetamine, 4-bromo-2,5-dimethoxyamphetamine, 2,5-dimethoxy-4-ethylamphetamine, mescaline, methoxamine

KEY WORDS
SPE

REFERENCE
Helmlin,H.-J.; Brenneisen,R. Determination of psychotropic phenylalkylamine derivatives in biological matrices by high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1992**, *593*, 87–94.

Phentolamine



Molecular formula: $C_{17}H_{19}N_3O$
Molecular weight: 281.36
CAS Registry No.: 50-60-2, 73-05-2 (HCl), 65-28-1 (mesylate)
Merck Index: 7417
Lednicer No.: 1 242

SAMPLE

Matrix: blood, tissue
Sample preparation: Serum. 200-450 μL Serum + 20 μL 50-100 μg/mL yohimbine in water + 800 μL 1 M pH 9.2 Delory King carbonate buffer, vortex, add 5 mL diethyl ether, mix for 30 s, centrifuge at 1000 g for 2 min. Remove the organic phase and add it to 100 μL 100 mM HCl, mix for 30 s, centrifuge at 1000 g for 2 min, discard the ether, volatilize residual ether from the aqueous phase under a stream of nitrogen, inject a 10-70 μL of the aqueous phase. Liver. Homogenize 1 g of liver in 3 mL ice cold 1 M pH 9.2 Delory King carbonate buffer, add 2 g yohimbine, add 5 mL diethyl ether, mix for 30 s, centrifuge at 1000 g for 2 min. Remove the organic phase and add it to 100 μL 100 mM HCl, mix for 30 s, centrifuge at 1000 g for 2 min, discard the ether, volatilize residual ether from the aqueous phase under a stream of nitrogen, inject a 10-70 μL of the aqueous phase.

HPLC VARIABLES

Column: 100 × 3.2 3 μm Phase-2 ODS
Mobile phase: MeCN:15 mM pH 3.0 monochloroacetate buffer 25:75 containing 350 mg/L EDTA
Flow rate: 0.6
Injection volume: 10-70
Detector: E, Bioanalytical Systems LC-4B, LC-17 oxidative flow cell, TL-5 glassy carbon electrode + 900 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 4.75
Internal standard: yohimbine (3.0)
Limit of detection: 10 ng/mL (liver homogenate), 5 ng/mL (serum)

KEY WORDS

serum; liver; mouse; pharmacokinetics

REFERENCE

Kerger,B.D.; James,R.C.; Roberts,S.M. An assay for phentolamine using high performance liquid chromatography with electrochemical detection, *Anal.Biochem.*, **1988**, *170*, 145–151.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquin-

amide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 2.41

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 5 μm Nova Pak C18

Mobile phase: MeCN:50 mM pH 5.5 phosphate buffer 25:75
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.3
Internal standard: papaverine (8.9)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating; buffer

REFERENCE
Wang,D.-P.; Tu,Y.-H.; Allen,L.V.,Jr. Degradation kinetics of phentolamine hydrochloride in solution, *J.Pharm.Sci.*, **1988**, *77*, 972–976.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 21.88

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 9.05 (A), 4.79 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

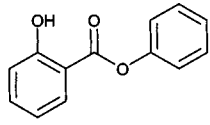
KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Phenyl salicylate



Molecular formula: $C_{13}H_{10}O_3$
Molecular weight: 214.22
CAS Registry No.: 118-55-8
Merck Index: 7464

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES

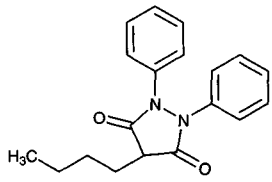
Guard column: 40 × 5 RP-18-MPLC (Brownlee)
Column: 250 × 2.6 ODS-HC-SIL-X (Perkin-Elmer)
Mobile phase: MeOH:50 mM phosphoric acid 70:30 (Flush column with MeOH at the end of each day.)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 256

CHROMATOGRAM
Retention time: 5.25

OTHER SUBSTANCES
Simultaneous: hydrocortisone, iodochlorhydroxyquin

REFERENCE
Ezzedeen,F.W.; Stohs,S.J.; Masoud,A.N. High-performance liquid chromatographic analysis of iodochlorhydroxyquin and hydrocortisone in ointments and creams, *J.Pharm.Sci.*, **1983**, *72*, 1036–1039.

Phenylbutazone



Molecular formula: $C_{19}H_{20}N_2O_2$
Molecular weight: 308.38
CAS Registry No.: 50-33-9
Merck Index: 7431
Lednicer No.: 1 236

SAMPLE
Matrix: blood
Sample preparation: Filter serum and inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: present but not defined
Column: 150 × 4.6 SPS-5PM-S5-100-C18 semipermeable surface column (Regis Chemical, IL) (A two phase column with an outer hydrophilic polyoxyethylene polymer bonded to the silica surface and an inner hydrophobic C18 phase, see J.Chromatogr. 1991, 544, 13-23.)
Mobile phase: MeCN:50 mM pH 7.5 phosphate buffer 15:85
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 11.3

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Haque,A.; Stewart,J.T. Direct injection HPLC method for the determination of phenylbutazone and oxyphenylbutazone in serum using a semipermeable surface column, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 287–293.

SAMPLE
Matrix: blood
Sample preparation: Filter serum and inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: present but not defined
Column: 150 × 4.6 GFF-S5-80 ISRP high efficiency column (A two phase column with an outer hydrophilic diol-glycine layer bonded to the silica surface and an inner hydrophobic diol-tripeptide phase, see J.Chromatogr. 1991, 544, 13-23.)
Mobile phase: MeCN:200 mM pH 7.0 phosphate buffer 2.5:97.5
Flow rate: 1

Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Haque,A.; Stewart,J.T. Direct injection HPLC method for the determination of phenylbutazone and oxyphenylbutazone in serum using a semipermeable surface column, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 287–293.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma or serum + 4 mL 250 ng/mL naproxen in MeCN, vortex for 30 s, centrifuge at 1000 g for 15 min. Remove 4 mL of the supernatant and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 mm long 30 μm pellicular ODS
Column: 250 mm long 5 μm Spherisorb ODS I
Mobile phase: MeCN:MeOH:1% pH 3.0 acetate buffer 30:20:50
Flow rate: 1.2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 14.2
Internal standard: naproxen (7.7)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: flunixin, oxyphenbutazone

KEY WORDS
plasma; serum; horse; pharmacokinetics; for dogs (see Am.J.Vet.Res. 1985; 46; 235)

REFERENCE
Hardee,G.E.; Lai,J.-W.; Moore,J.N. Simultaneous determination of flunixin, phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone in equine plasma by high-performance liquid chromatography: With application to pharmacokinetics, *J.Liq.Chromatogr.*, **1982**, *5*, 1991–2003.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 5 mL MeOH and 5 mL water. 500 μL Plasma + 50 μL 40 μg/mL indomethacin in MeOH, adjust to pH 3.4 with 345 mM citrate buffer, add to SPE cartridge, wash with water, dry, elute with 5 mL hexane:diethyl ether 50:50. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 6 × 4 4 μm Nova-Pack C18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:20 mM ammonium sulfate 55:45
Flow rate: 1.5

Injection volume: 25
Detector: UV 340

CHROMATOGRAM
Retention time: 10
Internal standard: indomethacin (7.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: oxyphenbutazone, suxibuzone

KEY WORDS
plasma; SPE

REFERENCE
Caturla,M.C.; Cusido,E. Solid-phase extraction for the high-performance liquid chromatographic determination of indomethacin, suxibuzone, phenylbutazone and oxyphenbutazone in plasma, avoiding degradation of compounds, *J.Chromatogr.*, **1992**, *581*, 101–107.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL MeCN, vortex for a few s, centrifuge at 2500 g for 5 min. Remove 100 μL of the supernatant and add it to 300 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-18
Mobile phase: EtOH containing 0.2% heptylamine:5 mM KH_2PO_4 30:70 (Place a 33 × 4.7 column of 37-53 μm pellicular ODS (Whatman) between pump and injector.)
Flow rate: 1.3
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 6
Internal standard: phenylbutazone

OTHER SUBSTANCES
Extracted: propyphenazone

KEY WORDS
phenylbutazone is IS; plasma

REFERENCE
Rouan,M.C.; Campestrini,J.; Lecaillon,J.B.; Godbillon,J. Rapid determination of propyphenazone in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *577*, 387–390.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1.5 mL MeOH, vortex, centrifuge for 15 min at 3000 g. Remove the supernatant and evaporate it to 500 μL using a vortex evaporator, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 10 μm RP-8 (Alltech)
Mobile phase: MeOH:1% acetic acid 70:30
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Internal standard: phenylbutazone

OTHER SUBSTANCES
Extracted: naproxen, napdice

KEY WORDS
dog; plasma; phenylbutazone is IS

REFERENCE
Samara,E.; Avnir,D.; Ladkani,D.; Bialer,M. Pharmacokinetic analysis of diethylcarbonate prodrugs of ibuprofen and naproxen, *Biopharm.Drug Dispos.*, **1995**, *16*, 201–210.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg 6 mL Isolute C18 non-endcapped SPE cartridge (International Sorbent Technology) with 2 mL MeOH and 2 mL water. 2 mL Plasma + 100 μL 100 μg/mL fenclofenac in MeOH + 1 mL 100 mM pH 7.2 phosphate buffer + 3 mL water, mix, add to the SPE cartridge, wash with 1 mL 100 mM pH 7.2 phosphate buffer, wash with 2 mL hexane, dry under vacuum for 2 min, elute with hexane:ethyl acetate 50:50. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH and 150 μL 100 mM pH 7.2 phosphate buffer, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 60:40 (Buffer was 100 mM acetic acid containing 0.01% heptanesulfonic acid.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 5
Internal standard: fenclofenac (7.5)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Extracted: oxyphenbutazone

KEY WORDS
horse; plasma; SPE

REFERENCE
Taylor,M.R.; Westwood,S.A. Quantitation of phenylbutazone and oxyphenbutazone in equine plasma by high-performance liquid chromatography with solid-phase extraction, *J.Chromatogr.A*, **1995**, *697*, 389–396.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 239

CHROMATOGRAM
Retention time: 6.51
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 50 μL 500 ng/mL mefenamic acid or indomethacin + 1 mL 100 mM HCl + 10 mL dichloromethane, rotate for 10 min, centrifuge at 1500 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°. Redissolve the residue in mobile phase, inject a 20 μL aliquot. Urine. 50 μL Urine + 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Supelcosil LC-8
Mobile phase: MeCN:50 mM phosphoric acid 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 4.0

Internal standard: mefenamic acid (8) or indomethacin (5)
Limit of detection: 50-250 ng/mL

OTHER SUBSTANCES
Simultaneous: naproxen, flunixin, thiosalicylic acid, ethacrynic acid

KEY WORDS
plasma

REFERENCE
Singh,A.K.; Jang,Y.; Mishra,U.; Granley,K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicyclic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *568*, 351–361.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 24.098

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: perfusate
Sample preparation: Inject an aliquot of perfusate. (Perfusion fluid contained 104 mM NaCl, 25 mM sodium bicarbonate, 2.3 mM sodium biphosphate, 10 mM sodium acetate, 1.2 mM calcium chloride, 1 mM magnesium sulfate, 5 mM KCl, 5 mM dextrose, and 5 mM alanine.)

HPLC VARIABLES
Column: 300 × 2 10 μm μBondapak C18
Mobile phase: MeOH:water 52:48
Flow rate: 0.13
Injection volume: 0.2

Detector: F ex 295 em 376 following post-column reaction. The column effluent mixed with 4 M NaOH pumped at 0.0013 mL/min and the mixture flowed through a 130 μL PTFE coil at 64° to the detector.

CHROMATOGRAM
Internal standard: phenylbutazone

OTHER SUBSTANCES
Extracted: indomethacin

KEY WORDS
post-column reaction; microbore; phenylbutazone is IS

REFERENCE
De Zeeuw,D.; Leinfelder,J.L.; Brater,D.C. Highly sensitive measurement of indomethacin using a high performance liquid chromatographic technique combined with post column in-line hydrolysis, *J.Chromatogr.*, **1986**, *380*, 157–162.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 26.7

OTHER SUBSTANCES
Simultaneous: acetophenone, amphetamine, desipramine, ethylmorphine, imipramine, mefenamic acid, methamphetamine, morphine, salicylic acid

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KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in the mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm Lichrosorb RP 18
Mobile phase: MeOH:water 50:50 containing 1% acetic acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 15.57

OTHER SUBSTANCES
Simultaneous: kebuzone, oxyphenbutazone, sulfinpyrazone

REFERENCE
Nivaud-Guernet,E.; Guernet,M.; Ivanovic,D.; Medenica,M. Effect of eluent pH on the ionic and molecular forms of the non-steroidal anti-inflammatory agents in reversed-phase high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2343–2357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:water 45:55, pH adjusted to 3.5 with acetic acid
Detector: UV 280

OTHER SUBSTANCES
Also analyzed: clomethacin, diclofenac, indomethacin

REFERENCE
Guterres,S.S.; Fessi,H.; Barratt,G.; Puisieux,F.; Devissaguet,J.-P. Poly(D,L-lactide) nanocapsules containing non-steroidal anti-inflammatory drugs: Gastrointestinal tolerance following intravenous and oral administration, *Pharm.Res.*, **1995**, *12*, 1545–1547.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 10.15 (A), 12.11 (B)

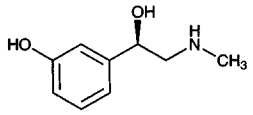
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline,

naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Phenylephrine



Molecular formula: $C_9H_{13}NO_2$
Molecular weight: 167.21
CAS Registry No.: 59-42-7, 61-76-7 (HCl)
Merck Index: 7440
Lednicer No.: 1 63

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut phenyl SPE cartridge with 2 mL MeOH, 3 mL water, and 3 mL buffer. Add 1-2 mL plasma to the SPE cartridge, let it sit on the column for 2 min, wash with 2 mL buffer, wash with 1 mL water, elute with three 200 μL aliquots of mobile phase, inject a 200 μL aliquot of the eluate. (Buffer was prepared by mixing 50 mL 50 mM sodium bicarbonate and 5 mL 100 mM NaOH, make up to 100 mL with water, adjust pH to 9.6 with 10 mM NaOH (if necessary).)

HPLC VARIABLES
Column: 300 mm long 10 μm μBondapak C18
Mobile phase: MeOH:1% acetic acid 10:90
Flow rate: 1
Injection volume: 200
Detector: F ex 270 em 305

CHROMATOGRAM
Retention time: 4.25
Limit of quantitation: 0.5 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Chien,D.-S.; Schoenwald,R.D. Fluorometric determination of phenylephrine hydrochloride by liquid chromatography in human plasma, *J.Pharm.Sci.*, **1985**, *74*, 562–564.

SAMPLE
Matrix: bulk

Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: k' 33.96, k' 40.07 (enantiomers)

OTHER SUBSTANCES
Simultaneous: epinephrine

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: formulations
Sample preparation: Capsules and Tablets. Leach 1 g of ground capsule or tablet with 250 mL 0.4 mg/mL 2,5-dihydroxybenzoic acid in water, sonicate for 10 min, centrifuge at 2500 rpm for 5 min, inject an aliquot. Liquid formulations. Dilute 4-25 mL of the formulation to 250 mL with 0.4 mg/mL 2,5-dihydroxybenzoic acid in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 10 C8
Mobile phase: MeOH:water:PIC-B5 300:675:25 (PIC-B5 (Waters) is 200 mM sodium pentanesulfonate in glacial acetic acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5
Internal standard: 2,5-dihydroxybenzoic acid (4.5)

OTHER SUBSTANCES
Simultaneous: phenylpropanolamine, guaifenesin, impurities, degradation products

KEY WORDS
capsules; tablets; liquid formulations; stability-indicating

REFERENCE
Schieffer,G.W.; Hughes,D.E. Simultaneous stability-indicating determination of phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and guaifenesin in dosage forms by reversed-phase paired-ion high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 55–59.

SAMPLE
Matrix: formulations

Sample preparation: Leach 1-1.3 g ground capsule or tablet with water and dilute to 250 mL, sonicate for 5 min, centrifuge at 2500 rpm for 5 min, inject an aliquot. Dilute 4-25 mL of liquid formulations to 250 mL with water, inject an aliquot.

HPLC VARIABLES
Column: Partisil-10 C8
Mobile phase: MeOH:MeCN:water:PIC-B5 50:170:755:25 (PIC-B5 (Waters) is 200 mM sodium pentanesulfonate in glacial acetic acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: benzoic acid, phenylpropanolamine, guaifenesin, impurities, degradation products

KEY WORDS
tablets; capsules; liquid formulations; stability-indicating

REFERENCE
Schieffer,G.W.; Smith,W.O.; Lubey,G.S.; Newby,D.G. Determination of the structure of a synthetic impurity in guaifenesin: modification of a high-performance liquid chromatographic method for phenylephrine hydrochloride, phenylpropanolamine hydrochloride, guaifenesin, and sodium benzoate in dosage forms, *J.Pharm.Sci.*, **1984**, *73*, 1856–1858.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 3 mL nasal spray to 50 mL with MeOH:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil PXS 10/25
Mobile phase: MeOH:10.6 mM phosphoric acid 30:70 containing 50 mg/L sodium octanesulfonate
Flow rate: 1.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: glutaric acid, benzyl alcohol (UV 254)

KEY WORDS
nasal sprays

REFERENCE
Wilson,T.D.; Forde,M.D.; Crain,A.V.R. Simultaneous liquid chromatographic determination of glutaric acid, phenylephrine, and benzyl alcohol in a prototype nasal spray with application to di- and tricarboxylic acids, *J.Pharm.Sci.*, **1985**, *74*, 312–315.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil-5 ODS-3

Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 19
Limit of detection: 40 ng

OTHER SUBSTANCES
Simultaneous: norepinephrine, epinephrine, levonordefrin, isoproterenol, metaraminol, impurities

KEY WORDS
tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE
Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE
Matrix: formulations
Sample preparation: Dilute syrup with mobile phase to a concentration of 5-100 μg/mL, shake, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 80 Å Ultrasphere CN
Mobile phase: MeCN:water:EtOH 60:38:2 containing 1 mM perchloric acid
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: Conductivity, zero suppression 2, range 1 or 10

CHROMATOGRAM
Retention time: 8.0

OTHER SUBSTANCES
Simultaneous: bromhexine, chlorpheniramine, codeine, dextromethorphan, diphenhydramine, ephedrine, papaverine

KEY WORDS
syrup; indirect conductometric detection; presence of compound causes a decrease in mobile phase conductivity

REFERENCE
Lau,O.-W.; Mok,C.-S. High-performance liquid chromatographic determination of active ingredients in cough-cold syrups with indirect conductometric detection, *J.Chromatogr.A*, **1995**, *693*, 45–54.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)

Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 7 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, dopamine, epinephrine, hydrochlorothiazide, isoproterenol, levodopa, methyldopa, norepinephrine

KEY WORDS
tablets; capsules; injections; ampules; sprays

REFERENCE
Villanueva Camañas,R.M.; Sanchis Mallols,J.M.; Torres Lapasió,J.R.; Ramis-Ramos,G. Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection, *Analyst*, **1995**, *120*, 1767–1772.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 700 mV

CHROMATOGRAM
Retention time: 7.60
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, hydralazine, isoproterenol, methoxamine, morphine, norepinephrine

KEY WORDS
rat; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W

Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.49

OTHER SUBSTANCES
Simultaneous: norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: trimethoxyamphetamine, pseudoephedrine, ephedrine, ethoheptazine, morphine-3-glucuronide, pholcodeine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 48

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol,

mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Acetic acid:triethylamine:water 1.5:0.5:98
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 0.38

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Make up a solution in 40 mM sodium formate and 62 mM formic acid buffer (pH 3.5), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 μm CN (Waters)
Column: 150 × 4.6 5 μm Ultrasphere CN
Mobile phase: MeOH:buffer 15:85 (Buffer was 40 mM sodium formate and 62 mM formic acid, pH 3.5.)
Flow rate: 1
Injection volume: 20
Detector: UV 258

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

Simultaneous: hydralazine, phthalazine

REFERENCE

Halasi,S.; Nairn,J.G. Quantitative determination of hydralazine hydrochloride and phthalazine in aqueous solutions by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1989**, *12*, 2397–2403.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Partisil ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM octanesulfonic acid in 0.2% acetic acid.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES

Simultaneous: epinephrine, isoproterenol, levonordefrin, metaraminol

REFERENCE

Phenomenex Catalog, **1994**, p. 1.077.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

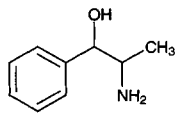
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone,

naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phenylpropanolamine



Molecular formula: $C_9H_{13}NO$
Molecular weight: 151.21
CAS Registry No.: 14838-15-4, 154-41-6 (HCl)
Merck Index: 7461

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM

Retention time: 5.015

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 40
Flow rate: 1
Detector: UV 210

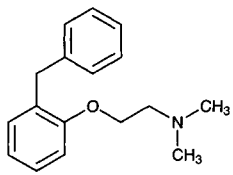
CHROMATOGRAM
Retention time: 2.95, 3.38

OTHER SUBSTANCES
Simultaneous: baclofen, levodopa, primaquine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

Phenyltoloxamine



Molecular formula: $C_{17}H_{21}NO$
Molecular weight: 255.36
CAS Registry No.: 92-12-6, 1176-08-5 (citrate)
Merck Index: 7469
Lednicer No.: 1 115

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL ammonia, extract twice with 7 mL pentane:diethyl ether 75:25. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 150 μL mobile phase, inject a 90 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb CN cyanopropyl
Mobile phase: MeCN:5 mM pH 6 phosphate buffer 40:60
Flow rate: 1
Injection volume: 90
Detector: E, Environmental Science Associates Coulochem model 5010, screen mode +0.55 V and +0.90 V

CHROMATOGRAM
Retention time: 19
Internal standard: phenyltoloxamine

OTHER SUBSTANCES
Extracted: carbinoxamine

KEY WORDS
plasma; phenyltoloxamine is IS

REFERENCE
Stockis,A.; Deroubaix,X.; Jeanbaptiste,B.; Lins,R.; Allemon,A.M.; Laufen,H. Relative bioavailability of carbinoxamine and phenylephrine from a retard capsule after single and repeated dose administration in healthy subjects, *Arzneimittelforschung*, **1995**, *45*, 1009–1012.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: thonzylamine, pheniramine, tripelennamine, chlorpheniramine, brompheniramine, phenindamine, clemizole

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 13.20 (A), 6.31 (B)

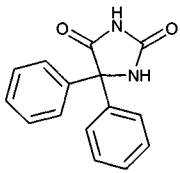
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, met-

ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Phenytoin



Molecular formula: $C_{15}H_{12}N_2O_2$
Molecular weight: 252.27
CAS Registry No.: 57-41-0, 630-93-3 (sodium salt)
Merck Index: 7475
Lednicer No.: 1 246

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 30 mg/L IS in water + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Zorbax Stable-Bond CN
Mobile phase: MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 10.5
Internal standard: cyheptamide (14)
Limit of detection: 20-35 ng/mL

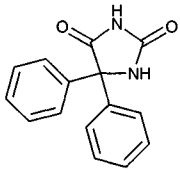
OTHER SUBSTANCES
Extracted: carbamazepine, carbamazepine diol, carbamazepine epoxide, lamotrigine, 5-(p-hydroxyphenyl)-5-phenylhydantoin

ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Phenytoin



Molecular formula: $C_{15}H_{12}N_2O_2$
Molecular weight: 252.27
CAS Registry No.: 57-41-0, 630-93-3 (sodium salt)
Merck Index: 7475
Lednicer No.: 1 246

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 30 mg/L IS in water + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Zorbax Stable-Bond CN
Mobile phase: MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 10.5
Internal standard: cyheptamide (14)
Limit of detection: 20-35 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, carbamazepine diol, carbamazepine epoxide, lamotrigine, 5-(p-hydroxyphenyl)-5-phenylhydantoin

Simultaneous: acetaminophen, N-acetylprocainamide, amikacin, caffeine, chlordiazepoxide, clonazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, digoxin, disopyramide, erythromycin, ethosuximide, felbamate, flurazepam, gabapentin, gentamicin, lidocaine, methotrexate, nitrazepam, oxazepam, phenylethylmalonamide, phenobarbital, primidone, quinidine, salicylate, temazepam, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
serum; SPE

REFERENCE
Lensmeyer,G.L.; Gidal,B.E.; Wiebe,D.A. Optimized high-performance liquid chromatographic method for determination of lamotrigine in serum with concomitant determination of phenytoin, carbamazepine, and carbamazepine epoxide, *Ther.Drug Monit.*, **1997**, *19*, 292–300.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, clonazepam, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, primidone

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood
Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 7.5
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: ethosuximide, primidone, phenobarbital, carbamazepine, valproic acid

KEY WORDS
human; plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: Dilute 20 μL serum with 100 μL pH 3.7 phosphate buffer, shake vigorously for 10 s, add to a 45 μL PTFE column packed with 50 μm ODS-silica (Asahi Chemicals, Tokyo) (Extrashot-ODS device), wash with 100 μL water, elute with 130 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:MeOH:pH 4.4 potassium phosphate buffer 14:21:65
Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 15.9

OTHER SUBSTANCES
Extracted: carbamazepine, phenobarbital

KEY WORDS
SPE

REFERENCE
Kouno,Y.; Ishikura,C.; Homma,M.; Oka,K. Extrashot-ODS, a syringe-type minicolumn sample injector for a reversed-phase high-performance liquid chromatographic column. Application to antiepileptics in human sera, *J.Chromatogr.B*, **1997**, *695*, 349–353.

SAMPLE
Matrix: blood, milk
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL water and MeOH: water 20:80. Add 80 μg/mL mephenytoin solution to 1 mL human breast milk or plasma, add 5 mL 0.5% pH 6.0 KH_2PO_4, mix briefly, add the sample to the SPE cartridge, elute with 5 mL MeOH, evaporate the eluate to dryness, dissolve the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Develosil C8-5 (Nomura Chemicals)
Mobile phase: MeCN:0.5% KH_2PO_4 buffer 30:70 (The pH of mobile phase was adjusted to 4.5 with 50% H_3PO_4.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Internal standard: mephenytoin (13)

KEY WORDS
cord blood plasma; human breast milk; maternal plasma; plasma; human; SPE

REFERENCE
Shimoyama,R.; Ohkubo,T.; Sugawara,K.; Ogasawara,T.; Ozaki,T.; Kagiya,A.; Saito,Y. Monitoring of phenytoin in human breast milk, maternal plasma and cord blood plasma by solid-phase extraction and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 863–869.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Inject a 5-20 μL aliquot onto the column with mobile phase A or B. Urine. Inject a 5 μL aliquot onto the column with mobile phase C.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 19:81 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer 14:76 for 5 min, to 25:75 over 1 min, to 30:70 over 2 min, to 50:50 over 3 min, maintain at 50:50 for 6 min (C)
Flow rate: 1
Injection volume: 5 (A, C), 20 (B)
Detector: UV 254 (serum); UV 230 (urine)

CHROMATOGRAM
Retention time: 2.88 (serum, A), 11.5 (serum, B), 13.5 (urine, C)
Limit of detection: 2 ng (urine)

OTHER SUBSTANCES
Simultaneous: acetaminophen (B), barbital (B), carbamazepine (B,C), phenobarbital (B,C), phenytoin (C), primidone (B), sulfapyridine (B)
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fritz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.288

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4 5 μm Phenyl Hypersil
Mobile phase: MeOH:buffer 35:65 (Buffer was 25 mM aqueous potassium phosphate monobasic solution adjusted to pH 3.8 with phosphoric acid.)
Column temperature: 50
Flow rate: 1.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: fosphenytoin

REFERENCE
Narisawa,S.; Stella,V.J. Increased shelf-life of fosphenytoin: solubilization of a degradant, phanytoin, through complexation with $(SBE)_{7m}$-β-CD, *J.Pharm.Sci.*, **1998**, *87*, 926–930.

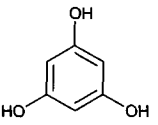
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spherisorb ODS-2
Mobile phase: MeCN:water 40:60
Flow rate: 0.8
Detector: UV 228

CHROMATOGRAM
Retention time: 8

REFERENCE
Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

Phloroglucinol



Molecular formula: $C_6H_6O_3$
Molecular weight: 126.11
CAS Registry No.: 108-73-6
Merck Index: 7482

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject

a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

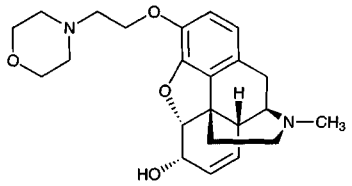
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 4.172

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pholcodine



Molecular formula: $C_{23}H_{30}N_2O_4$
Molecular weight: 398.50
CAS Registry No.: 509-67-1
Merck Index: 7484
Lednicer No.: 1 287

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

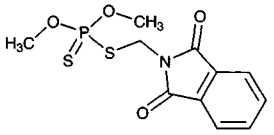
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 2.687

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Phosmet



Molecular formula: $C_{11}H_{12}NO_4PS_2$
Molecular weight: 317.33
CAS Registry No.: 732-11-6
Merck Index: 7492

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 150 mg phosmet in 5 mL THF, add 10 mL 1.3 mg/mL diphenylamine in THF, make up to 50 mL with n-hexane, add 1 g anhydrous sodium sulfate, mix well, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2 MicroPak CN-10
Mobile phase: n-Hexane:THF 8:92
Flow rate: 0.83
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 6
Internal standard: diphenylamine (2)

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Dulak,K.; Jonas,F. Determination of phosmet by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *396*, 433–436.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 229

CHROMATOGRAM
Retention time: 0.31

Limit of detection: 80 pg

OTHER SUBSTANCES
Also analyzed: amitraz (UV 313), chlorpyrifos (UV 313), coumaphos (UV 313), crotoxyphos (UV 229), permethrin (UV 229)

REFERENCE
Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.

SAMPLE
Matrix: solutions
Sample preparation: Equilibrate column A with 10 mL MeCN and 10 mL water (pH 7). Pump 200 mL drinking water through column A at 3 mL/min, back flush contents of column A onto column B with the mobile phase and start the gradient.

HPLC VARIABLES
Column: A 10 × 2.1 5 μm RP-18 octadecylsilica (E. Merck); B 150 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:water from 40:60 to 60:40 over 15 min
Injection volume: 200000
Detector: UV 254

CHROMATOGRAM
Retention time: 13.9
Limit of detection: 0.2 ng/mL

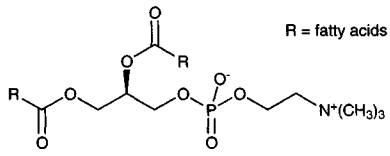
OTHER SUBSTANCES
Extracted: azinphos-methyl, carbaryl, parathion-methyl, azinphos-ethyl, fenitrothion, parathion, diazinon

KEY WORDS
drinking water; column-switching

REFERENCE
Driss,M.R.; Hennion,M.-C.; Bouguerra,M.L. Determination of carbaryl and some organophosphorus pesticides in drinking water using on-line liquid chromatographic preconcentration techniques, *J.Chromatogr.*, **1993**, *639*, 352–358.

Phosphatidylcholine

Merck Index: 5452



SAMPLE
Matrix: amniotic fluid
Sample preparation: Precipitate phospholipids from 5 mL amniotic fluid with cold acetone. Dissolve the precipitate in 30 μL chloroform, remove a 20 μL aliquot and add it to 40 μL chloroform:MeOH 2:1, inject a 10-30 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm SI 60 silica (Merck)
Column: 250 × 4.6 10 μm LiChrosorb DIOL
Mobile phase: Gradient. A was MeCN:water 80:20. B was MeCN. A:B 12.5:87.5 for 4.5 min, to 75:25 over 6.5 min.
Column temperature: 35
Flow rate: 2

Injection volume: 10-30
Detector: UV 203

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, sphingomyelin, lysolecithin

KEY WORDS
mobile phase temperature 40°

REFERENCE
Briand,R.L.; Harold,S.; Blass,K.G. High-performance liquid chromatographic determination of the lecithin/sphingomyelin ratio in amniotic fluid, *J.Chromatogr.*, **1981**, *223*, 277–284.

SAMPLE
Matrix: amniotic fluid
Sample preparation: Centrifuge amniotic fluid, remove a 5-10 mL aliquot and add it to an equal volume of MeOH, shake for 30 s, add twice the volume of chloroform, shake for 30 s, centrifuge at 1100 g for 5 min. Remove the lower chloroform layer and filter (Whatman GFF glass fiber), evaporate to dryness under a stream of nitrogen at 45°, add ice-cold acetone. Dry the precipitate thoroughly, take it up in 35 μL chloroform:MeOH 95:5, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: Corasil
Column: 300 × 4 10 μm μPorasil
Mobile phase: Chloroform:MeOH:water 178.5:64:5
Flow rate: 2
Injection volume: 25
Detector: RI

CHROMATOGRAM
Retention time: 20
Limit of detection: 3.7 μM

OTHER SUBSTANCES
Extracted: phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, sphingomyelin

KEY WORDS
normal phase

REFERENCE
Paton,R.D.; McGillivray,A.I.ir,T.F.; Whittle,M.J.; Whitfield,C.R.; Logan,R.W. HPLC of phospholipids in biological fluids --application to amniotic fluid for the prediction of fetal lung maturity, *Clin.Chim.Acta*, **1983**, *133*, 97–110.

SAMPLE
Matrix: amniotic fluid
Sample preparation: Centrifuge amniotic fluid, remove a 1.5 mL aliquot and add it to 1.5 mL MeOH, vortex for 30 s, add 6 mL chloroform, vortex for 30 s, centrifuge at 1500 g for 10 min. Remove the lower chloroform layer and evaporate it to dryness under a stream of nitrogen at 50°, chill the residue in a deep freeze for 10 min, add ice-cold acetone. Dry the precipitate thoroughly, take it up in 20 μL 22 μM gamma-capryloyl lysolecithin in chloroform:MeOH 2:1, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm SI 60 silica (Merck)

Column: 125 × 4.6 5 μm DIOL (Merck)
Mobile phase: Gradient. A was MeCN. B was MeCN:water 3.5:1. A:B 88:12 for 4.2 min, to 25: 75 over 8 min.
Column temperature: 38
Flow rate: 2
Injection volume: 10
Detector: UV 203

CHROMATOGRAM
Retention time: 13
Internal standard: gamma-capryloyl lysolecithin (16)
Limit of detection: 500 nM

OTHER SUBSTANCES
Extracted: phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, sphingomyelin

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KEY WORDS
mobile phase temperature 40°

REFERENCE
Andrews,A.G. Estimation of amniotic fluid phospholipids by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *336*, 139–150.

SAMPLE
Matrix: bile
Sample preparation: Shake 2 mL bile and 8 mL isopropanol, centrifuge. Purify 100 μL on a 10 × 10 cm Kieselgel 60 F254 TLC plate (Merck) by eluting with chloroform:MeOH:benzene: ammonia 65:30:10:6 (Caution! Benzene is a carcinogen!), visualize using iodine. Scrape off the band, elute with 5 mL chloroform:MeOH 4:3. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm mod HS-3 C18 (Perkin-Elmer)
Mobile phase: MeCN:MeOH:water 3:90:8 containing 20 mM choline chloride
Column temperature: 50
Flow rate: 1 for 2 min, increase to 2 over 20 min, maintain at 2 for 10 min
Injection volume: 10
Detector: UV 205

CHROMATOGRAM
Retention time: 12-25

REFERENCE
Cantafora,A.; Di Biase,A.; Alvaro,D.; Angelico,M.; Marin,M.; Attili,A.F. High performance liquid chromatographic analysis of molecular species of phosphatidylcholine--development of quantitative assay and its application to human bile, *Clin.Chim.Acta*, **1983**, *134*, 281–295.

SAMPLE
Matrix: bulk
Sample preparation: Emulsify 150 μg phosphatidylcholine in 8 mL 100 mM pH 5.5 acetate buffer and 1.5 mL 1 M calcium chloride, add 100 μg cabbage phospholipase D, add 4 mL diethyl ether, stir at rom temperature for 20 h, add 3 mL 500 mM EDTA, extract with chloroform/ MeOH. Dissolve the extracted phosphatidate in 2 mL chloroform:MeOH:water 63.5:31.5:5, add 500 μL 100 mM HCl, mix rapidly. Remove the lower phase and evaporate it to dryness, reconstitute the residue in 500 μL diethyl ether, add 1 mL diazomethane in diethyl ether, evaporate to dryness under a stream of nitrogen, dissolve the residue in 20 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:MeOH:isopropanol:water 50:18:27:5

Flow rate: 1.5
Detector: UV 205

CHROMATOGRAM
Retention time: 9.4-36.0 (depending on structure)

KEY WORDS
derivatization

REFERENCE
Nakagawa,Y.; Waku,K. Improved procedure for the separation of the molecular species of dimethylphosphatidate by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *381*, 225–231.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liposome dispersions 10-fold with chloroform:MeOH 60:40, centrifuge at 2700 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax amino
Mobile phase: MeCN:MeOH:buffer 64:28:8 (Buffer was 10 mM phosphoric acid adjusted to pH 4.8 with dilute ammonium hydroxide solution. To prepare mobile phase mix MeCN and MeOH and then add buffer.)
Flow rate: 1.5
Injection volume: 5-20
Detector: RI

CHROMATOGRAM
Retention time: 5
Limit of detection: 22 μg/mL

OTHER SUBSTANCES
Simultaneous: acyl lysophosphatidylcholine, acyl lysophosphatidylglycerol, phosphatidylglycerol

KEY WORDS
liposome dispersions

REFERENCE
Grit,M.; Crommelin,D.J.A.; Lang,J. Determination of phosphatidylcholine, phosphatidylglycerol and their lyso forms from liposome dispersions by high-performance liquid chromatography using high-sensitivity refractive index detection, *J.Chromatogr.*, **1991**, *585*, 239–246.

SAMPLE
Matrix: lung lavage fluid
Sample preparation: Centrifuge lung lavage fluid at 4° at 450 g for 10 min. Shake 10 mL supernatant and 40 mL chloroform:MeOH 2:1 at 4° for 3 min. Remove the lower organic phase and wash it with 2 mL 50 mM NaCl, centrifuge, dry under a stream of nitrogen at 45°, reconstitute with 500 μL mobile phase, vortex at 4° for 1 min, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Encapharm 100 spherical silica gel (Molnar, Berlin)
Column: 120 × 4.6 5 μm Encapharm 100 spherical silica gel (Molnar, Berlin)
Mobile phase: Gradient. A was chloroform:MeOH:ammonium hydroxide 80:19.5:0.5. B was chloroform:MeOH:water:ammonium hydroxide 60:34:5.5:0.5. A:B from 100:0 to 0:100 over 14 min, return to initial conditions over 7 min, re-equilibrate for 10 min.
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: evaporative light-scattering, SEDERE Sedex-45, evaporation temperature 50°, nebulization gas nitrogen, pressure 200 kPa, flow 6 L/min, response is non-linear but proportional to the power 1.7 of the mass and must be calibrated for each compound

CHROMATOGRAM
Retention time: 13.11
Limit of detection: 40 ng

OTHER SUBSTANCES
Extracted: diarachidoylphosphatidylcholine, dilinoleylphosphatidylcholine, dipalmitoylphosphatidylcholine, diphosphatidylglycerol, lysophosphatidylcholine, phosphatidic acid, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, sphingomyelin

KEY WORDS
normal phase

REFERENCE
Bünger,H.; Pison,U. Quantitative analysis of pulmonary surfactant phospholipids by high-performance liquid chromatography and light-scattering detection, *J.Chromatogr.B*, **1995**, *672*, 25–31.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 5 5 μm Hypersil silica
Column: 250 × 5 5 μm Hypersil silica
Mobile phase: MeCN:MeOH:water 50:45:6.5
Flow rate: 1
Detector: RI

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: degradation products, free fatty acids, lysophosphatidylcholine

KEY WORDS
eggs

REFERENCE
Christie,W.W.; Hunter,M.L. High-performance liquid chromatography in the analysis of the products of phospholipase A hydrolysis of phosphatidylcholine, *J.Chromatogr.*, **1984**, *294*, 489–493.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 3 μm Spherisorb silica
Mobile phase: Gradient. Isopropanol:hexane:water from 58:40:2 to 52:40:8 over 7 min, maintain at 52:40:8 for 8 min.
Flow rate: 1.25
Injection volume: 20
Detector: evaporative light-scattering detector

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: cholesterol, palmitic acid, phosphatidylethanolamine, phosphatidylserine, sphingomyelin

KEY WORDS
normal phase

REFERENCE
Supelco Catalog, **1993**, p. 760.

SAMPLE
Matrix: solutions
Sample preparation: Inject 100 mg soybean oil as a solution in hexane.

HPLC VARIABLES
Column: 250 × 4.6 15-40 μm silica gel 60
Mobile phase: Gradient. Hexane:isopropanol:water 55:44:4 for 22 min, 55:44:5.7 for 8 min, 55:44:7 for 70 min (step gradient).
Flow rate: 1
Detector: UV 214

CHROMATOGRAM
Retention time: 50

OTHER SUBSTANCES
Simultaneous: lysophosphatidylcholine, phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine

KEY WORDS
semi-preparative; also details of a preparative procedure; normal phase

REFERENCE
De Meulenaer,B.; Van der Meeren,P.; Vanderdeelen,J.; Baert,L. Optimization of a chromatographic method for the gram-scale preparative fractionation of soybean phospholipids, *Chromatographia*, **1995**, *41*, 527–531.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in chloroform:MeOH 98:2 on to column A and column B in series and elute with mobile phase A, after 10 min elute with mobile phase B, after 10 min remove column A from the circuit, monitor the effluent from column B, after 30 min elute column A with mobile phase B and monitor the effluent from column A.

HPLC VARIABLES
Column: A 250 × 4 5 μm LiChrosorb diol; B 250 × 4 5 μm LiChrospher Si100
Mobile phase: A MeCN; B MeCN:MeOH:phosphoric acid 93:5:1.5
Flow rate: 1
Injection volume: 50
Detector: UV 205

CHROMATOGRAM
Retention time: 46

OTHER SUBSTANCES
Simultaneous: lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin

KEY WORDS
column-switching

REFERENCE
Soudant,P.; Marty,Y.; Moal,J.; Samain,J.F. Separation of major polar lipids in Pecten maximus by high-performance liquid chromatography and subsequent determination of their fatty acids using gas chromatography, *J.Chromatogr.B*, **1995**, *673*, 15–26.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 30 mg/mL solution of lyophilized lipids in MeOH, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μPorosil (Waters)
Mobile phase: MeCN:MeOH:water 25:70:5
Flow rate: 2
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 3.23

OTHER SUBSTANCES
Simultaneous: cardiolipin, cholesterol

KEY WORDS
egg

REFERENCE
Choudhari,K.B.; Jayanthi,S.; Murty,R.B.; Matharu,R.P. A high-performance liquid chromatographic method for the analysis of lipids from lyophilized formulations, *J.Chromatogr.A*, **1996**, *724*, 343–347.

SAMPLE
Matrix: tissue
Sample preparation: Blend squid skin, add 5 volumes acetone, agitate at 25° for 2 h, filter, wash the solids three times with one volume of cold acetone. Keep the filtrate at -20° for 16 h, centrifuge at 2500 rpm for 10 min, discard the acetone supernatant, dry the pellet under a stream of nitrogen. Dissolve in the initial mobile phase, inject an aliquot. (All solvents contain 0.1% BHT.)

HPLC VARIABLES
Column: 250 × 7.2 5μm Lichrosorb Si 60
Mobile phase: Gradient. A:B:C from 42:52:6 to 32:52:16 over 20 min. A was hexane; B was isopropanol:chloroform (80:20); C was isopropanol:water (50:50).
Flow rate: 2.5
Injection volume: 250
Detector: ELSD (Cunow, France)

OTHER SUBSTANCES
Extracted: phosphatidylethanolamine, phosphatidylinotisol, phosphatidylserine, sphingomyelin

KEY WORDS
skin; squid; normal phase

REFERENCE
Baudimant,G.; Maurice,M.; Landrein,A.; Durand,G.; Durand,P. Purification of phosphatidylcholine with high content of DHA from squid Illex argentinus by countercurrent chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1793–1804.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Potter-Elvehjem) liver or lung with 20 volumes chloroform: MeOH 2:1, filter (paper), wash with a volume of 50 mM NaCl equal to one-fifth the volume of extract, centrifuge (J.Biol.Chem. 1957, 226, 497). Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in chloroform, add 100 μL 1 mM IS in chloroform. Purify by normal phase HPLC using hexane:isopropanol:water 42:56:6.3 at 1 mL/min on a 300 × 4.9 10 μm μPorasil column using UV 200 detection, collect fraction eluting between 26 and 32 min, evaporate, dissolve in the minimum amount of trifluoroethanol, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Apex ODS 2
Mobile phase: MeOH:water 92.5:7.5 containing 40 mM choline chloride

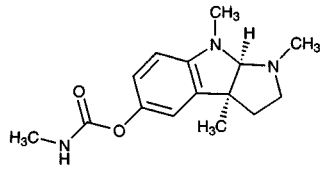
Column temperature: 50
Flow rate: 1
Detector: F ex 340 em 460 following post-column derivatization. The column effluent was mixed with the reagent pumped at 3 mL/min in a 15 μL mixing chamber and the mixture flowed through a 3 m × 0.5 mm i.d. PTFE coil at 50° to the detector. Reagent was water containing 150 μL/L 3 mM 1,6-diphenyl-1,3,5-hexatriene in THF:Tween 20 99.999:0.001

CHROMATOGRAM
Retention time: 35-57
Internal standard: phosphatidylcholine 14:0/14:0 (20)

KEY WORDS
rat; liver; lung; post-column reaction

REFERENCE
Postle,A.D. Method for the sensitive analysis of individual molecular species of phosphatidylcholine by high-performance liquid chromatography using post-column fluorescence detection, *J.Chromatogr.*, **1987**, *415*, 241–251.

Physostigmine



Molecular formula: $C_{15}H_{21}N_3O_2$
Molecular weight: 275.35
CAS Registry No.: 57-47-6, 57-64-7 (salicylate), 64-47-1 (sulfate)
Merck Index: 7540
Lednicer No.: 1 111

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 1 mg/mL neostigmine + 20 μL 1 μg/mL dimethylphysostigmine + 500 μL 100 mM pH 7 picric acid (Caution! Do not allow to dry! Dry picric acid is explosive!) + 500 μL 100 mM NaH_2PO_4, vortex thoroughly, add 10 mL water-saturated dichloromethane, mix vigorously by inverting and vortexing for 15 s, centrifuge at 1000 g for 10 min. Discard the upper aqueous phase and add 2 mL water-saturated dichloromethane, mix vigorously by inverting and vortexing for 15 s, centrifuge at 1000 g for 7 min. Remove the organic phase and add it to 200 μL 1 mM pH 1.8 tetrabutylammonium hydrogen sulfate, mix vigorously by inverting and vortexing for 15 s, centrifuge at 1000 g for 7 min, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 5 × 3.2 7 μm silica
Column: 250 × 4.6 5 μm Ultrasphere-Si
Mobile phase: MeCN:buffer 20:80 (Buffer was 10 mM NaH_2PO_4 containing 2.5 mM tetramethylammonium chloride, pH 3.0.)
Flow rate: 1
Injection volume: 50
Detector: F ex 240 em 360

CHROMATOGRAM
Retention time: 7.08
Internal standard: dimethylphysostigmine (9.63)
Limit of detection: 0.1 ng/mL
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: neostigmine

KEY WORDS
plasma

REFERENCE
Elsayed,N.M.; Ryabik,J.R.G.; Ferraris,S.; Wheeler,C.R.; Korte,D.W.,Jr. Determination of physostigmine in plasma by high-performance liquid chromatography and fluorescence detection, *Anal.Biochem.*, **1989**, *177*, 207–211.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 μg/mL neostigmine bromide in water, filter (Amicon Centricon, 10000 molecular mass cut-off) while centrifuging at 4° at 7000 g for 70 min, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 40:60, pH adjusted to 2.1 (Buffer was 5 mM NaH_2PO_4 containing 1% acetic acid and 0.5 mM 1-octanesulfonic acid.)
Flow rate: 1.5
Injection volume: 100
Detector: radioactivity (Radiomatic Instruments Flo-One B) (The column effluent mixed with Ultrafluor (National Diagnostics) pumped at 4 mL/min and passed to the flow cell.)

CHROMATOGRAM
Retention time: 10.1
Limit of detection: 0.05 ng/mL

OTHER SUBSTANCES
Extracted: eseroline

KEY WORDS
tritium labeled; guinea pig; plasma; pharmacokinetics

REFERENCE
Lukey,B.J.; Marlow,D.D.; Clark,C.R.; McCluskey,M.P.; Lieske,C.N. Application of a new radiometric high-performance liquid chromatographic assay to define physostigmine pharmacokinetics in guinea pigs, *J.Chromatogr.*, **1989**, *493*, 117–124.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 200 mg octadecyl SPE cartridge with 2 column volumes of MeOH and 2 column volumes of 100 mM pH 4 phosphate buffer. 1 mL Serum + 5 μL 1 mg/mL neostigmine bromide in 100 mM pH 4 NaH_2PO_4, vortex for 15 s, add 1 mL 1 mM reagent in 100 mM pH 4 phosphate buffer, mix for 30 s, add to the SPE cartridge, wash with 4 mL water, elute with 200 μL MeOH:water 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (Synthesize reagent, sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate, as follows. Add 5 mL 10% KOH in water to a stirred solution of 20 mmoles 3,4-(dimethoxyphenyl)acetonitrile and 20 mmoles 2-formylbenzenesulfonic acid, sodium salt hydrate (sodium benzaldehyde-2-sulfonate) in 50 mL EtOH at 50°, stir at 50° for 5 min, cool (evaporate to near dryness, if necessary), filter to obtain sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate (mp of p-toluidine salt is 218-223°) (J. Chem. Eng. Data 1975, 20, 215).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm diol (ES Industries, Marlton NJ)
Mobile phase: MeOH:50 mM pH 4 NaH_2PO_4 20:80 containing 500 μM sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate
Flow rate: 1
Injection volume: 50

Detector: F ex 243 em 418 (cutoff filter) following post-column extraction. The column effluent mixed with dichloromethane pumped at 1 mL/min and the mixture flowed through a 90 cm × 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector.

CHROMATOGRAM
Retention time: 6.42
Internal standard: neostigmine (11.16)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, eseroline
Also analyzed: amantadine, amphetamine, atropine, chlorpheniramine, clidinium bromide, N,N-dimethyl-N-benzyltetradecylammonium chloride, guanethidine, hydralazine, imipramine, malachite green, promazine, propantheline bromide
Noninterfering: chlordiazepoxide

KEY WORDS
post-column extraction; SPE; serum; silanize glassware; post-column reaction

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REFERENCE
Quinn,K.D.; Stewart,J.T. A high performance liquid chromatographic post-column fluorescent ion pair extraction system: application to physostigmine and its metabolite eseroline in human serum, *Biomed.Chromatogr.*, **1991**, *5*, 8–13.

SAMPLE
Matrix: blood
Sample preparation: Add physostigmine octylcarbamate to freshly-drawn blood in a final concentration of 500 nM, prepare plasma, add IS, purify by SPE, inject an aliquot. (Keep samples on ice throughout procedure.)

HPLC VARIABLES
Column: two 200 mm (?) long narrow-bore normal phase columns in series (Brownlee)
Mobile phase: MeCN:10 mM formic acid:50 mM Tris buffer 27:52:21
Flow rate: 0.15
Detector: F ex 250 em 345

CHROMATOGRAM
Internal standard: N-methylphysostigmine
Limit of detection: 0.055 ng/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Asthana,S.; Greig,N.H.; Hegedus,L.; Holloway,H.H.; Raffaele,K.C.; Schapiro,M.B.; Soncrant,T.T. Clinical pharmacokinetics of physostigmine in patients with Alzheimer's disease, *Clin.Pharmacol.Ther.*, **1995**, *58*, 299–309.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 500 mM sodium bicarbonate + 5 mL n-hexane, shake for 10 min, centrifuge at 1500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL MeCN:MeOH 50:50, inject a 70 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm silica (Violet)
Mobile phase: MeCN:MeOH:80 mM ammonium nitrate 50:40:10, pH 8.90
Flow rate: 1

Injection volume: 70
Detector: E, glassy carbon electrode +0.75 V

CHROMATOGRAM
Retention time: 6
Internal standard: physostigmine

OTHER SUBSTANCES
Extracted: eptastigmine

KEY WORDS
plasma; physostigmine is IS

REFERENCE
Imbimbo,B.P.; Licini,M.; Schettino,M.; Mosca,A.; Onelli,E.; Zecca,L.; Giustina,A. Relationship between pharmacokinetics and pharmacodynamics of eptastigmine in young healthy volunteers, *J.Clin.Pharmacol.*, **1995**, *35*, 285–290.

SAMPLE
Matrix: diffusate, tissue
Sample preparation: Homogenize (Polytron PCU-2) 150-200 mg skin and diazepam with 4 mL chloroform, repeat homogenization, filter (phase-separating paper) extracts. Make the residue alkaline with 2 mL 10% NaOH, extract twice with 4 mL portions of chloroform, wash the extracts twice with 2 mL portions of water, filter (phase-separating paper) the organic layer. Combine all the chloroform layers and evaporate them to dryness under a stream of air, reconstitute the residue in 1 mL mobile phase, filter (microfilter), inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 4 40 μm ODS (Valco)
Column: 150 × 4.6 5 μm Spherisorb ODS-I
Mobile phase: MeCN:water 52:48 containing 10 mM octanesulfonic acid and 1% acetic acid, pH 3.5
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: diazepam (6.0)
Limit of detection: 5 μg/g

OTHER SUBSTANCES
Extracted: tacrine

KEY WORDS
skin; pharmacokinetics; stability-indicating

REFERENCE
Lau,S.W.J.; Chow,D.; Feldman,S. Simultaneous determination of physostigmine and tetrahydroaminoacridine in a transdermal permeation study by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *526*, 87–95.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

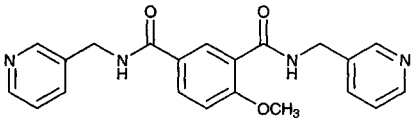
HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 35:1.5:0.5:63
Flow rate: 1.5
Injection volume: 10
Detector: UV 261

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: salicylic acid

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Picotamide



Molecular formula: $C_{21}H_{20}N_4O_3$
Molecular weight: 376.41
CAS Registry No.: 32828-81-2
Merck Index: 7560

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma + 20 μL 0.05 mg/mL bamifylline in MeOH + 60 μL 25% aqueous ammonia, mix, add 5 mL chloroform:isopropanol 95:5, shake for 20 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL 0.6 M HCl, add 600 μL ethyl acetate, vortex for 15 s, centrifuge at 2000 g for 3 min. Remove the acidic aqueous phase and evaporate it to dryness under vacuum, reconstitute the residue in 100 μL MeOH:water 50:50, inject a 25 μL aliquot. Urine. 0.1-1 mL Urine + 20 μL 2.5 mg/mL bamifylline in MeOH + 100 μL 25% aqueous ammonia, mix, add 5 mL chloroform:isopropanol 95:5, shake for 20 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL 1.2 M HCl, add 600 μL ethyl acetate, vortex for 15 s, centrifuge at 2000 g for 3 min. Remove the acidic aqueous phase and evaporate it to dryness under vacuum, reconstitute the residue in 100 μL MeOH: water 50:50, inject a 25 μL aliquot.

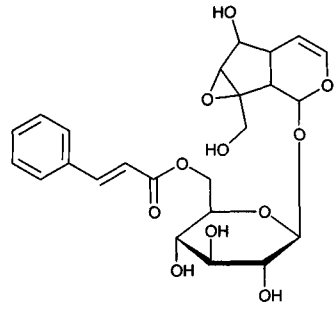
HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-Select B C8
Mobile phase: MeCN:50 mM pH 5.5 NaH_2PO_4 28:72
Flow rate: 1
Injection volume: 25
Detector: UV 230

CHROMATOGRAM
Retention time: 10
Internal standard: bamifylline (7)
Limit of quantitation: 1 μg/mL (urine), 5 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Fossati,T.; Parisi,S.; Abbiati,G.; Castiglioni,C. Determination of picotamide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *577*, 382–386.

Picroside



Molecular formula: $C_{24}H_{28}O_{11}$
Molecular weight: 492.47
CAS Registry No.: 27409-30-9

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL MeOH, vortex for 2 min, centrifuge at 3000 rpm for 10 min. Transfer the supernatant to another tube and evaporate the MeOH under a stream of nitrogen, extract 3 times with 500 μL portions of ethyl acetate. Evaporate the combined ethyl acetate layers to dryness. Reconstitute the residue with 50 μL MeOH, inject a 20 μL aliquot.

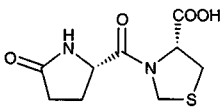
HPLC VARIABLES
Guard column: 4 × 4 5 μm C18
Column: 250 × 4 5 μm C18
Mobile phase: MeCN:100 mM acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 8.5
Limit of quantitation: 100 ng/ mL

KEY WORDS
plasma; rabbit

REFERENCE
Dwivedi,A.K.; Kulkarni,D.; Singh,S. Sensitive high-performance liquid chromatographic assay method for the determination of picroside I in plasma, *J.Chromatogr.B*, **1997**, *698*, 317–320.

Pidotimod



Molecular formula: $C_9H_{12}N_2O_4S$
Molecular weight: 244.27
CAS Registry No.: 121808-62-6
Merck Index: 7574

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 400 μg/mL oxiracetam + 100 μL 35% perchloric acid, vortex for 15 s, sonicate for 10 min, centrifuge at 12000 rpm for 10 min. Remove a 500 μL aliquot of the supernatant and add it to 500 μL mobile phase, vortex for 15 s, centrifuge at 12000 rpm for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 PRP (Brownlee)

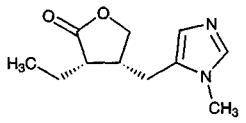
Column: 300 × 7.8 Aminex Ion-Exclusion HPX 874 (Bio-Rad)
Mobile phase: MeCN:0.05% sulfuric acid 12:88
Flow rate: 0.6
Injection volume: 40
Detector: UV 210

CHROMATOGRAM
Retention time: 16.5
Internal standard: oxiracetam (13.8)
Limit of detection: 100 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Dal Bo,L.; Broccali,G.P.; Silingardi,S.; Coppi,G. A new HPLC method for pidotimod plasma levels determination, *Boll.Chim.Farm.*, **1993**, *132*, 126–128.

Pilocarpine



Molecular formula: $C_{11}H_{16}N_2O_2$
Molecular weight: 208.26
CAS Registry No.: 92-13-7, 54-71-7 (HCl), 148-72-1 (nitrate)
Merck Index: 7578

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 500 μL 300 mM pH 8.4 potassium bicarbonate + 1 mL dichloromethane, vortex for 1 min, centrifuge at 2000 rpm for 5 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL 250 μg/mL p-nitrobenzyl bromide in MeCN, heat in a sealed tube at 40° for 24 h, cool, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 80:20 containing 1 mM sodium octanesulfonate
Flow rate: 1.6
Detector: UV 254

CHROMATOGRAM
Retention time: 11
Limit of detection: <50 ng/mL

OTHER SUBSTANCES
Simultaneous: isopilocarpine

KEY WORDS
derivatization; rabbit; silanize glassware with dimethyldichlorosilane

REFERENCE
Mitra,A.K.; Baustian,C.L.; Mikkelson,T.J. High-performance liquid chromatographic determination of pilocarpine in aqueous humor: derivatization by quaternization of methylimidazole tertiary amine group, *J.Pharm.Sci.*, **1980**, *69*, 257–261.

SAMPLE
Matrix: aqueous humor

Sample preparation: 200 μL Aqueous humor + 400 μL MeOH, centrifuge at 8000 g for 5 min. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5Ph (Nacalai Tesque)
Mobile phase: MeCN:0.1% acetic acid 5:95
Column temperature: 50
Flow rate: 1
Injection volume: 75
Detector: MS, Hitachi Model M-80B double-focusing, M-8093 APCI interface, nebulizer 350°, vaporizer 390°, drift voltage 140 V, corona discharge 12 μA, full scan m/z 1-600 in 4 s, SIM m/z 209 (Divert mobile phase to waste until pilocarpine elutes.)

CHROMATOGRAM
Retention time: 5.1
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: degradation products, isopilocarpic acid, isopilocarpine, pilocarpic acid

KEY WORDS
rabbit; pharmacokinetics

REFERENCE
Matsuura,K.; Kuwano,M.; Takashima,H. Determination of pilocarpine in aqueous humour by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1993**, *621*, 173–180.

SAMPLE
Matrix: blood
Sample preparation: Add 125 mg NaF to each 1 mL of blood collected. 500 μL Plasma + 500 μL clonidine in water + 3 mL dichloromethane, shake gently for 10 min, centrifuge at 2100 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 μL 1 mM HCl, vortex for 4 min, sonicate for 45 s, add 2 mL diethyl ether, vortex for 2 min, centrifuge at 2100 g for 5 min, discard the ether phase, apply a vacuum to the aqueous phase for 10 s, inject a 35-100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 15 × 4.6 37 μm Corasil C18
Column: 150 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeCN:MeOH:7 mM pH 4.0 potassium phosphate buffer 30:15:55
Flow rate: 1.2
Injection volume: 35-100
Detector: UV 214

CHROMATOGRAM
Retention time: 7.2
Internal standard: clonidine (12)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: pentobarbital

KEY WORDS
plasma; human; dog

REFERENCE
Weaver,M.L.; Tanzer,J.M.; Kramer,P.A. High-performance liquid chromatographic determination of pilocarpine in plasma, *J.Chromatogr.*, **1992**, *581*, 293–296.

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Mix 50 μL urine with 5 μL 10% sodium bicarbonate solution add 50 μL 500 ng/mL pilosine in MeOH. Mix 1.5 mL saliva with 90 μL 10% sodium bicarbonate, add 50 μL 500 ng/mL pilosine in MeOH. Mix 3 mL Plasma with 50 μL 500 ng/mL pilosine in MeOH. Extract these samples twice with 3 mL portions of chloroform. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 200 μL 0.08% 4-bromomethyl-7-methoxycoumarin in acetone, heat at 37° for 48 h, evaporate to dryness, reconstitute with 1 mL mobile phase, centrifuge at 20000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4.6 cyanopropyl silica (Brownlee)
Column: 220 × 4.6 cyanopropyl silica (Brownlee)
Mobile phase: MeCN:buffer 30:70 (Buffer was 3 mM diethylamine adjusted to pH 3.5 with 1 M phosphoric acid.)
Column temperature: 37
Flow rate: 1
Injection volume: 100
Detector: F ex 324 em 400

CHROMATOGRAM

Retention time: 13.86
Internal standard: pilosine (12.36)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Interfering: isopilocarpine

KEY WORDS

derivatization; silanize all glassware with dichlorodimethylsilane for 12 h; plasma; pharmacokinetics

REFERENCE

Aromdee,C.; Fawcett,J.P.; Ledger,R. Sensitive high-performance liquid chromatographic assay for pilocarpine in biological fluids using fluorescence derivatization, *J.Chromatogr.B*, **1996**, *677*, 313–318.

SAMPLE

Matrix: blood, urine

Sample preparation: Blood. 10 mL Whole blood + 250 μL 5% disodium EDTA, vortex, centrifuge at 1500 g for 10 min. Remove a 4 mL aliquot of the plasma and add it to 125 μL 5% disodium EDTA, mix. Remove a 1 mL aliquot and filter (Amicon Centrifree with 30000 Da cut-off), while centrifuging at 2000 g for 15 min, inject a 25 μL aliquot of the ultrafiltrate. Urine. Adjust urine to pH 5 with 100 mM HCl, dilute 20-fold with 200 mM pH 3.7 sodium acetate buffer, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:50 mM ammonium acetate buffer adjusted to pH 4.0 with trifluoroacetic acid 3:97
Flow rate: 1
Injection volume: 25
Detector: MS, PE Sciex API III plus, positive ion mode, multiple monitoring mode, APCI, nebulizer 500 °, m/z 209

CHROMATOGRAM

Retention time: 18
Limit of detection: 500 pg/mL (plasma), 10 ng/mL (urine)
Limit of quantitation: 2 ng/mL (plasma), 40 ng/mL (urine)

OTHER SUBSTANCES

Extracted: degradation products

KEY WORDS
plasma; ultrafiltrate; whole blood

REFERENCE
Van de Merbel,N.C.; Tinke,A.P.; Oosterhuis,B.; Jonkman,J.H.G.; Bohle,J.F. Determination of pilocarpine, isopilocarpine, pilocarpic acid and isopilocarpic acid in human plasma and urine by high-performance liquid chromatography with tandem mass spectrometric detection, *J.Chromatogr.B*, **1998**, *708*, 103–112.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM
Retention time: 4.622

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve ophthalmic gel in mobile phase so that the pilocarpine concentration is 40 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cyclobond β-cyclodextrin (Rainin)
Mobile phase: Water containing 40 g/L ammonium sulfate and 20 mL/L triethylamine, pH adjusted to 4.0 with phosphoric acid.
Flow rate: 1
Injection volume: 10
Detector: UV 214

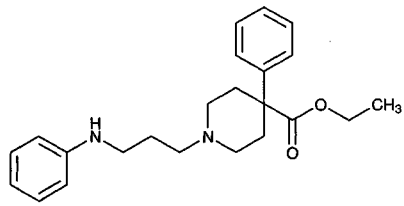
CHROMATOGRAM
Retention time: 7.5
Limit of detection: 0.2-0.3% (of pilocarpine present)

OTHER SUBSTANCES
Simultaneous: degradation products, isopilocarpic acid, isopilocarpine, pilocarpic acid

KEY WORDS
ophthalmic gel

REFERENCE
Sternitzke,K.D.; Fan,T.Y.; Dunn,D.L. High-performance liquid chromatographic determination of pilocarpine hydrochloride and its degradation products using a β-cyclodextrin column, *J.Chromatogr.*, **1992**, *589*, 159–164.

Piminodine



Molecular formula: $C_{23}H_{30}N_2O_2$
Molecular weight: 366.50
CAS Registry No.: 13495-09-5, 7081-52-9 (ethanesulfonate)
Merck Index: 7587
Lednicer No.: 1 301

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.8

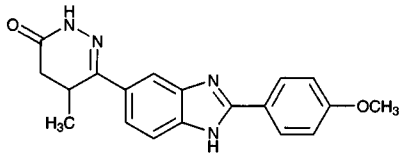
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine,

phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Pimobendan



Molecular formula: $C_{19}H_{18}N_4O_2$
Molecular weight: 334.38
CAS Registry No.: 118428-36-7, 74150-27-9
Merck Index: 7588
Lednicer No.: 5 117

SAMPLE

Matrix: blood
Sample preparation: Extract from plasma using SPE.

HPLC VARIABLES

Column: 5 μm ODS-Hypersil
Mobile phase: MeOH:water 59:46 containing 2.5 g/L ammonium acetate
Detector: F ex 332 em 405 following post-column reaction. The column effluent mixed with MeOH:85% orthophosphoric acid:water 60:20:20 pumped at 0.2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM

Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

pig; plasma; SPE; post-column reaction

REFERENCE

Verdouw,P.D.; Hartog,J.M.; Duncker,D.J.; Roth,W.; Saxena,P.R. Cardiovascular profile of pimobendan, a benzimidazole-pyridazinone derivative with vasodilating and inotropic properties, *Eur.J.Pharmacol.*, **1986**, *126*, 21–30.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 10 μg/mL carbamazepine in n-propanol + 5 mL MeCN, rotate for 10 min, centrifuge at 1880 g for 10 min. Remove the supernatant and evaporate it to 0.8-1 mL under a stream of air, add 500 μL 20 mM pH 5 ammonium hydrogen phosphate buffer, add 5 mL dichloromethane:n-propanol 70:30, vortex for 30 s, centrifuge at 1880 g for 5 min. Remove the organic layer and evaporate it to dryness, add 500 μL water and

3 mL diethyl ether to the residue, vortex for 30 s. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 350 μL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Spheri-5 silica
Column: 250 × 4.6 5 μm Spheri-5 silica + 250 × 4.6 10 μm Chiralcel OD in series
Mobile phase: n-Hexane:EtOH:diethylamine 75:25:0.1
Column temperature: 35
Flow rate: 1
Injection volume: 200
Detector: UV 328

CHROMATOGRAM
Retention time: 17.3 (+), 20.9 (-)
Internal standard: carbamazepine (13.7)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral; pharmacokinetics

REFERENCE
Chu,K.-M.; Shieh,S.-M.; Wu,S.H.; Hu,O.Y.-P. Enantiomeric separation of a cardiotonic agent pimobendan and its major active metabolite, UD-CG 212 BS, by coupled achiral-chiral normal-phase high-performance liquid chromatography, *J.Chromatogr.Sci.*, **1992**, *30*, 171–176.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut PH SPE cartridge with 3 mL MeOH and 3 mL water. 100 μL Plasma + 1 mL 100 mM pH 9.5 phosphate buffer, add to the SPE cartridge, wash with 3 mL water, dry by pulling air through the cartridge for 15 min, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL EtOH:n-hexane 50:50, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Sumchiral OA-4400 (Sumika)
Mobile phase: n-Hexane:EtOH:acetic acid 300:120:1
Column temperature: 40
Flow rate: 0.8
Injection volume: 100
Detector: F ex 330 em 415 following post-column reaction. The column effluent mixed with EtOH:acetic acid pumped at 0.3 mL/min and the mixture flowed to the detector.

CHROMATOGRAM
Retention time: 14.4 (-), 15.1 (+)
Limit of detection: 1.25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
chiral; post-column reaction; plasma; rat; SPE; pharmacokinetics

REFERENCE
Asakura,M.; Nagakura,A.; Tarui,S.; Matsumura,R. Simultaneous determination of the enantiomers of pimobendan and its main metabolite in rat plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 135–141.

SAMPLE
Matrix: cell incubations
Sample preparation: Inject 200 μL cell incubation on to column A with mobile phase A then switch to mobile phase B (start the gradient) and elute to waste, after 4 min direct the effluent from column A on to column B, after 30 min remove column A from the circuit.

HPLC VARIABLES
Column: A 40 × 4.6 37-75 μm Porasil B; B 30 mm long 5 μm Hypersil ODS + 125 × 4.6 5 μm Hypersil ODS
Mobile phase: A 1% pH 6.8 ammonium acetate buffer; B Gradient. A was water:25% ammonia 100:0.02. B was MeOH. A:B 100:0 for 6.9 min, to 95:5 over 0.1 min, to 85:15 over 11 min, to 0:100 over 9 min, maintain at 0:100 for 3 min, re-equilibrate at initial conditions for 2 min.
Column temperature: 28
Flow rate: 1
Injection volume: 200
Detector: F ex 332 em 405 following post-column reaction. The column effluent mixed with MeOH:water:85% orthophosphoric acid 60:20:20 pumped at 0.2 mL/min and flowed to the detector.

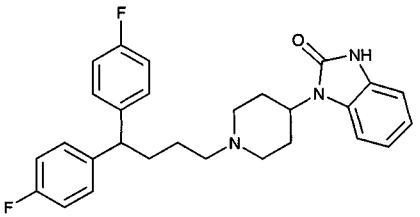
CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver; hepatocytes; post-column reaction; column-switching

REFERENCE
Pahernik,S.A.; Schmid,J.; Sauter,T.; Schildberg,F.W.; Koebe,H.G. Metabolism of pimobendan in long-term human hepatocyte culture: in vivo-in vitro comparison, *Xenobiotica*, **1995**, *25*, 811–823.

Pimozide



Molecular formula: $C_{28}H_{29}F_2N_3O$
Molecular weight: 461.55
CAS Registry No.: 2062-78-4
Merck Index: 7589
Lednicer No.: 2 290

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL whole blood with 500 ng IS, add 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 200 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 3.9 5 μm NovaPak-Phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 55:45, adjusted to pH 3.0
Flow rate: 1.5
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Internal standard: pentazocine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: sertraline

REFERENCE
McIntyre,I.M.; King,C.V.; Staikos,V.; Gall,J.; Drummer,O.H. A fatality involving moclobemide, sertraline, and pimozide, *J.Forensic Sci.*, **1997**, *42*, 951–953.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL 3-methylpimozide in 1% phosphoric acid + 1 mL 1 M NaOH + 5 mL n-hexane:isoamyl alcohol 98:2, shake reciprocally for 10 min, centrifuge at 1900 g for 5 min. Remove the organic layer and ad it to 100-200 μL 100 mM phosphoric acid, shake, centrifuge, discard the organic layer, inject almost all of the aqueous layer.

HPLC VARIABLES
Guard column: 10 × 4 5 μm TSK-GEL LS-410 (Toyo Soda)
Column: 150 × 4 5 μm TSK-GEL LS-410 (Toyo Soda)
Mobile phase: MeCN:buffer 48:52 (Buffer was 20 mM KH_2PO_4 adjusted to pH 2.5 with 20 mM phosphoric acid.)
Flow rate: 1
Injection volume: 100-200
Detector: F ex 210 em >320 or UV 280

CHROMATOGRAM
Retention time: 6
Internal standard: 3-methylpimozide (8)
Limit of detection: 0.3 ng/mL, 5 ng/mL (UV)

OTHER SUBSTANCES
Noninterfering: haloperidol, levomepromazine, sulpride, thioridazine, thiothixene

KEY WORDS
plasma

REFERENCE
Miyao,Y.; Suzuki,A.; Noda,K.; Noguchi,H. A sensitive assay method for pimozide in human plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1983**, *275*, 443–449.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 281

CHROMATOGRAM
Retention time: 8.77
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry by suction, wash with 2 mL acetone: chloroform 50:50, elute with 3 mL ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 50 µL MeOH, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 µm Nova-Pack C18

Mobile phase: MeOH:50 mM ammonium acetate 75:25 (Mix column effluent with 50 mM ammonium acetate pumped at 0.5 mL/min.)

Flow rate: 0.6

Injection volume: 10

Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, MAT TSP-2 interface, thermospray, selective reaction monitoring m/z 462-238, collision offset -27.5 V, repeller 100 V, vaporizer 130°, source 200°, filament on 200 µA, argon 2.5 mTorr, multiplier 1500 V, dynode 15 kV, scan time 1.20 s, MSMSC factor 10

CHROMATOGRAM

Retention time: 10.20

Limit of detection: 100 pg

OTHER SUBSTANCES
Extracted: benperidol, dextromoramide, droperidol, haloperidol, methadone, penfluridol, pipamperidone, propoxyphene (dextropropoxyphene)

KEY WORDS
SPE; LC/MS

REFERENCE
Verweij,A.M.; Hordijk,M.L.; Lipman,P.J. Quantitative liquid chromatographic thermospray-tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood, *J.Anal.Toxicol.*, **1995**, *19*, 65–68.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 17.192

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 14.36 (A), 7.96 (B)

OTHER SUBSTANCES

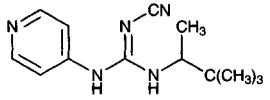
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-

azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Pinacidil



Molecular formula: $C_{13}H_{19}N_5$
Molecular weight: 245.32
CAS Registry No.: 60560-33-0, 85371-64-8 (monohydrate)
Merck Index: 7592
Lednicer No.: 4 102

SAMPLE
Matrix: blood
Sample preparation: 1-2 mL Plasma + 100 µL 1 µg/mL IS + 2 mL 100 mM pH 10 carbonate buffer, extract twice with 5 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 µL MeOH:water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 µm Lichrosorb RP18
Mobile phase: MeCN:10 mM $(NH_4)_2HPO_4$ 33:67
Flow rate: 1.2
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: N"-cyano-N-(1-ethyl-1-methylpropyl)-N'-4-pyridylguanidine (P 1149) (6.5)

Limit of detection: 3 ng/mL

KEY WORDS
rat; dog; human; plasma; pharmacokinetics

REFERENCE
Eilertsen,E.; Hart,J.W.; Magnussen,M.P.; Sorensen,H.; Arrigoni-Martelli,E. Pharmacokinetics and distribution of the new antihypertensive agent pinacidil in rat, dog and man, *Xenobiotica*, **1982**, *12*, 177–185.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 3 mL MeOH and 5 mL water. 1 mL Plasma + 50 μL 5 μg/mL IS in 10 mM HCl, vortex, add to the SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH:water 20:80, elute with 4 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Co-PELL ODS
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: MeOH:buffer 45:55 (Buffer was 550 mL 100 mM sodium acetate + 1 mL morpholine, adjust pH to 4.0 with glacial acetic acid.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 100
Detector: UV 284

CHROMATOGRAM
Retention time: 9
Internal standard: N'-cyano-N-(1-ethyl-1-methylpropyl)-N'-4-pyridinyl guanidine (11)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: minoxidil, triamterene
Noninterfering: allopurinol, atenolol, captopril, chlordiazepoxide, clidinium bromide, clonidine, dipyridamole, furosemide, hydralazine, hydrochlorothiazide, metoprolol, pilocarpine, propranolol, sulindac
Interfering: colchicine, prazosin

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Hamilton,M.; Farid,K.Z.; Henry,D.P. Liquid chromatographic determination of pinacidil, a new antihypertensive drug, and its major metabolite, pinacidil N-oxide, in plasma, *J.Chromatogr.*, **1986**, *375*, 359–367.

SAMPLE
Matrix: blood
Sample preparation: 0.3-1 mL Plasma + 2 μg phenacetin in MeOH, add to a Sep-Pak C18 SPE cartridge, wash with 7.5 mL water, elute with 5 mL MeOH, elute with 5 mL EtOH, evaporate the eluate, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Cosmosil-10-phenyl (Nacalai Tesque)
Column: 100 × 4.6 Cosmosil-5-phenyl (Nacalai Tesque)
Mobile phase: MeCN:50 mM pH 5.0 sodium acetate buffer 23:77
Flow rate: 1
Detector: UV 277

CHROMATOGRAM
Retention time: 11.3

Internal standard: phenacetin (8.9)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; plasma; pharmacokinetics; SPE

REFERENCE
Sakamoto,K.; Nakamura,Y. Stereoselective disposition and metabolism of pinacidil in rat, *Xenobiotica*, **1994**, *24*, 329–338.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 3-4 mL Plasma or 2 mL urine to a Sep-Pak C18 SPE cartridge, wash with 7.5 mL water, elute with 5 mL EtOH, inject an aliquot of the plasma extract. Purify the urine extract using a Silicagel 60 F254 (Merck) TLC plate with dichloromethane:EtOH as solvent 5:1.

HPLC VARIABLES
Guard column: 30 × 4.6 Chemcosorb-7-ODS-L (Chemco)
Column: 150 × 4.6 Chemcosorb-7-ODS-L (Chemco)
Mobile phase: Isopropanol:EtOH:50 mM pH 2.5 ammonium perchlorate 3:12:85 containing 3% γ-cyclodextrin and 20 mM sodium sulfate
Flow rate: 1
Detector: UV 277

CHROMATOGRAM
Retention time: 8.3 (-), 9.0 (+)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; plasma; pharmacokinetics; SPE; chiral

REFERENCE
Sakamoto,K.; Nakamura,Y. Stereoselective disposition and metabolism of pinacidil in rat, *Xenobiotica*, **1994**, *24*, 329–338.

SAMPLE
Matrix: microsomal incubations, urine
Sample preparation: Urine. Add 0.1-1 mL urine to a Sep-Pak C18 SPE cartridge, wash with 7.5 mL water, elute with 5 mL MeOH, elute with 5 mL EtOH, evaporate the eluate to dryness under reduced pressure, reconstitute in MeOH, inject an aliquot. Microsomal incubations. 3 mL Microsomal incubation + 2 mL water + 50 μL 200 μg/mL phenacetin in MeOH, homogenize. Remove a 2 mL aliquot and add it to 1 mL 10% perchloric acid, centrifuge at 3000 rpm for 5 min, add the supernatant to a Sep-Pak C18 SPE cartridge, wash with 7.5 mL water, elute with 5 mL MeOH, elute with 5 mL EtOH, evaporate the eluate to dryness under reduced pressure, reconstitute in MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Cosmosil-10-phenyl (Nakarai Tesque)
Column: 150 × 4.6 Chemcosorb-7-ODS-L (Chemco)
Mobile phase: MeCN:MeOH:50 mM pH 5.0 sodium acetate buffer 15:20:65
Flow rate: 1
Detector: UV 277

CHROMATOGRAM
Retention time: 17.6

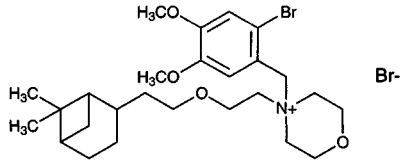
Internal standard: phenacetin (11.32)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; SPE; liver; rabbit; dog; mouse; monkey; human

REFERENCE
Sakamoto,K.; Nakamura,Y. Urinary metabolites of pinacidil: I. Isolation and identification of the metabolites in rat urine, *Xenobiotica*, **1993**, *23*, 391–400.

Pinaverium bromide



Molecular formula: $C_{26}H_{41}Br2NO_4$
Molecular weight: 591.42
CAS Registry No.: 53251-94-8
Merck Index: 7595

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

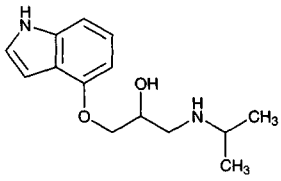
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 21.337

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pindolol



Molecular formula: $C_{14}H_{20}N_2O_2$
Molecular weight: 248.33
CAS Registry No.: 13523-86-9
Merck Index: 7597
Lednicer No.: 2 342

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 25 μL 5 μg/mL metoprolol in water + 100 μL 2 M NaOH + 4 mL dichloromethane, vortex for 10 s, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 80 μL aliquot.

HPLC VARIABLES

Guard column: NewGuard C18 (Brownlee)
Column: 250 × 4.6 5 μm Dynamax Microsorb C18
Mobile phase: MeCN:0.1% triethylamine in water adjusted to pH 3.5 with 85% phosphoric acid 20:80
Flow rate: 1
Injection volume: 80
Detector: F ex 215

CHROMATOGRAM

Retention time: 7.04
Internal standard: metoprolol (11.65)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

KEY WORDS

serum

REFERENCE

Chmielowiec,D.; Schuster,D.; Gengo,F. Determination of pindolol in human serum by HPLC, *J.Chromatogr.Sci.*, **1991**, *29*, 37–39.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut 40 μm cynaopropylsilica SPE cartridge with 1 mL MeOH at 6 mL/min and with 1 mL pH 7.4 buffer at 6 mL/min. Centrifuge plasma, add 1 mL plasma at 0.18 mL/min to the SPE cartridge, wash with 1 mL pH 7.4 buffer at 1.5 mL/min, elute with 300 μL MeOH:2-aminoheptane 99.9:0.1 at 1.5 mL/min, pass 700 μL pH 3.0 buffer through the cartridge at 1.5 mL/min. Mix both eluates, inject a 250 μL aliquot. (pH 7.4 Buffer was 250 mL 100 mM KH_2PO_4 and 195.5 mL 100 mM NaOH, made up to 1 L, if necessary pH adjusted to 7.4. pH 3.0 Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 4 μm Superspher 100 RP-18 (Merck)
Mobile phase: MeOH:buffer 30:70 containing 0.5% 2-aminoheptane (Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)
Column temperature: 37
Flow rate: 1.2
Injection volume: 250
Detector: F ex 255 em 315

CHROMATOGRAM

Retention time: 10

KEY WORDS
plasma; SPE

REFERENCE
Hubert,P.; Chiap,P.; Moors,M.; Bourguignon,B.; Massart,D.L.; Crommen,J. Knowledge-based system for the automated solid-phase extraction of basic drugs fom plasma coupled with their liquid chromatographic determination. Application to the biodetermination of β-receptor bocking agents, *J.Chromatogr.A*, **1994**, *665*, 87–99.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 265

CHROMATOGRAM
Retention time: 3.98
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvox-

amine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μL buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 12.5 mM NaOH in MeOH:water 50:50, inject a 50 μL aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

HPLC VARIABLES

Guard column: 4 × 4 30 μm LiChrocart Aluspher RP-select B (Merck)

Column: 125 × 4 5 μm Aluspher RP-select B (Merck)

Mobile phase: Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230, 254

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, haloperidol, nitrendipine, nordiazepam, nortriptyline, zolpidem

Also analyzed: acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyldopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripelennamine, tyramine, verapamil, yohimbine

REFERENCE

Lambert,W.E.; Meyer,E.; De Leenheer,A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J.Anal.Toxicol.*, **1995**, *19*, 73–78.

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute urine 1:10 with water. 500 μL Plasma or diluted urine + 500 μL buffer + 50 μL 2.2 μg/mL alprenolol in water, vortex, add 3 mL diethyl ether, shake for 10 min, centrifuge at 250 g for 4 min. Remove the ether layer and add it to 100 μL dilute sulfuric acid (pH 2.2), vortex for 1 min, centrifuge at 250 g for 4 min, inject a 20-75 μL aliquot of the aqueous phase. (Buffer was 5.3 g sodium bicarbonate and 4.2 g sodium carbonate in 100 mL water, pH 9.5.)

HPLC VARIABLES

Column: 300 × 4 10 μm Micropak CN-10 alkylnitrile (Varian) (Prepare column by rinsing with 100 mL dichloromethane, with 100 mL MeCN:water 50:50, and with mobile phase.)
Mobile phase: 10 mM KH_2PO_4 adjusted to pH 2.6 with concentrated phosphoric acid
Flow rate: 2
Injection volume: 20-75
Detector: UV 220

CHROMATOGRAM

Retention time: 5.5
Internal standard: alprenolol (9.5)
Limit of detection: 1.2 ng

OTHER SUBSTANCES

Simultaneous: atenolol, caffeine, disopyramide, nadolol, oxprenolol, practolol, procainamide, pronethalol, propranolol, sotalol, timolol
Interfering: N-acetylprocainamide, lidocaine, quinidine

KEY WORDS

plasma

REFERENCE

Shields,B.J.; Lima,J.J.; Binkley,P.F.; Leier,C.V.; MacKichan,J.J. Determination of pindolol in human plasma and urine by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1986**, *378*, 163–171.

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. Condition a 1 mL diol (2OH) SPE cartridge (Varian) with two volumes of MeOH and two volumes of water. Add 1 mL serum to the SPE cartridge, wash with 250 μL water, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. Urine. Centrifuge urine at 2000 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OD-R
Mobile phase: MeCN:300 mM sodium perchlorate 40:60
Flow rate: 0.5
Injection volume: 100
Detector: F ex 270 em 310

CHROMATOGRAM

Retention time: 10.5 (R-(+)), 18.5 (S-(-))
Limit of detection: 76 ng/mL (S, urine), 21 ng/mL (R, urine), 4.3 ng/mL (S, serum), 1.2 ng/mL (R, serum)

KEY WORDS

serum; chiral; SPE

REFERENCE

Zhang,H.; Stewart,J.T.; Ujhelyi,M. High-performance liquid chromatographic analysis of pindolol enantiomers in human serum and urine using a reversed-phase cellulose-based chiral column, *J.Chromatogr.B*, **1995**, *668*, 309–313.

SAMPLE

Matrix: blood, urine
Sample preparation: Filter (0.45 μm) plasma or urine. Inject a 20 (plasma) or 50 (urine) μL aliquot on to column A and elute to waste, after 5 min backflush the contents of column A on to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 5 min before the next run.

HPLC VARIABLES
Column: A 10 × 4.6 internal-surface phenylboronic acid (Yokogawa Analytical Systems); B 150 × 4.6 Capcell Pak C18 SG120 (Shiseido)
Mobile phase: A MeOH:50 mM Na_2HPO_4 5:95; B MeOH:50 mM pH 2.0 phosphate buffer 20:80
Flow rate: 1
Injection volume: 20-50
Detector: F ex 255 em 315 or UV 255

CHROMATOGRAM
Retention time: 6
Limit of detection: 100 nM

OTHER SUBSTANCES
Noninterfering: acetaminophen, caffeine, furosemide, hydrochlorothiazide, nalidixic acid, norfloxacin, pipemidic acid, phenylbutazone, salicylic acid, theophylline, tolbutamide, warfarin

KEY WORDS
plasma; column-switching

REFERENCE
Ohta,T.; Niida,S.; Nakamura,H. Selective extraction of β-blockers from biological fluids by column-switching high-performance liquid chromatography using an internal-surface phenylboronic acid precolumn, *J.Chromatogr.B*, **1996**, *675*, 168–173.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 215.8

CHROMATOGRAM
Retention time: 8.568

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk

Sample preparation: Dissolve 1.5 mg compound in 1 mL reagent, add 3 μL triethylamine, sonicate for 20 min, add 3 μL diethylamine, let stand for 15 min, inject an aliquot. (Reagent was 2 mg/mL (R)-(-)-(naphth-1-yl)ethylisocyanate solution in dry chloroform:DMF 80:20.)

HPLC VARIABLES
Column: 200 × 4.6 Silica 100 RP 18
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.50, k' 2.87 (enantiomers)

OTHER SUBSTANCES
Also analyzed: atenolol, methylphenidate, metipranolol, propranolol, propylhexedrine, talinolol

KEY WORDS
derivatization

REFERENCE
Jira,T.; Toll,C.; Vogt,C.; Beyrich,T. Zur Trennung einiger racemischer β-Blocker und α-Sympathikomimetika durch HPLC nach Derivatisierung [The separation of some racemic β-blockers and α-sympathomimetics with HPLC following derivatization], *Pharmazie*, **1991**, *46*, 432–434.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 85:15
Flow rate: 0.5
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: k' 3.00, k' 3.71 (enantiomers)

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexyl-isothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a

little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.91, k' 5.59 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, propranolol, xamoterol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: formulations
Sample preparation: Take up in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb C2
Mobile phase: MeCN:buffer 35:65 (1 mL 100 mM HCl + 1200 mL water + 5.84 g NaCl, mix to dissolve, add 700 mL MeOH, make up to 2 L, apparent pH 4.5.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.8

OTHER SUBSTANCES
Simultaneous: atenolol, nadolol, alprenolol, acebutolol, oxprenolol, metoprolol, practolol, propranolol, timolol
Interfering: sotalol

KEY WORDS
tablets

REFERENCE
Patel,B.R.; Kirschbaum,J.J.; Poet,R.B. High-pressure liquid chromatography of nadolol and other β-adrenergic blocking drugs, *J.Pharm.Sci.*, **1981**, *70*, 336–338.

SAMPLE
Matrix: saliva

Sample preparation: Condition a 100 mg 1 mL Bond-Elut C2 SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL pH 9.0 borate buffer. Centrifuge a cotton roll soaked with saliva at 1000 g for 5 min, remove the liquid supernatant. 1 mL Supernatant + 50 μL 100 μg/mL tertatolol, add to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL mobile phase, mix for 15 s, inject a 40 μL aliquot. (Acidified MeOH was 50 mL MeOH + 300 μL 96% acetic acid.)

HPLC VARIABLES
Guard column: RCSS silica guard-pack (Waters)
Column: 250 × 4.6 Chiralcel OD-H
Mobile phase: n-Hexane:EtOH:diethylamine 50:50:1
Flow rate: 1
Injection volume: 40
Detector: F ex 225 em 290 cut-off filter

CHROMATOGRAM
Internal standard: (R,S)-tertatolol

KEY WORDS
SPE; chiral

REFERENCE
Höld,K.M.; de Boer,D.; Zuidema,J.; Maes,R.A.A. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva, *J.Chromatogr.B*, **1995**, *663*, 103–110.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH, dilute with mobile phase.

HPLC VARIABLES
Column: 150 × 3.9 Novapak-phenyl-4
Mobile phase: MeOH:15 mM pH 6.5 sodium acetate buffer 81:19
Flow rate: 1.0
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: perphenazine

REFERENCE
Al-Obaid,A.M.; Hagga,M.E.M.; El-Khawad,I.E.; El-Mahi,O.H.M. Simultaneous quantitation of some phenothiazine drug substances and their monosulphoxide degrades by high performance liquid chromatography (HPLC), *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1369–1389.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 bidentate C18 silane column (Preparation is as follows. Reflux 60 g 7 μm Zorbax PSM300 silica in 600 mL 75 ppm HF in water for 72 h (Caution! HF is highly toxic!), wash with 1.5 L water, wash with 500 mL acetone, dry overnight under vacuum (30 in. Hg). Add to 570 mL water boil for 10 h, cool to room temperature, wash with 500 mL acetone, dry overnight at 110° under vacuum (30 in. Hg). Heat 6 g of this material at 110° under vacuum (30 in. Hg) and place it in a dry nitrogen atmosphere. Add 60 mL dry xylene, 240 μL pyridine, and 4.9 mL dichlorodimethyldioctadecyldisiloxane (?) (Petrarch Systems, Bristol, PA). Reflux under nitrogen for 80 h, cool, wash with 300 mL toluene, 300 mL dichloromethane, 300 mL MeOH, 300 mL MeOH:water 50:50, and 300 mL acetone. Dry at 110° under vacuum (30 in. Hg overnight) (cf. US Pat. 4 746 572).)

Mobile phase: MeCN:17 mM pH 11 K_3PO_4 buffer 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 5
Detector: not given

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Simultaneous: metoprolol

REFERENCE
Kirkland,J.J.; van Straten,M.A.; Claessens,H.A. Reversed-phase high-performance liquid chromatography of basic compounds at pH 11 with silica-based column packings, *J.Chromatogr.A*, **1998**, *797*, 111–120.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, qui-

nine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Detector: UV

CHROMATOGRAM

Retention time: k' 3.17 (of first (+) enantiomer)

KEY WORDS

chiral; α 5.07

REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE

Matrix: solutions
Sample preparation: Filter (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 internal surface reversed-phase silica (Pinkerton) (Regis Chemical)
Mobile phase: Isopropanol:100 mM pH 6.8 KH_2PO_4 10:90
Flow rate: 1
Injection volume: 10
Detector: UV 232-274 (wavelength of maximum absorption used)

CHROMATOGRAM

Retention time: 57.2

OTHER SUBSTANCES

Simultaneous: carteolol, atenolol, metoprolol, oxprenolol, acebutolol, alprenolol

REFERENCE

Ohshima,T.; Takagi,K.; Miyamoto,K.-I. High performance liquid chromatographic retention time of β-blockers as an index of pharmacological activity, *J.Liq.Chromatogr.*, **1993**, *16*, 3933–3939.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL Solution + 50 μL pH 7.4 PBS + 100 μL MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 25:75
Flow rate: 1

Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Internal standard: pindolol

OTHER SUBSTANCES
Simultaneous: carteolol

KEY WORDS
buffer; Earle's balanced salt solution; pindolol is IS

REFERENCE
Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil MOS C-8
Mobile phase: MeOH:water 70:30 containing 0.02% dimethyloctylamine, 25 mM sodium hexanesulfonate, and 20 mM acetic acid
Flow rate: 1
Injection volume: 40
Detector: F ex 275 em 305

CHROMATOGRAM
Retention time: 4.6

OTHER SUBSTANCES
Simultaneous: alprenolol, atenolol, propranolol (UV 288)

REFERENCE
Adson,A.; Burton,P.S.; Raub,T.J.; Barsuhn,C.L.; Audus,K.L.; Ho,N.F.H. Passive diffusion of weak organic electrolytes across Caco-2 cell monolayers: Uncoupling the contributions of hydrodynamic, transcellular, and paracellular barriers, *J.Pharm.Sci.*, **1995**, *84*, 1197–1204.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 3-chloro-4-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 9.50

KEY WORDS
narrow-bore; chiral; α 2.14

REFERENCE
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 20:80:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: k' 0.25, 1.66 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.85

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 7.02 (A), 4.07 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica
Mobile phase: Heptane:isopropanol:diethylamine 80:20:0.1
Flow rate: 1
Injection volume: 1000
Detector: UV 254

CHROMATOGRAM

Retention time: k' 5.51

KEY WORDS

chiral; α 2.84

REFERENCE

Oliveros,L.; Lopez,P.; Minguillon,C.; Franco,P. Chiral chromatographic discrimination ability of a cellulose 3,5-dimethylphenylcarbamate/10-undecenoate mixed derivative fixed on several chromatographic matrices, *J.Liq.Chromatogr.*, **1995**, *18*, 1521–1532.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.35

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 10 × 3.2 5 μm Partisil ODS3
Column: 100 × 4.6 5 μm Partisil ODS3
Mobile phase: MeCN:buffer 15:85 (Buffer was 60 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.6-1
Injection volume: 10-100
Detector: UV 270

REFERENCE
Palm,K.; Luthman,K.; Ungell,A.-L.; Strandlund,G.; Artursson,P. Correlation of drug absorption with molecular surface properties, *J.Pharm.Sci.*, **1996**, *85*, 32–39.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve salt of pindolol in MeOH:water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil RP-18
Mobile phase: Gradient. MeOH:buffer from 20:80 to 80:20 over 10 min. (Buffer was 2% acetic acid containing 1.1% sodium 1-heptanesulfonate.)
Column temperature: 40
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.8

OTHER SUBSTANCES
Simultaneous: benzoic acid (UV 273), 2-methoxyphenylacetic acid (UV 270)

REFERENCE
Pietiläinen,H.; Saesmaa,T. HPLC determination of pindolol benzoate and pindolol 2-methoxyphenylacetate, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 583–591.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate: benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane: acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25

mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 44:56:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM
Retention time: 30.9, 39.4 (enantiomers)
Limit of detection: 296-320 fmole

KEY WORDS
derivatization; chiral

REFERENCE
Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES
Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
Flow rate: A 1.25; B 1
Injection volume: 100
Detector: UV 220

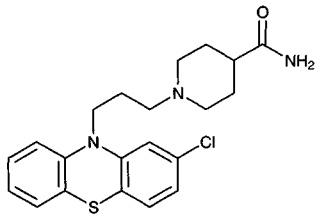
CHROMATOGRAM
Retention time: 10.3
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, propranolol, timolol

KEY WORDS
column-switching

REFERENCE
Saarinen,M.T.; Sirén,H.; Riekkola,M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J.Chromatogr.B*, **1995**, *664*, 341–346.

Pipamazine



Molecular formula: $C_{21}H_{24}ClN_3OS$
Molecular weight: 401.96
CAS Registry No.: 84-04-8
Merck Index: 7607
Lednicer No.: 1 385

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

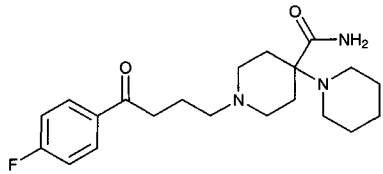
Retention time: 2.25

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Pipamperone



Molecular formula: $C_{21}H_{30}FN_3O_2$
Molecular weight: 375.49
CAS Registry No.: 1893-33-0, 2448-68-2 (2.HCl)
Merck Index: 7608
Lednicer No.: 2 288

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 246

CHROMATOGRAM

Retention time: 4.98
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine;

diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 10.918

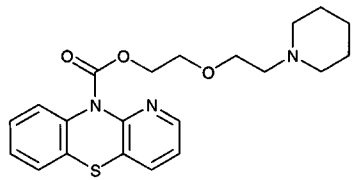
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pipazethate



Molecular formula: $C_{21}H_{25}N_3O_3S$
Molecular weight: 399.51
CAS Registry No.: 2167-85-3, 6056-11-7 (HCl)
Merck Index: 7609
Lednicer No.: 1 390

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 5.9

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Pipecuronium bromide

Molecular formula: $C_{35}H_{62}Br_2N_4O_4$
Molecular weight: 762.71
CAS Registry No.: 52212-02-9, 68399-57-5 (dihydrate)
Merck Index: 7612
Lednicer No.: 4 70

SAMPLE
Matrix: bulk

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: MeCN:MeOH:concentrated ammonia solution 43:43:14 containing 100 mM ammonium carbonate and 100 mM ammonium chloride
Flow rate: 1
Detector: UV 213

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
stability-indicating

REFERENCE
Szepesi,G.; Gazdag,M.; Mihályfi,K. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. III. Method validation, *J.Chromatogr.*, **1989**, *464*, 265–278.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 0.5% solution in the mobile phase, inject a 20 μL aliquot.

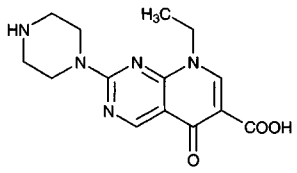
HPLC VARIABLES
Column: 250 × 4 5 μm SI 100 (Bio Separation Technologies)
Mobile phase: MeCN:100 mM sodium perchlorate 96:4
Flow rate: 1
Injection volume: 20
Detector: UV 213

CHROMATOGRAM
Retention time: 7
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: impurities, vecuronium, pancuronium

REFERENCE
Gazdag,M.; Babják,M.; Kemenes-Bakos,P.; Görög,S. Analysis of steroids. XLI. Ion-pair high-performance liquid chromatographic separation of quaternary ammonium steroids on silica, *J.Chromatogr.*, **1991**, *550*, 639–644.

Pipemidic acid



Molecular formula: $C_{14}H_{17}N_5O_3$
Molecular weight: 303.32
CAS Registry No.: 51940-44-4, 72571-82-5 (trihydrate)
Merck Index: 7613

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 13:87 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 275

CHROMATOGRAM
Retention time: 4.57
Internal standard: ofloxacin (8.51)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 100 μL plasma with 900 μL 100 mM phosphate buffer and 5 mL chloroform:ethyl chlorocarbonate 99:1, shake for 10 min, centrifuge at 1620 g for 5 min, evaporate the organic phase under reduced pressure, dissolve the residue in 100 μL MeOH:50 mM NaOH 2:1, inject a 20 μL aliquot. Tissue. Homogenate the cerebrum sample with 4 volumes of 100 mM phosphate buffer. Mix 1 mL homogenate with 5 mL dichloromethane, shake for 10 min, centrifuge at 1620 g for 5 min. Mix 4 mL 1 mM NaOH with 4 mL organic phase, shake for 10 min, centrifuge it at 1620 g for 5 min, collect 3 mL aqueous phase and treat in a manner similar to that for the plasma samples, except for the IS addition. Inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 5 C 18

Mobile phase: MeOH:5 mM sodium dodecylsulfate adjusted to pH 2.5 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: pipemidic acid

OTHER SUBSTANCES
Extracted: ciprofloxacin, foscarnet

KEY WORDS
plasma; brain; mouse; pipedimic acid is IS; derivatization

REFERENCE
Matsuo,H.; Ryu,M.; Nagata,A.; Uchida,T.; Kawakami,J.-I.; Yamamoto,K.; Iga,T.; Sawada,Y. Neurotoxicodynamics of the interaction between ciprofloxacin and foscarnet in mice, *Antimicrob.Agents Chemother.*, **1998**, *42*, 691–694.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Spherisorb ODS II
Mobile phase: MeCN containing 2 mM tetrabutyl ammonium hydrogen sulfate:100 mM citric acid buffer containing 5 mM ammonium perchlorate 87:13, adjusted to pH 2.2
Flow rate: 1.2
Injection volume: 5-50
Detector: F ex 290 em 460

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Simultaneous: fleroxacin

REFERENCE
Uehlinger,G.E.; Schaedeli,F.; Kinzig,M.; Sörgel,F.; Frey,F.J. Pharmacokinetics of fleroxacin after multiple oral dosing in patients receiving regular hemodialysis, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1903–1909.

SAMPLE
Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:0.4 mM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: F ex 270 em 440

CHROMATOGRAM
Retention time: 2.97
Limit of detection: 3.26 ng/mL

OTHER SUBSTANCES
Extracted: cinoxacin, oxolinic acid

REFERENCE
Durán Mer,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

SAMPLE
Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

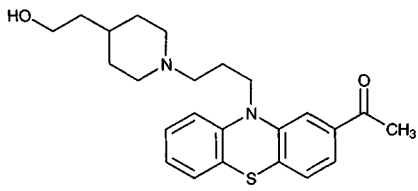
HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:400 μM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 2.97
Limit of detection: 1.15 μg/mL

OTHER SUBSTANCES
Simultaneous: cinoxacin, nalidixic acid, oxolinic acid, piromidic acid

REFERENCE
Durán Meré,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

Piperacetazine



Molecular formula: $C_{24}H_{30}N_2O_2S$
Molecular weight: 410.58
CAS Registry No.: 3819-00-9
Merck Index: 7615
Lednicer No.: 1 386

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.5

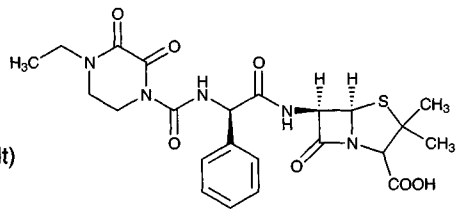
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine,

buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Piperacillin



Molecular formula: $C_{23}H_{27}N_5O_7S$
Molecular weight: 517.56
CAS Registry No.: 61477-96-1, 59703-84-3 (sodium salt)
Merck Index: 7616
Lednicer No.: 3 207; 4 179, 188

SAMPLE

Matrix: aqueous humor
Sample preparation: 150 μL Aqueous humor + 30 μL 2.5 μg/mL cephalothin + 50 μL 400 mM HCl, mix, add 700 μL chloroform:1-pentanol 3:1, mix by swirl-mixing for 5 min, centrifuge at 300 g for 5 min, discard the organic layer. Centrifuge the aqueous layer briefly, hold it at 4°, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm Ultrasphere RP-ODS
Mobile phase: MeOH:water:buffer 40:48:12, the final pH was adjusted to 6.7 with triethylamine (Buffer was 50 mM pH 6.7 morpholinopropanesulfonic acid (MOPS)-triethylamine.)
Column temperature: 32
Flow rate: 1

Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.86
Internal standard: cephalothin (4.74)
Limit of detection: 130 ng/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen, ampicillin, caffeine, salicylic acid, azlocillin, cefamandole, cefoxitin, cefuroxime, scopolamine, sulfamethoxazole, theophylline, ticarcillin, timolol
Noninterfering: acetazolamide, amitriptyline, atropine, carbachol, cefazolin, cefoperazone, cefotaxime, chlorpheniramine, codeine, diazepam, echothiophate, epinephryl borate, imipramine, prednisolone acetate, tropicamide, xylazine
Interfering: carbenicillin, mezlocillin

KEY WORDS
rabbit

REFERENCE
Riegel,M.A.; Ellis,P.P. High-performance liquid chromatographic assay for piperacillin in aqueous humor of the eye, *J.Chromatogr.*, **1988**, *424*, 177–181.

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 20:80 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 5.6
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, ticarcillin

KEY WORDS
serum

REFERENCE
Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Plasma, serum. 200 μL Plasma or serum + 200 μL 25 μg/mL penicillin G in 50 mM pH 6.0 sodium phosphate buffer + 800 μL MeCN, vortex for 30 s, centrifuge at 3000 g for 10 min. Remove the clear supernatant and add it to 2 mL dichloromethane, mix for 30 s, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot of the upper aqueous layer. Bile. 200

μL Bile + 400 μL 50 mM pH 7.0 sodium phosphate buffer + 2 mL MeCN, vortex for 30 s, centrifuge at 3000 g for 10 min. Remove the clear supernatant and add it to 2 mL dichloromethane, mix for 30 s, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot of the upper aqueous layer. Urine. 100 μL Urine + 50 μL 5 mg/mL penicillin G in water, vortex for 30 s, make up to 10 mL with 50 mM pH 6.0 sodium phosphate buffer, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Brownlee C 18 guard column
Column: 250 × 4.6 5 μm Hypersil ODS (Keystone)
Mobile phase: Gradient. A was MeCN:10 mM sodium phosphate adjusted to pH 2.7 with concentrated phosphoric acid 3:97. B was MeCN:10 mM sodium phosphate adjusted to pH 2.7 with concentrated phosphoric acid 90:10. A:B from 95:5 to 50:50 over 9 min and then to 95:5 over 1 min.
Flow rate: 1.5
Injection volume: 25
Detector: UV 220

CHROMATOGRAM
Retention time: 11.8
Internal standard: penicillin G (12.5)
Limit of quantitation: 50000 ng/mL (urine), 1000 ng/mL (plasma, serum, bile)

OTHER SUBSTANCES
Extracted: tazobactam
Simultaneous: amoxicillin, ampicillin, cefoperazine, cefometazole, cefotaxime, cefotetan, cefuroxime, mezlocillin

KEY WORDS
plasma; serum

REFERENCE
Ocampo,A.P.; Hoyt,K.D.; Wadgaonkar,N.; Carver,A.H.; Puglisi,C.V. Determination of tazobactam and piperacillin in human plasma, serum, bile and urine by gradient elution reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *496*, 167–179.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL 500 μg/mL mezlocillin + 200 μL 400 mM HCl + 3.5 mL dichloromethane, extract. Extract the organic phase with 200 μL 20 mM pH 6.2 phosphate buffer, inject a 30-60 μL aliquot.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:20 mM pH 3.0 sodium phosphate buffer 24:76
Flow rate: 1
Injection volume: 30-60
Detector: UV 254

CHROMATOGRAM
Internal standard: mezlocillin
Limit of quantitation: 250 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Harmsen,J.; Van Den Toorn,A. Determination of EDTA in water by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *249*, 379–384.

SAMPLE
Matrix: blood

Sample preparation: 500 μL Serum + methicillin + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex, centrifuge at 3000 g for 10 min, inject a 15 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 mm long μBondapak C18
Mobile phase: MeCN:100 mM pH 6.1 sodium phosphate buffer 25:75
Flow rate: 2.5
Injection volume: 15
Detector: UV 229

CHROMATOGRAM
Internal standard: methicillin
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: mezlocillin

KEY WORDS
serum; pharmacokinetics

REFERENCE
Martens,M.G.; Faro,S.; Feldman,S.; Cotton,D.B.; Dorman,K.; Riddle,G.D. Pharmacokinetics of the acyclureidopenicillins piperacillin and mezlocillin in the postpartum patient, *Antimicrob.Agents Chemother.*, **1987**, *31*, 2015–2017.

SAMPLE
Matrix: blood
Sample preparation: Condition a 55 × 5 100-200 mesh AG 50W-X8 (H^+) column (Bio-Rad) with 10 mL MeCN:water 50:50. 600 μL Serum + 600 μL MeCN, vortex for 1 min, centrifuge at 2000 g for 5 min, add a 1 mL aliquot of the supernatant to the column, discard the first 200 μL effluent, collect the rest of the effluent. Remove a 450 μL aliquot and add it to 50 μL 10% sodium carbonate solution, heat at 60° for 1 h (to hydrolyse the β-lactam ring), cool in an ice bath. Remove a 100 μL aliquot and add it to 15 μL 200 mM pH 6.0 phosphate buffer, add 35 μL 80 mM 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole in MeCN, heat at 60° for 10 min, cool in an ice bath, add 30 μL 1 M HCl, inject a 5-10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS-80TM (Tosoh)
Mobile phase: MeOH:100 mM pH 3.0 phosphate buffer 40:60
Flow rate: 1
Injection volume: 5-10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 7
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: methicillin, penicillin G
Interfering: penicilloic acid from piperacillin

KEY WORDS
derivatization; serum; SPE

REFERENCE
Iwaki,K.; Okumura,N.; Yamazaki,M.; Nimura,N.; Kinoshita,T. Precolumn derivatization technique for high-performance liquid chromatographic determination of penicillins with fluorescence detection, *J.Chromatogr.*, **1990**, *504*, 359–367.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL 500 μg/mL mezlocillin + 200 μL 400 mM HCl + 3.5 mL dichloromethane, extract. Extract the organic phase with 200 μL 20 mM pH 6.2 phosphate buffer, inject a 30-60 μL aliquot.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:20 mM pH 3.0 sodium phosphate buffer 24:76
Flow rate: 1
Injection volume: 30-60
Detector: UV 254

CHROMATOGRAM
Internal standard: mezlocillin
Limit of quantitation: 250 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Klepser,M.E.; Patel,K.B.; Nicolau,D.P.; Quintiliani,R.; Nightingale,C.H. Comparison of the bactericidal activities of ofloxacin and ciprofloxacin alone and in combination with ceftazidime and piperacillin against clinical strains of *Pseudomonas aeruginosa*, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2503–2510.

SAMPLE
Matrix: blood, dialysate
Sample preparation: Plasma. Mix 100 μL plasma with 200 μL MeOH containing 15 μg/mL IS, vortex for 15 s, centrifuge at 3000 rpm for 15 min, inject a 100 μL aliquot of the supernatant. Dialysate. Directly inject a 20 μL sample.

HPLC VARIABLES
Guard column: ODS
Column: 150 × 4.6 5 μm Regis C18
Mobile phase: MeCN:50 mM phosphate buffer 20:80, adjusted to pH 7.0
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Internal standard: p-aminobenzoic acid propyl ester
Limit of quantitation: 2 μg/mL

KEY WORDS
plasma; rat

REFERENCE
Nolting,A.; Dalla Costa,T.; Vistelle,R.; Rand,K.H.; Derendorf,H. Determination of free extracellular concentrations of piperacillin by microdialysis, *J.Pharm.Sci.*, **1996**, *85*, 369–372.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize tissue in water at 4° for 30 s, centrifuge at 15000 rpm (Sorvall centrifuge) for 10 min. 100 μL Plasma or tissue homogenate + 100 μL 100 mM pH 5.5 KH_2PO_4 + 400 μL 25 μg/mL mezlocillin in MeCN, mix, centrifuge at 15000 rpm (Sorvall centrifuge), extract with 1 mL dichloromethane, inject 40 μL of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher-C18
Mobile phase: MeCN:NaH_2PO_4 20:80, pH 5.5

Column temperature: 37.5
Flow rate: 1.7
Injection volume: 40
Detector: UV 220

CHROMATOGRAM
Retention time: 5.5
Internal standard: mezlocillin (7.7)
Limit of detection: 386 ng/mL

KEY WORDS
plasma; fatty tissue; muscle; skin; appendix; intestinal mucosa; pharmacokinetics

REFERENCE
Kinzig,M.; Sörgel,F.; Brismar,B.; Nord,C.E. Pharmacokinetics and tissue penetration of tazobactam and piperacillin in patients undergoing colorectal surgery, *Antimicrob.Agents Chemother.*, **1992**, *36*, 1997–2004.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 150 μL Plasma + 100 μL 400 μg/mL cloxacillin in water, vortex for 30 s, add 100 μL 10% trichloroacetic acid in water, vortex for 30 s, centrifuge at 3500 rpm for 5 min, inject a 200 μL aliquot of the supernatant. Tissue. Homogenize tissue in pH 7.4 Sörensen's buffer. 1 mL Tissue homogenate + 120 μL trichloroacetic acid, vortex for 30 s, centrifuge at 3500 rpm for 5 min. Remove the supernatant and add it to 5 mL chloroform, vortex for 1 min, centrifuge at 3500 rpm for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm reverse-phase RP-18
Mobile phase: MeCN:100 mM pH 6 potassium phosphate buffer 20:80
Flow rate: 2
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 3.20
Internal standard: cloxacillin (10.27)
Limit of detection: 500 ng/mL

KEY WORDS
plasma; brain

REFERENCE
Zarzuelo,A.; López,F.G.; Santos,M.; Lanao,J.M. Determination of piperacillin in biological samples by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 601–610.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Tissue (lung, gut). Cut tissue with a scalpel, homogenize with 1-3 mL buffer, centrifuge at 9600 g for 5 min three times, inject a 20 μL aliquot. Tissue (chondral). Cut tissue with a scalpel, homogenize with 3-6 mL buffer in an ice bath for 2-3 min, centrifuge at 9600 g for 5 min four or five times, inject a 100 μL aliquot. Dilute human pleural samples with buffer, centrifuge, inject a 20 μL aliquot. (Buffer was 66.6 mM K_2HPO_4 adjusted to pH 7.40 with KH_2PO_4.)

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil C18

Mobile phase: MeCN:buffer 23:77, adjusted to pH 5.2 with phosphoric acid (Buffer was 57.4 mM K_2HPO_4 adjusted to pH 5.2 with phosphoric acid.)
Flow rate: 1
Injection volume: 20-100
Detector: UV 220

CHROMATOGRAM
Retention time: 11
Limit of detection: 100 ng/mL

KEY WORDS
serum; plasma; lung; gut; pleural; chondral

REFERENCE
Knöller,J.; König,W.; Schönfeld,W.; Bremm,K.D.; Köller,M. Application of high-performance liquid chromatography of some antibiotics in clinical microbiology, *J.Chromatogr.*, **1988**, *427*, 257–267.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.758

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Solution + 4.9 mL MeOH:water 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Adsorbosphere C18
Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: MeOH:100 mM ammonium acetate 43:57
Flow rate: 1
Injection volume: 50

Detector: UV 254

CHROMATOGRAM
Retention time: 12.0

KEY WORDS
stability-indicating; injections; 5% dextrose

REFERENCE
Inagaki,K.; Gill,M.A.; Okamoto,M.P.; Takagi,J. Stability of ranitidine hydrochloride with aztreonam, ceftazidime, or piperacillin sodium during simulated Y-site administration, *Am.J.Hosp.Pharm.*, **1992**, *49*, 2769–2772.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 100:1 with saline, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere
Mobile phase: MeCN:10 mM NaH_2PO_4 40:60, pH adjusted to 3.3 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 6.3

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Choi,J.-S.; Burm,J.-P.; Jhee,S.S.; Chin,A.; Ulrich,R.W.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium and ranitidine hydrochloride in 0.9% sodium chloride injection during simulated Y-site administration, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2273–2276.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in mobile phase so that the concentration of piperacillin is 2 mg/mL, add 25 μg methyl benzoate, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Ultron ODS-X
Mobile phase: MeCN:MeOH:10 mM tetrabutylammonium hydroxide and 5 mM potassium sulfate adjusted to pH 4.1 with phosphoric acid 300:25:1000
Flow rate: 0.7
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 25.1
Internal standard: methyl benzoate (37.2)

OTHER SUBSTANCES
Extracted: YTR-830H, degradation products

REFERENCE
Tsukamoto,T.; Ushio,T. Determination of (2S,3S,5R)-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4, 4-dioxide (YTR-830H) and piperacillin in pharmaceutical preparations by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *678*, 69–76.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 100-fold with saline, filter (0.2 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: MeCN:100 mM sodium phosphate buffer 30:70 adjusted to pH 3.69 with 85% phosphoric acid
Flow rate: 1.2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 8-9

KEY WORDS
saline; injections; stability-indicating

REFERENCE
Chung,K.C.; Moon,Y.S.K.; Chin,A.; Ulrich,R.W.; Gill,M.A. Compatibility of ondansetron hydrochloride and piperacillin sodium tazobactam sodium during simulated Y-site administration, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1554–1556.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 25-100 μL sample with 10 mL saline, filter (0.2 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: MeCN:10 mM sodium phosphate 30:70 adjusted to pH 3.69 with 85% phosphoric acid
Flow rate: 1.2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 7.34

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; saline; 5% dextrose

REFERENCE
Moon,Y.S.K.; Chung,K.C.; Chin,A.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium in polypropylene syringes and polyvinyl chloride minibags, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 999–1001.

SAMPLE
Matrix: formulations
Sample preparation: Filter (0.2 μm) and inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: MeCN:10 mM sodium phosphate 40:60, pH adjusted to 3.3 with 85% phosphoric acid
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: tazobactam

KEY WORDS
stability-indicating; 5% dextrose; injections

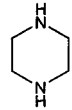
REFERENCE
Park,T.W.; Le-Bui,L.P.K.; Chung,K.C.; Rho,J.P.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium in peritoneal dialysis solutions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2022–2024.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate + 10 mM tetrabutylammonium bromide + 1% acetic acid 35:65
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 230

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Piperazine



Molecular formula: $C_4H_{10}N_2$
Molecular weight: 86.14
CAS Registry No.: 110-85-0, 18534-18-4 (phosphate monohydrate), 14538-56-8 (phosphate), 142-88-1 (adipate), 144-29-6 (citrate), 41372-10-5 (citrate hydrate), 12002-30-1 (edetate calcium), 50322-15-1 (edetate calcium dihydrate), 133-36-8 (tartrate)
Merck Index: 7617

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets to a fine powder, weigh out amount equivalent to about 200 mg piperazine citrate, dissolve in 50 mL water, sonicate for 15 min, make up to 100 mL with water, filter (0.45 μm). Remove a 3 mL aliquot and add it to 2 mL 4 mg/mL 1-benzylpiperazine in acetone:water 50:50, add 10 mL of a filtered 5 mg/mL solution of dansyl chloride in acetone, add 10 mL base solution, mix, sonicate for 10 min, let stand in the dark for 30 min, add 20 mL water, add 20 mL chloroform, shake vigorously for 1 min, filter the chloroform layer through anhydrous sodium sulfate, dilute 2 mL of the filtrate to 5 mL with mobile phase, inject an aliquot. Syrup. Dilute 5 mL syrup to 100 mL with water, mix, measure out an aliquot equivalent to about 200 mg piperazine citrate, make up to 100 mL with water, mix. Remove a 3 mL aliquot and add it to 2 mL 4 mg/mL 1-benzylpiperazine in acetone:water 50:50, add 10 mL of a filtered 5 mg/mL solution of dansyl chloride in acetone, add 10 mL base solution, mix, sonicate for 10 min, let stand in the dark for 30 min, add 20 mL water, add 20 mL chloroform, shake vigorously for 1 min, filter the chloroform layer through anhydrous sodium sulfate, dilute 2 mL of the filtrate to 5 mL with mobile phase, inject an aliquot. Granules, powders (effervescent). Weigh out amount equivalent to about 200 mg piperazine citrate, slowly

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: tazobactam

KEY WORDS
stability-indicating; 5% dextrose; injections

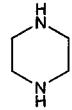
REFERENCE
Park,T.W.; Le-Bui,L.P.K.; Chung,K.C.; Rho,J.P.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium in peritoneal dialysis solutions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2022–2024.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate + 10 mM tetrabutylammonium bromide + 1% acetic acid 35:65
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 230

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Piperazine



Molecular formula: $C_4H_{10}N_2$
Molecular weight: 86.14
CAS Registry No.: 110-85-0, 18534-18-4 (phosphate monohydrate), 14538-56-8 (phosphate), 142-88-1 (adipate), 144-29-6 (citrate), 41372-10-5 (citrate hydrate), 12002-30-1 (edetate calcium), 50322-15-1 (edetate calcium dihydrate), 133-36-8 (tartrate)
Merck Index: 7617

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets to a fine powder, weigh out amount equivalent to about 200 mg piperazine citrate, dissolve in 50 mL water, sonicate for 15 min, make up to 100 mL with water, filter (0.45 μm). Remove a 3 mL aliquot and add it to 2 mL 4 mg/mL 1-benzylpiperazine in acetone:water 50:50, add 10 mL of a filtered 5 mg/mL solution of dansyl chloride in acetone, add 10 mL base solution, mix, sonicate for 10 min, let stand in the dark for 30 min, add 20 mL water, add 20 mL chloroform, shake vigorously for 1 min, filter the chloroform layer through anhydrous sodium sulfate, dilute 2 mL of the filtrate to 5 mL with mobile phase, inject an aliquot. Syrup. Dilute 5 mL syrup to 100 mL with water, mix, measure out an aliquot equivalent to about 200 mg piperazine citrate, make up to 100 mL with water, mix. Remove a 3 mL aliquot and add it to 2 mL 4 mg/mL 1-benzylpiperazine in acetone:water 50:50, add 10 mL of a filtered 5 mg/mL solution of dansyl chloride in acetone, add 10 mL base solution, mix, sonicate for 10 min, let stand in the dark for 30 min, add 20 mL water, add 20 mL chloroform, shake vigorously for 1 min, filter the chloroform layer through anhydrous sodium sulfate, dilute 2 mL of the filtrate to 5 mL with mobile phase, inject an aliquot. Granules, powders (effervescent). Weigh out amount equivalent to about 200 mg piperazine citrate, slowly

add 50 mL water with swirling, sonicate for 10 min, make up to 100 mL with water, filter (0.45 μm). Remove a 3 mL aliquot and add it to 2 mL 4 mg/mL 1-benzylpiperazine in acetone:water 50:50, add 10 mL of a filtered 5 mg/mL solution of dansyl chloride in acetone, add 10 mL base solution, mix, sonicate for 10 min, let stand in the dark for 30 min, add 20 mL water, add 20 mL chloroform, shake vigorously for 1 min, filter the chloroform layer through anhydrous sodium sulfate, dilute 2 mL of the filtrate to 5 mL with mobile phase, inject an aliquot. (Prepare base solution by dissolving 550 mg anhydrous sodium carbonate in 300 mL water, add 300 mL acetone, mix.)

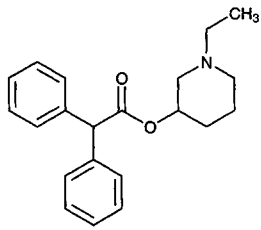
HPLC VARIABLES
Column: 250 × 4.6 5 μm CN5 SG cyanopropyl (Burdick & Jackson)
Mobile phase: Hexane:isopropanol 85:15
Flow rate: 1.5
Injection volume: 20
Detector: UV 335

CHROMATOGRAM
Retention time: 8.5
Internal standard: 1-benzylpiperazine (4.0)

KEY WORDS
derivatization; tablets; syrup; granules; powders

REFERENCE
Lau-Cam,C.A.; Roos,R.W. Normal-phase high performance liquid chromatographic method with dansylation for the assay of piperazine citrate in dosage forms, *J.Liq.Chromatogr.*, **1995**, *18*, 3347–3357.

Piperidolate



Molecular formula: $C_{21}H_{25}NO_2$
Molecular weight: 323.44
CAS Registry No.: 82-98-4, 129-77-1 (HCl)
Merck Index: 7623
Lednicer No.: 1 91

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.5

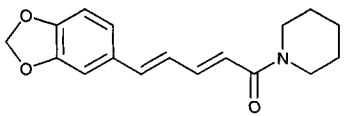
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cy-

clizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Piperine



Molecular formula: $C_{17}H_{19}NO_3$
Molecular weight: 285.34
CAS Registry No.: 94-62-2
Merck Index: 7625

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

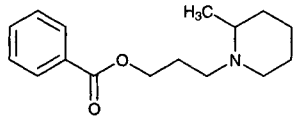
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 341.5

CHROMATOGRAM
Retention time: 20.373

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Piperocaine



Molecular formula: $C_{16}H_{23}NO_2$
Molecular weight: 261.36
CAS Registry No.: 136-82-3, 533-28-8 (HCl)
Merck Index: 7627
Lednicer No.: 1 13

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

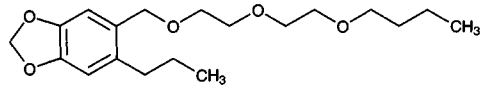
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam,

lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Piperonyl butoxide



Molecular formula: $C_{19}H_{30}O_5$
Molecular weight: 338.44
CAS Registry No.: 51-03-6
Merck Index: 7629

SAMPLE

Matrix: blood
Sample preparation: Dilute plasma with an equal volume of water. Inject a 20-100 μL aliquot onto column A and elute to waste with mobile phase A, after 5 min elute the contents of column A onto column B with mobile phase B, after 30 s remove column A from the circuit, elute column B with mobile phase B and monitor the effluent from column B. Wash column A with MeOH:water 10:90 for 10.5 min and with water for 1 min.

HPLC VARIABLES

Column: A 5 × 4 30-40 μm Perisorb RP-18 (Merck); B 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: A water; B MeOH:water 82:18
Flow rate: 0.5
Injection volume: 20-100
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Limit of detection: 130 ng/mL

OTHER SUBSTANCES

Extracted: pyrethrins

KEY WORDS

plasma; column-switching

REFERENCE
Wintersteiger,R.; Ofner,B.; Juan,H.; Windisch,M. Determination of traces of pyrethrins and piperonyl butoxide in biological material by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *660*, 205–210.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 27.627

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: fruit, grain, vegetables
Sample preparation: Homogenize (Polytron) 150 g high moisture sample and 300 mL MeOH at half speed for 30 s and full speed for 1 min, filter (paper) under vacuum, remove portion of filtrate equal to 100 g sample and make up to 100 mL with water. Homogenize (Polytron) 75 g low moisture sample and 300 mL MeOH at half speed for 30 s and full speed for 1 min, filter (paper) under vacuum, remove portion of filtrate equal to 50 g sample and make up to 100 mL with water. Concentrate samples to 75 mL under reduced pressure at 35°, add 15 g NaCl, add 75 mL MeCN, shake for 30 s, let stand for 5 min. Remove the aqueous phase and add it to 50 mL MeCN, shake for 20 s, let layers separate, discard the aqueous layer. Combine the MeCN layers, wash with 25 mL 20% NaCl, wash with 100 mL petroleum ether, extract petroleum ether layer with 10 mL MeCN. Combine the MeCN layers and add them to 50 mL 2% NaCl, extract with 100 mL dichloromethane, extract twice with 25 mL portions of dichloromethane. Combine the dichloromethane layers and pass them through a 22 mm i.d. column containing 5 g anhydrous sodium sulfate. Evaporate the eluate to dryness under reduced pressure at 35°, reconstitute in 10 mL dichloromethane, add to the charcoal column, rinse flask with 10 mL dichloromethane, rinse flask with 25 mL MeCN:toluene 75:25. Evaporate the eluate to dryness under reduced pressure at 35°, reconstitute with 5 mL MeOH, filter (5 μm), inject a 10 μL aliquot (J. Assoc. Off.Anal. Chem. 1980, 63, 1114). (Charcoal column was 5 g silanized Celite 545:Nuchar S-N 4:1 on top of 0.5 g silanized Celite 545 in a 300 × 22 glass column, wash with 50 mL MeCN:toluene 75:25, do not allow to go dry. Prepare silanized Celite 545 as follows. Boil 150 g Celite 545 in 1 L 6 M HCl with stirring for 10 min, cool, filter, wash with water until filtrate is neutral, wash with 500 mL MeOH, wash with 500 mL dichloromethane, air dry in hood, heat to 120° in a flask, cool in a desiccator, add 3 mL dichlorodimethylsilane, mix

well, let stand at room temperature for 4 h, add 500 mL MeOH, mix, let stand for 15 min, filter, wash with isopropanol until neutral, air dry in hood, dry at 105° for 2 h, cool in desiccator, store in stoppered container. Totally silanized Celite should float on water and appear yellow (not pink) in toluene saturated with methyl red (J. Assoc. Off.Anal. Chem. 1980, 63, 1114).)

HPLC VARIABLES
Guard column: 70 × 2.1 25-37 μm Co-Pell ODS
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: Gradient. MeCN:water from 12:88 to 70:30 over 30 min, 100:0 for 5 min.
Column temperature: 35
Flow rate: 1.5
Injection volume: 10
Detector: F ex 288 em 330 following post-column reaction. The column effluent mixed with 200 mM NaOH pumped at 0.5 mL/min and flowed through a 3 m × 0.48 mm stainless steel column to the detector.

CHROMATOGRAM
Retention time: 35
Limit of quantitation: 50 ppb

OTHER SUBSTANCES
Extracted: carbaryl, carbofuran, napropamide, phosalone

KEY WORDS
post-column reaction; pears; green beans

REFERENCE
Krause,R.T.; August,E.M. Applicability of a carbamate insecticide multiresidue method for determining additional types of pesticides in fruits and vegetables, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 234–240.

SAMPLE
Matrix: rice
Sample preparation: 30 g Rice + 50 mL acetone, let stand with occasional shaking for 48 h, evaporate a 1 mL aliquot to near dryness under a stream of nitrogen, shake the remaining few drops twice with 1 mL portions of hexane. Add the hexane extracts to a Florisil Sep-Pak SPE cartridge, elute with 3 mL acetone:hexane 15:85. Collect all the eluates and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL MeOH, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 30 s elute the contents of column A onto column B with mobile phase B, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A Guard Pak; B 150 × 3.9 Novapak C18
Mobile phase: A MeCN:water 40:60; B MeCN:water 75:25
Flow rate: 1
Injection volume: 100
Detector: UV 225

CHROMATOGRAM
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: bioremethrin, deltamethrin, fenvalerate, permethrin, phenothrin

KEY WORDS
column-switching; SPE

REFERENCE
Haddad,P.R.; Brayan,J.G.; Sharp,G.J.; Dilli,S.; Desmarchelier,J.M. Determination of pyrethroid residues on paddy rice by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *461*, 337–346.

SAMPLE
Matrix: rice
Sample preparation: 30 g Rice + 50 mL acetone, let stand with occasional shaking for 48 h, inject a 10 μL aliquot.

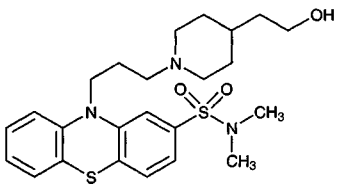
HPLC VARIABLES
Guard column: Guard Pak
Column: 150 × 3.9 Novapak C18
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 10
Detector: UV 225

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 1.7 μg/g

OTHER SUBSTANCES
Extracted: bioremethrin, deltamethrin, fenvalerate, permethrin, phenothrin

REFERENCE
Haddad,P.R.; Brayan,J.G.; Sharp,G.J.; Dilli,S.; Desmarchelier,J.M. Determination of pyrethroid residues on paddy rice by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *461*, 337–346.

Pipotiazine



Molecular formula: $C_{24}H_{33}N_3O_3S_2$
Molecular weight: 475.68
CAS Registry No.: 39860-99-6, 37517-26-3 (palmitic ester)
Merck Index: 7636

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

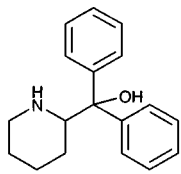
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 262.9

CHROMATOGRAM
Retention time: 14.695

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pipradrol



Molecular formula: $C_{18}H_{21}NO$
Molecular weight: 267.37
CAS Registry No.: 467-60-7, 71-78-3 (HCl)
Merck Index: 7638
Lednicer No.: 1 47

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

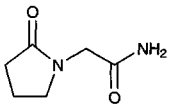
CHROMATOGRAM
Retention time: 2.0

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physo-

stigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Piracetam



Molecular formula: $C_6H_{10}N_2O_2$
Molecular weight: 142.16
CAS Registry No.: 7491-74-9
Merck Index: 7641

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Dilute sample with MeOH:water 50:50, inject a 100 μL aliquot. Serum. Mix 1 mL serum with 8 mL cold MeOH:dichloromethane 10:40, centrifuge at 3000-5000 rpm for 20 min. Remove the upper MeOH-water layer and add it to 9.5 mL cold MeOH. Cool a 2 mL sample at 8° for 20 min, centrifuge for 10 min, dry the clear MeOH/water residue at 60° under a stream of nitrogen. Extract the residue three times with 100 μL MeOH:dichloromethane 20:80, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 125 5 μm LiChrospher 60 RP-Select-B
Flow rate: 0.8
Injection volume: 100
Detector: UV 218, UV 212

CHROMATOGRAM
Limit of quantitation: 100 μg/mL (urine), 4 μg/mL (serum)

KEY WORDS
serum

REFERENCE
Bockhard,H.; Oelschläger,H.; Pooth,R. Rasche dünnschicht-densitometrische Bestimmung des Nootropikums Piracetam in biologischem Material [Fast detection of the nootropic drug piracetam in biological fluids], *Pharmazie*, **1997**, *52*, 357–361.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

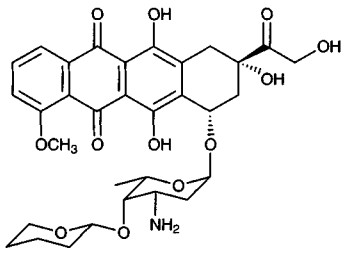
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.295

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pirarubicin



Molecular formula: $C_{32}H_{37}NO_{12}$
Molecular weight: 627.65
CAS Registry No.: 72496-41-4
Merck Index: 7642

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma or blood + 3 mL 100 mM pH 9.5 ammonia-ammonium chloride buffer + 20 ng daunorubicin + 13.5 mL chloroform:MeOH 2:1, shake mechanically for 30 min, centrifuge at 3000 g for 10 min, repeat the extraction with 9 mL chloroform. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30°, reconstitute the residue in 3 mL chloroform:MeOH 2:1, evaporate this mixture, reconstitute the residue in 300 μL mobile phase, centrifuge a 75 μL aliquot at 10000 g for 1 min, inject the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm STR ODS-M (Shimadzu)
Mobile phase: MeCN:buffer 30:70 (Buffer was 200 mM acetic acid-ammonium formate, pH 4.0.)
Column temperature: 22
Flow rate: 0.7
Injection volume: 75
Detector: F ex 470 em 550

CHROMATOGRAM
Retention time: 24.1
Internal standard: daunorubicin (16.2)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: doxorubicin

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Nagasawa,K.; Yokoyama,T.; Ohnishi,N.; Iwakawa,S.; Okumura,K.; Kosaka,Y.; Sano,K.; Murakami,R.; Nakamura,H. Pharmacokinetics of pirarubicin in pediatric patients, *J.Pharmacobiodyn.*, **1991**, *14*, 222–230.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 250 μL 100 ng/mL daunorubicin in mobile phase, extract with 3 mL MeCN for 10 min, add 100 mg NaCl, shake for 5 min, centrifuge at 995 g for 15 min, let stand at -20° for 1 h. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Spherisorb phenyl
Column: 250 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:30 mM citrate buffer adjusted to pH 4 with formic acid 30:70
Column temperature: 50
Flow rate: 1.5
Injection volume: 100
Detector: F ex 480 em 590

CHROMATOGRAM
Retention time: 10.8
Internal standard: daunorubicin (8.5)
Limit of detection: 1 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: doxorubicin, doxorubicinol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jacquet,J.M.; Galtier,M.; Bressolle,F.; Jourdan,J. A sensitive and reproducible HPLC assay for doxorubicin and pirarubicin, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 343–348.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 3 mL 10 mM pH 9.0 ammonium chloride buffer, adjust pH to 9.0 with NaOH, add 6 mL chloroform:MeOH 2:1, shake for 5 min, centrifuge at 12000 g at 4° for 10 min, remove the organic layer, re-adjust the pH of the aqueous layer, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Nova-Pak C18 phenyl
Mobile phase: MeCN:35 mM pH 3.0 ammonium formate buffer 35:65
Flow rate: 0.8
Detector: F ex 254 em 550

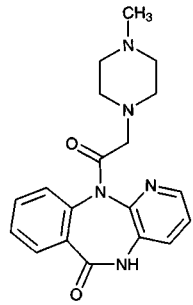
CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: doxorubicin, metabolites

KEY WORDS
plasma; SPE

REFERENCE
Raber,M.N.; Newman,R.A.; Lu,K.; Legha,S.; Gorski,C.; Benjamin,R.S.; Krakoff,I.H. Phase I clinical trial and pharmacokinetic evaluation of 4'-O-tetrahydropyranyladriamycin (THP-adriamycin), *Cancer Chemother.Pharmacol.*, **1989**, *23*, 311–315.

Pirenzepine



Molecular formula: $C_{19}H_{21}N_5O_2$
Molecular weight: 351.41
CAS Registry No.: 28797-61-7, 29868-97-1 (2.HCl)
Merck Index: 7646

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.3

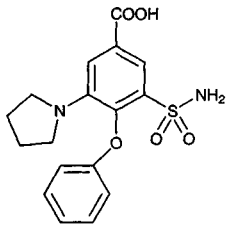
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metopro-

lol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Piretanide



Molecular formula: $C_{17}H_{18}N_2O_5S$
Molecular weight: 362.41
CAS Registry No.: 55837-27-9
Merck Index: 7647
Lednicer No.: 3 58

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

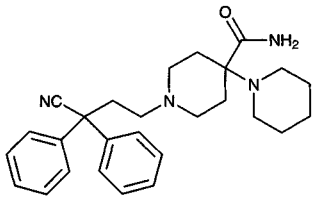
Retention time: 17.8

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Piritramide



Molecular formula: $C_{27}H_{34}N_4O$
Molecular weight: 430.59
CAS Registry No.: 302-41-0
Merck Index: 7653
Lednicer No.: 1 308

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.4

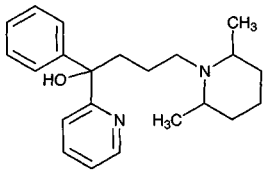
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, pro-

thipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Pirmenol



Molecular formula: $C_{22}H_{30}N_2O$
Molecular weight: 338.49
CAS Registry No.: 68252-19-7, 61477-94-9 (HCl)
Merck Index: 7656
Lednicer No.: 3 48

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 150 μL 1 M NaOH + 50 μL 40 μg/mL chlorodisopyramide in MeOH + 5 mL dichloromethane, shake (Labquake) for 10 min, centrifuge at 1000 g for 10 min. Remove the organic layer (avoid contamination with aqueous phase) and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm C18 (Altex) (At the beginning of each series of analyses use a conditioning injection of 4 μg pirmenol and IS.)
Mobile phase: MeCN:buffer 6:94 (Buffer was 10 mM K_2HPO_4 adjusted to pH 2.4 using phosphoric acid containing 375 μL nonylamine.)
Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Retention time: 2.9
Internal standard: chlorodisopyramide (3.6)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, carbamazepine, chloramphenicol, desipramine, digoxin, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, lithium, methotrexate, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, propranolol, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS

serum

REFERENCE

Hoyer,G.L. Pirmenol determination by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *565*, 497–503.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 100 μL 10 mM HCl + 100 μL 2.5 μg/mL (+)-propranolol in 10 mM HCl + 200 μL 1 M NaOH + 5 mL toluene, shake for 10 min, centrifuge at 750 g for 5 min, freeze in dry ice/acetone. Remove the toluene layer and add it to 500 μL 100 mM HCl, shake for 10 min, centrifuge at 420 g for 5 min, freeze in dry ice/acetone. Discard the toluene layer and thaw the aqueous layer, add 200 μL 1 M NaOH to the aqueous layer, add hexane, shake for 10 min, centrifuge at 420 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 175 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: Hexane:isopropanol:diethylamine 98.9:1:0.1
Column temperature: 65
Flow rate: 1
Injection volume: 175
Detector: UV 262

CHROMATOGRAM

Retention time: 7.4 (+), 9.2 (-)
Internal standard: (+)-propranolol hydrochloride (20.9)
Limit of quantitation: 20 ng/mL

KEY WORDS

dog; plasma; chiral; pharmacokinetics

REFERENCE

Janiczek,N.; Bockbrader,H.N.; Chang,T.; Amidon,G.L.; Smith,D.E. Stereoselective high-performance liquid chromatographic assay for pirmenol enantiomers in dog plasma, *J.Chromatogr.*, **1991**, *571*, 179–187.

SAMPLE

Matrix: blood, urine
Sample preparation: Blood. Mix 500 μL whole blood or plasma with 50 μL 20 μg/mL IS in 10 mM phosphoric acid, 100 μL 1 M NaOH, and 4 mL diethyl ether. Extract using a Labquake automatic shaker for 10 min, centrifuge at 1000 g for 5 min. Freeze the aqueous phase and remove the organic phase. Add 80 μL 100 mM phosphoric acid to the organic phase, vortex for 40 s, centrifuge, inject a 50 μL aliquot of the aqueous layer. Urine. Mix 500 μL urine with 50 μL 20 μg/mL IS in 10 mM phosphoric acid, 2 mL 100 mM sodium carbonate, and 4 mL diethyl ether. Extract using a Labquake automatic shaker for 10 min, centrifuge at 1000 g for 5 min. Freeze the aqueous phase and remove the organic phase. Add 80 μL 100 mM phosphoric acid to the organic layer, vortex for 40 s, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 6 μm Zorbax TMS
Mobile phase: MeCN:triethylamine:50 mM ammonium dihydrogen phosphate 15:0.5:85, adjusted to pH 2.6 with 1 M phosphoric acid
Flow rate: 1.1
Injection volume: 50
Detector: UV 262

CHROMATOGRAM

Retention time: 5
Internal standard: disopyramide (11)
Limit of quantitation: 100 ng/mL (blood, plasma), 5 μg/mL (urine)

KEY WORDS

plasma; whole blood

REFERENCE

Shand,D.G.; Verghese,C.; Barchowsky,A.; Hammill,S.C.; Pritchett,E.L.C. High-performance liquid chromatographic analysis of a new antiarrhythmic drug, pirmenol, in biological fluids, *J.Chromatogr.B*, **1981**, *224*, 343–347.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 500 μL 50 mM HCl + 500 μL 3.33 μg/mL IS in 50 mM HCl + 4 mL cyclohexane + 150 μL 1 M NaOH, shake horizontally for 10 min, centrifuge at 206 g for 5 min. Remove 3 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot. Urine. 1 mL Urine + 250 μL 50 mM HCl + 4 mL cyclohexane + 200 μL 1 M NaOH, shake horizontally for 10 min, centrifuge at 206 g for 5 min. Remove 3 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 1 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax TMS
Mobile phase: MeCN:buffer 15:85 (Buffer was 6.9 g $NaH_2PO_4.H_2O$ and 5 mL triethylamine in water, adjust pH to 2.6 with phosphoric acid, make up to 1 L with water.)
Flow rate: 1.5
Injection volume: 50-100
Detector: UV 254

CHROMATOGRAM
Retention time: 6.4
Internal standard: (±)-cis-2-[4-(2,6-dimethyl-1-piperidinyl)-1-phenylbutyl]pyridine monohydrochloride (9.5)
Limit of quantitation: 1 μg/mL (urine), 125 ng/mL (plasma)

OTHER SUBSTANCES
Simultaneous: aspirin, bretylium tosylate, chlorthalidone, naproxen, oxazepam, quinidine salicylic acid, triamterene, zomepirac
Noninterfering: acetaminophen, clonidine, diazepam, disopyramide, ethacrynic acid, fenoprofen, furosemide, hydralazine, hydrochlorothiazide, ibuprofen, methotrimeprazine, methyldopa, prazepam, prazosin, procainamide, propranolol, propoxyphene, reserpine, tolmetin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Johnson,E.L.; Pachla,L.A. Improved liquid chromatographic assay for the analysis of pirmenol in plasma and urine, *J.Pharm.Sci.*, **1984**, *73*, 754–756.

SAMPLE
Matrix: blood, urine
Sample preparation: Add IS to plasma and urine, extract with cyclohexane. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Zorbax TMS
Mobile phase: MeCN:buffer 15:85 (Buffer was 50 mM pH 2.58 sodium phosphate buffer containing 0.5% triethanolamine.)
Flow rate: 2
Detector: UV 262 (plasma), UV 254 (urine)

CHROMATOGRAM
Internal standard: PD-92038

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Stringer,K.A.; Cetnarowski,A.B.; Goldfarb,A.; Lebsack,M.E.; Chang,T.; Sedman,A.J. Enhanced pirmenol elimination by rifampin, *J.Clin.Pharmacol.*, **1988**, *28*, 1094–1097.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

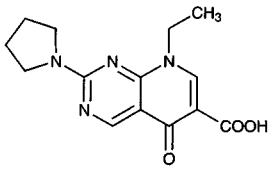
HPLC VARIABLES
Column: 150 × 6 YMC-PAK AM-312 ODS (YMC)
Mobile phase: Gradient. MeCN:50 mM pH 7.2 ammonium phosphate buffer 10:88:2 for 10 min, 17.5:80.5:2 for 5 min, 25:73:2 for 12 min, 40:58:2 for 13 min (step gradient (?)).
Column temperature: 45
Flow rate: 1 for 27 min then 1.2 for 13 min
Detector: UV 254

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Sakano,I.; Ishii,T.; Ichikawa,S.; Harasawa,K.; Minohara,K.; Yamamura,S.; Nishiyama,S. Isolation and structure elucidation of the major photodegradation products of pirmenol hydrochloride, *J.Pharm.Sci.*, **1994**, *83*, 1363–1366.

Piromidic acid



Molecular formula: $C_{14}H_{16}N_4O_3$
Molecular weight: 288.31
CAS Registry No.: 19562-30-2
Merck Index: 7660

SAMPLE
Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

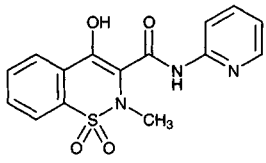
HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:400 μM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 8.51
Limit of detection: 1.02 μg/mL

OTHER SUBSTANCES
Simultaneous: cinoxacin, nalidixic acid, oxolinic acid, pipemidic acid,

REFERENCE
Durán Mer,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

Piroxicam



Molecular formula: $C_{15}H_{13}N_3O_4S$
Molecular weight: 331.35
CAS Registry No.: 36322-90-4, 87234-24-0 (cinnamic acid ester), 85056-47-9 (piroxicam olamine)
Merck Index: 7661
Lednicer No.: 4 173

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 40 μg/mL tenoxicam in MeOH + 1 mL pH 2 phosphate buffer + 10 mL diethyl ether, vortex for 1 min, centrifuge at 1300 g for 5 min. Evaporate the organic layer to dryness under a stream of nitrogen. Reconstitute the residue with 100 μL 10 mM HCl in MeOH, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak ODS
Mobile phase: MeOH:10 mM pH 2 phosphate buffer 45:55
Flow rate: 1.5
Injection volume: 40
Detector: UV 361

CHROMATOGRAM

Retention time: 9.85
Internal standard: tenoxicam (5.81)
Limit of detection: 20 ng/mL
Limit of quantitation: 200 ng/mL

KEY WORDS

plasma; rat

REFERENCE

Amanlou,M.; Dehpour,A.R. Rapid method for the determination of piroxicam in rat plasma using high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *696*, 317–319.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.57

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: perfusate
Sample preparation: Mix 1 mL perfusate with 100 μL 1 M HCl, 100 μL 1.5 mg/mL IS solution, and 8 mL diethyl ether. Vortex for 1 min, centrifuge at 3000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under nitrogen. Reconstitute the residue with 2 mL mobile phase. Inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Econosphere C18
Mobile phase: MeOH:40 mM pH 8.0 phosphate buffer 40:60
Injection volume: 30
Detector: UV 330

CHROMATOGRAM
Retention time: 5.2
Internal standard: naproxen (7.5)
Limit of detection: 1 μg/mL

REFERENCE
Takamatsu,N.; Welage,L.S.; Idkaidek,N.M.; Liu,D.Y.; Lee,P.I.-D.; Hayashi,Y.; Rhie,J.K.; Lennernäs,H.; Barnett,J.L.; Shah,V.P.; Lesko,L.; Amidron,G.L. Human intestinal permeability of piroxicam, propranolol, phenylalanine, and PEG 400 determined by jejunal perfusion, *Pharm.Res.*, **1997**, *14*, 1127–1132.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:10 mM Na_2HPO_4 buffer containing 10 mM citric acid 70:30
Flow rate: 1
Detector: UV 205

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230, UV 320

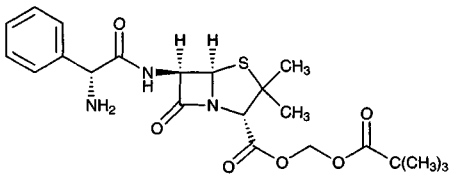
CHROMATOGRAM
Retention time: 5
Internal standard: indomethacin (18.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, fenbufen, flurbiprofen, ketoprofen, loxoprofen, mefenamic acid, naproxen, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

Pivampicillin



Molecular formula: $C_{22}H_{29}N_3O_6S$
Molecular weight: 463.55
CAS Registry No.: 33817-20-8, 26309-95-5 (HCl)
Merck Index: 7669
Lednicer No.: 1 414

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

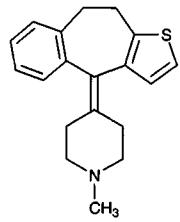
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.813

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pizotyline



Molecular formula: $C_{19}H_{21}NS$
Molecular weight: 295.45
CAS Registry No.: 15574-96-6, 73391-87-4 (HCl)
Merck Index: 7671
Lednicer No.: 2 420

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.197

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan,

benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

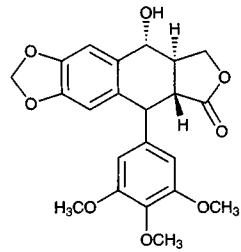
Retention time: k' 38.73

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Podofilox



Molecular formula: $C_{22}H_{22}O_8$
Molecular weight: 414.41
CAS Registry No.: 518-28-5
Merck Index: 7704

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 500 mg Bond-Elut PH SPE cartridge with 6 mL MeOH and 3 mL 20 mM pH 5.5 ammonium acetate. Mix 500 µL serum with 500 µL 20 mM pH 5.5 ammonium acetate and 50 µL 760 mM sodium dodecyl sulfate. Add to the SPE cartridge, wash with 3 mL 20 mM ammonium acetate and 3 mL MeOH:water 10:90. Elute with 2 mL MeOH, evaporate to dryness at 43° under reduced pressure, reconstitute in 150 µL MeOH:water 36:64.

HPLC VARIABLES
Column: 300 × 3.9 Bondclone 10 C18 (Phenomenex, Torrance, CA, USA)
Mobile phase: MeOH:40 mM pH 6.9 KH_2PO_4:0.14 mM 1-heptanesulfonic acid 40:60:0.6
Flow rate: 2
Detector: F ex 230 em 330

CHROMATOGRAM
Retention time: 28
Internal standard: podofilox
Limit of detection: 200 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: etoposide

KEY WORDS
serum; SPE; pharmacokinetics; podofilox is IS

REFERENCE
Manouilov,K.K.; McGuire,T.R.; Gordon,B.G.; Gwilt,P.R. Assay for etoposide in human serum using solid-phase extraction and high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1998**, *707*, 342–346.

SAMPLE
Matrix: plants
Sample preparation: Grind 10 g freeze-dried rhizomes to 20 mesh, add 100 mL water, stir, boil for 20 min, cool to 40°, centrifuge at 8000 g for 30 min, freeze-dry the residue. Shake 50 mg residue with 5 mL mobile phase for 5 min, centrifuge at 1500 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 32 × 5 Co:Pell ODS C18 pellicular
Column: 250 × 4.6 Partisil 10 ODS 3 C18
Mobile phase: MeCN:water 40:60
Flow rate: from 2 to 7 over 5 min (Waters program 8)

Detector: UV 254

CHROMATOGRAM
Retention time: k' 5.0

OTHER SUBSTANCES
Extracted: peltatin, picropodophyllotoxin, desoxypodophyllotoxin

REFERENCE
Bedows,E.; Hatfield,G.M. An investigation of the antiviral activity of *Podophyllum peltatum*, *J.Nat.Prod.*, **1982**, *45*, 725–729.

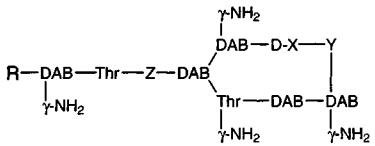
SAMPLE
Matrix: plants
Sample preparation: Extract from root and rhizome with 95% EtOH.

HPLC VARIABLES
Mobile phase: MeOH:water 50:50
Detector: UV 285

REFERENCE
Bai,Y.; Xu,J. [Determination of podophyllotoxin content in the root and rhizome of Podophyllum emodi by HPLC] (Chem.Abs. 1988, 109, 116127v), *Zhongyao Tongbao*, **1988**, *13*, 217–219.

Polymyxin

Molecular formula: $C_{56}H_{98}N_{16}O_{13}$ (B_1)
Molecular weight: 1203.49 (B_1)
CAS Registry No.: 1406-11-7, 1404-26-8 (B), 1405-20-5 (B sulfate)
Merck Index: 7734



DAB = L-α,γ-diaminobutyric acid

Polymyxin B_1	R = (+)-6-methyloctanoyl	X = Phe	Y = Leu	Z = DAB
B_2	R = 6-methylheptanoyl	X = Phe	Y = Leu	Z = DAB
D_1	R = (+)-6-methyloctanoyl	X = Leu	Y = Thr	Z = D-Ser
D_2	R = 6-methylheptanoyl	X = Leu	Y = Thr	Z = D-Ser

SAMPLE
Matrix: formulations
Sample preparation: Sandwich cream or ointment between two layers of 200 mesh silica gel, extract with carbon dioxide:MeOH 95:5 at 300 atmospheres at 55° at 2 mL/min for 75 min (restrictor 300°), sonicate the SPE tube, frits, and silica gel with MeOH:100 mM HCl 25:75 containing 0.1% Tween 80 for 15 min, filter (0.2 μm) inject an aliquot of the filtrate. (SFE removes the hydrocarbon base of the cream or ointment leaving behind the insoluble polymyxin.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Synchropak SCD
Mobile phase: MeCN:buffer 21.5:78.5 (Buffer was 100 mM KH_2PO_4 containing 0.1% trifluoroacetic acid.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Limit of quantitation: 0.016%

KEY WORDS
SFE; cream; ointment; SPE

REFERENCE
Moore,W.N.; Taylor,L.T. Analytical inverse supercritical fluid extraction of polar pharmaceutical compounds from cream and ointment matrices, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1227–1232.

SAMPLE
Matrix: solutions
Sample preparation: Inject 10 μL of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac TP C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.075% trifluoroacetic acid in MeCN. A:B from 90:10 to 20:80 over 20 min.
Flow rate: 1.2
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 12.0

OTHER SUBSTANCES
Simultaneous: degradation products.

REFERENCE
Vaara,M. Analytical and preparative high-performance liquid chromatography of the papain-cleaved derivative of polymyxin B, *J.Chromatogr.*, **1988**, *441*, 423–430.

SAMPLE
Matrix: solutions

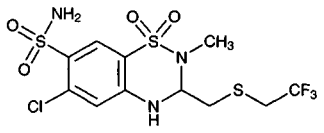
HPLC VARIABLES
Guard column: 45 × 4.7 Ultrasphere C18
Column: 250 × 4.7 Ultrasphere C18
Mobile phase: Gradient. A was 0.15% trifluoroacetic acid in water. B was 0.15% trifluoroacetic acid in MeCN. A:B from 100:0 to 50:50 over 25 min.
Flow rate: 2
Detector: UV 215

CHROMATOGRAM
Retention time: 18.5

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Danner,R.L.; Joiner,K.A.; Rubin,M.; Patterson,W.H.; Johnson,N.; Ayers,K.M.; Parrillo,J.E. Purification, toxicity, and antiendotoxin activity of polymyxin B nonapeptide, *Antimicrob.Agents Chemother.*, **1989**, *33*, 1428–1434.

Polythiazide



Molecular formula: $C_{11}H_{13}ClF_3N_3O_4S_3$
Molecular weight: 439.89
CAS Registry No.: 346-18-9
Merck Index: 7744
Lednicer No.: 1 360

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 500 μL 1 M citric acid, mix, inject onto column A with mobile phase A and elute to waste, elute column A to waste with mobile phase B, elute polythiazide from column A with mobile phase C into a mixing chamber where it is mixed with 50 mM trifluoroacetic acid pumped at 2.4 mL/min before flowing onto column B, elute column B with mobile phase D, monitor the effluent from column B. (Backflush column A with MeCN: 50 mM trifluoroacetic acid 80:20 then forward flush with 20 mM tris acetate.)

HPLC VARIABLES

Column: A 60 × 4 PRP-1 (Hamilton); B 30 × 4 silica ODS (Shandon) + 250 × 4 5 μm Hypersil ODS
Mobile phase: A 790 mL 20 mM Citric acid + 210 mL 20 mM LiOH, pH 3; B MeOH:buffer 42: 58 (Buffer was 20 mM pH 7 tris acetate lithium hydroxide.); C MeOH:buffer 58:42 (Buffer was pH 11 citric acid lithium hydroxide.); D Gradient. MeCN:50 mM trifluoroacetic acid from 20: 80 to 58:42
Flow rate: A 1; B 2.5; C 1.3; D ?
Injection volume: 1500
Detector: UV 269

CHROMATOGRAM

Retention time: 13
Limit of detection: 0.5 ng/mL

KEY WORDS

serum; column-switching

REFERENCE

Schöneshöfer,M.; Heilmann,P.; Rejaibi,R. Automated column liquid chromatographic determination of polythiazide in human serum, *J.Chromatogr.*, **1987**, *417*, 434–438.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 3 mL Plasma + 1.5 mL 10 mM NaOH + 800 μL 100 mM HCl + 10 mL dichloromethane, shake on a platform shaker for 20 min, centrifuge at -10° at 3000 rpm for 15 min, repeat extraction twice more. Combine all organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 μL, vortex, inject whole amount. Urine. 5 mL Urine + 2 mL 10 mM NaOH + 2 mL 0.68% KH_2PO_4 adjusted to pH 6.1 + 10 mL dichloromethane, shake on a platform shaker for 20 min, centrifuge at -10° at 3000 rpm for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:glacial acetic acid:water 35:2:63 (plasma) or MeCN:water 40:60 (urine)
Flow rate: 2
Injection volume: 20-50
Detector: UV 280

CHROMATOGRAM

Retention time: 9 (plasma), 8 (urine)

Internal standard: polythiazide

OTHER SUBSTANCES
Extracted: benzthiazide

KEY WORDS
polythiazide is IS; plasma

REFERENCE
Meyer,M.C.; Hwang,P.; Straughn,A.B.; Rotenberg,K. HPLC determination of benzthiazide in biologic material, *Biopharm.Drug Dispos.*, **1982**, *3*, 1–9.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM
Retention time: 14.5 (A), 15.2 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil
Interfering: bendroflumethiazide

REFERENCE
Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN: water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS

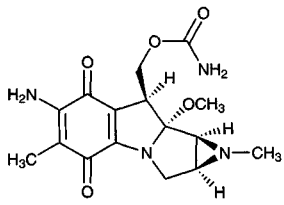
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 6.9
Internal standard: 7-propyltheophylline (4.5)

OTHER SUBSTANCES
Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, buthiazide, benzthiazide, canrenone, caffeine, clopamide, chlorthalidone, cyclothiazide, diclofenamide, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, probenecid, spironolactone, torsemide, triamterene
Interfering: bendroflumethiazide, ethacrynic acid

REFERENCE
Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

Porfiromycin



Molecular formula: $C_{16}H_{20}N_4O_5$
Molecular weight: 348.36
CAS Registry No.: 801-52-5
Merck Index: 7756

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 15800 g at 4° for 5 min. 500 µL Plasma + 1 mL MeCN, stir for 1 min, centrifuge at 15800 g for at 4° for 10 min. Lyophilize the supernatant in a vacuum centrifuge (Hetovac VR-1, Allerod, Denmark). Reconstitute the residue in 150 µL MeOH:10 mM pH 6.5 NaH_2PO_4 30:70. Inject a 50 µL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 µm Hypersil ODS
Mobile phase: MeOH:10 mM pH 6.5 NaH_2PO_4 30:70
Column temperature: 30
Flow rate: 1.3
Injection volume: 50
Detector: UV 365

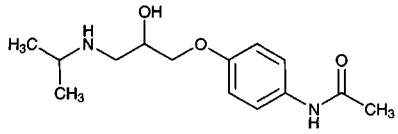
CHROMATOGRAM
Retention time: 6.64
Internal standard: porfiromycin

OTHER SUBSTANCES
Extracted: mitomycin
Noninterfering: dexamethasone, lorazepam, mezlocillin, ondansetron, meperidine

KEY WORDS
plasma; porfiromycin is IS

REFERENCE
Joseph,G.; Biederbick,W.; Woschée,U.; Theisohn,M.; Klaus,W. Sensitive and convenient high-performance liquid chromatographic method for the determination of mitomycin C in human plasma, *J.Chromatogr.B*, **1997**, *698*, 261–267.

Practolol



Molecular formula: $C_{14}H_{22}N_2O_3$
Molecular weight: 266.34
CAS Registry No.: 6673-35-4
Merck Index: 7882
Lednicer No.: 2 106

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

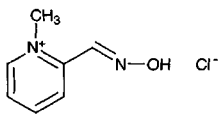
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.86

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Pralidoxime chloride



Molecular formula: $C_7H_9ClN_2O$
Molecular weight: 172.61
CAS Registry No.: 51-15-0, 94-63-3 (iodide), 154-97-2 (mesylate)
Merck Index: 7884

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 295

CHROMATOGRAM
Retention time: 2.863

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1 mL formulation to 25 mL with mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:buffer A:buffer B:water 10:1:9:80 containing 2.66 g/L sodium lauryl sulfate (Buffer A was 98 g orthophosphoric acid in 800 mL water, adjust to pH 3.0 with 25% trimethylamine solution, make up to 1 L with water. Buffer B was obtained by mixing 1 M NaH_2PO_4 and 1 M orthophosphoric acid to obtain a pH of 3.0.)
Column temperature: 30
Flow rate: 1.5
Injection volume: 3
Detector: UV 262

CHROMATOGRAM
Retention time: 12.5

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; buffer

REFERENCE
Utley,D. Analysis of formulations containing pralidoxime mesylate by liquid chromatography, *J.Chromatogr.*, **1987**, *396*, 237–250.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 25 μL formulation to 100 mL with mobile phase, inject a 45-60 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μPorasil
Mobile phase: MeCN:buffer 86:14 (Buffer was 52.5 mM acetic acid containing 8.36 mM tetraethylammonium chloride, pH 2.9.)
Flow rate: 1
Injection volume: 45-60
Detector: UV 295

CHROMATOGRAM
Retention time: 8.5

OTHER SUBSTANCES
Simultaneous: degradation products (UV 266.5)

KEY WORDS
stability-indicating; injections

REFERENCE
Schroeder,A.C.; DiGiovanni,J.H.; Von Bredow,J.; Heiffer,M.H. Pralidoxime chloride stability-indicating assay and analysis of solution samples stored at room temperature for ten years, *J.Pharm.Sci.*, **1989**, *78*, 132–136.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject a 20 μL aliquot.

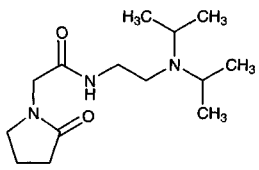
HPLC VARIABLES
Guard column: 12.5 × 4 5 μm Zorbax RX-C18
Column: 250 × 4.6 5 μm Zorbax RX-C18
Mobile phase: MeCN:buffer 3:97 (Buffer was 50 mM NaH_2PO_4 + 1 mM tetramethylammonium chloride + 1 mM 1-octanesulfonic acid adjusted to pH 3.5 with concentrated orthophosphoric acid.)
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 203

OTHER SUBSTANCES
Also analyzed: atropine, phenol, tropic acid, obidoxime, HI-6

KEY WORDS
nerve agent antidote mixtures

REFERENCE
Paddle,B.M.; Dowling,M.H. Simple high-performance liquid chromatographic method for assessing the deterioration of atropine-oxime mixtures employed as antidotes in the treatment of nerve agent poisoning, *J.Chromatogr.*, **1993**, *648*, 373–380.

Pramiracetam



Molecular formula: $C_{14}H_{27}N_3O_2$
Molecular weight: 269.39
CAS Registry No.: 68497-62-1, 75733-50-5 (HCl), 72869-16-0 (sulfate)
Merck Index: 7886

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 3 μg IS + 100 μL 100 mM NaOH + 5 mL chloroform, mix, centrifuge at 2000 g for 3 min. Remove the organic layer and evaporate it to dryness at 70°, reconstitute the residue in 500 μL 1 M (sic) HCl and 2 mL dichloromethane:isopropanol 90:10, centrifuge. Remove the aqueous phase and add it to 700 μL 100 mM NaOH and 5 mL chloroform, mix, centrifuge. Remove the organic layer and evaporate it to dryness at 70°, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 mm long 3 μm Spherisorb ODS
Mobile phase: MeCN:70 mM pH 5.5 KH_2PO_4 30:70
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Internal standard: LMC
Limit of detection: 100 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Auteri,A.; Blardi,P.; Celasco,G.; Segre,G.; Urso,R. Pharmacokinetics of pramiracetam in healthy volunteers after oral administration, *Int.J.Clin.Pharmacol.Res.*, **1992**, *12*, 129–132.

Pramlintide

Molecular formula: $C_{171}H_{267}N_{51}O_{53}S_2$
Molecular weight: 3949.47
CAS Registry No.: 151126-32-8

SAMPLE
Matrix: formulations
Sample preparation: Condition a 6 mL 200 mg C4 SPE cartridge (Baker) with MeCN and water. Add the liquid formulation to the SPE cartridge, wash with water, elute with MeCN:water 40:60 containing 0.1% trifluoroacetic acid. Evaporate the eluate to dryness and dissolve the residue in 30 mM pH 4.0 sodium acetate so as to obtain a 2 mg/mL solution. Inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm PolySULFOETHYL Aspartamide column (Poly LC, Columbia MD)
Mobile phase: Gradient. A was MeCN:5 mM potassium dihydrogen phosphate containing 5 mM sodium perchlorate 40:60, pH 5.8. B was MeCN:5 mM potassium dihydrogen phosphate containing sodium perchlorate 40:60, pH 5.8. A:B from 98:2 to 76:24 over 24 min, maintain at 76:24 for 37 min, to 12:88 over 20 min.
Column temperature: 40
Flow rate: 0.8
Detector: UV 220

CHROMATOGRAM
Retention time: 46

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
liquid formulations; SPE

REFERENCE
Hekman,C.M.; DeMond,W.; Dixit,T.; Mauch,S.; Nuechterlein,M.; Stepanenko,A.; Williams,J.D.; Ye,M. Isolation and identification of peptide degradation products of heat stressed pramlintide injection drug product, *Pharm.Res.*, **1998**, *15*, 650–659.

SAMPLE
Matrix: formulations
Sample preparation: Condition a 6 mL 200 mg C4 SPE cartridge (Baker) with MeCN and water. Add the liquid formulation to the SPE cartridge, wash with water, elute with MeCN:water 40:60 containing 0.1% trifluoroacetic acid. Evaporate the eluate to dryness and dissolve the residue in 30 mM pH 4.0 sodium acetate so as to obtain a 2 mg/mL solution. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Symmetry C8
Mobile phase: Gradient. A was MeCN:200 mM potassium dihydrogen phosphate 6.05:93.95, pH 3.0. B was MeCN:200 mM potassium dihydrogen phosphate 22.45:77.55, pH 3.0. C was MeCN:200 mM potassium dihydrogen phosphate 26.9:73.1, pH 3.0. A:B:C from 100:0:0 to 0:100:0 over 16 min, maintain at 0:100:0 for 69 min, to 0:0:100 over 15 min, maintain at 0:0:100 for 10 min
Column temperature: 55
Flow rate: 0.5
Detector: UV 220

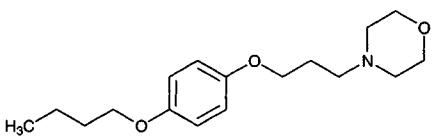
CHROMATOGRAM
Retention time: 57

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
liquid formulation; SPE

REFERENCE
Hekman,C.M.; DeMond,W.; Dixit,T.; Mauch,S.; Nuechterlein,M.; Stepanenko,A.; Williams,J.D.; Ye,M. Isolation and identification of peptide degradation products of heat stressed pramlintide injection drug product, *Pharm.Res.*, **1998**, *15*, 650–659.

Pramoxine



Molecular formula: $C_{17}H_{27}NO_3$
Molecular weight: 293.41
CAS Registry No.: 140-65-8, 637-58-1 (HCl)
Merck Index: 7888
Lednicer No.: 1 18

SAMPLE
Matrix: formulations
Sample preparation: Collect contents of an aerosol can using 100 mL ether:MeOH 5:1, extract with 100 mL 20% acetic acid, extract with 75 mL 20% acetic acid, combine the extracts, make up to 250 mL with water, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid:methanesulfonic acid 50:48.9:1:0.1
Flow rate: 2
Injection volume: 15
Detector: UV 286

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: methyl paraben

SAMPLE
Matrix: formulations
Sample preparation: Condition a 6 mL 200 mg C4 SPE cartridge (Baker) with MeCN and water. Add the liquid formulation to the SPE cartridge, wash with water, elute with MeCN:water 40:60 containing 0.1% trifluoroacetic acid. Evaporate the eluate to dryness and dissolve the residue in 30 mM pH 4.0 sodium acetate so as to obtain a 2 mg/mL solution. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Symmetry C8
Mobile phase: Gradient. A was MeCN:200 mM potassium dihydrogen phosphate 6.05:93.95, pH 3.0. B was MeCN:200 mM potassium dihydrogen phosphate 22.45:77.55, pH 3.0. C was MeCN:200 mM potassium dihydrogen phosphate 26.9:73.1, pH 3.0. A:B:C from 100:0:0 to 0:100:0 over 16 min, maintain at 0:100:0 for 69 min, to 0:0:100 over 15 min, maintain at 0:0:100 for 10 min
Column temperature: 55
Flow rate: 0.5
Detector: UV 220

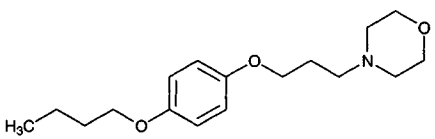
CHROMATOGRAM
Retention time: 57

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
liquid formulation; SPE

REFERENCE
Hekman,C.M.; DeMond,W.; Dixit,T.; Mauch,S.; Nuechterlein,M.; Stepanenko,A.; Williams,J.D.; Ye,M. Isolation and identification of peptide degradation products of heat stressed pramlintide injection drug product, *Pharm.Res.*, **1998**, *15*, 650–659.

Pramoxine



Molecular formula: $C_{17}H_{27}NO_3$
Molecular weight: 293.41
CAS Registry No.: 140-65-8, 637-58-1 (HCl)
Merck Index: 7888
Lednicer No.: 1 18

SAMPLE
Matrix: formulations
Sample preparation: Collect contents of an aerosol can using 100 mL ether:MeOH 5:1, extract with 100 mL 20% acetic acid, extract with 75 mL 20% acetic acid, combine the extracts, make up to 250 mL with water, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid:methanesulfonic acid 50:48.9:1:0.1
Flow rate: 2
Injection volume: 15
Detector: UV 286

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: methyl paraben

KEY WORDS
aerosol

REFERENCE
Weinberger,R.; Mann,B.; Posluszny,J. High-pressure liquid chromatographic analysis of pramoxine hydrochloride in high lipoid aerosol foam dosage form, *J.Pharm.Sci.*, **1980**, *69*, 475–477.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out cream or suppository containing 18 mg pramoxine hydrochloride, add 15 mL isopropanol, add 40 mL MeOH, warm until the sample dissolves, add 40 mL MeOH, add 5 mL 4 μL/mL dibutyl phthalate in MeOH, cool to 10° or lower, filter (0.4 μm polycarbonate), inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 C18 (Brownlee)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM pH 7.5 K_2HPO_4 buffer 11:10 (Use a 150 × 4 column of 32-63 μm silica (ICN) between pump and injector.)
Flow rate: 2
Injection volume: 20
Detector: UV 224

CHROMATOGRAM
Retention time: 12
Internal standard: dibutyl phthalate

KEY WORDS
cream; suppositories; stability-indicating

REFERENCE
Chang,Z.L.; Boller,J.P.; Pacenti,D.M.; Wong,C.F. Rapid high-performance liquid chromatographic determination of pramoxine hydrochloride in topical cream and suppositories, *J.Chromatogr.*, **1984**, *291*, 428–433.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6
Flow rate: 2
Injection volume: 5
Detector: UV 305

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: benzocaine, butamben, lidocaine, procaine, tetracaine

REFERENCE
Menon,G.N.; Norris,B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 569–570.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6

Injection volume: 25
Detector: UV 229

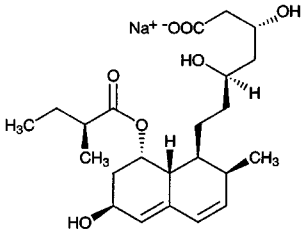
CHROMATOGRAM
Retention time: 11.16 (A), 6.25 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Pravastatin sodium



Molecular formula: $C_{23}H_{35}NaO_7$
Molecular weight: 446.52
CAS Registry No.: 81131-70-6, 81093-37-0 (free acid)
Merck Index: 7894

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Plasma. 2 mL Plasma + 4 mL MeCN, centrifuge at 700 g for 10 min, remove the supernatant and wash the precipitate twice with 2 mL MeCN:water 2:1. Combine the supernatants and evaporate them to dryness under vacuum, reconstitute the residue in 1 mL MeCN:water 2:1, centrifuge at 10000 g, remove the supernatant and add it to 500 µL

water, centrifuge at 10000 g, inject a 1 mL aliquot. Urine. Centrifuge at 10000 g, inject an aliquot. Feces. Homogenize feces, 1 g homogenate + 2 mL MeCN, sonicate for 5 min, shake in a wrist action shaker for 20 min, centrifuge at 700 g for 10 min. Remover the supernatant and wash the precipitate twice with 1 mL MeCN:water 2:1, combine the supernatants, inject a 500 μL aliquot.

HPLC VARIABLES

Guard column: present but not specified
Column: 500 × 9.4 Partisil 10 ODS-3 C18
Mobile phase: Gradient. MeCN:10 mM pH 7.2 potassium phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulphate at 25:75 for 20 min, then to 50:50 over 45 min, hold at 50:50 for 10 min
Flow rate: 4
Injection volume: 500-1000
Detector: Collect fractions and measure radioactivity (UV maximum absorbance at 245 nm)

CHROMATOGRAM

Retention time: 33

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; semi-preparative; radiolabeled starting material

REFERENCE

Everett,D.W.; Chando,T.J.; Didonato,G.C.; Singhvi,S.M.; Pan,H.Y.; Weinstein,S.H. Biotransformation of pravastatin sodium in humans, *Drug Metab.Dispos.*, **1991**, *19*, 740–748.

SAMPLE

Matrix: solutions
Sample preparation: Centrifuge at 2000 rpm, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak
Mobile phase: MeOH:water:triethylamine:glacial acetic acid 500:500:1:1
Column temperature: 30
Flow rate: 1.3
Detector: UV 238

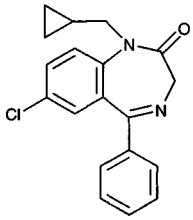
CHROMATOGRAM

Retention time: 17.5 (10.1 hydroxy acid form)
Limit of detection: 10 ng/mL
Limit of quantitation: 25 ng/mL

REFERENCE

Serajuddin,A.T.; Ranadive,S.A.; Mahoney,E.M. Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin, *J.Pharm.Sci.*, **1991**, *80*, 830–834.

Prazepam



Molecular formula: $C_{19}H_{17}ClN_2O$
Molecular weight: 324.81
CAS Registry No.: 2955-38-6
Merck Index: 7895
Lednicer No.: 2 405

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 4 mL diethyl ether and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL diethyl ether to extraction sample, mix. Evaporate combined organic layers to dryness under a stream of dry air at 50°. Purify extracts by partition between 1 mL MeCN and 2 mL heptane, separate MeCN layer, evaporate it to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot of the solution.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II ODS
Mobile phase: MeCN:MeOH:10 mM phosphoric acid:10 mM Na_2HPO_4 40:20:36:4
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 14.5
Internal standard: prazepam
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: diazepam, nitrazepam, oxazepam, temazepam

KEY WORDS

liver; lung; muscle; urine; pericardial fluid; prazepam is IS

REFERENCE

Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE

Matrix: blood
Sample preparation: 200 mg Extrelut + 400 μL blood + 100 μL MeOH, mix, let dry at room temperature for 1-2 h. Add to a 30 × 4.6 stainless steel extraction column, extract with carbon dioxide:ethyl acetate 95:5 at 2 mL/min, 65°, and 300 psi. for 10 min, collect by expansion into MeOH. Dry the collected extract at 65° under nitrogen. Reconstitute the residue in 50 μL mobile phase. Inject a 20 μL aliquot. Condition ca. 10 g Extrelut in a 10 mL plastic syringe with dichloromethane. Add 250 μL 5% ammonia to the top. Mix 900 μL blood with 100 μL MeOH. Add 1 mL pH 4 phosphate buffer and 250 μL 5% ammonia solution, mix thoroughly, add to the extraction column. After 5 min elute with diethyl ether under the influence of gravity. Collect 8 mL eluate, evaporate to dryness at 65° under nitrogen. Reconstitute the residue in 180 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Hypersil ODS
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:Na_2HPO_4 70:30

Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11.5
Internal standard: prazepam

OTHER SUBSTANCES
Extracted: temazepam
Also analyzed: diazepam, chlordiazepoxide, nordiazepam, oxazepam

KEY WORDS
SFE; SPE; prazepam is IS; whole blood

REFERENCE
Scott,K.S.; Oliver,J.S. Development of a supercritical fluid extraction method for the determination of temazepam in whole blood, *J.Anal.Toxicol.*, **1997**, *21*, 297–300.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C2 SPE cartridge with 1 volume MeOH and 1 volume 10 mM pH 8.0 phosphate buffer. 1 mL Plasma + 100 μL 1 M pH 8.0 potassium phosphate buffer, mix, add to the SPE cartridge, wash with 3 volumes of water, wash with 1 mL MeOH:water 30:70, wash with 1 mL water, elute with 1 mL MeOH:water 70:30, elute with 1 mL water. Evaporate the eluate to dryness, reconstitute with 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 35 × 4.6 5 μm Ultrabase C18
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 217

CHROMATOGRAM
Retention time: 8
Internal standard: prazepam

OTHER SUBSTANCES
Extracted: midazolam

KEY WORDS
plasma; SPE; prazepam is IS

REFERENCE
Berrueta,L.A.; Gallo,B.; Vincente,F. Rapid determination of midazolam in plasma using SPE and HPLC, *Am.Lab.*, **1993**, *25 (Dec.)*, 20R–20T.

SAMPLE
Matrix: blood
Sample preparation: Inject 100-200 μL plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

HPLC VARIABLES
Column: A 45 × 4 12 μm TSK-gel G 3 PW (Tosohass); B 75 × 4.6 Ultrasphere ODS C18 3 μm

Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.
Flow rate: 1
Injection volume: 100-200
Detector: UV 230

CHROMATOGRAM
Retention time: 29

OTHER SUBSTANCES
Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, desmethylclobazam, desmethyldiazepam, diazepam, estazolam, flunitrazepam, loflazepate, lorazepam, medazepam, nitrazepam, oxazepam, temazepam, tetrazepam, tofisopam, triazolam
Noninterfering: carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid

KEY WORDS
plasma; column-switching

REFERENCE
Lacroix,C.; Wojciechowski,F.; Danger,P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *617*, 285–290.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM
Retention time: 8.29
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine;

prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 17.73

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS

plasma; serum; column-switching

REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 16.92
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: 1 mL Blood, urine, or liver homogenate + 1 mL 1.15 M pH 6.4 phosphate buffer, add 25 μg prazepam, extract with 5 mL n-butyl chloride, centrifuge. Remove the organic layer and evaporate it in a vortex-evaporator. Reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil-10 ODS
Mobile phase: MeCN:1 mM pH 3.2 phosphate buffer 40:60
Column temperature: 50
Flow rate: 3
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 7.2
Internal standard: prazepam

OTHER SUBSTANCES
Simultaneous: temazepam

KEY WORDS
liver; prazepam is IS

REFERENCE
Martin,C.D.; Chan,S.C. Distribution of temazepam in body fluids and tissues in lethal overdose, *J.Anal.Toxicol.*, **1986**, *10*, 77–78.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 23.6

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 2.5 mL Microsomal incubation + 2.5 mL acetone, add 30 μL diazepam in MeOH, add 2.5 mL chloroform, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 6.2 7 μm Zorbax silica
Mobile phase: Hexane:EtOH:MeCN 95:3.33:1.67
Flow rate: 2
Detector: UV 232

CHROMATOGRAM
Retention time: 12
Internal standard: diazepam (16)

OTHER SUBSTANCES
Extracted: metabolites, oxazepam

KEY WORDS
human; liver; normal phase; pharmacokinetics

REFERENCE

Lu,X.-L.; Guengerich,F.P.; Yang,S.K. Stereoselective metabolism of prazepam and halazepam by human liver microsomes, *Drug Metab.Dispos.*, **1991**, *19*, 637–642.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 45:55 (Buffer was 800 mL 62.5 mM sodium acetate adjusted to pH 5.0 with 1 M NaOH and made up to 1 L.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 14.5

OTHER SUBSTANCES

Simultaneous: flurazepam, pentobarbital, cimetidine, clorazepate, diazepam, oxazepam, nordiazepam

REFERENCE

Colin,P.; Sirois,G.; Lelorier,J. High-performance liquid chromatography determination of dipotassium clorazepate and its major metabolite nordiazepam in plasma, *J.Chromatogr.*, **1983**, *273*, 367–377.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95: % for 2 min, to 0:100 over 15 min, maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: chlordiazepoxide, desalkylflurazepam, diazepam, flurazepam, norchlordiazepoxide, nordiazepam, oxazepam

REFERENCE

Rainin Catalog 1991-2, p. 3.26.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95: 5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50

Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: norchlordiazepoxide, chlordiazepoxide, nordiazepam, desalkylflurazepam, oxazepam, diazepam, flurazepam
Also analyzed: amitriptyline, amphetamine, chlorpromazine, desipramine, desmethyldoxepin, diethylpropion, doxepin, ephedrine, fenfluramine, imipramine, mesoridazine, methamphetamine, nortriptyline, phentermine, phenylpropanolamine, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, prednisolone, primidone, proben-

ecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Vydac C18
Mobile phase: MeCN:20 mM pH 7.0 phosphate buffer 55:45
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: temazepam

REFERENCE

Yang,S.K. Acid-catalyzed ethanolysis of temazepam in anhydrous and aqueous ethanol solutions, *J.Pharm.Sci.*, **1994**, *83*, 898–902.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10.49 (A), 12.77 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide,

ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 100 µL 5 mM pH 5.5 acetate buffer + 25 µL β-glucuronidase/arylsulfatase (0.235/0.065 U, Calbiochem), mix, heat at 37° for 16 h, add 50 µL MeOH, add 1 mL saturated trisodium phosphate, add 3 mL dichloromethane, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove a 2 mL aliquot of the organic layer and add it to 2 mL hexane and 2 mL 6 M HCl, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove 1 mL of the aqueous phase and adjust pH to 6 with 1 mL 6 M NaOH and 1 mL saturated trisodium phosphate, add 3 mLL dichloromethane, vortex for 2 min, centrifuge at 1610 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 150 µL mobile phase, inject a 60 µL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 µm LiChrospher 100 RP-18(e)
Mobile phase: MeOH:water:triethylamine 30:70:0.1 adjusted to pH 5.5 with phosphoric acid
Flow rate: 0.7
Injection volume: 60
Detector: UV 240

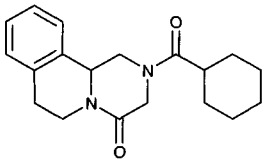
CHROMATOGRAM
Retention time: 17.0
Internal standard: prazepam

OTHER SUBSTANCES
Extracted: desmethyldiazepam, diazepam, oxazepam, temazepam
Simultaneous: amitriptyline, caffeine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, flunitrazepam, flurazepam, haloperidol, imipramine, levomepromazine, maprotiline, mianserin, nitrazepam, nortriptyline, perphenazine, phenobarbital, phenytoin, sulpride, thioridazine, triazolam

KEY WORDS
prazepam is IS

REFERENCE
Chiba,K.; Horii,H.; Chiba,T.; Kato,Y.; Hirano,T.; Ishizaki,T. Development and preliminary application of high-performance liquid chromatographic assay of urinary metabolites of diazepam in humans, *J.Chromatogr.B*, **1995**, *668*, 77–84.

Praziquantel



Molecular formula: $C_{19}H_{24}N_2O_2$
Molecular weight: 312.41
CAS Registry No.: 55268-74-1
Merck Index: 7896
Lednicer No.: 4 213

SAMPLE
Matrix: blood
Sample preparation: Acidify 1 mL plasma with 300 µL 1 M phosphoric acid, add 4 mL toluene, shake mechanically for 20 min, centrifuge at 1800 g, remove 3.5 mL of the organic layer. Add 1 mL water and 200 µL 1.5 M NaOH, shake in a mixer for 1 min, centrifuge. Remove a 3 mL aliquot of the organic layer and evaporate it under a stream of air. Dissolve the residue in 100 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 CN (Merck)
Column: 150 × 4.6 5 µm Chiralcel OD-H (Daicel, Chiral Technology)
Mobile phase: Hexane:EtOH 85:15
Flow rate: 1
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 9 (R-(-)), 12 (S-(+))
Limit of quantitation: 5 ng/mL (R-(-)-), 10 ng/mL (S-(+))

OTHER SUBSTANCES
Interfering: albendazole sulfone, albendazole metabolite, carbamazepine, clonazepam, lorazepam, triazolam

KEY WORDS
chiral

REFERENCE
Jabor,V.A.P.; Rocha,G.M.; Bonato,P.S. Enantioselective analysis of praziquantel in plasma samples, *J.Chromatogr.B*, **1997**, *696*, 307–311.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH, 1 mL water, and 5 mL 100 mM sodium carbonate. Mix 1.5 mL heparinized plasma with 1.5 mL 100 mM sodium carbonate, add to the SPE cartridge, wash with 5 mL 100 mM sodium carbonate, dry the cartridge in air for 30 s, elute with 750 µL MeCN:0.2% phosphoric acid 50:50, vortex the eluate (protect from light), inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 µm BST Nucleosil C18
Column: 150 × 3.9 4 µm Novapak C18
Mobile phase: MeCN:buffer 33:67 (Buffer was 50 mM KH_2PO_4 adjusted to pH 3.0 with 20% phosphoric acid.)

Flow rate: 1.5
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 8.2
Limit of detection: 4 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: fenbendazole

KEY WORDS
dog; pharmacokinetics; plasma; SPE

REFERENCE
Morovján,G.; Csokán,P.; Makranaszki,L.; Abdellah-Nagy,E.A.; Tóth,K. Determination of fenbendazole, praziquantel and pyrantel pamoate in dog plasma by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *797*, 237–244.

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Extract 1 mL serum or microsomal incubation three times with 2 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 15 μm RP-18
Column: 200 × 4 5 μm RP-18
Mobile phase: MeCN:water 50:50
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

KEY WORDS
serum; rat; human

REFERENCE
Masimirembwa,C.M.; Naik,Y.S.; Hasler,J.A. The effect of chloroquine on the pharmacokinetics and metabolism of praziquantel in rats and in humans, *Biopharm.Drug Dispos.*, **1994**, *15*, 33–43.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 250 μL Plasma + 100 μL water + 1 mL acetone, homogenize for 10 s, add 50 μL 1 M naOH, add 2 mL hexane:diethyl ether 40:60, mix for 10 s, centrifuge at 3000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeOH:buffer 70:30, add 500 μL hexane, mix, centrifuge for 3 min, discard the hexane layer. Remove 100 μL of the MeOH layer and add it to 100 μL 10 mM phosphoric acid, mix, filter (Costar Spin-X 0.2 μm nylon) while centrifuging at 5600 g for 4 min, inject a 25 μL aliquot of the filtrate. Muscle. 3 g Muscle + 300 μL water + 4.7 mL acetone, homogenize (Ultra-Turrax TP 18/2) for 6 s, centrifuge at 5000 rpm for 3 min. Remove 4 mL of the supernatant and add it to 5 mL hexane:diethyl ether 40:60, shake vigorously for 10 s, centrifuge at 3000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 μL MeOH:buffer 70:30, let stand at -20° for 5 min, centrifuge at 3000 rpm for 3 min. Remove 300 μL of the MeOH based phase and add it to 300 μL water, filter (Costar Spin-X 0.2 μm nylon) while centrifuging, inject a 25 μL aliquot of the filtrate. Liver. 3 g Liver + 300 μL water + 4.7 mL acetone, homogenize (Ultra-Turrax TP 18/2) for 6 s, centrifuge at 5000 rpm for 3 min. Remove 4 mL of the supernatant and add it to 5 mL hexane:diethyl ether 40:60, shake vigorously for 10 s, centrifuge at 3000 rpm for 3 min. Remove the organic layer and add it to 50 μL 1 M NaOH, shake vigorously for 5 s, centrifuge for 2 min. Remove the upper layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 600 μL MeOH:

buffer 70:30, add 1 mL hexane, mix, centrifuge for 3 min. Discard the hexane layer and add 240 μL of the MeOH based layer to 160 μL 20 mM phosphoric acid, mix for 3 s, filter (Costar Spin-X 0.2 μm nylon) while centrifuging, inject a 25 μL aliquot of the filtrate. (Buffer was 4.45 g sodium heptanesulfonate and 1.779 g Na_2HPO_4 in 750 mL water, adjust pH to 6 with 2 M phosphoric acid, make up to 1 L with water.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:water 40:60 (muscle) or 39:61 (liver, plasma) (After each injection wash with MeCN for 5 min at 1.5 mL/min, wash with mobile phase for 7 min at 1.8 mL/min and for 3 min at 1 mL/min.)
Flow rate: 1 for 3 min then 0.8 for 6 min
Injection volume: 25
Detector: UV 205

CHROMATOGRAM
Retention time: 8.5
Limit of quantitation: 15 ng/g (liver), 20 ng/mL (plasma), 5 ng/g (muscle)

KEY WORDS
plasma; fish; trout; muscle; liver

REFERENCE
Hormazal,V.; Yndestad,M. High-performance liquid chromatographic determination of praziquantel in plasma and tissues of cultured fish for residue and pharmacokinetic studies, *J.Liq.Chromatogr.*, **1995**, *18*, 589–597.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL 50 mM pH 5.0 phosphate buffer. 2 mL plasma, urine, or 10% liver homogenate + 100 μL 10 μg/mL IS + 1 mL 200 mM NaOH, vortex for 15 s, add to the SPE cartridge, wash with 20 mL 50 mM pH 5.0 phosphate buffer, wash with 8 mL MeOH, elute with two 3 mL aliquots of ethyl acetate:diisopropyl ether 70:30 (plasma, urine) or 30:70 (liver) (Caution! Diisopropyl ether readily forms explosive peroxides!). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Ultrasphere ODS C18
Mobile phase: MeCN:water 45:55 (plasma, urine) or 50:50 (liver)
Flow rate: 1.5
Injection volume: 20
Detector: UV 217

CHROMATOGRAM
Retention time: 6.1 (plasma, urine), 4.9 (liver)
Internal standard: 2-cycloheptylcarbonyl-4-oxo-1,2,3,6,7,11b-hexahydro-4H-pyrzino[2,1-a]isoquinoline (8.7 (plasma, urine), 6.5 (liver))
Limit of detection: 31.2 ng/mL

KEY WORDS
SPE; plasma; liver; human; rat

REFERENCE
González-Esquivel,D.F.; Okuno,C.M.; Sánchez Rodríguez,M.; Sotelo Morales,J.; Cook,H.J. Sensitive high-performance liquid chromatographic assay for praziquantel in plasma, urine and liver homogenates, *J.Chromatogr.*, **1993**, *613*, 174–178.

SAMPLE
Matrix: feed, sediment

Sample preparation: Feed. 0.5 g Ground feed + 6 mL acetone, mix for 5 s, let stand for 5 min, whirlmix for 5 s, make up to 50 mL with MeCN:water 40:60, blend, centrifuge at 3000 rpm for 3 min. Remove 0.5 mL of the supernatant and add it to 4.5 mL MeCN:water 40:60, blend, filter (Costar 0.2 μm nylon membrane Spin-X centrifuge filter) while centrifuging at 5600 g for 3 min, inject a 10 μL aliquot of the filtrate. Sediment. 2 g Sediment + 200 μL water + 6 mL acetone, mix for 5 s, let stand for 5 min, whirlmix for 5 s, centrifuge at 5000 rpm for 3 min. 4.1 mL Supernatant + 50 μL 1 M NaOH + 5 mL diethyl ether:hexane 60:40, shake vigorously for 5 s, centrifuge at 3000 rpm for 3 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL MeOH:buffer 70:30 and 1 mL hexane, whirlmix, centrifuge for 3 min, discard the hexane layer, add 2 mL MeCN:10 mM phosphoric acid 40:60, mix, filter a 500 μL aliquot (Costar 0.2 μm nylon membrane Spin-X centrifuge filter) while centrifuging at 5600 g for 3 min, inject a 20 μL aliquot of the filtrate. (Buffer was 4.45 g sodium 1-heptanesulfonate and 1.779 g $NaH_2PO_4.2H_2O$ in 750 mL water, adjust pH to 6 with 2 M phosphoric acid, make up to 1 L with water.)

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil ABZ
Column: 150 × 4.6 5 μm Supelcosil ABZ
Mobile phase: MeCN:water 40:60 (feed) or 39:61 (sediment)
Flow rate: 1 (feed) or 1 for 3 min, then 0.8 for 6 min (sediment)
Injection volume: 10-20
Detector: UV 205

CHROMATOGRAM

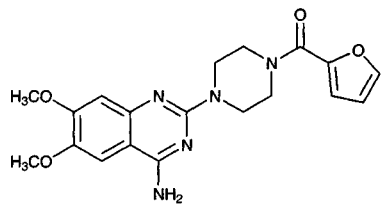
Retention time: 7 (feed), 9 (sediment)
Limit of quantitation: 500 ng/g (feed), 30 ng/g (sediment)

REFERENCE

Hormazal,V.; Yndestad,M. Determination of praziquantel in medicated fish feed and sediment by HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 1231–1238.

Prazosin

Molecular formula: $C_{19}H_{21}N_5O_4$
Molecular weight: 383.41
CAS Registry No.: 19216-56-9, 19237-84-4 (HCl)
Merck Index: 7897
Lednicer No.: 2 382



SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 M NaOH, extract with 5 mL pentane:dichloromethane 2:1. Remove the organic layer and evaporate it to dryness under a gentle stream of nitrogen, reconstitute the residue in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 10 μm Chiralpak AD (Chiral Technologies, Exton, PA)
Mobile phase: n-Hexane:isopropanol:diethylamine 70:30:0.1
Column temperature: 30
Flow rate: 0.2
Injection volume: 10
Detector: MS, SCIEX API 300 tandem mass, positive ion mode, nebulizer 440°, scan 384.0/247.0

CHROMATOGRAM

Retention time: 4.50
Internal standard: prazosin

OTHER SUBSTANCES

Extracted: doxazosin

KEY WORDS
plasma; small-bore; prazosin is IS

REFERENCE
Alebic-Kolbah; T.; Zavitsanos; A. P. Chiral bioanalysis by normal high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *759*, 65–77.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 50 mM pH 7.2 KH_2PO_4 buffer. Centrifuge whole blood at 1500 g for 10 min. Add 1 mL 50 mM pH 7.2 KH_2PO_4 buffer to 1 mL plasma, vortex for 5 s, add to the SPE cartridge, dry in a stream of air, wash with two 1 mL portions of 50 mM pH 7.2 KH_2PO_4 buffer, wash with 500 μL MeOH. Dry the cartridge in a stream of air, let stand for 15 min, elute with 1 mL MeCN:25% ammonium hydroxide 99:1, evaporate the eluate to dryness under a stream of nitrogen, dissolve the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Supelguard ABZ+plus C18 (Supelco)
Column: 250 × 4.6 Supelcosil ABZ+plus C18 (Supelco)
Mobile phase: MeCN:50 mM pH 6.5 KH_2PO_4 buffer 30:70
Flow rate: 1
Detector: E, ESA Coulochem II, 5011 model analytical cell, guard cell +950 mV, first electrode +600 mV, second electrode +900 mV

CHROMATOGRAM
Retention time: 6.14
Internal standard: prazosin

OTHER SUBSTANCES
Extracted: buspirone

KEY WORDS
plasma; prazosin is IS; SPE

REFERENCE
Ary,K.; Róna,K.; Ondi,S.; Gachályi,B. High-performance liquid chromatographic method with coulometric detection for the determination of buspirone in human plasma by means of a column-switching technique, *J.Chromatogr.A*, **1998**, *797*, 221–226.

SAMPLE
Matrix: blood
Sample preparation: Add 500 μL 5 M NaOH to 1 mL plasma, add 5 mL ethyl acetate, extract. Centrifuge at 700 g for 10 min, evaporate 4 mL of the organic phase to dryness under nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 69:31 (Mobile phase was 690 mL MeCN, 310 mL water, and 9 mL glacial acetic acid, adjusted to pH 5.0 with 5 M NaOH.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 362 em 414

CHROMATOGRAM
Retention time: 3.8
Internal standard: prazosin (3.8)

OTHER SUBSTANCES
Extracted: amiloride

KEY WORDS
plasma; prazosin is IS

REFERENCE
Jankowski,A.; Skorek-Jankowska,A.; Lamparczyk,H. Determination and pharmacokinetics of a furosemide-amiloride drug combination, *J.Chromatogr.B*, **1997**, *693*, 383–391.

SAMPLE
Matrix: blood
Sample preparation: Place 50 μL of a 100 μg/mL solution of doxazosin in MeOH into the bottom of a tube, evaporate to dryness under a stream of nitrogen at 37°, add 1 mL whole blood, mix thoroughly, add 5 mL diethyl ether, shake for 10 min, centrifuge at 2000 rpm for 5 min, freeze in acetone/dry ice. Remove the organic layer and add it to 100 μL 50 mM sulfuric acid, shake for 10 min, centrifuge at 2000 rpm for 5 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS
Mobile phase: MeOH:water 42:58 containing 10 mM pentane sodium sulfate and 11.6 mM tetramethylammonium chloride, adjusted to pH 3.4 with glacial acetic acid
Flow rate: 1.8
Injection volume: 20
Detector: F ex 254 em 400 (cut-off filter)

CHROMATOGRAM
Internal standard: doxazosin

OTHER SUBSTANCES
Extracted: trimazosin

KEY WORDS
whole blood

REFERENCE
Hughes,M.A.; Meredith,P.A.; Elliott,H.L. The determination of trimazosin and its metabolite CP23445 in whole blood by high performance liquid chromatography using fluorescence detection, *J.Pharmacol.Methods*, **1984**, *12*, 29–34.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL 1 M NaOH + 12.5 μL 100 μg/mL propyl hydroxybenzoate in MeOH + 7 mL diethyl ether, vortex for 1 min, centrifuge at 3000 rpm for 2 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 400 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere phenyl
Mobile phase: MeCN:50 mM phosphate buffer 30:70 adjusted to pH 3.3-3.4 with phosphoric acid
Flow rate: 1.5
Detector: F ex 247 em 394

CHROMATOGRAM
Retention time: 4.0
Internal standard: propyl hydroxybenzoate (6.0)
Limit of quantitation: 0.5 ng/mL

KEY WORDS
plasma

REFERENCE
Niazy,E.M.; El-Sayed,Y.M.; Khidr,S.H. Analysis of prazosin in plasma by high-performance liquid chromatography using fluorescence detection, *J.Liq.Chromatogr.*, **1995**, *18*, 977–987.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 200 μL MeCN, centrifuge at 9000 g for 5 min. Remove the supernatant and add it to 250 μL water-saturated n-hexane, vortex for 2 min, centrifuge at 10000 rpm for 5 min, discard the hexane layer, repeat the hexane ash twice more. Evaporate the MeCN layer to dryness under a stream of nitrogen, reconstitute with 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS Hypersil
Mobile phase: MeCN:50 mM Na_2HPO_4 40:60, pH 8.4
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem II, No. 5014 analytical cell, +500 mV on channel 1 (monitoring), channel 2 +0.00 mV, guard cell +300 mV

CHROMATOGRAM
Retention time: 5.7
Limit of detection: 2.5 ng/mL

KEY WORDS
serum; fetal; cow

REFERENCE
Rathinavelu,A.; Malave,A. High-performance liquid chromatography using electrochemical detection for the determination of prazosin in biological samples, *J.Chromatogr.B*, **1995**, *670*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 247

CHROMATOGRAM
Retention time: 3.89
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lor-

azepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 50 μL Plasma or urine + 100 μL saturated NaCl + 50 μL 2 μg/mL dimethothiazine mesylate in water + 50 μL 4 M NaOH, vortex for 10 s, add 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject a 110 μL aliquot of the organic phase.

HPLC VARIABLES

Column: 250 × 5 5 μm Spherisorb S5W

Mobile phase: MeOH:10 mM ammonium perchlorate adjusted to pH 6.7 with 1 mL/L methanolic NaOH (0.1 M)

Flow rate: 2

Injection volume: 110

Detector: F ex 370 em 370-700

CHROMATOGRAM

Retention time: 3

Internal standard: dimethothiazine mesylate (5)

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Simultaneous: N-acetylprocainamide, ajmaline, chlorpromazine, desipramine, dipyridamole, doxazosin, flecainide, flurazepam, gallopamil, imipramine, ketanserin, metoprolol, mexiletine, mianserin, nadolol, nitrazepam, orphenadrine, oxprenolol, penbutolol, pindolol, prajmalium, procainamide, propranolol, protriptyline, pyrimethamine, quinidine, quinine, terazosin, triamterene, trimipramine, verapamil

Noninterfering: amiodarone, atenolol, disopyramide, labetalol, lignocaine, lorcainide, methyldopa, nifedipine, prenalterol, propafenone, sotalol, timolol

KEY WORDS

plasma

REFERENCE

Bhamra,R.K.; Flanagan,R.J.; Holt,D.W. High-performance liquid chromatographic measurement of prazosin and terazosin in biological fluids, *J.Chromatogr.*, **1986**, *380*, 216–221.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 246.4

CHROMATOGRAM

Retention time: 10.608

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: perfusate

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 10 mL water. 1 mL Perfusate + 1 μg propranolol + 2 mL water, add to the SPE cartridge, wash with 10 mL water, dry under vacuum, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL mobile phase, centrifuge at 700 g for 5 min, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Alltima C18 (Alltech)

Mobile phase: MeOH:50 mM pH 5.8 phosphate buffer 55:45, final pH 5.1

Column temperature: 40

Flow rate: 1

Injection volume: 30

Detector: F ex 280 em 395

CHROMATOGRAM

Retention time: 4.9

Internal standard: propranolol (7.1)

Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES

Noninterfering: albuterol, alcuronium, aminophylline, atenolol, atropine, betamethasone, bupivacaine, cortisone, dexamethasone, diazepam, diltiazem, hydrocortisone, hyoscine, hyoscine-N-butylbromide, labetalol, lidocaine, methimazole, metoclopramide, norepinephrine, phenobarbital, L-phenylephrine, phenytoin, prednisolone, prednisone, promethazine, propylthiouracil, pyridoxine, ranitidine, verapamil

KEY WORDS
SPE

REFERENCE
Fletcher,A.J.; Addison,R.S.; Mortimer,R.H.; Cannell,G.R. Rapid determination of prazosin in perfusion media by HPLC with solid phase extraction, *J.Liq.Chromatogr.*, **1995**, *18*, 2911–2923.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in MeCN:water 40:60, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 3 μm silica (Phenomenex)
Mobile phase: MeCN:6.25 mM pH 3.0 phosphate buffer 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 9.31

OTHER SUBSTANCES

Also analyzed: atenolol, clonidine, diltiazem, metoprolol, nifedipine, propranolol, verapamil

REFERENCE

Simmons,B.R.; Stewart,J.T. HPLC separation of selected cardiovascular agents on underivatized silica using an aqueous organic mobile phase, *J.Liq.Chromatogr.*, **1994**, *17*, 2675–2690.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 39.26

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 7.38 (A), 4.21 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: tissue
Sample preparation: 300 mg Skin + 100 μL 10 μg/mL verapamil hydrochloride in PBS, let stand for 2 h, cut skin into small pieces, add 5 mL MeOH, homogenize, filter, homogenize residue again with MeOH four more times. Combine filtrates, evaporate to dryness, reconstitute in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 cyano (Alltech)
Mobile phase: MeCN:MeOH:water 45:5:50 containing 3 mM sodium heptanesulfonate
Flow rate: 1.5
Injection volume: 10
Detector: UV 230

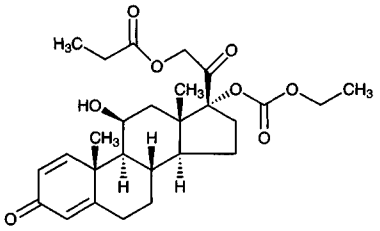
CHROMATOGRAM
Retention time: 4.4
Internal standard: verapamil (5.8)

Limit of detection: 50 ng/mL

KEY WORDS
skin

REFERENCE
Tenjarla,S.N.; Tseggai,A. High-performance liquid chromatographic assay of prazosin for transdermal screening studies, *J.Clin.Pharm.Ther.*, **1992**, *17*, 37–42.

Prednicarbate



Molecular formula: $C_{27}H_{36}O_8$
Molecular weight: 488.58
CAS Registry No.: 73771-04-7
Merck Index: 7899
Lednicer No.: 4 71

SAMPLE
Matrix: tissue
Sample preparation: Extract incubation suspension twice with 3 mL portions of ethyl acetate, vortex for 1 min, centrifuge at 1000 rpm for 5 min, dry the combined organic phases under nitrogen, reconstitute with 1 mL MeOH, vortex 1 min, dry in a conical tube, reconstitute with 100 µL MeOH, centrifuge, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 µm LiChrospher 100 RP-18
Mobile phase: Gradient. A was MeCN. B was water. A:B from 20:80 to 100:0 over 20 min
Flow rate: 1
Injection volume: 20
Detector: UV 254

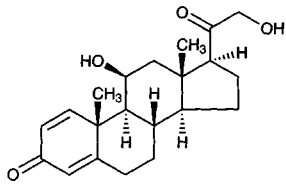
CHROMATOGRAM
Retention time: 15.1
Internal standard: betamethasone (9.4)
Limit of detection: 10 ng/mL
Limit of quantitation: 100 ng/mL-50 µg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics; keratinocytes; fibroplasts

REFERENCE
Gysler,A.; Lange,K.; Korting,H.C.; Schäfer-Korting,M. Prednicarbate biotransformation in human foreskin keratinocytes and fibroblasts, *Pharm.Res.*, **1997**, *14*, 793–797.

Prednisolone



Molecular formula: $C_{21}H_{28}O_5$
Molecular weight: 360.45
CAS Registry No.: 50-24-8, 52438-85-4 (sesquihydrate), 52-21-1 (acetate), 2920-86-7 (hemisuccinate), 125-02-0 (disodium phosphate), 302-25-0 (dihydrogen phosphate), 1715-33-9 (sodium succinate), 2920-86-7 (hydrogen succinate), 5060-55-9 (steaglate), 7681-14-3 (tebutate), 5626-34-6 (21-diethylaminoacetate), 1107-99- 9(21-trimethylacetate), 630-67-1 (sodium 21-m-sulfobenzoate)
Merck Index: 7901
Lednicer No.: 1 192

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge. Mix 1 mL plasma with 134.0 ng hydrocortisone-d_5 and 74.56 ng cortisone-d_5. Add the sample to the SPE cartridge, wash with 8 mL water, elute with 4 mL ethyl acetate, evaporate the eluate to dryness at 70° under a stream of nitrogen, dissolve the residue in 30 μL mobile phase, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.0 4 μm LiChroCART Superspher 100
Mobile phase: A MeOH:THF:50 mM ammonium formate 17:53:180; B MeCN:50 mM ammonium formate 35:65
Flow rate: 0.6 (A); 1.3 (B)
Injection volume: 20
Detector: MS, Shimadzu LCMS-QP1000EX Model 750 B, thermospray, vaporizer control 155°, vaporizer tip 195°, vapor 274°, ion source block 295°, tip heater 305°, m/z 361

CHROMATOGRAM

Retention time: 12 (A)
Internal standard: hydrocortisone-d_5, cortisone-d_5
Limit of detection: 1.2 ng

OTHER SUBSTANCES

Extracted: hydrocortisone, cortisone, prednisone

KEY WORDS

plasma; SPE

REFERENCE

Shibasaki,H.; Furuta,T.; Kasuya,Y. Quantification of corticosteroids in human plasma by liquid chromatography-thermospray mass spectrometry using stable isotope dilution, *J.Chromatogr.B*, **1997**, *692*, 7–14.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 4 mL 59.5 ng/mL triamcinolone acetonide in dichloromethane, shake at high speed for 15 min, centrifuge at 2000 rpm for 10 min, remove aqueous layer, add 5 mL saturated sodium bicarbonate solution to the organic layer, shake at high speed for 5 min, centrifuge at 2000 rpm for 10 min, remove aqueous layer. Place organic layer in a pointed tube and evaporate to dryness at 45° under a stream of nitrogen. Reconstitute with 50 μL mobile phase, inject 20 μL aliquot.

HPLC VARIABLES

Column: 10 μm Porasil
Mobile phase: Hexane:dichloromethane:ethanol:acetic acid 68.8:25:6:0.2
Flow rate: 2.5
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: triamcinolone acetonide (3)

OTHER SUBSTANCES
Simultaneous: hydrocortisone, prednisone

KEY WORDS
plasma; normal phase

REFERENCE
Agabeyoglu,I.T.; Wagner,J.G.; Kay,D.R. A sensitive high-pressure liquid chromatographic method for the determination of prednisone, prednisolone and hydrocortisone in plasma, *Res.Commun.Chem.Pathol.Pharmacol.*, **1980**, *28*, 163–176.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 μg/mL dexamethasone in EtOH:water 10:90 + 100 μL 250 mM NaOH + 7 mL ether:dichloromethane 60:40, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 100 μL dichloromethane:EtOH:water 95:4:1, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Partisil silica
Mobile phase: Dichloromethane:EtOH:water 95:4:1
Flow rate: 1.5
Injection volume: 50
Detector: UV 239

CHROMATOGRAM
Retention time: 19
Internal standard: dexamethasone (11.5)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, 11-deoxycortisol, hydrocortisone, 17-hydroxyprogesterone, 6α-methylprednisolone, prednisone, progesterone

KEY WORDS
plasma; normal phase

REFERENCE
Scott,N.R.; Chakraborty,J.; Marks,V. Determination of prednisolone, prednisone, and cortisol in human plasma by high-performance liquid chromatography, *Anal.Biochem.*, **1980**, *108*, 266–268.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 5 mL water + 1 mL 2 μg/mL equilenin in MeOH + 50 μL 0.1 M NaOH to adjust pH to 10, vortex briefly after each addition, shake with 10 mL dichloromethane for 10 min, centrifuge at 2000 g for 10 min. Wash organic layer twice with 2 mL water, centrifuge 5 min, evaporate 8 mL of organic phase to dryness at 40° under a stream of nitrogen, reconstitute residue in 150 μL mobile phase, inject 25 μL aliquot

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:buffer 60:40 (Buffer was 10 mL 200 mM acetic acid + 15 mL 200 mM sodium acetate made up to 1 L, pH 4.8.)

Flow rate: 1
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: equilenin (7.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: deoxycortisol, hydrocortisone, prednisone, triamcinolone, dexamethasone, betamethasone
Interfering: hydrocortisone

KEY WORDS
plasma

REFERENCE
Bouquet,S.; Brisson,A.M.; Gombert,J. Dosage du cortisol et du 11-désoxycortisol plasmatiques par chromatographie liquide haute performance [Cortisol and 11-desoxycortisol determination in blood by high performance liquid chromatography], *Ann.Biol.Clin.(Paris)*, **1981**, *39*, 189–191.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 10 μg/mL prednisolone in MeOH, add 1 mL 0.1 M NaOH, add 10 mL dichloromethane, shake for 10 min, centrifuge at 8400 g at 4° for 10 min. Remove organic layer and evaporate it at 40° under a stream of nitrogen. Dissolve residue in 100 μL mobile phase and inject.

HPLC VARIABLES
Column: 100 × 8 radial compression 10 μm Radialpack B
Mobile phase: Dichloromethane:MeOH:acetic acid 96:4:0.4
Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: prednisolone

OTHER SUBSTANCES
Simultaneous: hydrocortisone, corticosterone, dexamethasone

KEY WORDS
plasma; dog; normal phase; prednisolone is IS

REFERENCE
Alvinerie,M.; Toutain,P.L. Simultaneous determination of corticosterone, hydrocortisone, and dexamethasone in dog plasma using high-performance liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 816–818.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 150 ng dexamethasone + 1 mL 100 mM NaOH + 10 mL ether:dichloromethane 60:40, shake for 10 min, centrifuge at 300 g for 5 min. Remove the organic layer and add it to 1 mL 100 mM HCl, shake for 5 min, centrifuge at 300 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak/Corasil (Waters)

Column: 300 × 3.9 10 μm μPorasil (Waters)
Mobile phase: Dichloromethane:glacial acetic acid 99:1 (Prepare dichloromethane as follows. Stir 500 mL dichloromethane, 30 mL EtOH, and 30 mL water for 1 h, use the lower organic layer.)
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: dexamethasone (5)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: prednisone, hydrocortisone

KEY WORDS
plasma; normal phase; pharmacokinetics

REFERENCE
Hartley,R.; Brocklebank,J.T. Determination of prednisolone in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *232*, 406–412.

SAMPLE
Matrix: blood
Sample preparation: Prepare a Bond-Elut C18 SPE column by washing with 2 mL MeCN, 2 mL acetone:water 2:98, and 4 mL water. Do not allow column to run dry. 2 mL Plasma + 40 μL 5 μg/mL dexamethasone in MeOH, add to SPE cartridge, allow to sit for 15 min, wash twice with 2 mL water, wash twice with 2 mL acetone:water 2:98, pull a vacuum on the column for 15 min, elute with 1 mL MeCN under vacuum. Evaporate the eluate to dryness under a stream of nitrogen at 40°, dissolve the residue in 150 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb Si-60
Mobile phase: Dichloromethane:water-saturated dichloromethane:THF:MeOH:glacial acetic acid 664.5:300:10:25:0.5
Flow rate: 0.8
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 37 (prednisolone), 12.5 (prednisolone acetate)
Internal standard: dexamethasone (23.5)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: hydrocortisone, prednisone, cortisone

KEY WORDS
plasma; normal phase; pig; SPE

REFERENCE
Prasad,V.K.; Ho,B.; Haneke,C. Simultaneous determination of prednisolone acetate, prednisolone, prednisone, cortisone and hydrocortisone in swine plasma using solid-phase and liquid-liquid extraction techniques, *J.Chromatogr.*, **1986**, *378*, 305–316.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 μg/mL 11-deoxy-17-hydroxycorticosterone in MeOH, mix for 5 s, add 7 mL dichloromethane, shake on a mechanical shaker for 5 min. Remove the organic phase and add it to 2 mL 100 mM HCl, shake, centrifuge for 5 min, wash

the organic layer with 2 mL 200 mM NaOH, wash the organic layer with 2 mL water. Evaporate the organic layer to dryness, reconstitute the residue with 75 μL mobile phase, mix for 20 s, centrifuge at 10000 g for 2 min, inject 30 μL of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C6
Mobile phase: MeOH:water 40:60
Flow rate: 1.4
Injection volume: 30
Detector: UV 254

CHROMATOGRAM
Retention time: 14.0
Internal standard: 11-deoxy-17-hydroxycorticosterone (29.4)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: prednisone, aldosterone, corticosterone, hydrocortisone, cortisone, dexamethasone, ethinyl estradiol, methylprednisone, nandrolone, progesterone, testosterone, acetaminophen, allopurinol, amitriptyline, caffeine, calcitriol, cephalothin, chlordiazepoxide, chlorothiazide, diazepam, ephedrine, furosemide, ibuprofen, imipramine, indomethacin, metolazone, mechlorethamine, naproxen, phenacetin, phenobarbital, phenytoin, probenecid, propranolol, sulfasalazine, theophylline, vincristine

KEY WORDS
plasma

REFERENCE
Cheng,M.H.; Huang,W.Y.; Lipsey,A.I. Simultaneous liquid-chromatographic determination of prednisone and prednisolone in plasma, *Clin.Chem.*, **1988**, *34*, 1897–1899.

SAMPLE
Matrix: blood
Sample preparation: Prepare a Sep-Pak Plus Environmental C18 cartridge by washing with 15 mL MeOH then 15 mL water. 1 mL Serum + 100 μL 3 μg/mL betamethasone in isopropanol: MeCN 1:1 + 100 μL isopropanol:acetonitrile 1:1, mix, add to SPE cartridge, wash with 10 mL water, elute with 3 mL MeOH. Evaporate the eluate at 50° under a stream of nitrogen, reconstitute in 200 μL mobile phase A, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 guard column
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. A was isopropanol:50 mM pH 4.5 acetate buffer 10:90. B was isopropanol:50 mM pH 4.5 acetate buffer 30:70. A:B from 90:10 to 30:70 over 25 min, hold at 30:70 for 5 min, to 90:10 over 5 min, hold at 90:10 for 15 min before next injection.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 26.5
Internal standard: betamethasone (33)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, prednisone, cortisone, hydrocortisone

KEY WORDS
serum; SPE

REFERENCE
Hirata,H.; Kasama,T.; Sawai,Y.; Fike,R.R. Simultaneous determination of deflazacort metabolites II and III, cortisol, cortisone, prednisolone and prednisone in human serum by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 55–61.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 4 μg/mL betamethasone in EtOH + 15 mL dichloromethane, shake horizontally for 15 min, centrifuge at 1500 g for 15 min. Remove the organic layer and wash it with 100 μL 100 mM NaOH then 1 mL water. Remove the aqueous phase and dry the organic phase over 1 g of anhydrous sodium sulfate. Evaporate the organic phase to dryness under a stream of nitrogen at ≤ 37°, reconstitute in 200 μL mobile phase, inject a 175 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 30-38 μm HC Pellosil
Column: 250 × 4.6 5-6 μm Zorbax SIL
Mobile phase: Heptane:dichloromethane:glacial acetic acid:ethanol 350:600:10:35
Flow rate: 2
Injection volume: 175
Detector: UV 254

CHROMATOGRAM
Retention time: 20
Internal standard: betamethasone (12)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: hydrocortisone, prednisone
Noninterfering: cyclosporin, rapamycin, tacrolimus, ketoconazole, tenidap, ethinyl estradiol, levonorgestrel, tetrahydrocortisone

KEY WORDS
plasma; normal phase

REFERENCE
Jusko,W.J.; Pyszczynski,N.A.; Bushway,M.S.; D'Ambrosio,R.; Mis,S.M. Fifteen years of operation of a high-performance liquid chromatographic assay for prednisolone, cortisol and prednisone in plasma, *J.Chromatogr.B*, **1994**, *658*, 47–54.

SAMPLE
Matrix: blood
Sample preparation: 750 μL Serum + 75 μL MeOH + 100 μL 1.5 μg/mL dexamethasone in MeOH + 2 mL ethyl acetate, shake for 10 min, centrifuge at 2500 g for 10 min. Remove 1.9 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL ethyl acetate, inject a 17 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm LiChrosorb Si 60
Column: 250 × 4 5 μm LiChrosorb Si 60
Mobile phase: n-Hexane:dichloromethane:MeOH:acetic acid 266:120:26:0.8 (Prepare by mixing an aliquot of mobile phase with an aliquot of mobile phase saturated with water.)
Flow rate: 2
Injection volume: 17
Detector: UV 242

CHROMATOGRAM
Retention time: 15.1 (prednisolone), 5.23 (prednisolone acetate)
Internal standard: dexamethasone (11.43)
Limit of quantitation: 5 ng/mL (prednisolone acetate), 2 ng/mL (prednisolone)

OTHER SUBSTANCES
Extracted: hydrocortisone

KEY WORDS
serum; normal phase; pharmacokinetics

REFERENCE
Doppenschmitt,S.A.; Scheidel,B.; Harrison,F.; Surmann,J.P. Simultaneous determination of prednisolone, prednisolone acetate and hydrocortisone in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *674*, 237–246.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 2500 g for 10 min, mix the supernatant with an equal volume of 1 M pH 3.0 glycine buffer containing 0.2% Tween 20, centrifuge at 2500 g for 10 min, inject an aliquot of the supernatant on to column A and elute to waste with mobile phase, after 3 min divert the effluent from column A on to column B, after 3 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with mobile phase for 28 min.

HPLC VARIABLES
Column: A 30 × 2.1 Spherisorb C1 pH stable; B 150 × 2.1 Spherisorb C1 pH stable
Mobile phase: 5 mM pH 7.3 Tris-nitric acid buffer containing 0.1% Tween 20 and 150 mM sodium nitrate
Column temperature: 40
Flow rate: 0.2
Injection volume: 50
Detector: UV

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Extracted: cortisone, hydrocortisone

KEY WORDS
plasma; column-switching; heart-cut

REFERENCE
Lövgren,U.; Johansson,M.; Kronkvist,K.; Edholm,L.-E. Biocompatible sample pretreatment for immunochemical techniques using micellar liquid chromatography for separation of corticosteroids, *J.Chromatogr.B*, **1995**, *672*, 33–44.

SAMPLE
Matrix: blood
Sample preparation: Condition an Empore C8 extraction disc SPE cartridge (3M Co.) by adding 500 μL MeOH and forcing through three drops, discard the remaining liquid, add water, force through three drops, discard the water. 300 μL Serum + 150 μL IS solution, let stand at room temperature for 10 min, add 800 μL saturated sodium borate solution, mix, centrifuge at 12400 g for 3 min (if necessary), add to SPE cartridge, centrifuge at 100-120 g for 5 min, force through 200 μL water, force through 500 μL MeOH:water 18:82, elute with 50 μL MeCN then 150 μL water, mix the eluates, inject a 20 μL aliquot. (IS solution contained 0.5 mg/L fludrocortisone and 0.75 mg/L methylprednisolone in 400 mM HCl.) (The extraction disc permits use of lower volumes of eluate than a conventional SPE cartridge.)

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (Du Pont)
Column: 250 × 2 Ultrasphere C18 or 250 × 4.6 Ultrasphere C18
Mobile phase: THF:water 20:80 (Use a 150 × 4.6 37-53 μm silica gel (Whatman) saturating column (held at 55°) between the pump and the injector.)
Column temperature: 55

Flow rate: 0.18 (250 × 2 column) or 0.8 (250 × 4.6 column)
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Internal standard: fludrocortisone (15), methylprednisolone (20)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Extracted: hydrocortisone, cortisone, prednisone, corticosterone
Simultaneous: aldosterone, triamcinolone, metyrapone, 11-deoxycortisol, dexamethasone, 21-deoxycortisone, androsteindione, beclomethasone, 11-deoxycorticosterone, testosterone, 17-hydroxyprogesterone, progesterone, pregnenolone

KEY WORDS
serum; SPE; extraction disc

REFERENCE
Lensmeyer,G.L.; Onsager,C.; Carlson,I.H.; Wiebe,D.A. Use of particle-loaded membranes to extract steroids for high-performance liquid chromatographic analyses. Improved analyte stability and detection, *J.Chromatogr.A*, **1995**, *691*, 239–246.

SAMPLE
Matrix: blood
Sample preparation: Extract 1 mL plasma containing dexamethasone with 12 mL dichloromethane. Remove the organic phase and wash it with 2 mL 100 mM NaOH, wash with 1 mL water, dry over 1 g anhydrous sodium sulfate. Evaporate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb silica
Mobile phase: Hexane:dichloromethane:EtOH:glacial acetic acid 26:69:3.4:2
Flow rate: 0.75
Detector: UV 254

CHROMATOGRAM
Retention time: 7.7
Internal standard: dexamethasone (5.1)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: hydrocortisone, methylprednisolone

KEY WORDS
plasma; pharmacokinetics; normal phase

REFERENCE
Möllmann,H.; Hochhaus,G.; Rohatagi,S.; Barth,J.; Derendorf,H. Pharmacokinetic/pharmacodynamic evaluation of deflazacort in comparison to methylprednisolone and prednisolone, *Pharm.Res.*, **1995**, *12*, 1096–1100.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 83:17 over 5 min, to 75:25 over 12 min, to 70:30 over 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 14.77
Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
Limit of detection: 1-10 ng/mL (245 nm)

OTHER SUBSTANCES
Extracted: betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluendrenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE
Matrix: blood, formulations
Sample preparation: Blood. Centrifuge 200 μL fresh blood at 3000 rpm for 10 min. Inject an aliquot of the plasma. Formulations. Completely dissolve 50 mg sample in 20 mL MeOH, sonicate. Filter insoluble material and adjust filtrate to 50 mL with MeOH. Inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18-p (Nacalai Tesque, Japan)
Mobile phase: MeOH:water 60:40
Column temperature: 40
Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Internal standard: dexamethasone

KEY WORDS
freeze-dried formulations; plasma; rat; egg albumin; olive oil

REFERENCE
Tsuji,Y.; Kakegawa,H.; Miyataka,H.; Nishiki,M.; Matsumoto,H.; Satoh,T. Pharmaceutical properties of freeze-dried formulations of egg albumin, several drugs and olive oil, *Biol.Pharm.Bull.*, **1996**, *19*, 636–640.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Serum or urine + 500 μL 200 mM pH 3.85 acetate buffer (serum only) + 400 μL 2.5 μM IS in mobile phase, mix, centrifuge. Add the supernatant to the SPE cartridge, wash with 3 mL acetone:water 20:80, 3 mL water, and 3 mL hexane. Elute with 3 mL diethyl ether into tubes containing 1 mL 200 mM NaOH, vortex, centrifuge. Dry the organic layer under a stream of nitrogen. Reconstitute the residue in 250 μL mobile phase, mix for 5 min. Inject a 60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherex C18 (Phenomenex, USA)

Mobile phase: MeOH:THF:water 3:25:72
Flow rate: 1.0
Injection volume: 60
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Internal standard: fludrocortisone (15.9)
Limit of detection: 5 nM

OTHER SUBSTANCES
Extracted: 11-deoxycortisol, dexamethasone, hydrocortisone, methylprednisolone

KEY WORDS
serum; SPE

REFERENCE
McWhinney,B.C.; Ward,G.; Hickman,P.E. Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine, *Clin.Chem.*, **1996**, *42*, 979–981.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak Plus C18 SPE cartridge with 7 mL MeOH and 14 mL water. Add 40 ng 6β-hydroxycortisone to 400 μL plasma or urine, add the mixture to the SPE cartridge, wash with 6 mL water, 3 mL MeOH:water 12:88, and 3 mL petroleum ether, elute with 5 mL ethyl acetate. Dry the eluate under reduced pressure at 40°, add 200 μL MeCN: triethylamine 90:10 and MeCN:0.1% quinuclidine 20:80 to the residue, vortex. Add 200 μL 0.02% 9-anthroyl nitrile and a few molecular sieves (4A), let stand for 30 min, evaporate under reduced pressure at 40°, dissolve the residue in 200 μL acetone, dilute with 2 mL n-hexane. Add the mixture to a Sep-Pak Plus Silica SPE cartridge, wash with 14 mL 1,2-dichloroethane, elute with 5 mL ethyl acetate. Evaporate the eluate under reduced pressure at 40°, reconstitute the residue in 200 μL mobile phase, inject a 30-60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Japan)
Mobile phase: Dioxane:ethyl acetate:chloroform:n-hexane:pyridine 58.1:11.6:11.6:16.3:2.4 (Caution! Dioxane and chloroform are carcinogens!)
Flow rate: 1 for 45 min, to 1.2 over 5 min
Injection volume: 30-60
Detector: F ex 360 em 460

CHROMATOGRAM
Retention time: 32.5
Internal standard: 6β-hydroxycortisone (86)
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: cortisone, prednisone, hydrocortisone, 6β-hydroxycortisol, 6β-hydroxyprednisolone

KEY WORDS
derivatization; plasma; urine; SPE; normal phase

REFERENCE
Shibata,N.; Hayakawa,T.; Takada,K.; Hoshino,N.; Minouchi,T.; Yamaji,A. Simultaneous determination of glucocorticoids in plasma or urine by high-performance liquid chromatography with precolumn fluorimetric derivatization by 9-anthroyl nitrile, *J.Chromatogr.B*, **1998**, *706*, 191–199.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 246.4

CHROMATOGRAM
Retention time: 14.113

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: 100-300 mg Gel ointment + 3 mL MeOH, mix vigorously, filter (0.2 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7.2

OTHER SUBSTANCES
Extracted: 11-β-hydroxy-1,4-androsten-3,17-dione

KEY WORDS
ointment

REFERENCE
Yamamura,K.; Yamada,J.-I.; Yotsuyanagi,T. High-performance liquid chromatographic assay of antiinflammatory drugs incorporated in gel ointments. Separation and stability testing, *J.Chromatogr.*, **1985**, *331*, 383–388.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm SI-100 (Brownlee)
Mobile phase: Butyl chloride:THF:MeOH:glacial acetic acid 88:2.5:2.5:2.5 (Butyl chloride was 50% water saturated.)
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: bromoprednisolone acetate, isoflupredone, isoflupredone acetate, fluoroprednisone acetate

KEY WORDS
normal phase

REFERENCE
Kane,M.P.; Tsuji,K. Radiolytic degradation scheme for ^{60}Co-irradiated corticosteroids, *J.Pharm.Sci.*, **1983**, *72*, 30–35.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 6 (prednisolone), 7 (prednisolone succinate)

OTHER SUBSTANCES
Simultaneous: prednisone, hydrocortisone acetate, norethindrone, methyltestosterone, progesterone

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Dilute in an appropriate solvent, inject an aliquot.

HPLC VARIABLES
Guard column: RC18 Guardpak (Waters)
Column: 250 × 4.5 μBondapak C18
Mobile phase: MeCN:water 40:60
Flow rate: 1.5
Detector: UV 246

CHROMATOGRAM
Retention time: 3.7 (prednisolone), 6.9 (prednisolone acetate)

OTHER SUBSTANCES
Simultaneous: 20-α-dihydrofluorometholone, fluorometholone

REFERENCE
Richman,J.B.; Tang-Liu,D.D.-S. A corneal perfusion device for estimating ocular bioavailability in vitro, *J.Pharm.Sci.*, **1990**, *79*, 153–157.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate solution (eluate from preparative HPLC) to dryness under a stream of nitrogen, reconstitute with 10 μL 2 μg/mL 9-anthroylnitrile (Wako) in MeCN and 10 μL triethylamine:MeCN 30:70 under nitrogen, let stand at room temperature for 20 min, add 5 μL water, after 6 min add 50 μL 600 mM acetic acid in MeCN, evaporate to dryness under a stream of nitrogen at 37°, reconstitute with 90 μL MeOH:0.4 N NaH_2PO_4 60:40, add to a Cyclobond I silica-bonded β-cyclodextrin SPE cartridge (Astec), wash with 1 mL water, wash with 8 mL MeOH:water 25:75 containing 7.5 mM pH 7.0 phosphate buffer, elute with 1 mL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot on to column A and elute to waste with mobile phase, after the solvent front has passed through divert the effluent from column A on to column B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 2.1 silica (Brownlee); B 150 × 2 Hypersil
Mobile phase: Hexane:ethyl acetate 67:33 (half-saturated with water)
Flow rate: 0.5
Detector: F ex 305-395 em 430-470

CHROMATOGRAM
Retention time: 7.21

OTHER SUBSTANCES
Simultaneous: cortisone, hydrocortisone

KEY WORDS
derivatization; SPE; column-switching; normal phase

REFERENCE
Haegele,A.D.; Wade,S.E. Ultrasensitive differential measurement of cortisol and cortisone in biological samples using fluorescent ester derivatives in normal phase HPLC, *J.Liq.Chromatogr.*, **1991**, *14*, 1133–1148.

SAMPLE
Matrix: solutions
Sample preparation: Sample + 400 μL 5 mM DBD-PZ + 70 mM diethylphosphorocyanidate in MeCN, react for 6 h, inject a 1 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-

hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 45:55
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM
Retention time: 12 (prenisolone succinate)
Limit of detection: 12 fmol

OTHER SUBSTANCES
Simultaneous: hydrocortisone succinate, alprostadil
Interfering: dinoprost

KEY WORDS
derivatization

REFERENCE
Toyo'oka,T.; Ishibashi,M.; Takeda,Y.; Nakashima,K.; Akiyama,S.; Uzu,S.; Imai,K. Precolumn fluorescence tagging reagent for carboxylic acids in high-performance liquid chromatography: 4-substituted-7-aminoalkylamino-2,1,3-benzoxadiazoles, *J.Chromatogr.*, **1991**, *588*, 61–71.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin,

cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 25 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 ODS-1
Mobile phase: MeOH:water 55:45
Column temperature: 40
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM

Retention time: k' 1.033 (prednisolone), 1.519 (prednisolone acetate)

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisone (UV 240), pregnenolone (UV 210), progesterone (UV 240), testosterone (UV 240)

REFERENCE
Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

SAMPLE
Matrix: solutions
Sample preparation: Inject 20 μL aliquot of a MeOH solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil 5-ODS
Mobile phase: THF:water 23:77
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: k' 5.88

OTHER SUBSTANCES
Simultaneous: metabolites, betamethasone, corticosterone, cortisone, deflazacort, deoxycorticosterone, dexamethasone, fludrocortisone, fludrocortisone acetate, fluorocortisone, fluorocortisone acetate, hydrocortisone, 21-hydroxydeflazacort, 11α-hydroxyprogesterone, methylprednisolone, prednisone, triamcinolone acetonide, triamcinolone

REFERENCE
Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Extraction and high-performance liquid chromatographic separation of deflazacort and its metabolite 21-hydroxydeflazacort. Application to urine samples, *J.Chromatogr.B*, **1994**, *657*, 248–253.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water 40:60
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Limit of detection: 5 ng

KEY WORDS
rabbit; buffer

REFERENCE
Tang-Liu,D.D.-S.; Richman,J.B.; Weinkam,R.J.; Takruri,H. Effects of four penetration enhancers on corneal permeability of drugs in vitro, *J.Pharm.Sci.*, **1994**, *83*, 85–90.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 4 mL water then 3 mL MeOH. Add aqueous steroid solution to cartridge, elute with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 20

Detector: UV 242

CHROMATOGRAM
Limit of detection: 3500 ng/mL

OTHER SUBSTANCES
Also analyzed: hydrocortisone, hydrocortisone 21-acetate

KEY WORDS
SPE

REFERENCE
Valenta,C.; Janout,H. Corticosteroid analysis by HPLC with increased sensitivity by use of precolumn concentration, *J.Liq.Chromatogr.*, **1994**, *17*, 1141–1146.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 μM solution in MeOH.

HPLC VARIABLES
Column: 470 × 4.6 5 μm Spheri-5 RP-18
Mobile phase: MeOH:water 56:44
Flow rate: 0.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Simultaneous: cortisone, dehydrocorticosterone, methylprednisolone, prednisone, tetrahydrocortisol, tetrahydrocortisone
Interfering: hydrocortisone

REFERENCE
Lukulay,P.H.; McGuffin,V.L. Comparison of solvent modulation with premixed mobile phases for the separation of corticosteroids by liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 4039–4062.

SAMPLE
Matrix: urine
Sample preparation: Dilute 0.1-1 mL urine to 1 mL with water if necessary, add 50 μL 10 μg/mL dexamethasone in MeOH, vortex, add to a Chem-Elut cartridge (cat. no. 1003), allow to stand for 5 min, elute twice with 5 mL portions of ethyl acetate (which are first used to rinse the ample tube) at a 5 min interval, wash eluate twice with 1 mL 200 mM NaOH, add 1 g anhydrous sodium sulfate, let stand for 30 min. Evaporate the organic phase at 30° under a stream of nitrogen. Reconstitute the residue in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Beckman Si column
Mobile phase: Dichloromethane:MeOH:THF:glacial acetic acid 96.9:2:1:10.1
Flow rate: 1.3
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 12.4
Internal standard: dexamethasone (8.0)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: prednisone, 6β-hydroxyprednisolone

KEY WORDS
normal phase; SPE

REFERENCE
Teng,R.-L.; Benet,L.Z. Simultaneous measurement of prednisone, prednisolone and 6β-hydroxyprednisolone in urine by high-performance liquid chromatography using dexamethasone as the internal standard, *J.Chromatogr.*, **1989**, *493*, 421–423.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 1.5 μg betamethasone + 100 mg K_2HPO_4 + 500 mg anhydrous sodium sulfate + 5 mL diethyl ether, shake mechanically for 10 min, centrifuge at 2500 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. MeOH:water from 4:96 to 30:70 over 10 min, to 45:55 over 5 min, to 50:50 over 3 min
Column temperature: 40
Flow rate: 1
Injection volume: 15
Detector: UV 246

CHROMATOGRAM
Retention time: 4
Internal standard: betamethasone
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, cortisone, deoxycorticosterone, hydrocortisone, 11α-hydroxyprogesterone, prednisone, triamcinolone, triamcinolone acetonide

REFERENCE
Park,S.-J.; Kim,Y.-J.; Pyo,H.-S.; Park,J. Analysis of corticosteroids in urine by HPLC and thermospray LC/MS, *J.Anal.Toxicol.*, **1990**, *14*, 102–108.

SAMPLE
Matrix: urine
Sample preparation: Dilute, if necessary, 100 μL-1 mL urine to 1 mL with water, add 500 ng betamethasone, add to a Chem Elut high surface-area diatomaceous earth extraction column, after 5 min elute with two 6 mL portions of ethyl acetate, combine the eluates and wash them twice with 1 mL 200 mM NaOH. Dry the organic layer over 1 g anhydrous sodium sulfate, evaporate to dryness at 30° under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 6 37-53 μm HC-Pellocil
Column: 250 × 4.6 5-6 μm Zorbax SIL
Mobile phase: Dichloromethane:glacial acetic acid:MeOH 91.3:7.5:1.2
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Internal standard: betamethasone (8.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: prednisone, 20β-hydroxyprednisone, 6β-hydroxyprednisolone, 20α-hydroxyprednisolone, 20β-hydroxyprednisolone, hydrocortisone, 6β-hydroxycortisol, metabolites

KEY WORDS
normal phase; SPE

REFERENCE
Garg,V.; Jusko,W.J. Simultaneous analysis of prednisone, prednisolone and their major hydroxylated metabolites in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *567*, 39–47.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 µL 5 µg/mL IS in MeOH, inject 20 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS
Mobile phase: MeCN:water 32:68
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

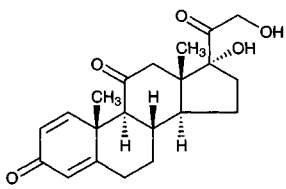
CHROMATOGRAM
Retention time: 5.5
Internal standard: methylprednisolone (9)

OTHER SUBSTANCES
Simultaneous: triamcinolone, triamcinolone acetonide, prednisone, dexamethasone, betamethasone, corticosterone, hydroxyprogesterone, fluorocortisone acetate
Interfering: cortisone, hydrocortisone, fluorocortisone

KEY WORDS
SPE also discussed

REFERENCE
Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Solvent and solid-phase extraction of natural and synthetic corticoids in human urine, *J.Chromatogr.B*, **1994**, *652*, 83–89.

Prednisone



Molecular formula: $C_{21}H_{26}O_5$
Molecular weight: 358.43
CAS Registry No.: 53-03-2, 125-10-0 (21-acetate)
Merck Index: 7904
Lednicer No.: 1 192

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge. Mix 1 mL plasma with 134.0 ng hydrocortisone-d_5 and 74.56 ng cortisone-d_5. Add the sample to the SPE cartridge, wash with 8 mL water, elute with 4 mL ethyl acetate, evaporate the eluate to dryness at 70° under a stream of nitrogen, dissolve the residue in 30 µL mobile phase, filter (0.45 µm), inject a 20 µL aliquot.

HPLC VARIABLES
Column: 125 × 4.0 4 µm LiChroCART Superspher 100

Mobile phase: A MeOH:THF:50 mM ammonium formate 17:53:180; B MeCN:50 mM ammonium formate 35:65
Flow rate: 0.6 (A); 1.3 (B)
Injection volume: 20
Detector: MS, Shimadzu LCMS-QP1000EX Model 750 B, thermospray, vaporizer control 155°, vaporizer tip 195°, vapor 274°, ion source block 295°, tip heater 305°, m/z 359

CHROMATOGRAM
Retention time: 9 (A)
Internal standard: hydrocortisone-d_5, cortisone-d_5
Limit of detection: 1.1 ng

OTHER SUBSTANCES
Extracted: hydrocortisone, cortisone, prednisolone

KEY WORDS
plasma; SPE

REFERENCE
Shibasaki,H.; Furuta,T.; Kasuya,Y. Quantification of corticosteroids in human plasma by liquid chromatography-thermospray mass spectrometry using stable isotope dilution, *J.Chromatogr.B*, **1997**, *692*, 7–14.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak Plus C18 SPE cartridge with 7 mL MeOH and 14 mL water. Add 40 ng 6β-hydroxycortisone to 400 μL plasma or urine, add the mixture to the SPE cartridge, wash with 6 mL water, 3 mL MeOH:water 12:88, and 3 mL petroleum ether, elute with 5 mL ethyl acetate. Dry the eluate under reduced pressure at 40°, add 200 μL MeCN:triethylamine 90:10 and MeCN:0.1% quinuclidine 20:80 to the residue, vortex. Add 200 μL 0.02% 9-anthroyl nitrile and a few molecular sieves (4Å), let stand for 30 min, evaporate under reduced pressure at 40°, dissolve the residue in 200 μL acetone, dilute with 2 mL n-hexane. Add the mixture to a Sep-Pak Plus Silica SPE cartridge, wash with 14 mL 1,2-dichloroethane, elute with 5 mL ethyl acetate. Evaporate the eluate under reduced pressure at 40°, reconstitute the residue in 200 μL mobile phase, inject a 30-60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Japan)
Mobile phase: Dioxane:ethyl acetate:chloroform:n-hexane:pyridine 58.1:11.6:11.6:16.3:2.4 (Caution! Dioxane and chloroform are carcinogens!)
Flow rate: 1 for 45 min, to 1.2 over 5 min
Injection volume: 30-60
Detector: F ex 360 em 460

CHROMATOGRAM
Retention time: 40
Internal standard: 6β-hydroxycortisone (86)
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: cortisone, prednisolone, hydrocortisone, 6β-hydroxycortisol, 6β-hydroxyprednisolone

KEY WORDS
derivatization; plasma; urine; SPE; normal phase

REFERENCE
Shibata,N.; Hayakawa,T.; Takada,K.; Hoshino,N.; Minouchi,T.; Yamaji,A. Simultaneous determination of glucocorticoids in plasma or urine by high-performance liquid chromatography with precolumn fluorimetric derivatization by 9-anthroyl nitrile, *J.Chromatogr.B*, **1998**, *706*, 191–199.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 241.7

CHROMATOGRAM

Retention time: 14.178

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare solutions in MeCN, dilute to an appropriate concentration with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 120 × 4.6 5 μm octadecyl Bakerbond
Mobile phase: MeCN:water 30:70 containing 16 mM β-cyclodextrin
Column temperature: 5
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 1.0

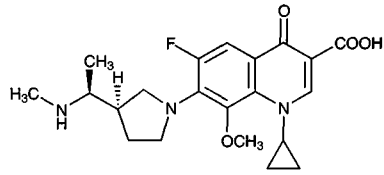
OTHER SUBSTANCES

Simultaneous: hydrocortisone, testosterone, cortisone, 17α-methyltestosterone, 17α-hydroxyprogesterone

REFERENCE

Zarzycki,P.K.; Wierzbowska,M.; Lamparczyk,H. The influence of temperature on the high performance liquid chromatographic separation of steroids using mobile phases modified with β-cyclodextrin, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1305–1311.

Premafloxacin



Molecular formula: $C_{21}H_{26}FN_3O_4$
Molecular weight: 403.45
CAS Registry No.: 143383-65-7

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 250-500 μg/mL solutions in mobile phase. Inject a 10 μL aliquot.

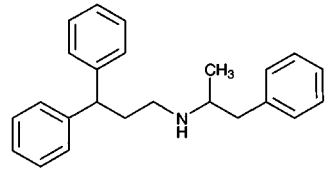
HPLC VARIABLES
Column: 100 × 4.6 3 μm YMC ODS-AQ
Mobile phase: A was MeCN:MeOH:THF:buffer 1.75:20.925:2.325:75 (Buffer was 100 mM phosphoric acid containing 46 mM tetrabutylammonium hydroxide, pH 3.)
Flow rate: 0.7
Injection volume: 10
Detector: UV 298

CHROMATOGRAM
Retention time: ca. 16

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Schinzer,W.C.; Bergren,M.S.; Aldrich,D.S.; Chao,R.S.; Dunn,M.J.; Jeganathan,A.; Madden,L.M. Characterization and interconversion of polymorphs of premafloxacin, a new quinolone antibiotic, *J.Pharm.Sci.*, **1997**, *86*, 1426–1431.

Prenylamine



Molecular formula: $C_{24}H_{27}N$
Molecular weight: 329.49
CAS Registry No.: 390-64-7, 69-43-2 (lactate)
Merck Index: 7919
Lednicer No.: 1 76

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.8

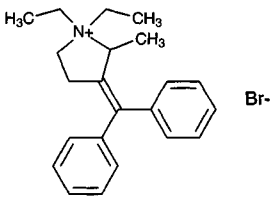
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Prifinium bromide



Molecular formula: $C_{22}H_{28}BrN$
Molecular weight: 386.38
CAS Registry No.: 4630-95-9
Merck Index: 7923

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

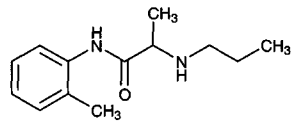
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.622

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Prilocaine



Molecular formula: $C_{13}H_{20}N_2O$
Molecular weight: 220.31
CAS Registry No.: 721-50-6, 1786-81-8 (HCl)
Merck Index: 7924
Lednicer No.: 1 17

SAMPLE
Matrix: blood
Sample preparation: Condition a diol AASP SPE cartridge (Jones Chromatography) with 1 mL MeOH, 1 mL water, 1 mL mobile phase, 0.5 mL MeOH, 0.5 mL toluene. 200 μL Plasma + 100 μL 1 μg/mL etidocaine in 100 mM K_2HPO_4, mix, add 1 mL toluene, vortex for 1 min, centrifuge at 12000 g for 1 min. Add 750 μL of the toluene layer to the SPE cartridge, wash with 500 μL MeCN, elute the contents of the SPE onto the column with mobile phase (MeCN used for purge (6 strokes) and afterwash (10 strokes), valve reset time 2 min).

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm Co:Pell ODS
Column: 150 × 4.6 Spherisorb 5 CN
Mobile phase: MeCN:water 40:60 containing 10 mM phosphoric acid
Flow rate: 1
Detector: E, Environmental Science Associates Model 5100A Coulochem, screen mode, electrode 1 +0.7 V, electrode 2 +0.9 V, palladium reference electrode, guard cell +1.2 V (before injection valve)

CHROMATOGRAM
Retention time: 8.0
Internal standard: etidocaine (10.8)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Whelpton,R.; Dudson,P.; Cannell,H.; Webster,K. Determination of prilocaine in human plasma samples using high-performance liquid chromatography with dual-electrode electrochemical detection, *J.Chromatogr.*, **1990**, *526*, 215–222.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 10 μL 15 mg/mL bupivacaine, mix, add 100 μL 2 M NaOH, vortex briefly, add 5 mL anhydrous ethyl ether, vortex for 30 s, rotate for 10 min, centrifuge at 1000 g for 5 min. Remove 4.5 mL ether and add to 250 μL 12.5 mM sulfuric acid, vortex for 30 s, rotate for 10 min, centrifuge for 5 min, inject a 50 μL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Octyl 1B (Keystone)
Mobile phase: MeCN:50 mM Na_2HPO_4 27:73 pH adjusted to 5.8 with 50% phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 3.32
Internal standard: bupivacaine (9.81)
Limit of detection: 4 ng/mL
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: lidocaine, o-toluidine

KEY WORDS
plasma; pig; pharmacokinetics

REFERENCE
Klein,J.; Fernandes,D.; Gazarian,M.; Kent,G.; Koren,G. Simultaneous determination of lidocaine, prilocaine and the prilocaine metabolite o-toluidine in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *655*, 83–88.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 227

CHROMATOGRAM
Retention time: 4.32
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 10 μL 100 μg/mL etidocaine, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL 10 mM HCl, add 3 mL diethyl ether, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 230

CHROMATOGRAM

Retention time: 7
Internal standard: etidocaine (14)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: lidocaine, bupivacaine, dibucaine
Also analyzed: procaine, butacaine, tetracaine, p-aminobenzoic acid, articaine, o-toluidine, caffeine, amphetamine, ephedrine, epinephrine, morphine, monoacetylmorphine, diamorphine, ethylmorphine, codeine, acetylcodeine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine,

thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 5.015

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, procaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993,** *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex Phase 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:acidified EtOH 75:20:5 (Acidified EtOH was EtOH:trifluoroacetic acid 20:1.)
Flow rate: 0.7
Detector: UV 242

KEY WORDS
chiral; $\alpha = 1.13$

REFERENCE

Phenomenex Catalog, **1994**, p. 1.038.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Partisil C18
Mobile phase: MeCN:10 mM pH 5.8 phosphate buffer 36:64
Detector: UV 214

CHROMATOGRAM
Retention time: 6.15

OTHER SUBSTANCES
Simultaneous: carbamazepine, lidocaine

REFERENCE
Verjee,Z.; Giesbrecht,E. Lidocaine and HPLC assay for anticonvulsants, *Clin.Chem.*, **1994**, *40*, 833–833.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 5-chloro-2-methylphenylcarbamate amylose in DMF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol:diethylamine 95:5:0.1
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.50

KEY WORDS
narrow-bore; chiral; α 1.38

REFERENCE
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE
Matrix: solutions

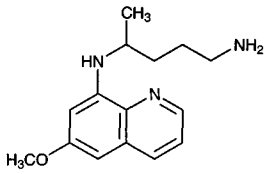
HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 75:20:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 11, 12 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Primaquine



Molecular formula: $C_{15}H_{21}N_3O$
Molecular weight: 259.35
CAS Registry No.: 90-34-6, 63-45-6 (phosphate)
Merck Index: 7925

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 500 µL 200 ng/mL IS in MeCN, mix, shake by hand for 2 min, let stand for 15 min with occasional shaking, centrifuge at 1200 g for 15 min. Filter (0.45 µm) the supernatant, inject a 20 µL aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Asahipak ODP-50 C18 polymer gel (Asahi Chemical)
Mobile phase: MeCN:70 mM pH 5.8 phosphate buffer 23:77 containing 10 µg/mL disodium EDTA
Column temperature: 40
Flow rate: 0.8
Injection volume: 20
Detector: E, Shimadzu L-ECD-6A, glassy carbon electrode +0.75 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.3
Internal standard: 2-methoxy-5-methylaniline (22.1)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

cow; plasma; calf; pharmacokinetics

REFERENCE

Endoh,Y.S.; Yoshimura,H.; Sasaki,N.; Ishihara,Y.; Sasaki,H.; Nakamura,S.; Inoue,Y.; Nishikawa,M. High-performance liquid chromatographic determination of pamaquine, primaquine and carboxy primaquine in calf plasma using electrochemical detection, *J.Chromatogr.*, **1992**, *579*, 123–129.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 50 µL 5 µg/mL IS in water + 50 µL 1 M KOH, add 2.5 mL hexane:isopropanol 97:3 containing 1 mL/L N,N'-dimethyloctylamine, vortex vigorously for 1 min, centrifuge at 4° at 1000 g for 10 min, freeze in liquid nitrogen, remove the organic layer, repeat extraction twice more. Combine the organic layers and evaporate under reduced pressure, reconstitute in 200 µL solvent containing 1 mL/L N,N'-dimethyloctylamine, inject an aliquot. (Solvent was MeCN:50 mM KH_2PO_4 60:40 adjusted to pH 7.5 with orthophosphoric acid. Rinse glass ware with hexane:isopropanol 97:3 containing 1 mL/L N,N'-dimethyloctylamine.)

HPLC VARIABLES

Guard column: 30 × 4.6 C18 (Pierce)
Column: 100 × 4.6 5 µm C18 (Pierce)
Mobile phase: MeCN:THF:buffer 60:1:39 containing 500 µL/L N,N'-dimethylactylamine (Buffer was 50 mM K_2HPO_4 adjusted to pH 6 with 20% orthophosphoric acid.)
Flow rate: 0.7
Injection volume: 50
Detector: UV 269

CHROMATOGRAM

Retention time: 3.9

Internal standard: 3-bromoprimaquine diphosphate (6.4)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: CDRI compound 80/53

KEY WORDS
serum

REFERENCE
Paliwal,J.K.; Gupta,R.C.; Grover,P.K. Simultaneous determination of a new antimalarial agent, CDRI compound 80/53, and its metabolite primaquine in serum by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *616*, 155–160.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 10 μg/mL IS in MeOH + 750 μL MeCN, mix, let stand at 4° in the dark for 1 h, centrifuge at 9500 g for 5 min. Remove the supernatant and evaporate under a stream of nitrogen for 45-60 min, reconstitute the residue in 100 μL mobile phase, centrifuge at 9500 g for 5 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:MeOH:buffer 24:20:56 (Buffer was 7 mM monochloroacetic acid containing 0.5 mM 1-decanesulfonic acid.)
Column temperature: 35
Flow rate: 1.4
Injection volume: 10
Detector: E, Bioanalytical Systems LC-4B, CC-4 cell, +0.82 V, clean electrode every 100 injections

CHROMATOGRAM
Retention time: 15
Internal standard: N1,N1-diethyl-N6-(6-methoxy-4-methyl-8-quinolinyl)-1,6-hexanediamine (WR6026) (35)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: carbenicillin, cefamandole, dicloxacillin, minocycline, moxalactam
Noninterfering: amikacin, amoxicillin, ampicillin, azlocillin, aztreonam, cefaclor, cefazolin, cefonicid, cefoperazone, cefotetan, cefoxitin, ceftazidime, ceftizoxime, ceftriaxone, cephalothin, cephalexin, chloramphenicol, chloroquine, ciprofloxacin, clindamycin, cloxacillin, erythromycin, gentamicin, imipenem, kanamycin, lincomycin, methicillin, nafcillin, neomycin, netilmicin, oxacillin, penicillin, piperacillin, rifampin, streptomycin, sulfamethoxazole, tetracycline, ticarcillin, trimethoprim, WR238605, WR242511, zidovudine

KEY WORDS
plasma; protect from light; pharmacokinetics

REFERENCE
Dean,R.A.; Ochieng,W.; Black,J.; Queener,S.F.; Bartlett,M.S.; Dumaual,N.G. Simultaneous determination of primaquine and carboxyprimaquine in plasma using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1994**, *655*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma or blood cells + 100 μL 0.5 μg/mL 6-methoxyprimaquine + 2 mL 25% ammonia (specific gravity 0.91), vortex for 2 min, add n-hexane:ethyl acetate 87.5:12.5, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness on

a vortex evaporator at 60°, reconstitute the residue in 100-250 μL mobile phase, inject a 20-250 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeCN:MeOH:1 M perchloric acid:water 30:9:1:95
Flow rate: 1.5
Injection volume: 20-250
Detector: UV 254

CHROMATOGRAM
Retention time: 12.5
Internal standard: 6-methoxyprimaquine (Walter Reed) (8)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: chloroquine, dapsone, pyrimethamine, quinine, sulfadoxine, sulfalene

KEY WORDS
plasma; blood cells

REFERENCE
Dua,V.K.; Kar,P.K.; Sarin,R.; Sharma,V.P. High-performance liquid chromatographic determination of primaquine and carboxyprimaquine concentrations in plasma and blood cells in *Plasmodium vivax* malaria cases following chronic dosage with primaquine, *J.Chromatogr.B*, **1996**, *675*, 93–98.

SAMPLE
Matrix: blood, saliva, urine
Sample preparation: Dilute 1 mL urine to 10 mL with water. 1 mL Plasma, saliva, or diluted urine + 200 μL perchloric acid, mix for 5 s, add 1 mL 5 M NaOH, add 4 mL diethyl ether, mix for 1 min, centrifuge at 3000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, mix for 1 min, centrifuge for 5 min, inject a 10 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:20 mM KH_2PO_4 10:15:75 containing 74 mM perchloric acid, pH 2.8
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10.8
Internal standard: primaquine

OTHER SUBSTANCES
Extracted: quinine
Noninterfering: acetaminophen, amodiaquine, chloroquine, chlorpheniramine, proguanil, promethazine, pyrimethamine

KEY WORDS
plasma; primaquine is IS

REFERENCE
Babalola,C.P.; Bolaji,O.O.; Dixon,P.A.F.; Ogunbona,F.A. Column liquid chromatographic analysis of quinine in human plasma, saliva and urine, *J.Chromatogr.*, **1993**, *616*, 151–154.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(4-tert-butylphenylcarbamate)
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: 22 (-), 33 (+)

KEY WORDS
chiral

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax-Sil
Mobile phase: Dichloromethane:MeOH:1 M perchloric acid 100:9:0.4
Flow rate: 0.8
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: chloroquine, dapsone, desethylchloroquine, dihydroquinidine, dihydroquinine, mefloquine, proguanil, pyrimethamine, quinidine, quinine, sulfadoxine, sulfalene, sulfamethoxazole
Interfering: amodiaquine

KEY WORDS
normal phase

REFERENCE
Dua,V.K.; Sarin,R.; Prakash,A. Determination of quinine in serum, plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 87–93.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:1 M perchloric acid:water 30:9:0.8:95
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.43

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, dapsone, pyrimethamine, quinidine, quinine, sulfadoxine, sulfalene, sulfamethoxazole

REFERENCE
Dua,V.K.; Sarin,R.; Sharma,V.P. Sulphadoxine concentrations in plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases after treatment with Fansidar using high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1317–1323.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 10, 12 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 40
Flow rate: 1
Detector: UV 210

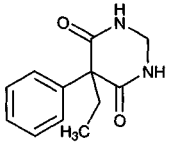
CHROMATOGRAM
Retention time: 35.17, 39.67

OTHER SUBSTANCES
Simultaneous: baclofen, levodopa, norephedrine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

Primidone



Molecular formula: $C_{12}H_{14}N_2O_2$
Molecular weight: 218.26
CAS Registry No.: 125-33-7
Merck Index: 7927
Lednicer No.: 1 276

SAMPLE
Matrix: blood

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 10, 12 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 40
Flow rate: 1
Detector: UV 210

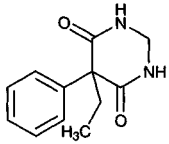
CHROMATOGRAM
Retention time: 35.17, 39.67

OTHER SUBSTANCES
Simultaneous: baclofen, levodopa, norephedrine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

Primidone



Molecular formula: $C_{12}H_{14}N_2O_2$
Molecular weight: 218.26
CAS Registry No.: 125-33-7
Merck Index: 7927
Lednicer No.: 1 276

SAMPLE
Matrix: blood

Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, clonazepam, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, phenytoin

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood
Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 3.0
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: ethosuximide, phenobarbital, phenytoin, carbamazepine, valproic acid

KEY WORDS
human; plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL heptabarbital in MeOH + 500 μL 400 mM pH 7.0 sodium phosphate buffer + 10 mL ethyl acetate, extract. Evaporate the extract to dryness at 50°, reconstitute the residue in 20 μL MeOH, inject a 3 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 Whatman Co:Pell ODS

Column: 125 × 4.5 5 μm SAS Hypersil
Mobile phase: MeCN:buffer 20:80 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 7.5 with phosphoric acid.)
Flow rate: 1.6
Injection volume: 3
Detector: UV 200

CHROMATOGRAM
Retention time: 4.2
Internal standard: heptabarbital (9.8)
Limit of quantitation: 2.5 μM

OTHER SUBSTANCES
Extracted: ethosuximide, phenobarbital, pheneturide, carbamazepine, phenytoin
Simultaneous: phenylethylmalonamide, sulthiame, sulfamethoxazole, ethotoin, butabarbital, pentobarbital, methsuximide, cyclobarbital, ethylphenacemide, amobarbital, glutethimide, secobarbital, barbital

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KEY WORDS
plasma; horse

REFERENCE
Christofides,J.A.; Fry,D.E. Measurement of anticonvulsants in serum by reversed-phase ion-pair liquid chromatography, *Clin.Chem.*, **1980**, *26*, 499–501.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 20 μg/mL IS in MeOH:water 10:90 + 75 μL glacial acetic acid, vortex for 30 s, add 5 mL chloroform, shake for 5 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 Permaphase ETH (DuPont)
Column: 250 × 4.6 CLC 1 C8 (DuPont)
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM KH_2PO_4 and 1 mM K_2HPO_4 adjusted to pH 5.6.)
Column temperature: 25
Flow rate: 2
Injection volume: 40
Detector: UV 220

CHROMATOGRAM
Retention time: 2.0
Internal standard: alphenal (5-allyl-5-phenylbarbituric acid) (4.4)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: ethosuximide, phenytoin, carbamazepine, phenobarbital
Simultaneous: amobarbital, barbital, chlordiazepoxide, codeine, cortisol, ethotoin, glutethimide, hexobarbital, mephenytoin, mephobarbital, metharbital, methsuximide, nitrazepam, pentobarbital, phenacetin, phensuximide, secobarbital
Noninterfering: acetaminophen, acetazolamide, amphetamine, bilirubin, caffeine, diazepam, dimenhydrinate, meperidine, meprobamate, methamphetamine, methaqualone, methylphenidate, nicotine, propoxyphene, theophylline, valproate

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KEY WORDS
plasma; serum

REFERENCE
Rydzewski,R.S.; Gadsden,R.H.; Phelps,C.A. Simultaneous rapid HPLC determination of anticonvulsant drugs in plasma and correlation with EMIT, *Ann.Clin.Lab.Sci.*, **1980**, *10*, 89–94.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 10.0
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS
serum

REFERENCE
Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Serum or plasma + 400 μL 10 μg/mL IS in acetone, vortex for 10 s, centrifuge at 4500-5000 g for 1 min, remove the supernatant to another tube, centrifuge for 30 s, inject a 5-7.5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:buffer 17:28:55, final pH 6.8-7.0 (Buffer was 400 μL 1 M KH_2PO_4 in 1 L water, pH adjusted to 6.0 with 900 mM phosphoric acid.)
Column temperature: 30
Flow rate: 0.7
Injection volume: 5-7.5
Detector: UV 195

CHROMATOGRAM
Retention time: 7.4
Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (13.8)

OTHER SUBSTANCES
Extracted: carbamazepine, N-desmethylmethsuximide, ethosuximide, phenobarbital, phenytoin
Simultaneous: acetaminophen, butalbital, caffeine, hexobarbital, methsuximide, phenacetin, phenylethylmalonamide, salicylic acid

KEY WORDS
plasma; serum

REFERENCE
Szabo,G.K.; Browne,T.R. Improved isocratic liquid-chromatographic simultaneous measurement of phenytoin, phenobarbital, primidone, carbamazepine, ethosuximide, and N-desmethylmethsuximide in serum, *Clin.Chem.*, **1982**, *28*, 100–104.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 10 μg/mL IS in MeCN, vortex for 10 s, centrifuge at 3000 g for 1 min, remove the supernatant and place it in another tube, centrifuge for 1 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 5 μm Nova Pak C18 Radial pak
Mobile phase: MeCN:MeOH:acetone:buffer 8:21:10:61 adjusted to pH 7.95 ± 0.02 with NaOH (Buffer was 1.36 g/L KH_2PO_4.)
Flow rate: 2.8
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 2.03
Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (4.89)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: ethosuximide, phenobarbital, carbamazepine, phenytoin, metabolites
Simultaneous: acetaminophen, N-acetylprocainamide, aspirin, ampicillin, caffeine, cephapirin, chloramphenicol, digoxin, disopyramide, hexobarbital, indomethacin, lidocaine, mephobarbital, methsuximide, nafcillin, pentobarbital, phenylethylmalonamide, procainamide, quinidine, salicylic acid, secobarbital, sulfamerazine, sulfamethazine, terbutaline, tetracycline, theobromine, theophylline
Noninterfering: acetazolamide, amikacin, cephalosporin C, gentamicin, propranolol, sulfadiazine, sulfamethoxazole, sulfisoxazole, tobramycin, valproic acid, verapamil

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Rognerud,C.L. Simultaneous measurement of ethosuximide, primidone, phenobarbital, phenytoin, carbamazepine, and their bioactive metabolites by liquid chromatography, *Clin.Chem.*, **1984**, *30*, 1667–1670.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 200 μL 1 M HCl saturated with ammonium sulfate, vortex for 20 s, add 60 μL 10 μg/mL 4-methylprimidone in MeCN, vortex for 20 s, centrifuge at 2700 g for 5 min, inject a 5-10 μL aliquot of the MeCN layer.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeOH:THF:50 mM pH 5.9 phosphate buffer 44:1:55
Column temperature: 50
Flow rate: 1.1
Injection volume: 5-10
Detector: UV 210

CHROMATOGRAM
Retention time: 3
Internal standard: 4-methylprimidone (5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, phenobarbital, phenytoin, valproic acid
Simultaneous: acetaminophen, salicylic acid, ethylphenylmalonamide, theophylline, caffeine, ethosuximide, chloramphenicol, methylphenobarbital, glutethimide, pentobarbital, lidocaine, diazepam

KEY WORDS
plasma

REFERENCE
Kushida,K.; Ishizaki,T. Concurrent determination of valproic acid with other antiepileptic drugs by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 131–139.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 1 mL 5 μg/mL 5-ethyl-5-tolylhydantoin in MeCN, agitate for 3 min. Remove the supernatant and evaporate it to dryness, dissolve the residue in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: pellicular reversed phase (Chrompack 28653)
Column: 100 × 3 octyl CP-tm-Spher C8 glass column (Chrompack)
Mobile phase: MeCN:50 mM NaH_2PO_4 25:75 adjusted to pH 2.2 with phosphoric acid
Flow rate: 0.9
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 1.7
Internal standard: 5-ethyl-5-tolylhydantoin (7.8)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: phenobarbital, ethylphenylmalonamide

KEY WORDS
serum

REFERENCE
Van Damme,M.; Molle,L.; Abi Khalil,F. Useful sample handlings for reversed phase high performance liquid chromatography in emergency toxicology, *J.Toxicol.Clin.Toxicol.*, **1985**, *23*, 589–614.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 2.71

Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, secobarbital, theophylline, thiopental

Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, procainamide, salicylamide, salicylic acid, sulfamethoxazole, tolmetin, trimethoprim, vancomycin

Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

Interfering: sulindac, phenylbutazone

KEY WORDS

serum

REFERENCE

Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE

Matrix: blood

Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM

Retention time: 1.61
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, caffeine, barbital, ethosuximide, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone

Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS

plasma; SPE

REFERENCE

Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 2 μg 10-methoxycarbamazepine + 25 μL 1 M NaOH + 1.2 mL dichloromethane, mix for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 20 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.9 10 μm LiChrosorb RP8
Mobile phase: MeCN:water 32:68
Flow rate: 1.8
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 2.7
Internal standard: 10-methoxycarbamazepine (9.3)

OTHER SUBSTANCES
Extracted: carbamazepine, oxcarbazepine, phenobarbital
Noninterfering: clobazam, clonazepam, diazepam, ethosuximide, phenytoin, valproic acid

KEY WORDS
plasma

REFERENCE
Elyas,A.A.; Goldberg,V.D.; Patsalos,P.N. Simple and rapid micro-analytical high-performance liquid chromatographic technique for the assay of oxcarbazepine and its primary active metabolite 10-hydroxycarbazepine, *J.Chromatogr.*, **1990**, *528*, 473–479.

SAMPLE
Matrix: blood
Sample preparation: Inject 20 μL serum onto column A with mobile phase A and elute to waste, after 1.5 min backflush the contents of column A onto column B with mobile phase B, after 1 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 30 × 4.6 ISRP silica (for preparation see Anal. Chem. 1989, 61, 2445); B 150 × 4.6 5 μm Nucleosil C18
Mobile phase: A 14 mM NaH_2PO_4 containing 6 mM Na_2HPO_4; B MeCN:MeOH:14 mM NaH_2PO_4 containing 6 mM Na_2HPO_4 15:20:65
Flow rate: 0.8
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Extracted: carbamazepine, phenobarbital, phenytoin

KEY WORDS
serum; column-switching

REFERENCE
Haginaka,J.; Wakai,J.; Yasuda,H.; Kimura,Y. Determination of anticonvulsant drugs and methyl xanthine derivatives in serum by liquid chromatography with direct injection: column-switching method using a new internal-surface reversed-phase silica support as a precolumn, *J.Chromatogr.*, **1990**, *529*, 455–461.

SAMPLE
Matrix: blood
Sample preparation: Add two volumes of MeCN to the mouse serum, mix, centrifuge at 1500 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Sentry (Waters)
Column: 150 × 4.6 Nova-Pak C18
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110
Column temperature: 40
Flow rate: 0.5
Injection volume: 5
Detector: UV 214

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Extracted: phenylethyl malonamide, phenobarbital, carbamazepine, phenytoin, carbamazepine-10,11-epoxide

KEY WORDS
serum; mouse

REFERENCE
Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE
Matrix: blood, saliva, urine
Sample preparation: Serum. 100 μL Serum + 200 μL MeCN, vortex for 10 s, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Saliva. 250 μL Saliva + 50 μL MeCN, centrifuge at 1500 g for 5 min, inject a 2 μL aliquot of the supernatant. Urine. Condition a Sep-Pak SPE cartridge with 5 mL MeCN then 20 mL water. Add 2 mL urine to the cartridge, wash with 20 mL water, elute with 500 μL MeCN, inject 2 μL of the eluent.

HPLC VARIABLES
Guard column: 20 × 2 3 μm ODS-Hypersil
Column: 250 × 2 3 μm ODS-Hypersil
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 50:30:110
Column temperature: 40
Flow rate: 0.2
Injection volume: 2
Detector: UV 200

CHROMATOGRAM
Retention time: 5.0
Limit of quantitation: 780 ng/mL

OTHER SUBSTANCES
Simultaneous: p-hydroxyphenobarbital, phenylethylmaleimide, phenobarbital, dihydrodihydroxycarbamazepine, 5-(p-hydroxyphenyl)-5-phenylhydantoin, carbamazepine, 5-(m-hydroxyphenyl)-5-phenylhydantoin, phenytoin, carbamazepine-10,11-epoxide, hexobarbital, nitrazepam, clonazepam
Noninterfering: oxazepam, nordiazepam, cyheptamide, diazepam, prezepam, temazepam, lorazepam, chlordiazepoxide

KEY WORDS
serum; SPE

REFERENCE
Liu,H.; Delgado,M.; Forman,L.J.; Eggers,C.M.; Montoya,J.L. Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1993**, *616*, 105–115.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize 20-200 mg brain tissue with 1 mL 1.5 μg/mL IS in 1% ammonium acetate + 1% sodium azide buffer:MeCN 99:1, flush apparatus with 1 mL extraction buffer, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 μL MeOH, add 50 μL water, inject a 10-25 μL aliquot. Serum. 100 μL Serum + 1 mL 1.5 μg/mL IS in 1% ammonium acetate + 1% sodium azide buffer:MeCN 99:1,mix, add 1 mL extraction buffer, mix, add 1 mL acetone, shake for 5 min, centrifuge for 10 min. Add sample to an Extrelut-3 SPE cartridge (Kieselguhr), add 1 mL extraction buffer, wait for 10 min, elute with 15 mL extraction solvent. Evaporate the eluate, take up residue in 50 μL MeOH, add 50 μL water, inject a 10-25 μL aliquot. (Extraction buffer was 20 g $NaH_2PO_4.2H_2O$ + 4.5 g $Na_2HPO_4.2H_2O$ + 1.5 NaN_3 in 1 L water, pH 6. Extraction solvent was dichloromethane:isopropanol 97:3.)

HPLC VARIABLES
Column: 200 × 2.1 5 μm Hypersil ODS
Mobile phase: Gradient. A was MeCN:50 mM $(NH_4)H_2PO_4$ (pH 4.4) 10:90. B was MeCN:50 mM $(NH_4)H_2PO_4$ (pH 4.4) 60:40. A:B from 85:15 to 55:45 over 9.5 min, keep at 55:45 for 0.5 min, return to 85:15 over 0.5 min.
Column temperature: 65
Flow rate: 0.3
Injection volume: 10-25
Detector: UV 207

CHROMATOGRAM
Retention time: 3.73
Internal standard: 5-ethyl-5-(p-tolyl)barbituric acid (9.07)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: phenobarbital, N-desmethylmethsuximide, carbamazepine-10,11-epoxide, phenytoin, carbamazepine

KEY WORDS
serum; SPE; brain

REFERENCE
Juergens,U.; Rambeck,B. Sensitive analysis of antiepileptic drugs in very small portions of human brain by microbore HPLC, *J.Liq.Chromatogr.*, **1987**, *10*, 1847–1863.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Inject a 5 or 20 μL aliquot directly onto the column with mobile phase A or C. Urine. Inject a 5 μL aliquot directly onto the column with mobile phase C.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 10:90 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer 14:86 for 5 min, to 25:75 over 1 min, to 30:70 over 2 min, to 50:50 over 3 min, maintain at 50:50 for 6 min (C)
Flow rate: 1
Injection volume: 5 (A, C), 20 (B)
Detector: UV 254 (serum); UV 230 (urine)

CHROMATOGRAM
Retention time: 2.16 (A, serum), 9.7 (B, serum), 4.5 (C, urine)
Limit of detection: 1 ng (urine)

OTHER SUBSTANCES
Extracted: acetaminophen (B), barbital (B), carbamazepine (B,C), ethosuximide (A), methamphetamine (A), phenobarbital (B,C), phenytoin (B,C), sulfamethoxazole (A), sulfapyridine (B)
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.13

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30

Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Pristinamycin

Merck Index: 7933

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 227.5

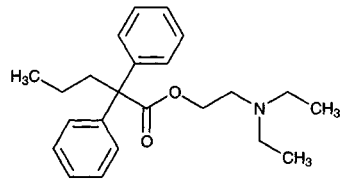
CHROMATOGRAM
Retention time: 17.235

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Proadifen

Molecular formula: $C_{23}H_{31}NO_2$
Molecular weight: 353.50
CAS Registry No.: 302-33-0, 62-68-0 (HCl)



SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

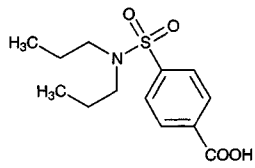
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cy-

clizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Probenecid



Molecular formula: $C_{13}H_{19}NO_4S$
Molecular weight: 285.36
CAS Registry No.: 57-66-9
Merck Index: 7934
Lednicer No.: 1 135

SAMPLE

Matrix: blood
Sample preparation: Filter plasma (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm GFF-S5-80 internal-surface reversed phase "Pinkerton" (Regis)
Mobile phase: THF:100 mM potassium phosphate 5:95, pH 7.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7.8

KEY WORDS

plasma; direct injection

REFERENCE

Nakagawa,T.; Shibukawa,A.; Shimono,N.; Kawashima,T.; Tanaka,H.; Haginaka,J. Retention properties of internal-surface reversed-phase silica packing and recovery of drugs from human plasma, *J.Chromatogr.*, **1987**, *420*, 297–311.

SAMPLE

Matrix: blood

Sample preparation: 50 μL Plasma + 350 μL MeOH, mix thoroughly, centrifuge at 10000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeCN:20 mM pH 7.5 phosphate buffer 25:75
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 10
Limit of quantitation: 1000 ng/mL

KEY WORDS

plasma

REFERENCE

Terashita,S.; Sawamoto,T.; Deguchi,S.; Tokuma,Y.; Hata,T. Sex-dependent and independent renal excretion of nilvadipine metabolites in rat: evidence for a sex-dependent active secretion in kidney, *Xenobiotica*, **1995**, *25*, 37–47.

SAMPLE

Matrix: blood

Sample preparation: 150 μL Plasma + 150 μL MeCN, vortex, rotate at 20 rpm for 10 min; centrifuge at 1000 g for 10 min. Transfer supernatant to another tube and add 7 volumes dichloromethane, equilibrate for 10 min, rotate at 20 rpm for 10 min; centrifuge at 1000 g for 10 min, inject an aliquot of the upper aqueous layer (J.Chromatogr. 1987, 413, 109).

HPLC VARIABLES

Guard column: C18
Column: 150 × 1.6 Spherisorb S5-ODS2 C18
Mobile phase: MeOH:100 mM pH 3 acetate buffer 50:50
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Limit of detection: 300 ng/mL

KEY WORDS

plasma; rat

REFERENCE

Gimeno,M.J.; Martínez,M.; Granero,L.; Torres-Molina,F.; Peris,J.-E. Influence of probenecid on the renal excretion mechanisms of cefadroxil, *Drug Metab.Dispos.*, **1996**, *24*, 270–272.

SAMPLE

Matrix: blood, CSF

Sample preparation: Mix 100 μL plasma or 50 μL CSF with an equal volume of 5 μg/mL n-butyl p-hydroxybenzoate in MeCN, centrifuge at 14000 rpm, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4 LiChrospher RP-18e

Mobile phase: MeCN:water:acetic acid 50:49.9:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 244

CHROMATOGRAM
Internal standard: n-butyl p-hydroxybenzoate

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Seki,T.; Sato,N.; Hasegawa,T.; Kawaguchi,T.; Juni,K. Nasal absorption of zidovudine and its transport to cerebrospinal fluid in rats, *Biol.Pharm.Bull.*, **1994**, *17*, 1135–1137.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Plasma. Add 500 μL MeCN to 500 μL plasma while mixing on a Whirlmixer, rotate for 10 min, centrifuge at 1000 g for 5 min. Remove a 700 μL aliquot of the supernatant and ad it to 3.5 mL dichloromethane, mix for 30 s, centrifuge at 1000 g for 1 min, inject a 20 μL aliquot of the aqueous layer. Urine. Dilute with Sörensen buffer, inject an aliquot. CSF. Inject an aliquot directly.

HPLC VARIABLES
Column: 100 × 3 5 μm MOS-Hypersil C8
Mobile phase: MeCN:MeOH:buffer 12:26:62 containing 3 mM tetrabutylammonium bromide (Buffer was 5 mM pH 5.0 sodium acetate.)
Column temperature: 22
Flow rate: 1
Injection volume: 20
Detector: UV 231

CHROMATOGRAM
Retention time: 11
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: penicillin G

KEY WORDS
plasma

REFERENCE
van Gulpen,C.; Brokerhof,A.W.; van der Kaay,M.; Tjaden,U.R.; Mattie,H. Determination of benzylpenicillin and probenecid in human body fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *381*, 365–372.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 350 μL 2 μg/mL Naproxen in 10 mM pH 6.0 phosphate buffer containing 0.05% MeOH + 650 μL pH 6 phosphate buffer + 100 μL plasma + 0.5 mL 1 M pH 2 phosphate buffer + 10 mL diethyl ether, vortex 1 min, centrifuge at 2000 g for 3 min. Remove organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 250 μL mobile phase, vortex for 15 s, inject aliquot. Urine. 350 μL 20 μg/mL Naproxen in 10 mM pH 6.0 phosphate buffer containing 0.5% MeOH + 650 μL pH 6 phosphate buffer + 100 μL urine + 1 mL 0.5 M pH 7 phosphate buffer + 10 mL diethyl ether, vortex 1 min, centrifuge at 2000 g for 3 min. Remove organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 250 μL mobile phase, vortex for 15 s, inject aliquot.

HPLC VARIABLES
Guard column: 40 × 3.2 30-44 μm Vydac reverse-phase

Column: 40 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 6:94 to 8:92
Flow rate: 2
Injection volume: 5-200
Detector: UV 262

CHROMATOGRAM
Retention time: 18
Internal standard: naproxen (10)
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: ketoprofen, fenoprofen, salicylic acid

KEY WORDS
plasma

REFERENCE
Upton,R.A.; Buskin,J.N.; Guentert,T.W.; Williams,R.L.; Riegelman,S. Convenient and sensitive high-performance liquid chromatography assay for ketoprofen, naproxen and other allied drugs in plasma or urine, *J.Chromatogr.*, **1980**, *190*, 119–128.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 100 μL 100 μg/mL indoprofen in water + 100 μL 600 mM sulfuric acid + 5 mL isooctane:isopropanol 95:5, vortex 30 s, centrifuge at 1800 g for 5 min. Remove organic layer and add 5 mL water to it. Vortex for 30 s, centrifuge for 3 min. Remove organic layer and evaporate it to dryness on a Speed Vac concentrator. Reconstitute residue in 100 μL 50 mM triethylamine in MeCN, vortex 30 s, add 50 μL 60 mM ethyl chloroformate in MeCN, let stand 30 s, add 50 μL 1 M L-leucinamide hydrochloride and 1 M triethylamine in MeOH, let stand 2 min, add 50 μL water, inject 10-60 μL aliquots. Urine. 100 μL Urine + 25 μL 1 M NaOH, add 125 μL 600 mM sulfuric acid, proceed as for plasma.

HPLC VARIABLES
Guard column: 50 × 5 37-53 μm C18 material
Column: 100 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:60 mM KH_2PO_4:triethylamine 35:65:0.1
Flow rate: 1
Injection volume: 10-60
Detector: UV 275

CHROMATOGRAM
Retention time: 18
Internal standard: indoprofen (6(R), 7(S))
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: ketoprofen
Also analyzed: fenoprofen, cicloprofen, pirprofen, flurbiprofen, indoprofen, carprofen

KEY WORDS
plasma; rat; derivatization

REFERENCE
Palylyk,E.L.; Jamali,F. Simultaneous determination of ketoprofen enantiomers and probenecid in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *568*, 187–196.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 100 μL 600 mM sulfuric acid + 4 mL isooctane: isopropanol 95:5, vortex 30 s, centrifuge at 1800 g for 5 min. Remove organic layer and add 4

mL water to it. Vortex for 30 s, centrifuge for 3 min. Remove organic layer and evaporate it to dryness on a Speed Vac concentrator. Reconstitute residue in 200 μL MeOH, vortex 30 s, add 100 μL 100 μg/mL indoprofen in water, 20 μL aliquots. Urine. 100 μL Urine + 25 μL 1 M NaOH, add 125 μL 600 mM sulfuric acid, proceed as for plasma.

HPLC VARIABLES
Guard column: 50 × 5 37-53 μm C18 material
Column: 100 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:60 mM KH_2PO_4:triethylamine 25:75:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 6.5
Internal standard: indoprofen (3.4)

OTHER SUBSTANCES
Simultaneous: ketoprofen

KEY WORDS
plasma; rat

REFERENCE
Palylyk,E.L.; Jamali,F. Simultaneous determination of ketoprofen enantiomers and probenecid in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *568*, 187–196.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spheri-5 RP-8
Mobile phase: MeOH:buffer 30:70 (Prepare buffer by mixing 4 mM Na_2HPO_4 and 7 mM KH_2PO_4 to achieve pH 7.)
Flow rate: 1
Injection volume: 50
Detector: F ex 355 em 460 (408 nm cutoff filter) following post-column extraction. The column effluent mixed with 50 μg/mL reagent in mobile phase pumped at 0.5 mL/min and then with chloroform pumped at 1 mL/min and the mixture flowed through a 1.8 m × 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector. (Synthesize the reagent, α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate, as follows. Stir 20 mmoles 3,4-dimethoxyphenylacetonitrile and 20 mmoles p-toluamide in 50 mL EtOH at 50°, add 5 mL 50% aqueous KOH slowly, stir at 50° for 5 min, cool to room temperature, filter, dry the precipitate of α-(3,4-dimethoxyphenyl)-4'- methylcinnamonitrile. Dissolve 20 mmoles α-(3,4-dimethoxyphenyl)-4'-methylcinnamonitrile, 20 mmoles N-bromosuccinimide, and 20 mg benzoyl peroxide in 100 mL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), reflux with stirring for 1.5 h , cool, filter, evaporate to dryness under reduced pressure, recrystallize from MeOH to give α-(3,4-dimethoxyphenyl)-4'-bromomethylcinnamonitrile. Vigorously stir 30 mmoles anhydrous dimethylamine in 100 mL dry benzene (Caution! Benzene is a carcinogen!) at 0°, very slowly add 10 mmoles α-(3,4-dimethoxyphenyl)-4'-bromomethylcinnamonitrile while stirring at 0°, stir at room temperature overnight, add 150 mL water, remove the organic phase, extract the aqueous phase twice with 100 mL portions of diethyl ether, wash the organic layers with saturated NaCl solution, dry over anhydrous magnesium sulfate, evaporate under reduced pressure to give α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile (J.Chem.Eng.Data 1987, 32, 387). Reflux 10 mmoles α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile, 20 mmoles dimethyl sulfate (Caution! Dimethyl sulfate is a carcinogen and acutely toxic!), and 5 g potassium carbonate in 50 mL acetone for 1 h, cool to room temperature, filter, dry the precipitate under vacuum at room temperature overnight, recrystallize from chloroform containing 2-3 drops of 95% EtOH to give α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate (mp 212-215°). Protect solutions from light.)

CHROMATOGRAM
Retention time: k' 1.2791
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: ibuprofen, ketoprofen, mefenamic acid, naproxen, salicylic acid, valproic acid

KEY WORDS
post-column extraction; post-column reaction

REFERENCE
Kim,M.; Stewart,J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α-phenylcinnamonitrile quaternary ammonium salt as a new fluorescent ion-pair reagent, *J.Liq.Chromatogr.*, **1990**, *13*, 213–237.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinna-

mine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 7.49

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 15.78 (A), 16.1 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 5000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, spironolactone, canrenone, flumethiazide, bumetanide, ethacrynic acid

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 15:15:70:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM

Retention time: 3.2

Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES

Extracted: bumetanide, ethacrynic acid, spironolactone

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 19.9
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN: water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 7.3
Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, clopamide, chlorthalidone, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, spironolactone, torsemide, triamterene

REFERENCE
Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE
Matrix: urine
Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 Hypersil ODS-C18
Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

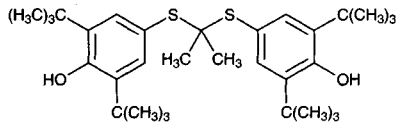
CHROMATOGRAM
Retention time: 10.3
Limit of detection: 200 ng/mL.

OTHER SUBSTANCES
Simultaneous: bumetanide, ethacrynic acid, acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, spironolactone, triamterene

KEY WORDS
column-switching

REFERENCE
Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Probucol



Molecular formula: $C_{31}H_{48}O_2S_2$
Molecular weight: 516.85
CAS Registry No.: 23288-49-5
Merck Index: 7935
Lednicer No.: 2 126

SAMPLE
Matrix: blood
Sample preparation: Dilute 1 mL serum 0.5-5 times with saline. Add 1 mL 10 μg/mL IS in EtOH to 1 mL diluted serum dropwise while vortexing, add 1.5 mL n-heptane, vortex for 1 min, centrifuge at 3000 rpm (Labofuge) for 15 min. Remove 1.3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 40 μL MeCN:THF 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil

Mobile phase: MeCN:water:THF 81.3:5.7:13
Column temperature: 40
Flow rate: 0.4
Injection volume: 5
Detector: UV 244

CHROMATOGRAM
Retention time: 2.763
Internal standard: 2-pentanone bis(3,5-di-tert)mercaptole (3.666)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: vitamin E (α-tocopherol), gamma-tocopherol, vitamin A (retinol), lycopene, α-carotene, β-carotene, metabolites

KEY WORDS
serum

REFERENCE
Schäfer Elinder,L.; Walldius,G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants, *J.Lipid Res.*, **1992**, *33*, 131–137.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 400 μL 20 μg/mL IS in EtOH + 800 μL isooctane + 500 μL water, vortex for 20 s, centrifuge at 960 g for 5 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL MeCN, inject a 10 μL aliquot.

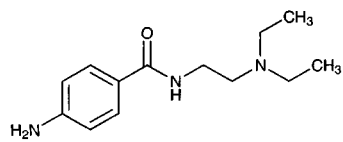
HPLC VARIABLES
Column: 200 × 3 5 μm 5 μm Hypersil ODS
Mobile phase: MeCN:water 96:4
Flow rate: 1
Injection volume: 10
Detector: UV 241

CHROMATOGRAM
Retention time: 5
Internal standard: 4,4'-[1-methylbutylidene-bis(thio)]-bis[2,6-bis(1,1-dimethylethyl)]phenol (MDL 27272) (7)
Limit of detection: 500 ng/mL

KEY WORDS
plasma; rabbit

REFERENCE
Nourooz-Zadeh,J.; Gopaul,N.K.; Forster,L.A.; Ferns,G.A.; Änggård,E.E. Measurement of plasma probucol levels by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 55–60.

Procainamide



Molecular formula: $C_{13}H_{21}N_3O$
Molecular weight: 235.33
CAS Registry No.: 51-06-9, 614-39-1 (HCl)
Merck Index: 7936
Lednicer No.: 1 14

SAMPLE
Matrix: activated neutrophils
Sample preparation: Centrifuge, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 mm long 5 μm Spherisorb ODS2
Mobile phase: MeCN:water:acetic acid:triethylamine 20:80:1:0.05
Flow rate: 1
Injection volume: 100
Detector: UV

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Uetrecht,J.P.; Zahid,N.; Whitfield,D. Metabolism of vesnarinone by activated neutrophils: Implications for vesnarinone-induced agranulocytosis, *J.Pharmacol.Exp.Ther.*, **1994**, *270*, 865–872.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL buffer + 50 μL 10 μg/mL N-propionylprocainamide in MeCN + 3 mL chloroform:isopropanol 95:5, mix for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot. (Buffer was 2.9 g/L sodium bicarbonate in water, adjusted to pH 11.0 with NaOH.)

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:buffer 9.75:90.25 (Buffer was 100 mM KH_2PO_4 adjusted to pH 4.0 with phosphoric acid.) (At the end of each day clean with water for 20 min and MeOH for 30 min.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.8
Internal standard: N-propionylprocainamide (6)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: N-acetylprocainamide
Simultaneous: dyphylline, theophylline, caffeine, aspirin, salicylic acid, acetaminophen
Noninterfering: benzoic acid

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Frawley,V.L. Theophylline, dyphylline, caffeine, acetaminophen, salicylate, acetylsalicylate, procainamide, and N-acetylprocainamide determined in serum with a single liquid-chromatographic assay, *Clin.Chem.*, **1982**, *28*, 2157–2160.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 5 mL MTBE, place on a reciprocating shaker at low speed for 15 min, centrifuge at 1250 g for 10 min. Remove organic layer and evaporate under a stream of nitrogen. Reconstitute in 200 μL mobile phase, inject 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelco Pelliguard C18
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:7 mM sodium heptanesulfonate 18:82, containing 1% glacial acetic acid and 0.035% triethylamine
Flow rate: 0.8
Injection volume: 100
Detector: UV 272

CHROMATOGRAM
Retention time: 10.1
Internal standard: procainamide.HCl

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide
Noninterfering: aspirin, acetaminophen, ibuprofen

KEY WORDS
plasma; procainamide is IS

REFERENCE
Azumaya,C.T. Sensitive liquid chromatographic method for the determination of hydrochlorothiazide in human plasma, *J.Chromatogr.*, **1990**, *532*, 168–174.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum or plasma + 100 μL 500 mM sodium carbonate + 100 μL 15 μg/mL N-propionylprocainamide in water, vortex for 5 s, add 0.5 (procainamide) or 1 (tocainide) mL dichloromethane, vortex for 30 (procainamide) or 60 (tocainide) s, centrifuge at 9500 g for 1 min. Remove the lower organic layer and add it to 200 μL 10 mM HCl, vortex for 15 s, centrifuge, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 100 × 5 NovaPak cyano HP radial compression
Mobile phase: MeCN:buffer 10:90, final pH adjusted to 6.0 (Buffer was 5 mM acetate buffer containing 0.05% triethylamine.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3.7
Internal standard: N-propionylprocainamide (6.1)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: N-acetylprocainamide, tocainide
Simultaneous: disopyramide, lidocaine, mexiletine, quinidine
Noninterfering: carbamazepine, desmethyldoxepin, digoxin, doxepin, ethosuximide, lithium, phenobarbital, phenytoin, primidone, propranolol, theophylline, valproic acid

KEY WORDS
serum; plasma

REFERENCE
vasBinder,E.; Annesley,T. Liquid chromatographic analysis of mexiletine in serum, with alternate application to tocainide, procainamide, and N-acetylprocainamide, *Biomed.Chromatogr.*, **1991**, *5*, 19–22.

SAMPLE
Matrix: blood

Sample preparation: Condition a 100 mg LRC Bond Elut unendcapped cyanopropyl SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Serum or plasma + 250 μL buffer, vortex, centrifuge at 1300 rpm for 10 min (plasma only), add 1 mL sample to SPE cartridge, wash twice with 1 mL water, dry with nitrogen for 30 s, elute with two 250 μL aliquots of MeOH: buffer 60:40, inject 100 μL aliquot of buffer. (Buffer was 50 mM KH_2PO_4 and Na_2HPO_4 50:50 v/v adjusted to pH 6.0 with phosphoric acid.)

HPLC VARIABLES
Guard column: 15 × 4.6 7 μm Brownlee RP-18
Column: 250 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 50 mM KH_2PO_4 and Na_2HPO_4 50:50 v/v adjusted to pH 6.0 with phosphoric acid.)
Column temperature: 48
Flow rate: 1.25
Injection volume: 100
Detector: UV 320

CHROMATOGRAM
Retention time: 5.4
Internal standard: procainamide

OTHER SUBSTANCES
Simultaneous: ranitidine

KEY WORDS
serum; plasma; robotic sample preparation; procainamide is IS; SPE

REFERENCE
Lloyd,T.L.; Perschy,T.B.; Gooding,A.E.; Tomlinson,J.J. Robotic solid phase extraction and high performance liquid chromatographic analysis of ranitidine in serum or plasma, *Biomed.Chromatogr.*, **1992**, *6*, 311–316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 287

CHROMATOGRAM
Retention time: 3.65
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihy-

dralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 25 μL MeOH + 15 μL 6 M NaOH + 2 mL ethyl acetate: isopropanol 96:4, shake mechanically for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeOH, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:10 mM pH 5.1 KH_2PO_4 8:92
Flow rate: 2.5
Injection volume: 75
Detector: UV 330

CHROMATOGRAM

Retention time: 3
Internal standard: procainamide

OTHER SUBSTANCES

Extracted: ranitidine
Simultaneous: lidocaine
Noninterfering: brompheniramine, chlorpheniramine, cimetidine, diazepam, diclofenac, glyburide, ibuprofen, ketoprofen, metoclopramide, naproxen, phenylbutazone, verapamil

KEY WORDS

plasma; procainamide is IS

REFERENCE

al-Khamis,K.I.; El-Sayed,Y.M.; Al-Rashood,K.A.; Bawazir,S.A. High-performance liquid chromatographic determination of ranitidine in human plasma, *J.Liq.Chromatogr.*, **1995**, *18*, 277–286.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 2 mL Plasma + 100 μL water + 660 μL 2 M perchloric acid, shake briefly, centrifuge at 3000 rpm for 5 min. Remove 1.5 mL of the supernatant and adjust the pH to 9 with 150 μL 4 M NaOH and 4 mL 500 mM boric acid/KCl buffer, add 12 mL water-saturated n-pentanol:chloroform 20:60, shake for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic phase and dry it over anhydrous sodium sulfate, add 8 mL to 300 μL 100 mM HCl, shake for 10 min, centrifuge at 3000 rpm for 10 min, inject a 25-100 μL aliquot of the aqueous phase. Urine. Dilute urine with pH 9 borate buffer, add 12 mL water-saturated n-pentanol:chloroform 20:60, shake for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic phase and dry it over anhydrous sodium sulfate, add 8 mL to 300 μL 100 mM HCl, shake for 10 min, centrifuge at 3000 rpm for 10 min, inject a 25-100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:water:acetic acid 38:61:1 containing 1-heptanesulfonic acid (PIC B7)
Flow rate: 1.5
Injection volume: 25-100
Detector: F ex 235 em no filter

CHROMATOGRAM
Retention time: 7
Internal standard: procainamide

OTHER SUBSTANCES
Extracted: sotalol

KEY WORDS
plasma; procainamide is IS

REFERENCE
Lefebvre,M.A.; Girault,J.; Saux,M.C.; Fourtillan,J.B. Fluorometric high-performance liquid chromatographic determination of sotalol in biological fluids, *J.Pharm.Sci.*, **1980**, *69*, 1216–1217.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Sample + 9 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:500 mM KH_2PO_4:water 22:10:68 adjusted to pH 7.1 with 10 M NaOH
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: milrinone, degradation products

KEY WORDS
stability-indicating; 5% dextrose; injections

REFERENCE
Riley,C.M. Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1988**, *45*, 2079–2091.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL sample to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeCN:water:500 mM KH_2PO_4 15:75:10, pH 6.8
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: amrinone

KEY WORDS
injections; stability-indicating; 5% dextrose; 0.45% NaCl

REFERENCE
Riley,C.M.; Junkin,P. Stability of amrinone and digoxin, procainamide hydrochloride, propranolol hydrochloride, sodium bicarbonate, potassium chloride, or verapamil hydrochloride in intravenous admixtures, *Am.J.Hosp.Pharm.*, **1991**, *48*, 1245–1252.

SAMPLE
Matrix: formulations
Sample preparation: Make up 1 mL syrup to 50 mL with water. Remove a 2 mL aliquot and add it to 2 mL 0.1% procaine hydrochloride in water, make up to 100 mL with water, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil ODS-3 C18
Mobile phase: MeCN:buffer:triethanolamine:water 15:80:0.2:4.8, pH adjusted to 4.5 with glacial acetic acid (Buffer was 4.72 g sodium acetate and 1.8 mL acetic acid in 1 L water.)
Flow rate: 1.2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4.6
Internal standard: procaine (7.5)

KEY WORDS
syrup; stability-indicating

REFERENCE
Alexander,K.S.; Pudipeddi,M.; Parker,G.A. Stability of procainamide hydrochloride syrups compounded from capsules, *Am.J.Hosp.Pharm.*, **1993**, *50*, 693–698.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: MeOH:21 mM pH 4.4 NaH_2PO_4 13.5:86.5
Flow rate: 1.5
Detector: UV 266

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Simultaneous: metabolites, N-acetylprocainamide

REFERENCE
Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

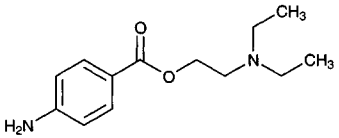
CHROMATOGRAM
Retention time: 6.32 (A), 3.52 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Procaine



Molecular formula: $C_{13}H_{20}N_2O_2$
Molecular weight: 236.31
CAS Registry No.: 59-46-1, 51-05-8 (HCl), 149-13-3 (borate)
Merck Index: 7937
Lednicer No.: 1 9

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 μL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 μg sodium acetate, inject a 40 μL aliquot. (The sodium acetate was measured out by adding 50 μL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES

Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 20:80, adjusted to pH 2.1
Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM

Retention time: 10
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: lidocaine

KEY WORDS

plasma; rabbit

REFERENCE

Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE

Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN: water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: Gradient A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 11
Limit of detection: 0.20 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS
whole blood

REFERENCE
Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL caffeine + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:MeOH:0.5 mM phosphoric acid 6:2.5:91.5
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Internal standard: caffeine

KEY WORDS
plasma; rat

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: blood, urine
Sample preparation: 200 μL Serum or urine + 100 μL 0.072 μg/mL procaine hydrochloride (+ 100 μL 6.25% $NaHCO_3$ solution for urine samples) + 5 mL dichloromethane, vortex 210 s. Evaporate organic phase to dryness under nitrogen, take up in 100 μL mobile phase, inject 20 μL.

HPLC VARIABLES
Column: 250 × 4.6 Partisil-10 ODS-3
Mobile phase: MeCN:10 mM pH 4.8 potassium phosphate buffer 7:93
Flow rate: 2
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 12.9
Internal standard: procaine
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: cimetidine
Noninterfering: cimetidine sulfoxide, caffeine, minoxidil, furosemide, propranolol, sulfinpyrazone, flurazepam, diazepam, methyldopa, acetaminophen, digoxin, quinidine
Interfering: procainamide, tolazamide

KEY WORDS
serum; procaine is IS

REFERENCE
Guay,D.R.; Bockbrader,H.N.; Matzke,G.R. High-performance liquid chromatographic analysis of cimetidine in serum and urine, *J.Chromatogr.*, **1982**, *228*, 398–403.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Adjust pH of urine to 5 before freezing. 5 mL Urine + 3-30 μg tetracaine, adjust pH to 9.5 with borax buffer, extract twice with 7 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL MeOH, inject a 20-100 μL aliquot. Blood. 4 mL Plasma or whole blood + 1 μg tetracaine, adjust pH to 9.5 with borax buffer, extract twice with 7 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 0.2-2 mL MeOH, inject a 125 μL aliquot.

HPLC VARIABLES
Column: 10 μm Radial Pak C18
Mobile phase: MeCN:16.5 mM triethylamine 85:15, pH adjusted to 3 with concentrated phosphoric acid
Flow rate: 2
Injection volume: 20-125
Detector: UV 288

CHROMATOGRAM
Internal standard: tetracaine
Limit of detection: 10 ng/mL (urine), 1 ng/mL (plasma)

KEY WORDS
horse; plasma; whole blood; pharmacokinetics

REFERENCE
Stevenson,A.J.; Weber,M.P.; Todi,F.; Mendonca,M.; Fenwick,J.D.; Young,L.; Kwong,E.; Chen,F.; Beaumier,P.; Timmings,S.; Woodard,W.; Kacew,S. Determination of procaine in equine plasma and urine by high-performance liquid chromatography, *J.Anal.Toxicol.*, **1992**, *16*, 93–96.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 50 μL 10 μg/mL butacaine, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: 4

Internal standard: butacaine (10)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: tetracaine, p-aminobenzoic acid
Also analyzed: articaine, prilocaine, o-toluidine, lidocaine, bupivacaine, etidocaine, dibucaine, caffeine, amphetamine, ephedrine, epinephrine, morphine, diamorphine, ethylmorphine, acetylcodeine
Interfering: codeine, monoacetylmorphine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 292.6

CHROMATOGRAM
Retention time: 5.218

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1

Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 4.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine

REFERENCE
Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets, add 3-20 mL MeCN:water 15:85, sonicate for 10 min, filter, make up to 100 mL with MeCN:water 15:85. Remove a 500 μL aliquot and add it to 300 μL 250 μg/mL procaine hydrochloride in water, make up to 10 mL with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm ASI chromosphere 3869 octadecylsilane (Analytical Sciences, Inc.)
Mobile phase: MeCN:50 mM NaH_2PO_4 30:70 containing sodium pentanesulfonate, pH adjusted to 2.5 with concentrated phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: E, Metrohm model E-611, Bioanalytical Systems Kel F cell, glassy carbon electrode + 1300 mV, auxiliary platinum electrode, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5.7
Internal standard: procaine
Limit of quantitation: 1250 ng/mL

OTHER SUBSTANCES
Simultaneous: guanethidine, hydrochlorothiazide

KEY WORDS
tablets; not stability-indicating; procaine is IS

REFERENCE
Stewart,J.T.; Clark,S.S. Liquid chromatographic determination of guanethidine salts and hydrochlorothiazide using electrochemical detection and ion-pair techniques, *J.Pharm.Sci.*, **1986**, *75*, 413–415.

SAMPLE
Matrix: formulations
Sample preparation: Make up 1 mL syrup to 50 mL with water. Remove a 2 mL aliquot and add it to 2 mL 0.1% procaine hydrochloride in water, make up to 100 mL with water, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil ODS-3 C18
Mobile phase: MeCN:buffer:triethanolamine:water 15:80:0.2:4.8, pH adjusted to 4.5 with glacial acetic acid (Buffer was 4.72 g sodium acetate and 1.8 mL acetic acid in 1 L water.)

Flow rate: 1.2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: procaine

OTHER SUBSTANCES
Simultaneous: procainamide

KEY WORDS
syrup; procaine is IS

REFERENCE
Alexander,K.S.; Pudipeddi,M.; Parker,G.A. Stability of procainamide hydrochloride syrups compounded from capsules, *Am.J.Hosp.Pharm.*, **1993**, *50*, 693–698.

SAMPLE
Matrix: perfusate
Sample preparation: Adjust pH of 5-10 mL perfusate to 5 with 180 μL 2.5 M HCl, extract twice with an equal volume of ethyl acetate. Combine the organic layers, add 1 mL water, evaporate them to 1 mL under vacuum, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water containing 30 μL/L triethylamine, adjusted to pH 2.3 with phosphoric acid 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Extracted: p-aminohippuric acid, aminobenzoic acid, 4-acetamidobenzoic acid

KEY WORDS
rabbit; chinchilla; pharmacokinetics

REFERENCE
Henrikus,B.M.; Kampffmeyer,H.G. Ester hydrolysis and conjugation reactions in intact skin and skin homogenate, and by liver esterase of rabbits, *Xenobiotica*, **1992**, *22*, 1357–1366.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher100RP-18
Column: 125 × 4 5 μm Spherisorb ODS 2
Mobile phase: Gradient. MeOH:buffer from 10:90 to 30:70 over 5 min, to 10:90 over 2 min. (Buffer was 20 mM sodium acetate containing 0.28% triethylamine, adjusted to pH 4.5 with acetic acid.)
Flow rate: 1.5
Detector: UV 260

CHROMATOGRAM
Retention time: k' 3.5
Internal standard: caffeine

OTHER SUBSTANCES
Simultaneous: 4-aminobenzoic acid

REFERENCE
Yang,H.; Thyrion,F.C. Determination of six pharmaceuticals and their degradation products in reversed-phase high performance liquid chromatography by using amine additives, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1347–1357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 45 × 4.6 5 μm Ultrasphere ODS
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:2.5 mM hexanesulfonic acid 35:40:25, adjusted to pH 6.0 with 100 mM acetic acid
Flow rate: 2
Detector: UV 310

CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Simultaneous: tetracaine

REFERENCE
Asavapichayont,P.; Hu,J.; Foldvari,M. Development of an HPLC method for simultaneous analysis of tetracaine and its metabolite in dosage forms and biological fluids, with comparison to capillary electrophoresis method (Abstract 3307), *Pharm.Res.*, **1997**, *14*, S565.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl,

isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 1.15

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Retention time: 2.241

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 216, 290

CHROMATOGRAM

Retention time: 1.5
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 7.25 (A), 4.03 (B)

OTHER SUBSTANCES

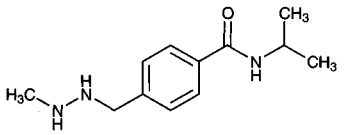
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flur-

azepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Procarbazine



Molecular formula: $C_{12}H_{19}N_3O$
Molecular weight: 221.30
CAS Registry No.: 671-16-9, 366-70-1 (HCl)
Merck Index: 7938
Lednicer No.: 2 27

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM
Retention time: 3.69
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, microsomal incubation

Sample preparation: Rat plasma, microsomal incubation. 1 mL Rat plasma or microsomal incubation, extract with five 3 mL portions of cold diethyl ether. Evaporate combined ether extracts to dryness under a stream of nitrogen. Redissolve the residue in 250-500 μL MeOH, add 10 μL 670 μg/mL IS in MeOH. Vortex, centrifuge at 12000 g for 1 min, inject a 15 μL aliquot. Human plasma. 1 mL Human plasma, extract with three 4 mL portions of diethyl ether. Evaporate combined ether extracts to dryness under a stream of nitrogen. Re-dissolve the residue in 20 μL MeOH, add 5 μL 670 μg/mL IS. Centrifuge at 12000 g for 1 min, inject a 15-20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 2.0
Injection volume: 15-20
Detector: UV 254

CHROMATOGRAM

Internal standard: 4-methylacetophenone
Limit of detection: 2 nmol/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; plasma; rat; liver; pharmacokinetics

REFERENCE
Shiba,D.A.; Weinkam,R.J. Quantitative analysis of procarbazine, procarbazine metabolites and chemical degradation products with application to pharmacokinetic studies, *J.Chromatogr.*, **1982**, *229*, 397–407.

SAMPLE
Matrix: blood, urine
Sample preparation: Filter (0.45 μm) plasma or urine, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil PXS 10/25 PAC
Mobile phase: MeOH:100 mM pH 7 $(NH_4)H_2PO_4$ 10:90
Flow rate: 0.4
Injection volume: 10
Detector: E, Bioanalytical systems model TL-3, carbon paste working electrode +0.75 V, Ag/AgCl reference electrode, resurface electrode daily (glassy carbon electrode may be superior)

CHROMATOGRAM
Retention time: 14
Limit of detection: 2 ng

KEY WORDS
protect from light; plasma

REFERENCE
Rucki,R.J.; Ross,A.; Moros,S.A. Application of an electrochemical detector to the determination of procarbazine hydrochloride by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *190*, 359–365.

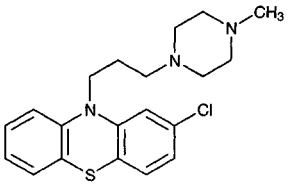
SAMPLE
Matrix: reaction mixtures
Sample preparation: Centrifuge, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:0.4 g/L $(NH_4)H_2PO_4$ + 0.1% triethylamine (pH 10.0) 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.4
Limit of detection: 32000 ng/mL

REFERENCE
Lunn,G.; Sansone,E.B. Reductive destruction of dacarbazine, procarbazine hydrochloride, isoniazid, and iproniazid, *Am.J.Hosp.Pharm.*, **1987**, *44*, 2519–2524.

Prochlorperazine



Molecular formula: $C_{20}H_{24}ClN_3S$
Molecular weight: 373.95
CAS Registry No.: 58-38-8, 84-02-6 (maleate), 1257-78-9 (edisylate)
Merck Index: 7942
Lednicer No.: 1 381

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 1 mL water + 500 μL saturated sodium carbonate solution, vortex for 5 s, add 5 mL pentane:isopropanol 97:3, shake for 15 min, centrifuge at 1725 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness at 65°, reconstitute the residue in 200 μL MeCN, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:100 mM ammonium acetate buffer 90:10
Flow rate: 4
Injection volume: 100
Detector: E, Bioanalytical Systems, +0.9 V

CHROMATOGRAM

Retention time: 5.56
Internal standard: prochlorperazine

OTHER SUBSTANCES

Extracted: trimeprazine

KEY WORDS

plasma; prochlorperazine is IS

REFERENCE

McKay,G.; Cooper,J.K.; Midha,K.K.; Hall,K.; Hawes,E.M. Simple and sensitive high-performance liquid chromatographic procedure with electrochemical detection for the determination of plasma concentrations of trimeprazine following single oral doses, *J.Chromatogr.*, **1982**, *233*, 417–422.

SAMPLE

Matrix: blood
Sample preparation: 2-5 mL Plasma + 10-25 ng methotrimeprazine + 1 mL 1 M NaOH + 8 mL diethyl ether:chloroform 4:1, shake for 10 min, centrifuge. Remove the upper organic layer and evaporate it to dryness at 50° under a stream of nitrogen. Dissolve the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb nitrile-bonded silica
Mobile phase: MeCN:MeOH:100 mM K_2HPO_4 adjusted to pH 6.5 with orthophosphoric acid 6:4:7
Detector: E, Model LCA 15-EDT Research, glassy carbon electrode +0.85 V

CHROMATOGRAM

Retention time: 6
Internal standard: methotrimeprazine (4)
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES

Noninterfering: chlorpromazine

KEY WORDS
plasma

REFERENCE
Sankey,M.G.; Holt,J.E.; Kaye,C.M. A simple and sensitive H.P.L.C. method for the assay of prochlorperazine in plasma, *Br.J.Clin.Pharmacol.*, **1982**, *13*, 578–580.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 1 mL water, vortex for 10 s, add 500 μL saturated sodium carbonate, vortex, add 5 mL pentane:isopropanol 97:3, mix for 20 min, centrifuge at 1725 g for 5 min, remove the organic layer and repeat the extraction. Combine the organic layers and evaporate them to dryness at 65° after adding a few anti-bumping granules. Cool, add 200 μL MeCN, mix for 20 s, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spherisorb CN
Mobile phase: MeCN:100 mM ammonium acetate 90:10
Flow rate: 4
Injection volume: 100
Detector: E, Bioanalytical Systems Model LC4A, glassy carbon electrode +0.9 V, fixed 10 nA feed

CHROMATOGRAM
Retention time: 4.4
Internal standard: prochlorperazine

OTHER SUBSTANCES
Simultaneous: chlorpromazine

KEY WORDS
plasma; prochlorperazine is IS

REFERENCE
Cooper,J.K.; McKay,G.; Midha,K.K. Subnanogram quantitation of chlorpromazine in plasma by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Sci.*, **1983**, *72*, 1259–1262.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 30 ng chlorpromazine in MeOH + 200 μL 5 M NaOH + 10 mL chloroform, shake for 10 min, stand in an ice bath for at least 30 min, centrifuge at 4° at 2800 rpm (RCF = 1578) for 10 min. Remove the organic layer and evaporate it under nitrogen at 40°. Dissolve the residue in 200 μL mobile phase, inject.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb nitrile
Mobile phase: MeCN:100 mM $(NH_4)H_2PO_4$ + 50 mg/L EDTA adjusted to pH 6.5 with ammonia 60:40
Flow rate: 2
Injection volume: 200
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode 0.85 V

CHROMATOGRAM
Retention time: 6
Internal standard: chlorpromazine (4)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma

REFERENCE
Fowler,A.; Taylor,W.; Bateman,D.N. Plasma prochlorperazine assay by high-performance liquid chromatography-electrochemistry, *J.Chromatogr.*, **1986**, *380*, 202–205.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of 1 mL plasma to 9.0 with 1 M NaOH, add 6 mL n-hexane: ethyl acetate 1:2, shake for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and add it to 100 μL pH 2.4 KH_2PO_4, shake for 30 s, centrifuge at 3000 g for 3 min, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 120 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer 26:4:70 (Buffer was 10 mM KH_2PO_4 containing 5 mM tetramethylammonium chloride adjusted to pH 2.4 with 85% phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem Model 5100A, detector 1 +0.20 V, detector 2 +0.73 V, guard cell 0.75 V

CHROMATOGRAM
Retention time: 17.80
Internal standard: prochlorperazine

OTHER SUBSTANCES
Extracted: perphenazine
Simultaneous: alprazolam, chlorpheniramine, diltiazem, lorazepam, mesoridazine, nifedipine, ranitidine
Noninterfering: doxepin, metoprolol, nordoxepin, nortriptyline, propranolol, theophylline, trifluoperazine, trihexyphenidyl, verapamil

KEY WORDS
plasma; protect from light; prochlorperazine is IS

REFERENCE
Foglia,J.P.; Sorisio,D.; Kirshner,M.A.; Mulsant,B.H.; Perel,J.M. Quantitative determination of perphenazine and its metabolites in plasma by high-performance liquid chromatography and coulometric detection, *J.Chromatogr.B*, **1995**, *668*, 291–297.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 257

CHROMATOGRAM
Retention time: 18.25
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents, tissue, urine, vitreous humor

Sample preparation: Homogenize tissue with 4 volumes water. Extract 3 mL blood, gastric contents, urine, vitreous humor, or homogenized tissue with 1.5 mL saturated pH 9.5 ammonium chloride buffer and 5 mL chloroform:2-propanol:n-heptane 25:10:65 for 10 min. (Caution! Chloroform is a carcinogen!). Centrifuge at 3500 g for 10 min, evaporate the organic layer at 45°. Reconstitute with 30μL MeOH. Centrifuge at 10000 g for 5 min, remove 20 μL of the supernatant, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:THF:10 mM pH 2.6 KH_2PO_4 buffer 65:5:30
Flow rate: 0.8
Injection volume: 7
Detector: UV 228

CHROMATOGRAM

Internal standard: prochlorperazine

OTHER SUBSTANCES

Extracted: zuclopenthixol

KEY WORDS
liver; kidney; lung; brain; skeletal muscle; prochlorperazine is IS

REFERENCE
Tracqui,A.; Kintz,P.; Cirimele,V.; Berthault,F.; Mangin,P.; Ludes,B. HPLC-DAD and HPLC-MS findings in a fatality involving (Z)-cis-clopenthixol (zuclopenthixol), *J.Anal.Toxicol.*, **1997**, *21*, 314–318.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Pulverize tablet, add 1 L water, stir for 2 h, filter. Remove 9 mL filtrate, add 1 mL 100 μg/mL imipramine hydrochloride in water, mix, inject a 200 μL aliquot. Injections. Dilute a 2 mL aliquot to 1 L with water, mix. Remove a 9 mL aliquot, add 1 mL 100 μg/mL imipramine hydrochloride in water, mix, inject a 200 μL aliquot. Syrup. Dilute a 1 mL aliquot to 100 mL with water, mix. Remove a 9 mL aliquot, add 1 mL 100 μg/mL imipramine hydrochloride in water, mix, inject a 200 μL aliquot. Suppositories. Weigh out amount equivalent to about 1 mg prochlorperazine, add 80 mL pentane:isopropanol 97:3, shake thoroughly, stir for 15 min, make up to 100 mL with pentane:isopropanol 97:3, mix thoroughly, filter. Remove an aliquot, add imipramine, evaporate to dryness under a stream of nitrogen, reconstitute in 200 μL mobile phase, inject whole sample.

HPLC VARIABLES
Guard column: Guard Pak CN
Column: μBondapak CN (RCM 8 × 10)
Mobile phase: MeCN:18 mM sodium acetate 95:5
Flow rate: 4
Injection volume: 200
Detector: UV 250

CHROMATOGRAM
Retention time: 6
Internal standard: imipramine (4)

KEY WORDS
injections; saline; 5% dextrose; tablets; syrup; suppositories; protect from light

REFERENCE
el-Yazigi,A.; Wahab,F.A.; Afrane,B. Stability study and content uniformity of prochlorperazine in pharmaceutical preparations by liquid chromatography, *J.Chromatogr.A*, **1995**, *690*, 71–76.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 45:55 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 254

OTHER SUBSTANCES
Also analyzed: albendazole, epirizole

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: mesoridazine, promethazine, acetophenazine, chlorpromazine, thioridazine, butaperazine, thiethylperazine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-8
Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

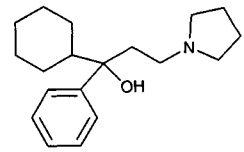
CHROMATOGRAM
Retention time: 8.18

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Procyclidine



Molecular formula: $C_{19}H_{29}NO$
Molecular weight: 287.45
CAS Registry No.: 77-37-2, 1508-76-5 (HCl)
Merck Index: 7944
Lednicer No.: 1 47

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperido-

late, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 2 Deltabond C8 (Keystone)
Mobile phase: MeCN:2-butanone:50 mM pH 7.0 phosphate buffer 27:13:60
Flow rate: 0.15
Injection volume: 1
Detector: Chemiluminescence following post-column reaction. A 1 mM solution of Ru(2,2'-bipyridine)$_3^{++}$ in 50 mM sodium sulfate(continuously sparged with helium) was oxidized to Ru(2,2'-bipyridine)$_3^{+++}$ using a Princeton Applied Research Model 174A polarographic analyzer with a platinum gauze working electrode, a platinum wire auxiliary electrode, and a silver wire reference electrode. The Ru solution at 0.3 mL/min was mixed with the column effluent in the flow cell of the detector, a fluorescence detector with the light source removed.

CHROMATOGRAM

Retention time: 5
Limit of detection: 0.1-1 μg/mL

OTHER SUBSTANCES

Simultaneous: dicyclomine

REFERENCE

Holeman,J.A.; Danielson,N.D. Microbore liquid chromatography of tertiary amine anticholinergic pharmaceuticals with tris(2,2'-bipyridine)ruthenium(III) chemiluminescence detection, *J.Chromatogr.Sci.*, **1995**, *33*, 297–302.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 RP-8
Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 4.65

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

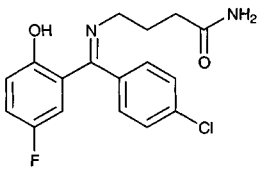
KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Progabide



Molecular formula: $C_{17}H_{16}ClFN_2O_2$
Molecular weight: 334.78
CAS Registry No.: 62666-20-0
Merck Index: 7955
Lednicer No.: 4 47

SAMPLE

Matrix: blood

Sample preparation: Evaporate 20 μL of a 20 μg/mL solution of IS in MeCN into the bottom of a tube using a stream of nitrogen, add 500 μL plasma, add 500 μL 2 M sodium acetate adjusted to pH 4.9 with HCl, add 9 mL hexane:isopropanol 96:4, shake for 5 min, centrifuge. Remove the organic layer and add it to a glass tube (pretreated with MeOH:triethylamine 80:20), add 500 μL 0.4% sodium borohydride in EtOH, vortex, let stand at room temperature for 10 min, add 2 mL 250 mM sodium citrate (adjusted to pH 2 with HCl), shake for 5 min, centrifuge, discard the organic layer, add 9 mL hexane:isopropanol, shake for 5 min, centrifuge, discard the organic layer. Add 200 μL 5 M NaOH to the aqueous layer, add 500 μL 1 M sodium citrate adjusted to pH 4.8 with HCl, add 9 mL dichloromethane, shake for 5 min, centrifuged.

Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spheri-5 RP-18
Column: 100 × 9.4 5 μm RAC Partisil 5 ODS-3
Mobile phase: MeOH:buffer 70:30 (Buffer was 33.3 mM KH_2PO_4 adjusted to pH 5.06 with 33.3 mM K_2HPO_4.)
Flow rate: 2
Injection volume: 10
Detector: E, Bioanalytical Systems BAS LC-4A, TL-5 glassy carbon electrode 1 V

CHROMATOGRAM
Retention time: 4.5
Internal standard: 4-[[(4-chlorophenyl)(5-chloro-2-hydroxyphenyl)methylene]amino]butanamide (SL 78050) (6.5)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
derivatization; pharmacokinetics; plasma

REFERENCE
Yonekawa,W.; Kupferberg,H.J.; Lambert,T. Measurement of progabide and its deaminated metabolite in plasma by high-performance liquid chromatography and electrochemical detection, *J.Chromatogr.*, **1983**, *276*, 103–110.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 70 μL 10 μg/mL thiopental in water + 1.2 mL buffer, mix gently, add 10 mL toluene, shake for 5 min (break any emulsion with sonication), centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL MeOH, inject a 50 μL aliquot. (Buffer was 100 mM acetic acid:100 mM sodium acetate 76:24, pH 4.5.)

HPLC VARIABLES
Column: 300 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer:150 mM NaCl 27:27:36:10 (Buffer was 9.00 g KH_2PO_4 and 140 mg $Na_2HPO_4.7H_2O$ in 1 L water, pH 5.05.)
Flow rate: 2.5
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 7.9
Internal standard: thiopental (4)
Limit of detection: 20 ng/mL

KEY WORDS
plasma

REFERENCE
Decourt,J.P.; Mura,P.; Papet,Y.; Piriou,A.; Reiss,D. Simultaneous determination of progabide and its acid metabolite by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 214–219.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma, whole blood, urine + 50 μL 10 μg/mL IS in MeOH + 500 μL 2 M pH 4.5 acetate buffer + 8 mL toluene, shake on a rotary shaker for 20 min, centrifuge

at 4° at 1000 g for 10 min. Remove the organic layer and add it to 500 μL 0.5% sodium borohydride in EtOH, vortex vigorously, let stand at room temperature for 20 min, add 2 mL 250 mM pH 1.8 citrate buffer, extract for 20 min. Remove the aqueous layer and add 200 μL 5 M NaOH, add 500 μL 1 M pH 7.7 citrate buffer, add 7 mL freshly distilled diethyl ether, extract for 20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL MeOH:15 mM pH 7.1 phosphate buffer 40:60, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:MeOH:33 mM pH 5.05 phosphate buffer:1.5 M NaCl 30:30:40:9
Column temperature: 54
Flow rate: 1
Injection volume: 100
Detector: E, Kipp Analytica Model 9205, +850 mV, Ag/AgCl reference electrode

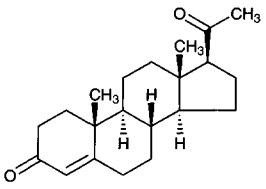
CHROMATOGRAM
Retention time: 4.1
Internal standard: 4-[[(4-chlorophenyl)(5-chloro-2-hydroxyphenyl)methylene]amino]butanamide (SL 78050) (5.9)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: carbamazepine, carbamazepine epoxide, ethosuximide, phenobarbital, phenytoin, valproic acid

KEY WORDS
plasma; whole blood; derivatization

REFERENCE
Padovani,P.; Deves,C.; Bianchetti,G.; Thenot,J.P.; Morselli,P.L. Determination of progabide and its main acid metabolite in biological fluids using high-performance liquid chromatography and electrochemical detection. Application to the measurement of blood/plasma partition ratio, *J.Chromatogr.*, **1984**, *308*, 229–239.

Progesterone



Molecular formula: $C_{21}H_{30}O_2$
Molecular weight: 314.47
CAS Registry No.: 57-83-0
Merck Index: 7956
Lednicer No.: 2 164

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 μg/mL dexamethasone in EtOH:water 10:90 + 100 μL 250 mM NaOH + 7 mL ether:dichloromethane 60:40, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 100 μL dichloromethane:EtOH:water 95:4:1, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Partisil silica
Mobile phase: Dichloromethane:EtOH:water 95:4:1
Flow rate: 1.5
Injection volume: 50
Detector: UV 239

CHROMATOGRAM
Retention time: 2
Internal standard: dexamethasone (11.5)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, 11-deoxycortisol, hydrocortisone, 17-hydroxyprogesterone, 6α-methylprednisolone, prednisolone, prednisone

KEY WORDS
plasma; normal phase

REFERENCE
Scott,N.R.; Chakraborty,J.; Marks,V. Determination of prednisolone, prednisone, and cortisol in human plasma by high-performance liquid chromatography, *Anal.Biochem.*, **1980**, *108*, 266–268.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE column by suspending 250 mg 80-120 mesh Carbopack B (Supelco) in chloroform and adding to a 150 × 6 glass column, wash with 5 mL MeOH, wash with 10 mL water. 1 mL Serum + 9 mL MeOH:water 35:65, add to the SPE column, rinse vial with two 5 mL portions of water, add rinses to SPE column, wash with 15 mL MeCN, wash with 1 mL MeCN:chloroform 70:30, elute with 5 mL MeOH:chloroform 10:90. Collect 4.5 mL eluate (as soon as eluant is added to column), evaporate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 μL MeCN:MeOH 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm C18 (PoliConsult Scientifica, Rome)
Column: 250 × 4.6 5 μm C18 (PoliConsult Scientifica, Rome)
Mobile phase: MeCN:water 46:54
Flow rate: 1.6
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 13.6
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES
Simultaneous: adrenosterone, aldosterone, androstenedione, corticosterone, cortisone, 11-dehydrocorticosterone, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisol, 20α-dihydroprogesterone, hydrocortisone, 19-hydroxyandrostenedione, 18-hydroxycorticosterone, 6α-hydroxyprogesterone, 16α-hydroxyprogesterone, 17α-hydroxyprogesterone, 7α-hydroxytestosterone, prednisolone, prednisone, testosterone
Noninterfering: acebutolol, allopurinol, aminophylline, amitriptyline, aspirin, caffeine, carbamazepine, diazepam, digoxin, disopyramide, doxepin, indapamide, meprobamate, methyldopa, oxazepam, phenobarbital, propranolol, theophylline, ascorbic acid

KEY WORDS
serum; SPE

REFERENCE
Laganà,A.; D'Ascenzo,G.; Marino,A.; Tarola,A.M. Liquid-chromatographic determination of progesterone in serum, with spectrophotometric detection, *Clin.Chem.*, **1986**, *32*, 508–510.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with MeOH and water. Add 500 μL plasma to the SPE cartridge, wash with 2 mL water, wash with 2 mL MeOH:water 20:80, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 1 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 25:25:50
Flow rate: 0.1
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 17
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: androstenedione, 20α-hydroxy-4-pregnen-3-one, 17α-hydroxyprogesterone, norethindrone, testosterone

KEY WORDS
microbore; rat; plasma; SPE

REFERENCE
Taylor,R.B.; Kendle,K.E.; Reid,R.G.; Hung,C.T. Chromatography of progesterone and its major metabolites in rat plasma using microbore high-performance liquid chromatography columns with conventional injection and detection systems, *J.Chromatogr.*, **1987**, *385*, 383–392.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 500 μL water + 100 μL 10 μg/mL 3,7-dimethoxyflavone in EtOH + 8 mL diethyl ether, shake, centrifuge at 4° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:water 40:60, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 3 μm NS-Gel C18
Mobile phase: Gradient. MeOH:water from 40:60 to 55:45, maintain at 55:45 for 24 min, to 80:20 over 25 min
Column temperature: 50
Flow rate: 1
Injection volume: 50
Detector: UV 210, UV 240

CHROMATOGRAM
Retention time: 52.25
Internal standard: 3,7-dimethoxyflavone (47)

OTHER SUBSTANCES
Extracted: aldosterone, androstenedione, dehydroepiandrosterone, deoxycorticosterone, 11-deoxycortisol, estradiol, estrone, hydrocortisone, 17-hydroxyprogesterone, pregnenolone

KEY WORDS
serum

REFERENCE
Ueshiba,H.; Segawa,M.; Hayashi,T.; Miyachi,Y.; Irie,M. Serum profiles of steroid hormones in patients with Cushing's syndrome determined by a new HPLC/RIA method, *Clin.Chem.*, **1991**, *37*, 1329–1333.

SAMPLE
Matrix: blood
Sample preparation: Extract 1 mL serum twice with 5 volumes ether by vortexing for 2 min, evaporate extracts to dryness under a stream of nitrogen at 35°, reconstitute in 100 μL MeOH.

HPLC VARIABLES
Column: 240 × 4.5 Bio-Rad ODS-5S
Mobile phase: Gradient. MeOH:MeCN:water at 20:60:20 for 3 min then to 5:85:10 over 26 min
Flow rate: 1
Injection volume: 50
Detector: UV 230

OTHER SUBSTANCES
Simultaneous: estradiol, androstenedione, testosterone

KEY WORDS
serum

REFERENCE
Yu,F.H.; Yun,Y.W.; Yuen,B.H.; Moon,Y.S. Effects of hydroxyflutamide on rats treated with a superovulatory dose of pregnant mare serum gonadotropin, *Can.J.Physiol.Pharmacol.*, **1991**, *69*, 185–190.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 25.66
Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
Limit of detection: 1-10 ng/mL (245 nm)

OTHER SUBSTANCES
Extracted: betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluendrenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 242.9

CHROMATOGRAM
Retention time: 23.835

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 0.5 mL of nanocapsules suspension 1:200 with MeCN, filter, inject an aliquot. Alternatively, evaporate 5 mL of a nanocapsule suspension to dryness and dissolve the residue in 150 mL dichloromethane or ethyl acetate, dry over anhydrous sodium sulfate. Evaporate to dryness under reduced pressure, take up the residue in 50 mL MeOH, dilute 1:20 with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN: water 75:25
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.01
Limit of detection: 500 ng/ml

OTHER SUBSTANCES
Simultaneous: benzyl benzoate
Noninterfering: poly-ϵ-caprolactone

KEY WORDS
nanocapsules

REFERENCE
Benali,S.; Tharasse-Bloch,C.; André, D; Vérité, P.; Duclos,R.; Lafont,O. Determination of progesterone in nanocapsules by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3233–3243.

SAMPLE
Matrix: formulations
Sample preparation: Injections. Extract 2 mL with EtOH:water 85:15, make up extracts to 100 mL with EtOH:water 85:15, remove a 2 mL aliquot and add it to 1 mL 1 mg/mL hydrocortisone in EtOH. Dilute this mixture to 50 mL with EtOH:water 50:50, inject an aliquot. Suspensions.

Dilute 2 mL suspension to 100 mL with EtOH, filter (if necessary), remove a 2 mL aliquot and add it to 1 mL 1 mg/mL hydrocortisone in EtOH. Dilute this mixture to 50 mL with EtOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak CN
Mobile phase: MeOH:20 mM KH_2PO_4 30:70
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: hydrocortisone (2.5)

OTHER SUBSTANCES
Simultaneous: medroxyprogesterone acetate
Noninterfering: polyethylene glycol 4000, mystristyl-gamma-picolinium chloride, methylcellulose, thimerosal
Interfering: benzyl benzoate

REFERENCE
Das Gupta,V. Quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone by reversed-phase high-pressure liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 294–297.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets, weigh out amount equivalent to 0.1 mg digitoxin, add 500 μL MeOH:water 50:50, sonicate for 5 min, add 5 mL 12.6 μg/mL progesterone in acetone:EtOH 90:10, sonicate for 10 min, centrifuge at 1400 g for 5 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL MeOH, inject a 0.5 μL aliquot.

HPLC VARIABLES
Column: 102 × 0.5 5 μm SC-01 ODS in a PTFE tube (Japan Spectroscopic)
Mobile phase: MeCN:MeOH:water 10:20:17
Flow rate: 0.008
Injection volume: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 23
Internal standard: progesterone

OTHER SUBSTANCES
Simultaneous: digitoxigenin, digitoxigenin monodigitoxoside, digitoxigenin bisdigitoxoside, digitoxin

KEY WORDS
microbore; tablets; progesterone is IS

REFERENCE
Fujii,Y.; Ikeda,Y.; Yamazaki,M. Determination of cardiac glycosides in digitoxin tablets and deslanoside injections by micro-HPLC, *J.Chromatogr.Sci.*, **1990**, *28*, 288–291.

SAMPLE
Matrix: formulations
Sample preparation: Place a 100 mg capsule in 1 mL water, heat to dissolve, add 2 mL 2 mg/mL progesterone in MeOH, make up to 100 mL with MeOH, allow to settle, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 4.6 10 μm 100 Å Chromegabond C-22 (ES Industries)
Mobile phase: MeCN:water 70:30 containing 5 mM tetrabutylammonium phosphate (Pic A)
Flow rate: 1.8
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 6.8
Internal standard: progesterone

OTHER SUBSTANCES
Simultaneous: docusate
Noninterfering: ferrous fumarate, casanthranol

KEY WORDS
capsules; progesterone is IS

REFERENCE
Hogue,D.R.; Zimmardi,J.A.; Shah,K.A. High-performance liquid chromatographic analysis of docusate sodium in soft gelatin capsules, *J.Pharm.Sci.*, **1992**, *81*, 359–361.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 100 mg hydrogel in 50 mL MeOH for 15 min, cool to room temperature, make up to 100 mL with MeOH, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 5 μm Nucleosil C18
Mobile phase: MeOH:water 90:10
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 3.9

KEY WORDS
hydrogels

REFERENCE
Valenta,C.; Schmatzberger-Wagerer,M. Stabilitätsuntersuchungen von Progesteron-Hydrogelen [Stability testing of progesterone hydrogels], *Pharmazie*, **1995**, *50*, 69–70.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL MeCN, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 45 mm long 5 μm Ultrasphere C18
Column: 150 mm long 5 μm Ultrasphere C8
Mobile phase: MeCN:MeOH:water 27.5:27.5:45
Flow rate: 1.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 22.6

OTHER SUBSTANCES
Extracted: deoxycorticosterone

KEY WORDS
rat; liver

REFERENCE
Cribb,A.E.; Spielberg,S.P.; Griffin,G.P. N_4-Hydroxylation of sulfamethoxazole by cytochrome P450 of the cytochrome P4502C subfamily and reduction of sulfamethoxazole hydroxylamine in human and rat hepatic microsomes, *Drug Metab.Dispos.*, **1995**, *23*, 406–414.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:water 60:40
Column temperature: 37
Flow rate: 1.5
Detector: UV 226

CHROMATOGRAM
Retention time: 4.51

OTHER SUBSTANCES
Simultaneous: estradiol

REFERENCE
Kim,D.-D.; Kim,J.L.; Chien,Y.W. Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid, *J.Pharm.Sci.*, **1996**, *85*, 1191–1195.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Simultaneous: prednisone, prednisolone, prednisolone succinate, hydrocortisone acetate, norethindrone, methyltestosterone

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: Radial-PAK μBondapak C18

Mobile phase: MeCN:water 50:50
Flow rate: 2
Injection volume: 100
Detector: UV 254 or 214

CHROMATOGRAM
Retention time: 16.3

OTHER SUBSTANCES
Simultaneous: estrone, estriol, estradiol, testosterone

REFERENCE
Erkoc,F.U.; Özsar,S.; Güven,B.; Kalkandelen,G.; Ugrar,E. High-performance liquid chromatographic analysis of steroid hormones, *J.Chromatogr.Sci.*, **1989**, *27*, 86–90.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: MeOH:THF:water 26:18:56
Column temperature: 48
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 23.4

OTHER SUBSTANCES
Simultaneous: cortisone, estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, testosterone, 17α-hydroxyprogesterone, dexamethasone acetate, estradiol, estrone

REFERENCE
Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, *4*, 34–38.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 27.6

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 25 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 ODS-1
Mobile phase: MeOH:water 55:45
Column temperature: 40
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM

Retention time: k' 7.649

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone acetate (UV 240), prednisolone (UV 240), prednisone (UV 240), pregnenolone (UV 210), testosterone (UV 240)

REFERENCE

Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 60:40 containing 0.3% Tween 80, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 50
Detector: UV 248

OTHER SUBSTANCES

Simultaneous: levonorgestrel

REFERENCE

Gao,Z.-H.; Shukla,A.J.; Johnson,J.R.; Crowley,W.R. Controlled release of a contraceptive steroid from biodegradable and injectable gel formulations: In vitro evaluation, *Pharm.Res.*, **1995**, *12*, 857–863.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES

Column: 250 × 4 7 μm LichroCART RP-8 (Merck)
Mobile phase: MeCN:MeOH:water 32:37:31
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: fluoxymesterone, medrogestone, mestranol, norethindrone, testosterone propionate

REFERENCE
Gau,Y.S.; Sun,S.W.; Chem,R.R.-L. Optimization of high-performance liquid chromatographic separation for progestogenic, estrogenic, and androgenic steroids using factorial design, *J.Liq.Chromatogr.*, **1995**, *18*, 2373–2382.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL 200 μg/mL progesterone in toluene + 100 μL 2.5% trifluoroacetic acid (?) in toluene + 100 μL 1 mg/mL acenaphthene-5-sulfonyl hydrazine in EtOH:toluene 10:90, evaporate to dryness under reduced pressure at 60°, reconstitute with 100 μL MeCN, inject a 20 μL aliquot. (Preparation of acenaphthene-5-sulfonyl hydrazine is as follows. Dissolve 20 g acenaphthene in 100 g nitrobenzene, cool to 0°, add 9 mL chlorosulfonic acid dropwise with stirring, maintain the temperature below 5°, when the addition is complete allow the temperature to rise to 20° over 30 min, add 500 mL water. Remove the aqueous layer and neutralize it with solid sodium carbonate, heat and add NaCl until precipitation occurs, cool in an ice bath for 1 h, filter, heat at 140° to remove traces of water and nitrobenzene to give acenaphthene-5-sulfonic acid sodium salt as a pale yellow solid (mp >300°). Grind 10 g acenaphthene-5-sulfonic acid sodium salt with 3.5 g phosphorus pentachloride in a mortar for 3 min, add ice and water, extract with 100 mL ethyl acetate. Wash the ethyl acetate layer with 5% sodium bicarbonate and with water until neutral, dry over anhydrous sodium sulfate, evaporate the ethyl acetate under a stream of nitrogen, chromatograph on a 300 × 20 column of silica gel H with toluene to give acenaphthene-5-sulfonyl chloride (mp 98-101°) as the first yellow band to elute. Cool a solution of 1 g acenaphthene-5-sulfonyl chloride in 3 mL THF to 10° and pass nitrogen through the solution, add 400 μL 85% hydrazine hydrate dropwise with stirring, maintain the temperature between 10° and 15°, stir for a further 15 min. Filter the upper THF layer through Celite, wash the Celite with 1 mL THF. Stir the filtrate vigorously and add two 10 mL portions of water, cool in a refrigerator for 1 h, filter the precipitate, wash with water, dry, recrystallize from EtOH to give acenaphthene-5-sulfonyl hydrazine (mp 132-4°).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: Gradient. A was 0.5 g/L pH 7 Tris buffer. B was MeCN:water 90:10. A:B from 50:50 to 30:70 over 10 min.
Flow rate: 2
Injection volume: 20
Detector: F ex 230 em 350

KEY WORDS
derivatization

REFERENCE
Gifford,L.A.; Owusu-Daaku,F.T.K.; Stevens,A.J. Acenaphthene fluorescence derivatization reagents for use in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *715*, 201–212.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 100 ppm solution.

HPLC VARIABLES
Column: 150 × 4.6 Develosil ODS-5
Mobile phase: Gradient. MeOH:water from 50:50 to 90:10 over 15 min
Flow rate: 1
Injection volume: 10

Detector: MS, JEOL JMS-SX102A reversed geometry (BE), accelerating voltage +5 kV, air pressure chemical ionization APCI, nebulizer 290°, ion source chamber 400°, discharge electrode, skimmer 1 aperture 300 μm, skimmer 2 aperture 400 μm, no nebulizer gas

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: corticosterone, cortisone, hydrocortisone

REFERENCE
Nojima,K.; Fujimaki,S.; Hertsens,R.C.; Morita,T. Application of liquid chromatography-atmospheric pressure chemical ionization mass spectrometry to a sector mass spectrometer, *J.Chromatogr.A*, **1995**, *712*, 17–19.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH: water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, add 1 mL MeOH to column, collect all eluates from column, evaporate to dryness under nitrogen at 40°, take up in 200 μL MeOH:water 50:50, add 25 μL 10 μg/mL p-chlorophenol, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: Gradient. MeCN:water from 40:60 to 65:35 in 30 min
Flow rate: 1.2
Injection volume: 50
Detector: UV 242

CHROMATOGRAM
Retention time: 26
Internal standard: p-chlorophenol (7)
Limit of detection: 1 ng/g

OTHER SUBSTANCES
Simultaneous: testosterone, trenbolone

KEY WORDS
muscle; liver; chicken; ox

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: urine
Sample preparation: Add 10 mL urine to a Supelclean LC-18 SPE tube at a flow rate of 2 mL/min, wash with 4 mL 25 mM sodium borate buffer, wash with 4 mL 40% MeOH, wash with 4 mL 20% acetone, elute with two 500 μL aliquots of 73% MeOH, evaporate under nitrogen at 40°, reconstitute with 1 mL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb silica

Mobile phase: Cyclohexane:ethyl acetate 40:60
Injection volume: 200
Detector: F ex 247 em 547, after post-column reaction with 30 mM $Tb(NO_3)_3$ in ethyl acetate using a 50 cm tightly coiled capillary tube to ensure mixing

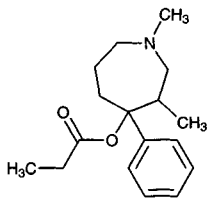
CHROMATOGRAM
Retention time: 12
Limit of detection: 80 pg/mL

OTHER SUBSTANCES
Extracted: testosterone acetate, bolasterone, testosterone
Simultaneous: 17-methyltestosterone

KEY WORDS
SPE; normal phase; post-column reaction

REFERENCE
Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by senstized terbium fluorescence, *Anal.Chem.*, **1993**, *65*, 2346–2351.

Proheptazine



Molecular formula: $C_{17}H_{25}NO_2$
Molecular weight: 275.39
CAS Registry No.: 77-14-5
Merck Index: 7959

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl,

Mobile phase: Cyclohexane:ethyl acetate 40:60
Injection volume: 200
Detector: F ex 247 em 547, after post-column reaction with 30 mM $Tb(NO_3)_3$ in ethyl acetate using a 50 cm tightly coiled capillary tube to ensure mixing

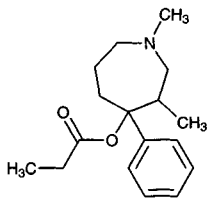
CHROMATOGRAM
Retention time: 12
Limit of detection: 80 pg/mL

OTHER SUBSTANCES
Extracted: testosterone acetate, bolasterone, testosterone
Simultaneous: 17-methyltestosterone

KEY WORDS
SPE; normal phase; post-column reaction

REFERENCE
Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by senstized terbium fluorescence, *Anal.Chem.*, **1993**, *65*, 2346–2351.

Proheptazine



Molecular formula: $C_{17}H_{25}NO_2$
Molecular weight: 275.39
CAS Registry No.: 77-14-5
Merck Index: 7959

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.9

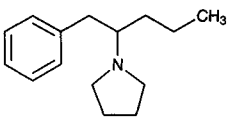
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl,

isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Prolintane



Molecular formula: $C_{15}H_{23}N$
Molecular weight: 217.35
CAS Registry No.: 493-92-5, 1211-28-5 (HCl)
Merck Index: 7964
Lednicer No.: 1 70

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

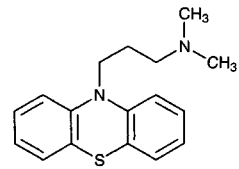
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextro-

propoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Promazine



Molecular formula: $C_{17}H_{20}N_2S$
Molecular weight: 284.43
CAS Registry No.: 58-40-2, 53-60-1 (HCl)
Merck Index: 7966
Lednicer No.: 1 377

SAMPLE
Matrix: blood
Sample preparation: 1-5 mL Plasma + 1 mL 1 M NaOH + hexanes, extract for 30 min, centrifuge. Remove a 9 mL aliquot of the organic phase and evaporate it to dryness at 30° under a stream of nitrogen. Dissolve the residue in 100 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 10 µm Micropak CN (Varian)
Mobile phase: MeCN:5 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 27.5
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, fluphenazine, promethazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, trifluoperazine, chlorpromazine, thiothixene, thioridazine, triflupromazine, trihexyphenidyl, trimeprazine, metabolites

KEY WORDS
plasma

REFERENCE
Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C18 column with 2 volumes MeOH then 2 volumes water. Add 1 mL serum then MeOH:0.1 M HCl 13:87 to each column, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with MeOH/water, add 200 μL 10 mM ammonium acetate in MeOH, wait for 30 s, elute with vacuum, repeat elution process two more times. Combine eluates and evaporate them to dryness at 56-8° under compressed air. Reconstitute with 200 μL mobile phase, vortex 10 s, inject 75-100 μL aliquot. (MeOH/water was 500 mL MeOH:water 65:35 plus 25 μL concentrated HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco silica
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Mix 1 gallon EtOH with 77 mL MeCN and 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 5.2
Internal standard: promazine

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, desmethyldoxepin, doxepin, imipramine, nortriptyline, protriptyline
Simultaneous: thioridazine, hydroxyamoxapine, meperidine, chlorpromazine, disopyramide, amphetamine, 2-hydroxyimipramine, iprindole, pyrilamine, promethazine, prolixin, amoxapine, N-acetylprocainamide, procainamide, zimeldine, morphine, codeine, trifluoperazine, desmethyldisopyramide, 10-hydroxynortriptyline, prochlorperazine, oxaprotiline, 2-hydroxydesipramine, chlorpheniramine, maprotiline, norzimeldine, iminostilbene, desmethylchlordiazepoxide, buprion, diazepam, demoxepam, chlordiazepoxide, propoxyphene, dextropropoxyphene, cocaine, oxapam, trimipramine, mianserin, trimeprazine, loxepin, fluphenazine, methadone, trifluopromazine, phenteramine, chlorimipramine, perphenazine, quinidine
Noninterfering: thiopropazine

KEY WORDS
serum; normal phase; promazine is IS

REFERENCE
Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L

methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 3.9

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, imipramine, lidocaine, maprotiline, methadone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, pentazocine, propoxyphene, propranolol, quinidine, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: desipramine, methaqualone, norverapamil, ibuprofen, propafenone, protriptyline

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Grind 5 tablets to a fine powder, dissolve in 100 mL MeOH:0.5% acetic acid 1:1, filter (paper), inject an aliquot. Suppositories. Cut up 3 suppositories, add to 100 mL MeOH:0.5% acetic acid 1:1, heat at 40° until all the fat melted, shake, filter (paper), inject a 25 μL aliquot. Liquid formulations. Dilute 10 mL formulation to 100 mL with MeOH: 0.5% acetic acid 1:1, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 μBondapak phenyl
Mobile phase: Gradient. A was 10 mM heptanesulfonic acid in 1 mM acetic acid. B was 10 mM heptanesulfonic acid and 1 mM acetic acid in MeOH. A:B from 60:40 to 25:75 over 30 min
Column temperature: 35
Flow rate: 1.75
Injection volume: 25
Detector: UV 225

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: diphenhydramine, dipyrone (metamizol), adiphenine, ethyldiphenacetate, drofenine, impurities

KEY WORDS
tablets; suppositories; liquid formulations

REFERENCE
Facchini,G.; Zaccheo,F.; Nannetti,M. Simultaneous determination of hydrochloride salts of adiphenine, diphenhydramine, ethyldiphenacetate, drofenine and promazine by ion-pair HPLC, *Boll.Chim.Farm.*, **1983**, *122*, 405–411.

SAMPLE
Matrix: formulations
Sample preparation: Extract ground tablets containing 1 mg with 10 mL MeOH, shake for 30 min, centrifuge at 2000 rpm for 5 min. Remove a 5 mL aliquot of the supernatant and add it to 10 mL 1.25 mg/mL norephedrine hydrochloride in MeOH, make up to 25 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeOH:MeCN:25 mM pH 4.5 acetate buffer 30:40:30
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4.45
Internal standard: norephedrine (2.38)

OTHER SUBSTANCES
Interfering: desipramine, fluphenazine

KEY WORDS
tablets

REFERENCE
Beaulieu,N.; Gagné,C.; Lovering,E.G. Liquid chromatographic determination of identity, content, and content uniformity of desipramine, fluphenazine, and promazine, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 178–179.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve crushed tablets, suspensions, or injections in water to give a promazine concentration of 200 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Spherisorb silica
Mobile phase: MeOH:buffer 75:25 (Buffer was 5% ammonium acetate adjusted to pH 9.5 with ammonia solution.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 3
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
tablets; injections; suspensions

REFERENCE
Tebbett,I.R. Analysis of promazine in pharmaceutical preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *356*, 227–229.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Column: 125 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Mobile phase: MeCN:buffer 10:90 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3.39

OTHER SUBSTANCES
Simultaneous: amiodarone

REFERENCE
Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, *728*, 307–310.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 251

OTHER SUBSTANCES
Also analyzed: amitriptyline, chlorpromazine, clomipramine, promethazine, thymol

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 6.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.92

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM

Retention time: 13.5

OTHER SUBSTANCES

Simultaneous: mesoridazine, thiothixene, chlorpromazine, trifluoperazine, thioridazine
Also analyzed: amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 247

CHROMATOGRAM

Retention time: 3.3
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl

Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Simultaneous: triflupromazine, carphenazine, methotrimeprazine, perphenazine, chlorprothixene, deserpidine, thiothixene, reserpine
Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 14.24 (A), 6.34 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital,

sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

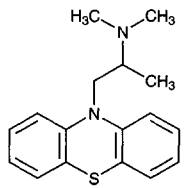
CHROMATOGRAM
Retention time: k' 7.94

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Promethazine



Molecular formula: $C_{17}H_{20}N_2S$
Molecular weight: 284.43
CAS Registry No.: 60-87-7, 58-33-3 (HCl), 17693-51-5 (teoclate)
Merck Index: 7970
Lednicer No.: 1 373

SAMPLE
Matrix: blood

Sample preparation: Attach PLUS™-MP3 extraction discs (15 mg/3 cc size, Ansys. Inc., CA) to a vacuum manifold and condition with 200 μL MeOH followed by 400 μL 100 mM pH 6.0 KH_2PO_4. Dilute 1 mL serum containing 6000 ng verapamil with 1.5 mL MeOH:100 mM pH 6.0 KH_2PO_4 40:100, mix. Add the sample to the extraction disc by applying a vacuum of about 2 kPa. After aspirating the sample through the disc wash with two 300 μL portions of MeOH: water 1:2, dry the disc under full vacuum for at least 5 min, elute with four 300 μL portions of freshly prepared MeCN:triethylamine 100:2, evaporate the eluate to dryness under nitrogen, redissolve the residue in 800 μL mobile phase, inject 100 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 1 RP C8 (Optimize Technologies, OR)
Column: 150 × 4.6 5 μm Chiralcel OJ-R (Chiral Technologies, PA)
Mobile phase: MeCN:500 mM sodium perchlorate 37:63
Flow rate: 0.5
Injection volume: 100
Detector: UV 249

CHROMATOGRAM

Retention time: 12.2 (R(+)), 14.1 (S(-))
Internal standard: verapamil (9.5)
Limit of detection: 2 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

chiral; serum; SPE

REFERENCE

Liu,J.; Stewart,J.T. Quantitation of promethazine enantiomers in human serum using a chiralcel OJ-R column and mixed-mode disc solid-phase extraction, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 303–309.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 251.1

CHROMATOGRAM

Retention time: 14.482

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

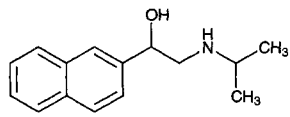
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 251

OTHER SUBSTANCES
Also analyzed: amitriptyline, chlorpromazine, clomipramine, promazine, thymol

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

Pronethalol



Molecular formula: $C_{15}H_{19}NO$
Molecular weight: 229.32
CAS Registry No.: 54-80-8. 51-02-5 (HCl)
Merck Index: 7974
Lednicer No.: 1 66

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.0

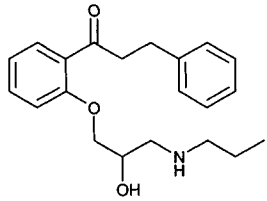
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, hal-

operidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Propafenone



Molecular formula: $C_{21}H_{27}NO_3$
Molecular weight: 341.45
CAS Registry No.: 54063-53-5, 34183-22-7 (HCl)
Merck Index: 7978
Lednicer No.: 5 17

SAMPLE

Matrix: blood
Sample preparation: Mix 400 µL serum with 20 µL 10 µg/mL IS in MeOH and 50 µL 10% sodium carbonate. Add 4 mL diisopropyl ether, shake vigorously for 4 min, centrifuge, freeze at -20°. Mix the organic layer with 100 µL 10 mM HCl, vortex carefully for 45 s using a microshaker, centrifuge, evaporate the aqueous phase to dryness under a stream of argon in a 56° water bath. Reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot. (Caution! Diisopropyl ether readily forms explosive peroxides!)

HPLC VARIABLES

Guard column: 20 × 4.6 5 µm Supelguard LC-CN
Column: 150 × 4.6 5 µm Supelcosil LC-CN
Mobile phase: MeCN:water:500 mM KH_2PO_4 36:62:2
Flow rate: 1.8
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 9.3
Internal standard: LU41616 (2'-[2-hydroxy-3-(3"-hydroxy-3"-methylbutylamino)propoxy]-3-phenylpropiophenone hydrochloride) (7.7)
Limit of detection: 5 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, diltiazem, mexiletine
Simultaneous: acebutolol, amiodarone, aprobarbital, atenolol, bupranolol, celiprolol, clobazam, debrisoquine, diazepam, flecainide, gallopamil, hexobarbiltal, lidocaine, mephenytoin, metopolol, nadolol, pentobarbital, phenacetin, prazosin, procainamide, progesterone, propranolol, quinidine, sotalol, theophylline, verapamil

KEY WORDS
serum

REFERENCE
Kunicki,P.K.; Sitkiewicz,D. High performance liquid chromatographic analysis of some antiarrhythmic drugs in human serum using cyanopropyl derivatized silica phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1169–1181.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL 500 mg ENVI-18 (Supelco) SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 25 μL 100 μg/mL IS, add to the SPE cartridge, wash with 5 mL water and 1 mL MeOH, dry under vacuum for 10 min, elute with 2 mL 700 mM ammonium hydroxide in MeOH, evaporate the eluate to dryness under a stream of air. Reconstitute the residue in 100 μL mobile phase and 100 μL hexane, vortex for 1 min, centrifuge at 1800 g for 5 min, inject a 20 μL aliquot of the lower phase.

HPLC VARIABLES
Guard column: 4 × 4 RP-8 endcapped (Merck)
Column: 250 × 4.6 10 μm Chiracel OD-R
Mobile phase: MeCN:250 mM sodium perchlorate adjusted to pH 4.0 with perchloric acid 40:60
Flow rate: 0.7
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 16.5 (S), 18 (R)
Internal standard: propranolol (9, 12 (enantiomers))
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Simultaneous: atenolol, bromazepam, carbamazepine, clobazam, clonazepam, dexamethasone, diazepam, diclofenac, flunitrazepam, flurazepam, lidocaine, lorazepam, mebendazole, metoprolol, oxyphenbutazone, phenytoin, praziquantel, procainamide, propoxyphene, salicylic acid, triazolam, trimethoprim, verapamil, warfarin
Noninterfering: acetaminophen, albendazole, albuterol, alprazolam, cimetidine, disopyramide, fenfluramine, mexiletine, phenobarbital, pindolol, primidone
Interfering: amitriptyline, diltiazem, haloperidol, imipramine

KEY WORDS
plasma; chiral; SPE; pharmacokinetics

REFERENCE
de Gaitani,C.M.; Lanchote,V.L.; Bonato,P.S. Enantioselective analysis of propafenone in plasma using a polysaccharide-based chiral stationary phase under reversed-phase conditions, *J.Chromatogr.B*, **1998**, *708*, 177–183.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Brownlee cyano spheri-5

Column: 250 × 4.6 5 μm Altex ultrasphere cyano
Mobile phase: MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5
Column temperature: 20
Flow rate: 1.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 6.5
Internal standard: minaprine (5.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: amiodarone, desethylamiodarone, diltiazem, verapamil, nortriptyline, amitriptyline
Also analyzed: haloperidol, desipramine, imipramine, clomipramine

KEY WORDS
serum

REFERENCE
Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 μg/mL (-)-ephedrine in water + 200 μL saturated sodium carbonate + 4 mL hexane:isopropanol:heptafluoro-1-butanol 95:5:1.25, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL reagent, vortex for 5 s, let stand at room temperature for 3 min, add 100 μL bupranolol solution, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 400 μL hexane, add 200 μL 100 mM HCl, vortex for 15 s, centrifuge at 1800 g for 5 min, inject a 100 μL aliquot of the upper organic layer. (Prepare reagent by diluting commercially available R-(-)-1-(1-naphthyl)ethyl isocyanate 10-fold with hexane, pass through a 50 mm column of silica, dilute the eluate with hexane to a concentration of 0.1%, store in amber containers at -30°. Prepare working reagent immediately before use by diluting to 0.005% with hexane:isopropanol 95:5. Prepare bupranolol solution by dissolving bupranolol hydrochloride in 1 mL water, add 500 μL saturated sodium carbonate solution, extract with 25 mL hexane, use the hexane solution.)

HPLC VARIABLES
Guard column: 50 × 4.6 pellicular silica (Whatman)
Column: 100 × 4.6 5 μm Partisil 5 silica
Mobile phase: Hexane:isopropanol:isobutanol 96:2:2
Flow rate: 1.5
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 8.3 (-), 10.1 (+)
Internal standard: (-)-ephedrine (16.4)
Limit of quantitation: 6.25 ng/mL

OTHER SUBSTANCES
Simultaneous: alprenolol, methoxamine, mexiletine, pindolol, propranolol, tocainide

KEY WORDS
plasma; normal phase; derivatization; pharmacokinetics; chiral

REFERENCE
Mehvar,R. Liquid chromatographic analysis of propafenone enantiomers in human plasma, *J.Chromatogr.*, **1990**, *527*, 79–89.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 200 μL 1 M NaOH + 5 mL dichloromethane, mix for 10 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in hexane:isopropanol 90:10, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralpak AD (J.T. baker)
Mobile phase: n-Hexane:isopropanol:diethylamine 75:25:0.2
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 6.5 (R-(-)), 9 (S-(+))

KEY WORDS

plasma; chiral

REFERENCE

Hollenhorst,T.; Blaschke,G. Direct separation of the enantiomers of propafenone, diprafenone and their major metabolites by high-performance liquid chromatography on modified cellulose and amylose chiral stationary phases, *J.Chromatogr.*, **1991**, *585*, 329–332.

SAMPLE

Matrix: blood

Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 4.2

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, imipramine, lidocaine, maprotiline, methadone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, pentazocine, propoxyphene, propranolol, quinidine, temazepam, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide,

primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: desipramine, methaqualone, norverapamil, ibuprofen, promazine, protriptyline

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 1439 g for 10 min. 1 mL Plasma + 50 μL n-hexane: isopropanol containing 8 μg/mL R,S-propranolol hydrochloride and 20 μg/mL R,S-metoprolol tartrate + 200 μL pH 11 ammonium chloride/ammonium hydroxide buffer + 3 mL dichloromethane, shake horizontally for 10 min, centrifuge at 1439 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL n-hexane:isopropanol 75:25, centrifuge at 8160 g for 5 min, inject a 70 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm LiChrosorb RP8
Column: 250 × 4.6 10 μm Chiralpak AD
Mobile phase: n-Hexane:isopropanol 83.4:16.6
Column temperature: 28
Flow rate: 0.9 for 3 min, 0.65 for 7 min, 0.9 for 9 min, 1 for 2 min, 0.9 for 3 min
Injection volume: 70
Detector: UV 270 for 11.5 min, UV 305 for 1.65 min, UV 248 for 10.85 min

CHROMATOGRAM
Retention time: 12.55 (R), 16.44 (S)
Internal standard: R,S-propranolol (6.84), R-metoprolol (7.75), S-metoprolol (8.55)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral

REFERENCE
Böhm,R.; Ellrich,R.; Koytchev,R. Quantitation of R- and S-propafenone and of the main metabolite in plasma, *Pharmazie*, **1995**, *50*, 542–545.

SAMPLE
Matrix: blood
Sample preparation: Make serum alkaline with 10% sodium carbonate, extract with diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!). Remove the organic layer and extract it with 10 mM HCl, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: MeCN:water:500 mM KH_2PO_4 36:62:2
Flow rate: 1.8
Detector: UV 210

CHROMATOGRAM
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, diltiazem, mexiletine

KEY WORDS
serum

REFERENCE
Kunicki,P.K.; Sitkiewicz,D. High-performance liquid chromatographic determination of some antiarrhythmic drugs using cyanopropyl derivatized silica phase (Abstract 43), *Ther.Drug Monit.*, **1995**, *17*, 394–394.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 7.38
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide;

imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 100 μL saturated sodium carbonate + 2 mL hexane: propanol:heptafluorobutanol 95:5:1.25, shake for 30 min, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 0.005% R-(-)-1-(1-naphthyl)ethylisocyanate in hexane, vortex, let stand at room temperature for a short time, add 100 μL bupranolol solution, evaporate to dryness, reconstitute in 200 μL hexane, add 200 μL 100 mM HCl, mix, centrifuge, inject a 100 μL aliquot of the upper organic phase. (The bupranolol solution was obtained by dissolving 5 mg bupranolol hydrochloride in 1 mL water, adding 500 μL saturated sodium carbonate, and extracting with 25 mL hexane. Use the hexane layer.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm 60 Å Nova-Pak silica
Mobile phase: Hexane:isopropanol:isobutanol 96:2:2
Flow rate: 1.5
Injection volume: 100
Detector: UV 220

CHROMATOGRAM

Retention time: 7.5 (R(-)), 9.5 (S(+))
Internal standard: bupranolol (16)
Limit of detection: 20 ng/mL

KEY WORDS

plasma; chiral; derivatization; normal phase; pharmacokinetics

REFERENCE

Volz,M.; Mitrovic,V.; Thiemer,J.; Schlepper,M. Steady-state plasma kinetics of slow-release propafenone, its two isomers and its main metabolites, *Arzneimittelforschung*, **1995**, *45*, 246–249.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 15.128

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Hypersil ODS
Mobile phase: MeCN:20 mM ammonium acetate 70:30
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 5.07, k' 5.98 (enantiomers)

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 5 mL dichloromethane + 100 μL 200 μg/mL oxprenolol in MeOH, extract. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 6 CLC-ODS (Shimadzu)
Mobile phase: MeCN:MeOH:15 mM sulfuric acid 32.5:4:63.5 containing 20 mM triethylamine

Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 9.8
Internal standard: oxprenolol (2.9)

OTHER SUBSTANCES
Extracted: metabolites, 5-hydroxypropafenone

KEY WORDS
mouse; liver

REFERENCE
Morita,K.; Mizuochi,M.; Yamaji,A.; Yokoyama,T. Stereoselectivity in the hydroxylation of propafenone enantiomers in mouse hepatic microsomes, *Biol.Pharm.Bull.*, **1994**, *17*, 531–534.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 20
Detector: F ex 296 em 356

CHROMATOGRAM
Retention time: 27.8 (R), 29.8 (S)

KEY WORDS
derivatization; chiral

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1

Detector: UV 204, 244

CHROMATOGRAM
Retention time: 3.8
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES
Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE
Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 12.20 (A), 6.37 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmoles sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

HPLC VARIABLES
Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 100
Detector: UV 230, F ex 270 em 350

CHROMATOGRAM
Retention time: k' 9.5 (R-(-)), 10.2 (S-(+))

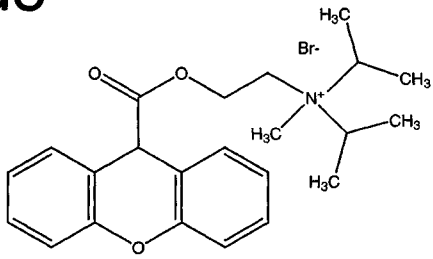
OTHER SUBSTANCES
Simultaneous: carvedilol, flecainide (no enantiomeric separation)

KEY WORDS
derivatization; chiral; F not much more sensitive than UV; $\alpha = 1.07$

REFERENCE
Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

Propantheline bromide

Molecular formula: $C_{23}H_{30}BrNO_3$
Molecular weight: 448.40
CAS Registry No.: 50-34-0
Merck Index: 7989
Lednicer No.: 1 394



SAMPLE
Matrix: bulk
Sample preparation: 50 mg Bulk drug + 5 mL 1.2% diethyl phthalate in MeCN + 45 mL MeCN, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 3 TSK 410 (Toyo Soda)
Mobile phase: MeCN:50 mM pH 2.5 phosphate buffer 40:60
Flow rate: 1
Injection volume: 5
Detector: UV 258

CHROMATOGRAM
Retention time: 5
Internal standard: diethyl phthalate (10)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Yoshioka,S.; Uchiyama,M. Kinetics and mechanism of the solid-state decomposition of propantheline bromide, *J.Pharm.Sci.*, **1986**, *75*, 92–96.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out capsule contents, dissolve in 30 mL MeOH, add 1 mL 100 μg/mL isothipendyl hydrochloride in MeOH, filter (paper), wash filter with MeOH, make up filtrate to 50 mL with MeOH. Dilute a 1 mL aliquot to 10 mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Econosphere C18
Mobile phase: MeOH:water:triethylamine 80:20:0.15, pH adjusted to 7.8 with phosphoric acid
Flow rate: 1.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Internal standard: isothipendyl (7)
Limit of quantitation: 60 μg/mL

OTHER SUBSTANCES
Simultaneous: haloperidol

KEY WORDS
capsules

REFERENCE

Sane,R.T.; Ghadge,J.K.; Jani,A.B.; Vaidya,A.J.; Kotwal,S.S. Simultaneous high-performance liquid chromatographic determination of haloperidol with propantheline bromide, nalidixic acid with phenazopyridine hydrochloride, and dipyridamole with aspirin in combined dosage (forms), *Indian Drugs*, **1992**, *29*, 240–244.

SAMPLE

Matrix: membrane suspensions

Sample preparation: 200 μL Membrane suspension + 10 mL ice-cold 1 mM pH 7.5 Tris-HCl buffer containing 150 mM NaCl, filter (0.45 μm), wash filter with 15 mL ice-cold 1 mM pH 7.5 Tris-HCl buffer containing 150 mM NaCl. Remove the filter and add it to 4 mL 67 mM pH 7 phosphate buffer, heat in a boiling water bath for 40 min, remove the filter, add 500 μL 1 M HCl to the solution, add 5 mL chloroform, shake gently for 15 min, centrifuge at 1500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 μL n-butyl p-aminobenzoate in MeOH, inject a 15-20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Hitachi gel 3053 ODS
Mobile phase: MeCN:50 mM pH 2.5 KH_2PO_4 36:64
Column temperature: 55
Flow rate: 0.8
Injection volume: 15-20
Detector: UV 200

CHROMATOGRAM

Retention time: 10 (as xanthene 9-carboxylic acid)
Internal standard: n-butyl p-aminobenzoate (12)

KEY WORDS

propantheline is hydrolyzed to xanthene 9-carboxylic acid which is chromatographed

REFERENCE

Saitoh,H.; Kobayashi,Y.; Miyazaki,K.; Arita,T. A highly sensitive HPLC method for the assay of propantheline used to measure its uptake by rat intestinal brush border membrane vesicles, *J.Pharm.Pharmacol.*, **1987**, *39*, 9–12.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 7.08

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flur-

azepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50-200 μL aliquot of a solution in pH 7.4 Tyrode's buffer.

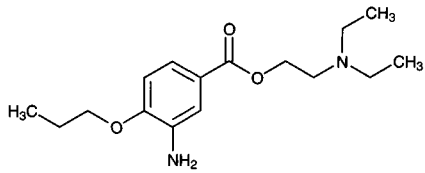
HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C-18
Mobile phase: MeCN:50 mM phosphoric acid:triethylamine 40:60:0.1
Column temperature: 35
Flow rate: 0.6
Injection volume: 50-200
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: chlorpromazine, verapamil

KEY WORDS
buffer

REFERENCE
Saitoh,H.; Aungst,B.J. Possible involvement of multiple P-glycoprotein-mediated efflux systems in the transport of verapamil and other organic cations across rat intestine, *Pharm.Res.*, **1995**, *12*, 1304–1310.

Proparacaine



Molecular formula: $C_{16}H_{26}N_2O_3$
Molecular weight: 294.39
CAS Registry No.: 499-67-2, 5875-06-9 (HCl)
Merck Index: 7991
Lednicer No.: 1 11

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.9

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

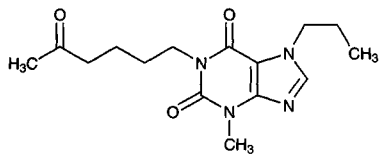
HPLC VARIABLES
Column: 150 × 4.5 5 μm Ultrasphere octyl
Mobile phase: MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, pH 4.35
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 2.33
Internal standard: naproxen (3.89)

OTHER SUBSTANCES
Simultaneous: bacitracin, cortisone acetate, diazepam, diclofenac, fluorometholone, flurbiprofen, hydrocortisone acetate, imipramine, indomethacin, ketoprofen, ketorolac tromethamine, levobunolol, meclofenamic acid, neomycin, prednisolone acetate, salicylic acid, sulfacetamide, suprofen
Noninterfering: acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide
Interfering: metipranolol, propranolol

REFERENCE
Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

Propentofylline



Molecular formula: $C_{15}H_{22}N_4O_3$
Molecular weight: 306.36
CAS Registry No.: 55242-55-2
Merck Index: 7997

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL tetracaine hydrochloride + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:MeOH:0.5 mM phosphoric acid 21:20:59
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Internal standard: tetracaine hydrochloride

KEY WORDS
plasma; rat

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

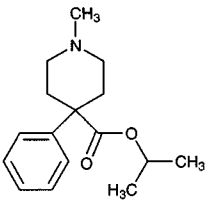
HPLC VARIABLES
Column: 150 × 6 5 μm 3-aminopropylsilyl silica gel with the amino group derivatized with 1,8-naphthalic anhydride (Bunseki Kagaku 1993, 42, 817)
Mobile phase: MeOH:buffer 50:50 (Prepare buffer by dissolving 6.183 g boric acid and 1.461 g NaCl in 500 mL water, adjust pH to 6.4 with sodium borate solution.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 18
Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)

OTHER SUBSTANCES
Simultaneous: caffeine, hypoxanthine, pentoxifylline, theobromine, theophylline, uric acid, xanthine

REFERENCE
Nakashima,K.; Inoue,K.; Mayahara,K.; Kuroda,N.; Hamachi,Y.; Akiyama,S. Use of 3-(1,8-naphthalimido)propyl-modified silyl silica gel as a stationary phase for the high-performance liquid chromatographic separation of purine derivatives, *J.Chromatogr.A*, **1996**, *722*, 107–113.

Properidine



Molecular formula: $C_{16}H_{23}NO_2$
Molecular weight: 261.36
CAS Registry No.: 561-76-2
Lednicer No.: 1 299

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

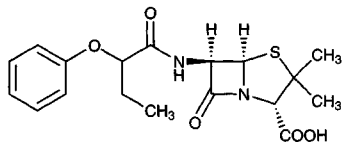
HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Propicillin



Molecular formula: $C_{18}H_{22}N_2O_5S$
Molecular weight: 378.45
CAS Registry No.: 551-27-9, 1245-44-9 (potassium salt)
Merck Index: 8002

SAMPLE
Matrix: perfusate
Sample preparation: Vortex perfusate, centrifuge at 11600 g for 5 min, inject an aliquot of the supernatant.

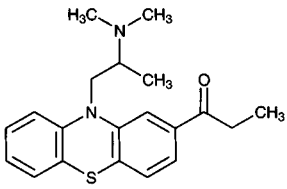
HPLC VARIABLES
Guard column: 20 × 2 5 μm Hypersil ODS

Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:50 mM pH 7 KH_2PO_4 buffer 30:70
Flow rate: 1
Injection volume: 100
Detector: UV 218

CHROMATOGRAM
Retention time: 4.1
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, *705*, 63–69.

Propiomazine



Molecular formula: $C_{20}H_{24}N_2OS$
Molecular weight: 340.49
CAS Registry No.: 362-29-8, 1240-15-9 (HCl)
Merck Index: 8007
Lednicer No.: 1 376

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

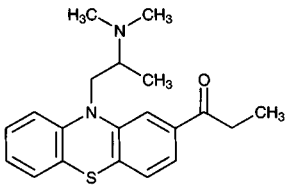
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine,

Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:50 mM pH 7 KH_2PO_4 buffer 30:70
Flow rate: 1
Injection volume: 100
Detector: UV 218

CHROMATOGRAM
Retention time: 4.1
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, *705*, 63–69.

Propiomazine



Molecular formula: $C_{20}H_{24}N_2OS$
Molecular weight: 340.49
CAS Registry No.: 362-29-8, 1240-15-9 (HCl)
Merck Index: 8007
Lednicer No.: 1 376

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine,

methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 amylose tris(3,4,5-trimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 98:2
Flow rate: 0.5
Detector: UV

CHROMATOGRAM

Retention time: 28 (-), 41 (+)

KEY WORDS

chiral

REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 2.48

OTHER SUBSTANCES

Simultaneous: triflupromazine, carphenazine, methotrimeprazine, promazine, perphenazine, thiothixene, reserpine, acetophenazine, ethopropazine, deserpidine, methotrimeprazine
Interfering: promethazine, chlorprothixene

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 14.08 (A), 7.10 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with

continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 μL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 μL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES
Guard column: 10 × 2.1 37-50 μm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 18
Limit of detection: 4 ng/g

OTHER SUBSTANCES
Extracted: azaperol, carazolol, acepromazine, xylazine, azaperone, haloperidol, chlorpromazine

KEY WORDS
SPE; pig; kidney

REFERENCE
Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Cut pig kidney or liver into small pieces and homogenize. 5 g Homogenate + 10 mL MeCN, shake, vortex for 30 s, sonicate for 3 min, vortex for 30 s, sonicate for 3 min, centrifuge at 10000 g for 20 min. Add 7.5 mL supernatant + 40 mL 10% NaCl to the SPE cartridge at about 1 mL/min, do not allow cartridge to dry out, wash with 850 μL 10 mM sulfuric acid, dry with air, elute with 3.5 mL acidic MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL 10 mM sulfuric acid, vortex briefly, add 1 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, inject an aliquot of the aqueous layer. (Acidic MeCN was 1 mL 50 mM sulfuric acid in 100 mL MeCN.)

HPLC VARIABLES
Guard column: Hypersil 5 μm SAS C1
Column: 250 mm long 5 μm Hypersil SAS C1
Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate
Flow rate: 2
Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

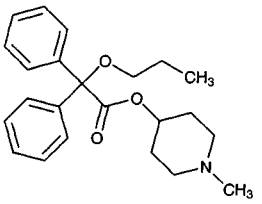
CHROMATOGRAM
Retention time: 25
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: azaperol, acepromazine, carazolol, azaperone, xylazine, haloperidol, chlorpromazine

KEY WORDS
SPE; pig; kidney; liver

REFERENCE
Rose,M.D.; Shearer,G. Determination of tranquilisers and carazolol residues in animal tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *624*, 471–477.

Propiverine



Molecular formula: $C_{23}H_{29}NO_3$
Molecular weight: 367.49
CAS Registry No.: 60569-19-9
Merck Index: 8018

SAMPLE
Matrix: blood, perfusate, tissue
Sample preparation: Homogenize skin with EtOH:water 70:30. 100 μL Plasma, perfusate, or skin homogenate + 200 μL 2.5 μg/mL phenytoin in MeCN, mix, centrifuge. Filter (0.45 μm) the supernatant, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 38:62 containing 0.6% nonylamine
Injection volume: 10
Detector: UV 200

CHROMATOGRAM
Internal standard: phenytoin

OTHER SUBSTANCES
Extracted: terodiline

KEY WORDS
rat; plasma; skin

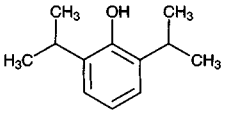
REFERENCE
Ogiso,T.; Iwaki,M.; Hirota,T.; Tanino,T.; Muraoka,O. Comparison of the in vitro skin penetration of propiverine with that of terodiline, *Biol.Pharm.Bull.*, **1995**, *18*, 968–875.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4.6 10 μm LiChrosorb RP-18
Column: 200 × 4.6 10 μm LiChrosorb RP-18
Mobile phase: MeOH:water 60:40
Flow rate: 1.5
Detector: UV 254

REFERENCE
Göber,B.; Dressler,K.; Franke,P.; Alder,L. Zur Analytik und Stabilität von Propiverinhydrochlorid (Mictonorm) [Analysis and stability of propiverine hydrochloride (Mictonorm)], *Pharmazie*, **1986**, *41*, 840–842.

Propofol



Molecular formula: $C_{12}H_{18}O$
Molecular weight: 178.27
CAS Registry No.: 2078-54-8
Merck Index: 8020

SAMPLE

Matrix: blood

Sample preparation: To 2 mL whole blood or plasma containing 20 μL thymol in MeOH, solid blood elements obtained by centrifuging 2 mL blood containing 20 μL thymol in MeOH, solid blood elements without plasma (obtained by centrifuging 2 mL blood containing 20 μL thymol in MeOH and by washing with four portions of 0.9% NaCl), or lysed solid elements (obtained by centrifuging 2 mL blood containing 20 μL thymol in MeOH and by washing with four portions of 0.9% NaCl, and mixing with three volumes of water), add 1 mL 100 mM NaH_2PO_4 and 5 mL cyclohexane. Shake vigorously at 200 rpm for 15 min, centrifuge at 1200 g for 5 min. Add 50 μL tetraethylammonium hydroxide solution to ca. 5 mL of the cyclohexane layer, evaporate to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot. (Prepare tetraethylammonium hydroxide solution by mixing a 25% solution of tetraethylammonium hydroxide in EtOH with EtOH in the ratio of 3:37.)

HPLC VARIABLES

Column: 250 × 4 RP octadecyl silane (prepared as described in J. Chromatogr. Sci. 1995, 33, 377-382)
Mobile phase: MeCN:buffer 67:33 (Buffer was water adjusted to pH 4.0 with acetic acid.)
Detector: UV (wavelength not given)

CHROMATOGRAM

Retention time: 12
Internal standard: thymol (7.5)

KEY WORDS

whole blood; plasma

REFERENCE

Dawidowicz,A.J.; Fijalkowska,A. Possibilities of propofol analysis in various blood components by means of HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1423–1435.

SAMPLE

Matrix: blood

Sample preparation: Add 30 μL 10 ng/mL or 70 μL 100 ng/mL methyldopa solution and 50 μL 2 M HCl to 50 μL plasma, make up to 170 μL with water, centrifuge at 1400 rpm for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 8 4 μm NovaPak C18
Mobile phase: MeCN:MeOH:10 mM pH 3 sodium acetate buffer 47.25:15.75:37
Flow rate: 2
Detector: F ex 276 em 310

CHROMATOGRAM

Retention time: 8.20
Internal standard: methyldopa (5.15)

OTHER SUBSTANCES

Noninterfering: albuterol, ampicillin, amoxicillin, amphotericin B, bleomycin, ceftazidime, cefoxitin, cephalexin, ciprofloxacin, dobutamine, dopamine, epinephrine, erythromycin, esmolol, fluconazole, gentamicin, labetalol, metoclopramide, miconazole, nitroglycerin, nitroprusside, norepinephrine, paclitaxel, penicillin G benzathine, ranitidine, streptomycin, tetracycline

KEY WORDS
plasma

REFERENCE
el-Yazigi,A.; Hussein,R.F. Microdetermination of propofol in plasma by a rapid and sensitive liquid chromatographic method, *J.Pharm.Biomed.Anal.*, **1996**, *15*, 99–104.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma, serum. 20-100 μL Plasma or serum + 100-200 μL IS in MeOH:water 50:50. Make up to 1 mL with water. Add 4 mL pentane, rock at 40 cycles per min for 15 min, centrifuge at 1500 g for 5-10 min. Remove the organic layer and add it to 1 mL 100 mM HCl, rock for 10 min, centrifuge at 1500 g for 5 min. Remove the organic layer and add it to 500 μL isopropanol:100 mM NaOH 90:10, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 140-240 μL mobile phase. Add 10 μL 600 mM phosphoric acid, inject an aliquot. Tissue. Homogenize 0.5-1.5 g tissue divided into 2 × 2 mm pieces with MeCN:50 mM pH 7.0 phosphate buffer 50:50 equal to four times the tissue weight. Mix the homogenate from 20-100 μg tissue with 100 μL 500 mM pH 7.0 phosphate buffer and 100-200 μL IS in MeOH:water 50:50. Make up to a total volume 1 mL with water. Add 4 mL pentane, rock at 40 cycles per min for 15 min, centrifuge at 1500 g for 5-10 min. Remove the organic layer and add it to 1 mL 100 mM HCl, rock for 10 min, centrifuge at 1500 g for 5 min. Remove organic layer and add it to 500 μL isopropanol:100 mM NaOH 90:10, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 490 μL mobile phase. Add 10 μL 600 mM phosphoric acid, inject an aliquot. Fat, skin. 100 mg fat or skin + 20 mL 5 mM sodium deoxycholate + 1 mL 500 mM phosphate buffer + 150 μL 10 (skin) or 100 (fat) μg/mL IS in MeOH:water 50:50, homogenize, steam-distill immediately at a rate of 2 mL/min. Collect 8 mL distillate in a tube containing 8 mL pentane, add 4 mL pentane, rock on a LabQuake for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and add it to 2 mL 500 mM NaOH, rock for 10 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to about 4-5 mL under a stream of nitrogen, add 500 μL isopropanol:100 mM NaOH 90:10, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 990 μL mobile phase and 10 μL 600 mM phosphoric acid for skin and 10 mL mobile phase and 10 μL 600 mM phosphoric acid for fat, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Rainin Microsorb MV phenyl RP
Mobile phase: MeOH:50 mM pH 2.8 phosphate buffer 60:40
Column temperature: 40
Flow rate: 0.8
Injection volume: 50-100
Detector: E, ESA Coulochem model 5100A, Model 5020 guard cell +850 mV, Model 5010 dual analytical cell, detector 1 +300 m V, detector 2 +800 m V (monitored)

CHROMATOGRAM
Retention time: 9-10
Internal standard: 2,6-tert-butylmethylphenol (7.5-8)
Limit of quantitation: 5 ng/mL (serum), 50 ng/g (tissue)

KEY WORDS
human; plasma; serum; rat ; fat; skin; liver; stomach; intestine; pharmacokinetics

REFERENCE
Dowrie; R.H.; Ebling; W.F.; Mandema; J.W.; Stanski; D.R. High-performance liquid chromatographic assay of propofol in human and rat plasma and fourteen rat tissues using electrochemical detection, *J.Chromatogr.B.*, **1996**, *678*, 279–288.

SAMPLE
Matrix: formulations
Sample preparation: Dilute equal volume 10 mg/mL propofol and 25 mg/mL thiopental injections 1:200 with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax SB
Mobile phase: MeCN:buffer 45:55 (Buffer was 10 mM KH_2PO_4, adjusted to pH 4.0 with 10% phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: thiopental

KEY WORDS
stability-indicating; injections

REFERENCE
Chernin,E.L.; Stewart,J.T.; Smiler,B. Stability of thiopental sodium and propofol in polypropylene syringes at 23 and 4°C, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1576–1579.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in 0.9% sodium chloride.

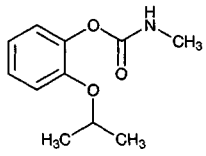
HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax SB phenyl (A) or 300 × 4.6 10 μm T-Bondapak phenyl (B)
Mobile phase: MeCN:buffer 45:55 (A) or 50:50 (B) (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid)
Flow rate: 1
Injection volume: 20
Detector: UV 235 (A), UV 268 (B)

CHROMATOGRAM
Retention time: 12.0 (A), 10 (B)
Limit of detection: 1210 ng/mL (A), 117 ng/mL (B)

OTHER SUBSTANCES
Simultaneous: ondansetron (B), thiopental (A)

REFERENCE
King,D.T.; Stewart,J.T.; Venkateshwaran,T.G. HPLC determination of propofol-thiopental sodium and propofol-ondansetron mixtures, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2285–2294.

Propoxur



Molecular formula: $C_{11}H_{15}NO_3$
Molecular weight: 209.25
CAS Registry No.: 114-26-1
Merck Index: 8022

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize tissue with an equal volume of water, treat with a saturated solution of calcium chloride, let stand overnight, filter. Extract filtrate, blood, or other body fluid with an equal volume of ether. Adjust pH of aqueous layer to 2 with 2 M HCl, extract with an equal volume of ether. Combine the ether layers, evaporate to dryness, reconstitute in a suitable solvent, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax cyano
Mobile phase: Iso-octane:ethyl acetate 80:20
Flow rate: 1
Injection volume: 20
Detector: RI

CHROMATOGRAM

Retention time: 7.56
Limit of detection: 100 ng

OTHER SUBSTANCES

Extracted: methyl parathion, dichlorvos, monocrotophos, quinalphos, malathion, phosphamidon, carbaryl

KEY WORDS

liver; lung

REFERENCE

Sharma,V.K.; Jadhav,R.K.; Rao,G.J.; Saraf,A.K.; Chandra,H. High performance liquid chromatographic method for the analysis of organophosphorus and carbamate pesticides, *Forensic Sci.Int.*, **1990**, *48*, 21–25.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 17.293

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: fruit, vegetables
Sample preparation: Homogenize (Omni-Mixer) 100 g chopped sample with 250 mL MeOH at half-speed for 5 min, filter (Whatman No. 1 PS paper), make up filtrate to 500 mL with MeOH. Remove 100 mL filtrate and add it to 125 mL 4% aqueous sodium sulfate, shake well, extract mixture with 75, 50, and 50 mL portions of dichloromethane with 30 s shaking each time, drain organic layers through anhydrous sodium sulfate. Combine the organic layers and evaporate them to 1 mL under reduced pressure at 30°, transfer residue to a tube with two 2 mL rinses of dichloromethane:cyclohexane 50:50, make volume up to 10 mL with dichloromethane:cyclohexane 50:50, filter (0.45 μm), add 5 mL to a 600 × 25 tube containing 60 g 200-400 mesh BioBeads SX-3 resin (Analytical BioChemistry Laboratories), pump through at 5 mL/min with dichloromethane:cyclohexane 50:50 mobile phase, discard mobile phase for 24 min, collect fraction containing the compound for 12 min, evaporate under reduced pressure at 30° to low volume, add 15 mL MeOH, evaporate to about 1 mL, filter (0.45 μm), inject a 20 μL aliquot. Alternatively, run output from BioBeads column through a column containing 0.5 g of a mixture of Nuchar S-N(Fisher):Celite 545 1:4, at the end of the chromatography elute this column with 10 mL MeCN:toluene 75:25, evaporate the eluate under reduced pressure at 30° to low volume, add 15 mL MeOH, evaporate to about 1 mL, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Pellicular ODS (Whatman)
Column: 250 × 4.6 5 μm Apex ODS (Jones Chromatography)
Mobile phase: Gradient. MeOH:water from 10:90 to 90:10 over 23 min, to 10:90 over 4 min, re-equilibrate at 10:90 for 10 min
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 455, following post-column derivatization. The column effluent is mixed with 200 mM NaOH at 0.8 mL/min and the mixture flows through a 1 mL coil at 95° and is mixed with 500 mg/L o-phthalaldehyde and 1 mL/L 2-mercaptoethanol in 50 mM sodium tetraborate pumped at 0.8 mL/min. The mixture flows through a 0.5 mL coil at ambient temperature to the detector.

CHROMATOGRAM
Retention time: 24
Limit of detection: 5-10 ppb

OTHER SUBSTANCES
Extracted: oxamyl, methomyl, aldicarb, carbaryl, carbofuran, methiocarb

KEY WORDS
apples; broccoli; cabbage; cauliflower; potatoes; post-column reaction

REFERENCE
Chaput,D. Simplified multiresidue method for liquid chromatographic determination of N-methyl carbamate insecticides in fruits and vegetables, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 542–546.

SAMPLE
Matrix: fruit, vegetables
Sample preparation: Blend (Waring) 100 g chopped fruit or vegetable with 200 mL acetone at low speed for 1 min, filter. 80 mL Filtrate + 100 mL petroleum ether + 100 mL dichloromethane,

shake vigorously, filter the organic phase through anhydrous sodium sulfate. Saturate the aqueous phase with 7 g NaCl, extract with 100 mL dichloromethane, filter the organic layer through anhydrous sodium sulfate. Wash the anhydrous sodium sulfate with 50 mL dichloromethane, combine the organic layers, evaporate to about 4 mL through a Snyder column on a steam bath, add 40 μL 1 mg/mL IS solution, adjust volume to 4 mL, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 220 × 4.6 5 μm Spheri-5 C18
Mobile phase: Gradient. MeCN:water:13 mM ammonium acetate from 20:65:15 to 80:5:15 over 30 min.
Flow rate: 1
Injection volume: 50
Detector: MS, Vestec Model 301 thermospray, positive ion discharge mode, vaporizer tip 225-235°, SIM, m/z 210

CHROMATOGRAM

Retention time: 17
Internal standard: 2-fluoro-9-fluorenone (25)
Limit of detection: 0.25 ppm

OTHER SUBSTANCES

Extracted: aldicarb, aldicarb sulfoxide, bufencarb, carboxin, chlorbromuron, diuron, linuron, methiocarb, methomyl, metobromuron, monuron, neburon, oxamyl, thiodicarb

KEY WORDS

apples; beans; lettuce; peppers; potatoes; tomatoes

REFERENCE

Liu,C.-H.; Mattern,G.C.; Yu,X.; Rosen,R.T.; Rosen,J.D. Multiresidue determination of nonvolatile and thermally labile pesticides in fruits and vegetables by thermospray liquid chromatography/mass spectrometry, *J.Agric.Food Chem.*, **1991**, *39*, 718–723.

SAMPLE

Matrix: solutions
Sample preparation: Pass 100 mL water through column A at 5 mL/min then elute the contents of column A onto column B with the mobile phase, elute column B with the mobile phase and monitor the effluent from column B.

HPLC VARIABLES

Column: A 30 × 4.6 5 μm Spherisorb ODS C18; B 250 × 4.6 5 μm Supelcosil LC-8 C8
Mobile phase: Gradient. MeCN:water 30:70 for 5 min, to 60:40 over 10 min, maintain at 60:40 for 10 min, to 30:70 over 5 min, maintain at 30:70 for 5 min and inject next sample.
Flow rate: 1.5
Injection volume: 100000
Detector: UV 220

CHROMATOGRAM

Retention time: 16.00
Limit of detection: 65 pg/mL

OTHER SUBSTANCES

Simultaneous: carbaryl, carbofuran, captan, propham, chloropropham, barban, butylate

KEY WORDS

water; drinking water; column-switching

REFERENCE

Marvin,C.H.; Brindle,I.D.; Hall,C.D.; Chiba,M. Development of an automated high-performance liquid chromatographic method for the on-line pre-concentration and determination of trace concentrations of pesticides in drinking water, *J.Chromatogr.*, **1990**, *503*, 167–176.

SAMPLE
Matrix: solutions
Sample preparation: Condition a 10 × 4 55 mg 40 μm C18/OH Bondesil SPE cartridge (Varian/Analytichem) with 1 mL MeOH and 1 mL water, pass through 5 mL test water at 1 mL/min, pass through 500 μL pure water, elute the contents of the SPE cartridge onto the analytical column with mobile phase.

HPLC VARIABLES
Guard column: 10 × 4 4 μm Supersphere RP-8 (Merck)
Column: 250 × 4 4 μm Supersphere RP-8 (Merck)
Mobile phase: Gradient. A was MeCN:water 20:80 containing 2.5 mM sodium acetate. B was MeOH:water 20:80 containing 2.5 mM sodium acetate. C was MeCN:water 60:40 containing 2.5 mM sodium acetate. A:B:C 75:25:0 for 5 min, to 0:0:100 over 20 min, maintain at 0:0:100 for 5 min, re-equilibrate at initial conditions for 15 min.
Column temperature: 35
Flow rate: 0.75
Injection volume: 100
Detector: F ex 340 em 445 following post-column reaction. The column effluent flowed through a 50 × 4 Aminex A-27 (Bio-Rad) column at 120-140° and was mixed with reagent pumped at 1 mL/min, this mixture flowed through a 200 × 0.12 PTFE tube to the detector. (Reagent was prepared by adding 2 mL 25 mg/mL o-phthalaldehyde in MeCN and 100 μL 2-mercaptoethanol to 200 mL 5 mg/mL disodium tetraborate in water then making up to 250 mL with water.)

CHROMATOGRAM
Retention time: 23.09
Internal standard: trimethacarb (26.12)
Limit of detection: 0.03-0.05 ng/mL

OTHER SUBSTANCES
Simultaneous: aldicarb, bendiocarb, bufencarb, butocarboxim, carbanolate, carbaryl, carbofuran, cloethocarb, dioxacarb, ethiofencarb, fenobucarb, isoprocarb, methiocarb, methomyl, oxamyl, promecarb, thiofanox, tranid

KEY WORDS
water; SPE; post-column reaction

REFERENCE
Hiemstra,M.; de Kok,A. Determination of N-methylcarbamate pesticides in environmental water samples using automated on-line trace enrichment with exchangeable cartridges and high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *667*, 155–166.

SAMPLE
Matrix: solutions
Sample preparation: Flush column A with 5 mL MeOH and 5 mL MeOH:pH 5.0 ammonium acetate, pass a 100 mL sample through the column at 4 mL/min, backflush the contents of column A onto column B and start the gradient, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 2 15-25 μm PLRP-S styrene-divinylbenzene co-polymer (Spark Holland); B 200 × 4 5 μm Spherisorb ODS2
Mobile phase: MeOH:100 mM pH 5.0 ammonium acetate from 30:70 to 88:12 over 34 min.
Column temperature: 40
Flow rate: 0.4
Injection volume: 100000
Detector: UV 280 or MS, Hewlett-Packard 5989 A, dual EI/chemical ionization source, ion source block 250°, quadrupole 100°, m/z 64-400, desolvation chamber 65°, helium nebulizer 50 psi, second-stage momentum separator 0.5 Torr, ion source chamber 15 μTorr

CHROMATOGRAM
Retention time: 20
Limit of detection: <1 ng/mL

OTHER SUBSTANCES
Simultaneous: carbaryl, aldicarb, atrazine, barban, carbofuran, cyanazine, diuron, fluometuron, linuron, methomyl, monuron, oxamyl, simazine

KEY WORDS
water; column-switching

REFERENCE
Marcé,R.M.; Prosen,H.; Crespo,C.; Calull,M.; Borrull,F.; Brinkman,U.A.T. On-line trace enrichment of polar pesticides in environmental waters by reversed-phase liquid chromatography-diode array detection-particle beam mass spectrometry, *J.Chromatogr.A*, **1995**, *696*, 63–74.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 5 μm Kromasil-100-C18 (Akzo Nobel)
Column: 150 × 4 5 μm Kromasil-100-C18 (Akzo Nobel)
Mobile phase: MeCN:buffer 28:72 (Buffer was 820 mg/L sodium tetraborate decahydrate containing 50 μg/mL phthalaldehyde and 0.06 μL/mL 2-mercaptoethanol, adjusted to pH 8.5 with 100 mM HCl.)
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 460 following post-column reaction. The column effluent flowed through a 3 m × 0.51 mm ID stainless steel tube at 140° to the detector. (Although the reagents are in the mobile phase the derivatization reaction does not take place until the post-column reactor where the insecticides are hydrolyzed to methylamine that is then derivatized. This procedure avoids the use of a second pump for the post-column reagent.)

CHROMATOGRAM
Retention time: 14
Limit of detection: 400 pg

OTHER SUBSTANCES
Simultaneous: aldicarb, butocarboxim, carbaryl, carbofuran, dioxacarb, methomyl

KEY WORDS
post-column reaction

REFERENCE
Sabala,A.; Portillo,J.L.; Broto-Puig,F.; Comellas,L. Development of a new high-performance liquid chromatography method to analyse N-methylcarbamate insecticides by a simple post-column derivatization system and fluorescence detection, *J.Chromatogr.A*, **1997**, *778*, 103–110.

SAMPLE
Matrix: tissue
Sample preparation: 21 g Liver + 60 g anhydrous sodium sulfate, mix with spatula, add 200 mL dichloromethane, mix with spatula, homogenize (VirTis 45) for 2 min at medium speed, filter through 5 g anhydrous sodium sulfate, re-extract tissue and sodium sulfate with 100 mL dichloromethane, filter, wash out flask with 25 mL dichloromethane, filter. Combine filtrates and filter them through 2 g anhydrous sodium sulfate, rinse flask with 20 mL dichloromethane, wash filter with 10 mL dichloromethane. Concentrate filtrate to 1-2 mL under reduced pressure at 30° (do not allow to go dry), transfer residue to a tube with 1-2 mL cyclohexane, wash in with dichloromethane:cyclohexane 50:50, make volume in tube 7.5 mL, filter (0.45 μm), add 5 mL to a 600 × 25 tube containing 60 g 200-400 mesh BioBeads SX-3 resin (Analytical Bio-Chemistry Laboratories), pump through at 5 mL/min with dichloromethane:cyclohexane 50:50 mobile phase, collect fraction containing the compound, evaporate under reduced pressure at 30° to about 1 mL, make up to 2 mL with dichloromethane, add 1 mL to a 1 mL 100 mg Bond Elut aminopropyl SPE cartridge (previously conditioned with 1 mL dichloromethane), elute with 3-5 mL dichloromethane:MeOH 98.5:1.5, evaporate eluate to dryness at 30° under reduced

pressure (do not over dry), reconstitute in 200 μL MeOH, vortex for 5 s, filter (0.45 μm), inject a 20-30 μL aliquot.

HPLC VARIABLES
Guard column: Guard-PAK (Waters no. 88070)
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. MeCN:water from 12:88 to 70:30 over 30 min, to 80:20 over 1 min, maintain at 80:20 for 8 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1.5
Injection volume: 20-30
Detector: F ex 340 em 418, following post-column derivatization. The column effluent is mixed with 50 mM NaOH at 0.27 mL/min and the mixture flows through a 1 mL coil at 80° and is mixed with 140 μg/mL o-phthalaldehyde and 1 mL/L mercaptoethanol in 50 mM pH 10.5 potassium borate buffer pumped at 0.27 mL/min. The mixture flows through a 1 mL coil at 40° to the detector.

CHROMATOGRAM
Retention time: 22.1
Limit of quantitation: 5 ppb

OTHER SUBSTANCES
Extracted: aldicarb, bendiocarb, bufencarb, carbaryl, carbofuran, dioxacarb, isoprocarb, methiocarb, methomyl, oxamyl, promecarb

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KEY WORDS
liver; pig; cow; duck; SPE; post-column reaction

REFERENCE
Ali,M.S.; White,J.D.; Bakowski,R.S.; Stapleton,N.K.; Williams,K.A.; Johnson,R.C.; Phillippo,T.; Woods,R.W.; Ellis,R.L. Extension of a liquid chromatographic method for *N*-methylcarbamate pesticides in cattle, swine, and poultry liver, *J.AOAC Int.*, **1993**, *76*, 907–910.

SAMPLE
Matrix: water
Sample preparation: Extract 500 mL water with two 25 mL portions of dichloromethane, combine the extracts and dry them over anhydrous sodium sulfate for 10 min, evaporate to dryness under a stream of air, reconstitute with 40 μL acetone, add 300 μL 100 mM sodium carbonate, heat at 45-50° for 30-40 min, cool, add 300 μL acetone, add 100 μL 0.2% dansyl chloride in acetone, mix well, heat at 45° for 20 min, cool, evaporate the acetone under a stream of air, extract with 300 μL benzene (Caution! Benzene is a carcinogen!). Remove the organic layer and dry it over anhydrous sodium sulfate, inject a 1-10 μL aliquot.

HPLC VARIABLES
Column: 1000 × 2.4 Zipax coated with 0.5% β,β'-oxydipropionitrile
Mobile phase: Hexane:EtOH 95:5
Injection volume: 1-10
Detector: F primary filter Turner 810, secondary filter Turner 827

CHROMATOGRAM
Retention time: k' 0.67

OTHER SUBSTANCES
Simultaneous: aldicarb (Temik), carbaryl (Sevin), carbofuran, Carzol, dimethylamine, methomyl, methylamine, Mobam

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KEY WORDS
lake water; derivatization

REFERENCE
Frei,R.W.; Lawrence,J.F.; Hope,J.; Cassidy,R.M. Analysis of carbamate insecticides by fluorigenic labelling and high-speed liquid chromatography, *J.Chromatogr.Sci.*, **1974**, *12*, 40–44.

SAMPLE
Matrix: water
Sample preparation: Filter, inject a 400 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3 μm HS-3C18 (Perkin Elmer)
Mobile phase: Gradient. MeCN:water from 5:95 to 20:80 over 13 min, to 65:35 over 15 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 8 min.
Flow rate: 1
Injection volume: 400
Detector: F ex 340 em 460 following post-column reaction. The column effluent mixed with the reagent pumped at 0.1 mL/min and the mixture flowed through a 500 μL reaction coil at 95° to the detector. (Prepare the reagent by adding 1.25 mL 10 M NaOH to 100 mL water, add 10 mL 18 mg/mL N,N-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor; Pickering Laboratories, Mountain Vie CA), add 2.5 mL 10 mg/mL o-phthalaldehyde in MeOH, make up to 250 mL with water, filter (0.45 μm nylon), degas with helium for 10 min before use. Prepare fresh each day.)

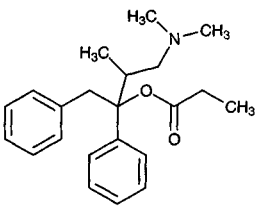
CHROMATOGRAM
Retention time: 28.35
Internal standard: 4-bromo-3,5-dimethylphenyl N-methylcarbamate (34)
Limit of detection: 0.6 ng/mL

OTHER SUBSTANCES
Simultaneous: aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbaryl, carbofuran, 3-hydroxycarbofuran, methiocarb, methomyl, oxamyl

KEY WORDS
post-column reaction

REFERENCE
Simon,V.A.; Pearson,K.S.; Taylor,A. Determination of N-methylcarbamates and N-methylcarbamoyloximes in water by high performance liquid chromatography with the use of fluorescence detection and a single o-phthalaldehyde post-column reaction, *J.Chromatogr.*, **1993**, *643*, 317–320.

Propoxyphene



Molecular formula: $C_{22}H_{29}NO_2$
Molecular weight: 339.48
CAS Registry No.: 469-62-5, 1639-60-7 (HCl), 26570-10-5 (napsylate monohydrate), 55557-30-7 (l-form napsylate monohydrate), 2338-37-6 (l form), 17140-78-2 (l-form napsylate anhydrous)
Merck Index: 8024
Lednicer No.: 1 50, 298; 2 57

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 259

CHROMATOGRAM
Retention time: 7.24
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

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REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.82

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phena-

zocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

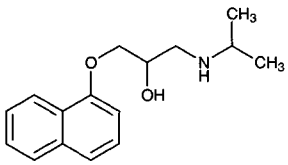
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenyl-

butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Propranolol



Molecular formula: $C_{16}H_{21}NO_2$
Molecular weight: 259.35
CAS Registry No.: 525-66-6, 318-98-9 (HCl)
Merck Index: 8025
Lednicer No.: 1 117; 2 105, 212

SAMPLE

Matrix: bile, perfusate
Sample preparation: 500 μL Perfusate or 100 μL bile + 50 μL 50 μg/mL labetalol + 1 mL 1 M pH 10.3 carbonate buffer + 5 mL acid-washed diethyl ether, vortex, centrifuge. Remove the organic layer and add it to 125 μL 0.5% phosphoric acid, extract, inject a 10 μL aliquot of the aqueous layer. (Deconjugate 500 μL perfusate with 250 μL 8000 U/mL β-D-glucuronidase/aryl sulfatase in 200 mM pH 4.5 sodium acetate buffer, heat at 40° for 1 h, proceed as above.)

HPLC VARIABLES

Column: 100 × 8 4 μm Novapak phenyl radial compression
Mobile phase: MeCN:water:triethylamine 23:77:1 adjusted to pH 3.6 with concentrated phosphoric acid
Flow rate: 3
Injection volume: 10
Detector: F ex 295 em 360

CHROMATOGRAM

Retention time: 5.5
Internal standard: labetalol (6.9)
Limit of quantitation: 62.5 ng/mL

KEY WORDS

sheep; liver; pharmacokinetics

REFERENCE

Ring,J.A.; Ghabrial,H.; Ching,M.S.; Shulkes,A.; Smallwood,R.A.; Morgan,D.J. Fetal hepatic propranolol metabolism. Studies in the isolated perfused fetal sheep liver, *Drug Metab.Dispos.*, **1995**, *23*, 190–196.

SAMPLE

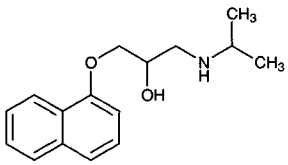
Matrix: blood
Sample preparation: Prepare a silica SPE cartridge. Fill 3 mL cartridge with 500 mg Silica gel 60 (Merck). Condition it with 2.5 mL MeOH and with 2.5 mL water. Add 500 μL plasma or serum to the SPE cartridge. Wash with 1 mL water, elute with 3 mL MeOH (added dropwise).

butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Propranolol



Molecular formula: $C_{16}H_{21}NO_2$
Molecular weight: 259.35
CAS Registry No.: 525-66-6, 318-98-9 (HCl)
Merck Index: 8025
Lednicer No.: 1 117; 2 105, 212

SAMPLE

Matrix: bile, perfusate
Sample preparation: 500 μL Perfusate or 100 μL bile + 50 μL 50 μg/mL labetalol + 1 mL 1 M pH 10.3 carbonate buffer + 5 mL acid-washed diethyl ether, vortex, centrifuge. Remove the organic layer and add it to 125 μL 0.5% phosphoric acid, extract, inject a 10 μL aliquot of the aqueous layer. (Deconjugate 500 μL perfusate with 250 μL 8000 U/mL β-D-glucuronidase/aryl sulfatase in 200 mM pH 4.5 sodium acetate buffer, heat at 40° for 1 h, proceed as above.)

HPLC VARIABLES

Column: 100 × 8 4 μm Novapak phenyl radial compression
Mobile phase: MeCN:water:triethylamine 23:77:1 adjusted to pH 3.6 with concentrated phosphoric acid
Flow rate: 3
Injection volume: 10
Detector: F ex 295 em 360

CHROMATOGRAM

Retention time: 5.5
Internal standard: labetalol (6.9)
Limit of quantitation: 62.5 ng/mL

KEY WORDS

sheep; liver; pharmacokinetics

REFERENCE

Ring,J.A.; Ghabrial,H.; Ching,M.S.; Shulkes,A.; Smallwood,R.A.; Morgan,D.J. Fetal hepatic propranolol metabolism. Studies in the isolated perfused fetal sheep liver, *Drug Metab.Dispos.*, **1995**, *23*, 190–196.

SAMPLE

Matrix: blood
Sample preparation: Prepare a silica SPE cartridge. Fill 3 mL cartridge with 500 mg Silica gel 60 (Merck). Condition it with 2.5 mL MeOH and with 2.5 mL water. Add 500 μL plasma or serum to the SPE cartridge. Wash with 1 mL water, elute with 3 mL MeOH (added dropwise).

Evaporate eluates to dryness under a gentle stream of nitrogen. Reconstitute residue in 200 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Keystone ODS2
Mobile phase: MeCN:tetrahydrofuran:50 mM pH 5.00 phosphate buffer 24:1:75
Flow rate: 1
Injection volume: 30
Detector: UV 204

CHROMATOGRAM
Retention time: 21
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: clindamycin

KEY WORDS
plasma; serum; SPE; propranolol is IS

REFERENCE
Liu,C.-M.; Chen,Y.-K.; Yang,T.-H.; Hsieh,S.-Y.; Hung,M.-H.; Lin,E.T. High-performance liquid chromatographic determination of clindamycin in human plasma or serum: application to the bioequivalency study of clindamycin phosphate injections, *J.Chromatogr.B*, **1997**, *696*, 298–302.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma or serum at 11300 g for 7 min, inject a 200 μL aliquot onto column A, elute to waste with mobile phase A, after 10 min backflush the contents of column A onto column B with mobile phase B, after 6 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Reequilibrate column A with mobile phase A for 5 min.)

HPLC VARIABLES
Column: A 20 × 4.0 BioTrap 500 C18 (ChromTech); B 10 × 4.0 5 μm Hypersil Elite C18 + 150 × 4.6 5 μm Hypersil Elite C18
Mobile phase: A 2-Propanol:20 mM pH 7.0 sodium phosphate buffer containing 5 mM sodium octanesulfonic acid 4:96; B MeCN:116 mM pH 2.8 sodium phosphate buffer containing 2 mM sodium octanesulfonic acid 33:67
Flow rate: A 0.8; B 1
Injection volume: 500
Detector: F ex 220 em 340

CHROMATOGRAM
Retention time: 16.5

KEY WORDS
plasma; serum; column-switching

REFERENCE
Hermansson,J.; Grahn,A.; Hermansson,I. Direct injection of large volumes of plasma/serum on a new biocompatible extraction column for the determination of atenolol, propranolol and ibuprofen. Mechanisms for the improvement of chromatographic performance, *J.Chromatogr.A*, **1998**, *797*, 251–263.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL 500 mg ENVI-18 (Supelco) SPE cartridge with 2 mL MeOH and 2 mL water. Add 1 ml plasma to the SPE cartridge, wash with 5 mL water and 1 mL MeOH, dry under vacuum for 10 min, elute with 2 mL 700 mM ammonium hydroxide in MeOH, evaporate to dryness under a stream of air. Reconstitute the residue in 100 μL mobile

phase and 100 μL hexane, vortex for 1 min, centrifuge at 1800 g for 5 min, inject a 20 μL aliquot of the lower phase.

HPLC VARIABLES
Guard column: 4 × 4 RP-8 endcapped (Merck)
Column: 250 × 4.6 10 μm Chiracel OD-R
Mobile phase: MeCN:250 mM sodium perchlorate adjusted to pH 4.0 with perchloric acid 40:60
Flow rate: 0.7
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 9, 12 (enantiomers)
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: propafenone
Simultaneous: amitriptyline, atenolol, bromazepam, clobazam, clonazepam, dexamethasone, diazepam, diclofenac, diltiazem, flunitrazepam, haloperidol, imipramine, lidocaine, mebendazole, metoprolol, phenytoin, praziquantel, procainamide, propoxyphene, salicylic acid, triazolam, trimethoprim, warfarin
Noninterfering: acetaminophen, albendazole, albuterol, alprazolam, cimetidine, disopyramide, fenfluramine, mexiletine, phenobarbital, pindolol, primidone
Interfering: carbamazepine, flurazepam, lorazepam, oxyphenbutazone, verapamil

KEY WORDS
plasma; chiral; SPE; propranolol is IS

REFERENCE
de Gaitani,C.M.; Lanchote,V.L.; Bonato,P.S. Enantioselective analysis of propafenone in plasma using a polysaccharide-based chiral stationary phase under reversed-phase conditions, *J.Chromatogr.B*, **1998**, *708*, 177–183.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 60 μL 1 μg/mL IS in MeCN:water 50:50 + 500 μL 1 M pH 10 potassium phosphate, vortex for 1 min, add 6 mL dichloromethane, rotate for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen. Reconstitute with 1 mL dry dichloromethane, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL dimethoxypropane, evaporate to dryness under a stream of nitrogen. Add 150 μL 100 μg/mL 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate in MeCN to the residue, vortex for 1 min, let stand overnight. Evaporate the reaction mixture to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN:water 50:50, inject a 70 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Altex Ultrasphere C18
Mobile phase: MeCN:water:phosphoric acid:triethylamine 58:42:0.1:0.06
Flow rate: 1
Injection volume: 70
Detector: F ex 280 em 340

CHROMATOGRAM
Retention time: 9 (S-(-)), 10.5 (R-(+))
Internal standard: 4-methylpropranolol hydrochloride (14.65)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites (F ex 325 em 400)

KEY WORDS
plasma; human; chiral; pharmacokinetics; derivatization

REFERENCE
Wu,S.T.; Chang,Y.P.; Gee,W.L.; Benet,L.Z.; Lin,E.T. Stereoselective high-performance liquid chromatography determination of propranolol and 4-hydroxypropranolol in human plasma after pre-column derivatization, *J.Chromatogr.B*, **1997**, *692*, 133–140.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 4 μg/mL labetalol in water + 100 μL 20% sodium metabisulfite (freshly prepared) + 1 mL 1 M pH 10.2 sodium carbonate + 8 mL ether, shake gently for 10 min on a reciprocating shaker, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, centrifuge for 4 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 10 mM potassium phosphate adjusted to pH 3.4 with 5 M HCl.)
Injection volume: 50
Detector: F ex 295 em 360

CHROMATOGRAM
Retention time: 6
Internal standard: labetalol (F ex 310 em 380 (filter)) (4.8)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 4-hydroxypropranolol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Drummer,O.H.; McNeil,J.; Pritchard,E.; Louis,W.J. Combined high-performance liquid chromatographic procedure for measuring 4-hydroxypropranolol and propranolol in plasma: Pharmacokinetic measurements following conventional and slow-release propranolol administration, *J.Pharm.Sci.*, **1981**, *70*, 1030–1032.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 25 mg/mL ascorbic acid in water (prepare fresh daily) + 500 μL buffer, vortex, add 6 mL hexane:n-butanol 96:4, shake vigorously for 2 min, centrifuge briefly. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL MeOH:21 mM pH 5.5 ammonium acetate buffer 50:50, vortex, inject a 50 μL aliquot. (Buffer was prepared by mixing saturated sodium carbonate and saturated sodium bicarbonate to pH 9.4.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeOH:THF:buffer 60:6:34 (Buffer was 0.8 g ammonium acetate in 340 mL water, pH adjusted to 5.5 with acetic acid (if necessary).)
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: F ex 275 (slit width 6 nm) em 324 (slit width 10 nm)

CHROMATOGRAM
Retention time: 8
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: penbutolol
Simultaneous: metoprolol, pergolide, physostigmine

Noninterfering: acetaminophen, aspirin, atenolol, bromocriptine, chloroquine, doxorubicin, hydrochlorothiazide, indomethacin, 17-methyltestosterone, nadolol, nandrolone, practolol, quinidine, salicyluric acid, sulfadiazine, sulfamerazine, sulfamethazine, timolol, triamterene, vinzolidine, warfarin
Interfering: carazolol

KEY WORDS
propranolol is IS; plasma

REFERENCE
Miner,D.J.; Binkley,D.A.; Bechtol,L.D. Liquid-chromatographic determination of penbutolol and its principal metabolites in plasma and urine, *Clin.Chem.*, **1984**, *30*, 717–723.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 100 μL 20 μg/mL pronethalol in MeOH + 1 mL pH 10 carbonate buffer + 10 mL diethyl ether, shake for 10 min, centrifuge. Remove the ether layer and cool it to 0°, add 10 μL 12.5% phosgene in toluene, vortex for 30 s, centrifuge. Remove the ether layer and evaporate it to dryness under a stream of nitrogen, dissolve the residue in 50 μL dichloromethane, inject.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Pirkle Type 1-A with gamma-aminopropyl packing modified with (R)-N-(3,5-dinitrobenzoyl)phenylglycine (Regis)
Mobile phase: Hexane:isopropanol:MeCN 97:3:1
Column temperature: 20
Flow rate: 2
Injection volume: 50
Detector: F ex 290 em 335

CHROMATOGRAM
Retention time: k' 57 (S), k' 62 (R)
Internal standard: pronethalol (k' 16)
Limit of quantitation: 0.5 ng/mL

KEY WORDS
whole blood; derivatization; chiral

REFERENCE
Wainer,I.W.; Doyle,T.D.; Donn,K.H.; Powell,J.R. The direct enantiomeric determination of (-) and (+)-propranolol in human serum by high-performance liquid chromatography on a chiral stationary phase, *J.Chromatogr.*, **1984**, *306*, 405–411.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma or 0.5 mL plasma water + 50 μL 120 μg/mL penbutolol in EtOH + 1 mL 1 M NaOH + 12 mL n-heptane:isoamyl alcohol 98.5:1.5, shake mechanically for 10 min, centrifuge at 1680 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 50 μL EtOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hitachi Gel 3013 spherical styrene-divinylbenzene
Mobile phase: EtOH:buffer 65:35 (Buffer was 20 mM pH 2.0 perchloric acid/sodium perchlorate.)
Column temperature: 30
Flow rate: 0.2
Injection volume: 10
Detector: F ex 285 em 340

CHROMATOGRAM
Retention time: 14
Internal standard: penbutolol (18)

Limit of quantitation: 2 ng/mL (plasma water), 1 ng/mL (plasma)

OTHER SUBSTANCES
Simultaneous: quinidine, reserpine
Noninterfering: allopurinol, benzbromarone, diazepam, digoxin, diltiazem, dipyridamole, disopyramide, furosemide, isosorbide dinitrate, maprotiline, nifedipine, nitrazepam, trichlormethiazide, verapamil

KEY WORDS
plasma; plasma water; pharmacokinetics

REFERENCE
Yamamura,Y.; Uchino,K.; Kotaki,H.; Isozaki,S.; Saitoh,Y. Quantitative determination of propranolol in plasma and plasma water from normal subjects and patients with angina pectoris by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 311–319.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 100 μL 0.5% ascorbic acid solution + 1 mL 1 M pH 10 carbonate buffer + 100 μL 0.05 μg/mL 4-methylpropranolol in MeOH + 3 mL diethyl ether, vortex for 1 min, centrifuge at 1500 g at 4° for 10 min. Remove the organic layer and add it to 500 μL 100 mM orthophosphoric acid, vortex for 1 min, centrifuge, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelco C18
Mobile phase: MeOH:MeCN:0.1% triethylamine in water 25:25:50, pH adjusted to 2.5 with 1 M orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: F ex 300 em 375

CHROMATOGRAM
Retention time: 5
Internal standard: 4-methylpropranolol (8)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; rat

REFERENCE
Qureshi,S.A.; Buttar,H.S. High-performance liquid chromatographic determination of propranolol and its metabolites in rat serum, *J.Chromatogr.*, **1988**, *431*, 465–470.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Whole blood + 75 ng IS + 6 mL diethyl ether + 2 mL 1 M pH 9.9 sodium carbonate, shake horizontally for 25 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 250 μL 1.2 M triethylamine in dichloromethane, add 200 μL reagent, vortex briefly, let stand at room temperature for 1 h, evaporate under a stream of nitrogen at 30° for 17 min, reconstitute the residue in 2 mL 100 mM NaOH, agitate for 5 min, add 6 mL diethyl ether, shake for 15 min, centrifuge at 2000 g. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, cool the tubes to 0°, reconstitute the residue in 250 μL trifluoroacetic acid, vortex briefly, let stand at 0°, after 7 min add 2 mL 2 M NaOH, add 6 mL diethyl ether. Remove the organic layer and add it to 150 μL 100 mM orthophosphoric acid, extract, inject a 20 μL aliquot of the aqueous phase. (Prepare the reagent by mixing 2 mL 250 mM 1,3-dicyclohexylcarbodiimide in dichloromethane with 1 mmole N-tert-butoxycarbonyl-L-

leucine (Boc-L-Leu) in 3 mL dichloromethane, vortex briefly, react at 0° for 75 min, filter, use the filtrate as the reagent. Store at 0°.)

HPLC VARIABLES
Guard column: 30 × 2.1 5 μm Spheri-5-RP-18
Column: 250 × 2 5 μm Ultrasphere ODS
Mobile phase: MeOH:20 mM pH 2.8 $(NH_4)H_2PO_4$ 72:28
Flow rate: 0.2
Injection volume: 20
Detector: F ex 228 em 290 (cutoff filter)

CHROMATOGRAM
Retention time: 18 (S-(-)), 29 (S-(+))
Internal standard: cyclopentyldesisopropylpropranolol (Pierce) (33, 54 (enantiomers))
Limit of detection: 2.5 ng/mL

KEY WORDS
derivatization; chiral; rat; whole blood; pharmacokinetics

REFERENCE
Guttendorf,R.J.; Kostenbauder,H.B.; Wedlund,P.J. Quantification of propranolol enantiomers in small blood samples from rats by reversed-phase high-performance liquid chromatography after chiral derivatization, *J.Chromatogr.*, **1989**, *489*, 333–343.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 600 ng/mL IS in water + 200 μL 10% sodium bicarbonate + 5 mL diethyl ether, shake at 20 rpm for 15 min, centrifuge at 400 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature. Add 50 μL 5 μL/mL R(+)-phenylethylisocyanate in diethyl ether to the residue, vortex vigorously for 30 s, keep at 4° for 30 min, allow to warm to room temperature, vortex for 15 s, evaporate under nitrogen, reconstitute in 100 μL mobile phase, let stand at room temperature for 20 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 25-37 μm Whatman Co:Pell ODS pellicular C18
Column: 250 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeOH:isopropanol:dichloromethane:water 67:7.5:1:25.5
Flow rate: 0.7
Injection volume: 20
Detector: F ex 220 em 300 (cut-off filter)

CHROMATOGRAM
Retention time: 13.7 (S(-)), 14.9 (R(+))
Internal standard: (±)-N-cyclopentyldesisopropylpropranolol (19.3, 21.2)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; human; rat; chiral; derivatization

REFERENCE
Laganière,S.; Kwong,E.; Shen,D.D. Stereoselective high-performance liquid chromatographic assay for propranolol enantiomers in serum, *J.Chromatogr.*, **1989**, *488*, 407–416.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 0.5 N pH 10 sodium bicarbonate/sodium carbonate buffer + 100 μL 300 ng/mL IS, vortex for 10 s, add to a 150 × 14 column packed with a 45

mm layer of Extrelut on top of a 25 mm layer of anhydrous sodium sulfate. elute with 15 mL diethyl ether. Add the eluate to 100 μL 10 mM trichloroacetic acid in dry dichloromethane, add 100 μL 250 mM (R,R)-O,O-diacetyltartaric acid anhydride in acetic acid:dichloromethane 20:80, mix, heat at 40° for 4 h, evaporate to dryness under a stream of nitrogen, wash the tube down with 1 mL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with 20 μL acetic acid, add 20 μL MeOH, add 60 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4 10 μm Lichrosorb RP18
Column: 125 × 4 5 μm C18 Hypersil
Mobile phase: MeCN:2% aqueous acetic acid 70:30, adjusted to pH 4.0 with concentrated ammonia
Flow rate: 1
Injection volume: 50
Detector: F ex 290 em 335

CHROMATOGRAM
Retention time: 4 (R), 6 (S)
Internal standard: N-tert-butylpropranolol (Synthesize as follows. Reflux 2.9 g 1-naphthol, 30 mL epichlorohydrin (Caution! Epichlorohydrin is a carcinogen!), and 4.4 g (ca. 22 mequiv OH^-, Merck) ion-exchange resin for 4 h, filter, evaporate to dryness, take the residue up in toluene, evaporate to dryness, take the residue up in toluene, evaporate to dryness, take up the residue in hot petroleum ether, evaporate to dryness. Reflux the residue with 30 mL tert-butylamine for 16 h, evaporate to dryness, take up the residue in 30 mL diethyl ether, wash with two 15 mL portions of water, add 4.5 mL 4 M HCl. Remove the organic phase and the product crystallizes after several h, recrystallize from water to give N-tert-butylpropranolol hydrochloride (mp 180°).) (5 (R), 7 (S))
Limit of detection: 0.5 ng/mL (R), 1 ng/mL (S)

KEY WORDS
derivatization; plasma; chiral; pharmacokinetics

REFERENCE
Lindner,W.; Rath,M.; Stoschitzky,K.; Uray,G. Enantioselective drug monitoring of (*R*)- and (*S*)-propranolol in human plasma via derivatization with optically active (*R,R*)-O,O-diacetyl tartaric acid anhydride, *J.Chromatogr.*, **1989**, *487*, 375–383.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL pH 10 boric acid/KCl buffer + 5 mL toluene, shake on a mechanical shaker for 30 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute with 50 μL MeOH:triethylamine 99:1, add 20 μL 0.1% NAPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (NAPIC is (-)-(S)-naproxen isocyanate; synthesis is as follows (protect from light). Dissolve 1 g (+)-(S)-naproxen in 30 mL acetone, cool to 0°, add a solution of 700 μL triethylamine in 2 mL acetone dropwise, add a solution of 450 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 310 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain NAPIC as an oil that crystallized in the desiccator (mp 48°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 20
Detector: F ex 276 em 356

CHROMATOGRAM
Retention time: 18.6 (R), 20.3 (S)

KEY WORDS
derivatization; chiral; plasma; pharmacokinetics

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL 200 mM pH 10.5 phosphate buffer + 5 mg ascorbic acid + 4 mL ethyl acetate, shake vigorously for 10 min, centrifuge at 1500 g for 15 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 500 μL chloroform, add 30 μL triethylamine, add 2 μL R-(+)-phenylethylisocyanate, shake, let stand at room temperature for 30 min, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeOH:water 60.5:39.5
Flow rate: 1.2
Injection volume: 50
Detector: F ex 228 em 340 (cutoff filter)

CHROMATOGRAM
Retention time: 27 (-), 31 (+)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
derivatization; chiral; plasma; pharmacokinetics

REFERENCE
Schaefer,H.G.; Spahn,H.; Lopez,L.M.; Derendorf,H. Simultaneous determination of propranolol and 4-hydroxypropranolol enantiomers after chiral derivatization using reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 351–359.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 100 mg C18 Bond Elut SPE cartridge with 1. mL MeOH and 1.5 mL water. 1 mL Whole blood + 1 mL water + 100 μL 400 ng/mL methyl 4-propranolol hydrochloride in water + 3 mL 1 M sodium carbonate + 10 mL heptane:isopropanol 98:2, shake at 80-100 strokes/min for 15 min, centrifuge at 1500 g at 4° for 5 min. Remove 7 mL of the organic phase and evaporate it to dryness at 50° under a stream of nitrogen. Take up the residue in 150 μL acetone, vortex for 30 s, add 100 μL pH 7.85 borate buffer, add 50 μL (+)-1-(9-fluorenyl)ethyl chloroformate 500 μg/mL in acetone, mix for 30 s, let stand for 5 min at room temperature. Add 800 μL water and the reaction mixture to the SPE cartridge, wash with 1.5 mL isooctane, elute with 500 μL dichloromethane. Evaporate the eluate to dryness and take up the residue in 35 μL MeCN, vortex vigorously for 15 s, add 75 μL water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 Nova-Pak C18 radial compression
Mobile phase: MeCN:water 75:25
Flow rate: 2
Injection volume: 100

Detector: F ex 260 em 340

CHROMATOGRAM
Retention time: 10.5 (S(-)), 11.3 (R(+))
Internal standard: methyl 4-propranolol hydrochloride (14.5 (S), 15.5 (R))
Limit of quantitation: 0.5 ng/mL

KEY WORDS
whole blood; chiral; derivatization; SPE

REFERENCE
Roux,A.; Blanchot,G.; Baglin,A.; Flouvat,B. Liquid chromatographic analysis of propranolol enantiomers in human blood using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate, *J.Chromatogr.*, **1991**, *570*, 453–461.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 ng (+)-bufuralol + 4 mL 500 mM pH 7.0 sodium phosphate buffer, mix, add to a Sep-Pak C18 SPE cartridge, wash with 5 mL water, wash with 5 mL EtOH:water 30:70, elute with 8 mL EtOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL 2 mg/mL (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide in MeCN containing 0.01% quinuclidine, heat at 60° for 20 min, add 50 μL MeOH, evaporate to dryness, reconstitute with 1 mL EtOH:water 90:10, add to an 18 × 6 column containing 100 mg carboxymethyl Sephadex LH-20, wash with EtOH:water 90:10 at 0.2 mL/min, elute with 3 mL 100 mM methylamine in EtOH:water 90:10. Evaporate the eluate to dryness, reconstitute the residue in 50-100 μL mobile phase, inject a 10-20 μL aliquot. (Synthesis of (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide is as follows. Reflux 210 g 1-bromo-2-methylnaphthalene, 160 g N-bromosuccinimide, 1 g benzoyl peroxide, and 250 mL carbon tetrachloride for 2.5 h, add 250 mL carbon tetrachloride, filter while warm, wash the residue several times with solvent. Concentrate and cool the filtrate to give 1-bromo-2-bromomethylnaphthalene (mp 230-240°) (J. Org. Chem. 1949, 14, 375). Dissolve 90 g 1-bromo-2-bromomethylnaphthalene in 400 mL chloroform, reflux, add 46.5 g powdered hexamine in portions, remove the hexaminium salt by filtration. Reflux this salt in 650 mL 50% acetic acid for 1 h, add 105 mL concentrated HCl, reflux for 5 min, cool, obtain 1-bromo-2-naphthaldehyde (mp 119-120°) by filtration. Heat 11 g 1-bromo-2-naphthaldehyde in 275 mL acetone at 60-68°, add a hot solution of 14 g potassium permanganate in 330 mL water over 30 min, heat for another 30 min, pass in sulfur dioxide (sodium metabisulfite ?) until the solution is clear, pour into water to give 1-bromo-2-naphthoic acid, purify by forming the ammonium salt and reprecipitating. Reflux 1-bromo-2-naphthoic acid in MeOH in the presence of sulfuric acid to give methyl 1-bromo-2-naphthoate. Heat methyl-1-bromo-2-naphthoate with copper bronze at 270-280° for 20 min, while still hot extract with toluene, cool to obtain dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate, obtain more crystals by evaporating some of the solvent, recrystallize from EtOH to give dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate (mp 158°) (J. Chem. Soc. 1955, 1242). Add 8 g lithium tri-tert-butoxyaluminohydride in portions to 2.8 g dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate in 150 mL anhydrous benzene:ether 50:50 (Caution! Benzene is a carcinogen!), heat at 80° for 2 h, acidify with 5% HCl. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness, chromatograph on 50 g silica gel with hexane:ethyl acetate 80:20, recrystallize the product from hexane/acetone to give methyl 2-hydroxymethyl-1,1'-binaphthalene-2'-dicarboxylate (mp 117.5-118.5°). Add 5 mL 30% hydrogen bromide in acetic acid to 2 g methyl 2-hydroxymethyl-1,1'-binaphthalene-2'-dicarboxylate in 10 mL acetic acid, stir at 50° for 10 min, pour into ice-water, filter, chromatograph the solid on 40 g silica gel with hexane:ethyl acetate 30:1 to give methyl 2-bromomethyl-1,1'-binaphthalene-2'-dicarboxylate as pale yellow needles (mp 137-138°). Add 400 mg sodium borohydride to 1.9 g methyl 2-bromomethyl-1,1'-binaphthalene-2'-dicarboxylate in 10 mL DMSO, stir at 60° for 15 min, pour into ice-water, acidify with concentrated HCl, chromatograph the crude product on 40 g silica gel with hexane:ethyl acetate 10:1, recrystallize from MeOH to give methyl 2-methyl-1,1'-binaphthalene-2'-carboxylate as colorless needles mp 97-98°. Add 30 mL 10% KOH to 1.2 g methyl 2-methyl-1,1'-binaphthalene-2'-carboxylate in 50 mL MeOH, reflux for 3 h, pour into ice-water, filter, recrystallize from hexane/ethyl acetate to give 2-methyl-1,1'-binaphthalene-2'-carboxylic acid as colorless needles (mp 232-233°). Add 4.1 g (-)-brucine in 20 mL EtOH to 3.3 g 2-methyl-1,1'-binaphthalene-2'-carboxylic acid dissolved in 60 mL EtOH, allow to stand overnight, filter, recrystallize the precipitate several times from EtOH. Add 5% HCl to the salt and extract with ethyl acetate, wash the organic layer with water, dry over anhydrous sodium sulfate, evaporate to dryness, recrystallize from hexane/acetone to give (-)-2-methyl-1,1'-binaphthalene-2'-carbox-

ylic acid as colorless needles (mp 229-229.5°; $[\alpha]_D^{20}$ -41.3° (c = 0.58 in chloroform). Add 3 mL oxalyl chloride to 500 mg (-)-2-methyl-1,1'-binaphthalene-2'-carboxylic acid in 30 mL anhydrous dichloromethane, stir at room temperature for 2 h, evaporate to give an oily residue, take up in 10 mL dichloromethane, add 2 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, chromatograph on 5 g silica gel with hexane to give (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide as a yellow oil ($[\alpha]_D^{20}$ -42.8° (c = 1.05 in chloroform) (Anal. Sci. 1990, 6, 261).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm spherical silica (Waters)
Mobile phase: Hexane:ethyl acetate:MeOH 90:6:1.8
Injection volume: 10-20
Detector: F ex 318 em 408

CHROMATOGRAM
Retention time: 9 (-), 11 (+)
Internal standard: (+)-bufuralol (5)
Limit of detection: 100 pg

KEY WORDS
derivatization; plasma; SPE; chiral; normal phase

REFERENCE
Shao,G.; Goto,J.; Nambara,T. Separation and determination of propranolol enantiomers in plasma by high-performance liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.*, **1991**, *14*, 753–763.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL pH 10 boric acid/KCl buffer + 500 mg NaCl + 5 mL toluene, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL dichloromethane, add 50 μL 1% triethylamine in MeOH, add 20 μL 0.1% FLOPIC in dichloromethane, mix, let stand at room temperature for 30 min, add 50 μL 1% ethanolamine in MeOH, mix, let stand at room temperature for 15 min, evaporate to dryness, reconstitute with 1% acetic acid in mobile phase, inject an aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure (Chirality 1989, 1, 223).)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeOH:water 75:25
Flow rate: 1
Detector: F ex 305 em 355

CHROMATOGRAM
Retention time: 18, 20 (enantiomers)
Internal standard: pronethalol (15, 17 (enantiomers))
Limit of detection: 1-2 ng/mL

KEY WORDS
derivatization; plasma; chiral; comparison with other derivatization reagents

REFERENCE
Spahn-Langguth,H.; Podkowik,B.; Stahl,E.; Martin,E.; Mutschler,E. Improved enantiospecific RP-HPLC assays for propranolol in plasma and urine with pronethalol as internal standard, *J.Anal.Toxicol.*, **1991**, *15*, 209–213.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 200 mg RP 18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 1 mL pH 9 borate buffer, add to SPE cartridge, wash with 5 mL pH 3.15 phosphate buffer, wash with 3 mL water, wash with 500 µL MeCN:water:phosphate buffer 62:32:6, elute with 1 mL MeCN:water:phosphate buffer 62:32:6, inject a 100 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Spherisorb ODS
Mobile phase: MeCN:water:phosphate buffer 62:32:6
Flow rate: 1
Injection volume: 100
Detector: F ex 290 em 350

CHROMATOGRAM
Retention time: 12
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: celiprolol

KEY WORDS
plasma; SPE; propranolol is IS

REFERENCE
Rostock,G.; Günzel,R.; Glöckl,D. Solid-phase extraction and direct high-performance liquid chromatographic determination of celiprolol in plasma, *Int.J.Clin.Pharmacol.Ther.Toxicol.*, **1992**, *30*, 512–513.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 µL 5 µg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 µL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 µL 1 M pH 10.3 carbonate buffer and 25 µL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 µL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Supelcosil LC-18
Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 µL/L orthophosphoric acid + 600 µL/L n-butylamine
Flow rate: 2
Injection volume: 25-40
Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM
Retention time: 5.94
Internal standard: maprotiline (12.8)

OTHER SUBSTANCES
Simultaneous: fluoxetine, fluvoxamine, clovoxamine, fenfluramine, amoxapine, desipramine, protriptyline, nortriptyline, sertraline, norfluoxetine
Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine

KEY WORDS
plasma

REFERENCE
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 3 μg/mL propranolol hydrochloride in water + 6 mL MTBE, shake 15 min, centrifuge at 1500 g for 15 min. Remove organic layer and add it to 100 μL 0.05 M sulfuric acid, shake 15 min, centrifuge at 1500 g at 4° for 10 min, discard organic layer, inject 50 μL aliquots of aqueous layer.

HPLC VARIABLES
Column: 100 × 2 5 μm ODS Hypersil
Mobile phase: MeCN:10 mM Na_2HPO_4 40:60 containing 40 mM sodium dodecyl sulfate and 3 mM tetrabutylammonium bromide, adjusted to pH 2 with orthophosphoric acid
Flow rate: 0.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 10.5
Internal standard: propranolol

OTHER SUBSTANCES
Simultaneous: diltiazem, diltiazem metabolites

KEY WORDS
plasma; microbore; propranolol is IS

REFERENCE
Zoest,A.R.; Hung,C.T.; Wanwimolruk,S. Diltiazem: a sensitive HPLC assay and application to pharmacokinetic study, *J.Liq.Chromatogr.*, **1992**, *15*, 1277–1287.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 1 M NaOH + 5 mL dichloromethane, shake, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL dichloromethane, vortex for 5 s, add 10 μL 0.01% S-(+)-1-(1-naphthyl)ethyl isocyanate, heat at 37° for 2 h, add 20 μL tert-butylamine, evaporate under a stream of nitrogen, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 240 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeOH:THF:200 mM pH 3.6 acetate buffer 51:14:35
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: F ex 226 em 333

CHROMATOGRAM
Retention time: 25.2 (R-(+)), 28.3 (S-(-))
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: oxprenolol

KEY WORDS
plasma; chiral; derivatization; propranolol is IS

REFERENCE
Laethem,M.E.; Rosseel,M.T.; Wijnant,P.; Belpaire,F.M. Chiral high-performance liquid chromatographic determination of oxprenolol in plasma, *J.Chromatogr.*, **1993**, *621*, 225–229.

SAMPLE
Matrix: blood
Sample preparation: Inject sample onto column A with mobile phase A and elute for 3 min. Backflush contents of column A onto column B with mobile phase B for 6 min and elute column B with mobile phase B and monitor eluant.

HPLC VARIABLES
Column: A 10 × 3 BioTrap Amine C18 (ChromTech); B 10 × 3 CT-sil C8 guard column + 100 × 4.6 5 μm CT-sil C8 (ChromTech)
Mobile phase: A 48 mM pH 7.0 phosphate buffer; B MeCN:120 mM pH 3.0 phosphate buffer 28:72
Flow rate: A 0.55; B 1
Injection volume: 50
Detector: F ex 220 em 340

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 4.5 ng/mL

KEY WORDS
plasma; column-switching; direct injection

REFERENCE
Hermansson,J.; Grahn,A. Determination of drugs by direct injection of plasma into a biocompatible extraction column based on a protein-entrapped hydrophobic phase, *J.Chromatogr.A*, **1994**, *660*, 119–129.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut 40 μm cyanopropylsilica SPE cartridge with 1 mL MeOH at 6 mL/min and with 1 mL pH 7.4 buffer at 6 mL/min. Centrifuge plasma, add 1 mL plasma at 0.18 mL/min to the SPE cartridge, wash with 1 mL pH 7.4 buffer at 1.5 mL/min, elute with 240 μL MeOH:2-aminoheptane 99.7:0.3 at 1.5 mL/min, pass 410 μL pH 3.0 buffer through the cartridge at 1.5 mL/min. Mix both eluates, inject a 250 μL aliquot. (pH 7.4 Buffer was 250 mL 100 mM KH_2PO_4 and 195.5 mL 100 mM NaOH, made up to 1 L, if necessary pH adjusted to 7.4. pH 3.0 Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 4 μm Superspher 100 RP-18 (Merck)
Mobile phase: MeCN:buffer 30:70 containing 0.5% 2-aminoheptane (Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)
Column temperature: 37
Flow rate: 1.2
Injection volume: 250
Detector: F ex 225 em 340

CHROMATOGRAM
Retention time: 14
Limit of detection: 1.3 ng/mL
Limit of quantitation: 4.5 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Hubert,P.; Chiap,P.; Moors,M.; Bourguignon,B.; Massart,D.L.; Crommen,J. Knowledge-based system for the automated solid-phase extraction of basic drugs fom plasma coupled with their liquid chromatographic determination. Application to the biodetermination of β-receptor bocking agents, *J.Chromatogr.A*, **1994**, *665*, 87–99.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 2.1
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, promazine, propafenone, propoxyphene, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: dextromethorphan, lidocaine, pentazocine

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: 500 μL + NaOH + 3 mL MTBE, extract. Remove the organic layer and evaporate it to dryness, reconstitute the residue in equal parts of MeCN:triethylamine 99.6:

0.4 and 0.025% 2,3,4,5-tetra-O-acetyl-α-D-glucopyranosyl isothiocyanate in MeCN. Evaporate to dryness, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:75 mM pH 3 ammonium phosphate 50:50
Flow rate: 1.4
Injection volume: 100
Detector: F ex 216 em 340

CHROMATOGRAM
Limit of quantitation: 2.5 ng/mL

KEY WORDS
plasma; derivatization; chiral; pharmacokinetics

REFERENCE
Bleske,B.E.; Welage,L.S.; Rose,S.; Amidon,G.L.; Shea,M.J. The effect of dosage release formulations on the pharmacokinetics of propranolol stereoisomers in humans, *J.Clin.Pharmacol.*, **1995**, *35*, 374–378.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 mM pH 7.4 phosphate buffer + 500 μL 2% zinc sulfate in MeOH:water 50:50, mix, centrifuge at 13000 rpm for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 SynChropak bulk support (Knauer)
Column: 120 × 4.6 5 μm Spherisorb ODS1 C18
Mobile phase: MeCN:MeOH:pH 4.5 acetate buffer (ratio not given)
Flow rate: 1
Detector: UV 232

CHROMATOGRAM
Retention time: 6.2

OTHER SUBSTANCES
Extracted: cyclopropane carboxylic acid ester prodrug

KEY WORDS
plasma

REFERENCE
Hovgaard,L.; Brondsted,H.; Buur,A.; Bundgaard,H. Drug delivery studies in Caco-2 monolayers. Synthesis, hydrolysis, and transport of O-cyclopropane carboxylic acid ester prodrugs of various β-blocking agents, *Pharm.Res.*, **1995**, *12*, 387–392.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 600 μL 100 mM pH 8.9 borate buffer + 500 mg NaCl + 100 μL 1 mg/mL 4-methylpropranolol + 6 mL hexane:butanol 96:4, shake for 10 min, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 180 μL 5 mM sulfuric acid, shake for 10 min, centrifuge at 2500 g for 10 min, inject a 50 μL aliquot of the aqueous layer. Alternatively, to determine unbound propranolol filter (Amicon centricon-30) plasma while centrifuging at 5500 g for 45-50 min. 1.4 mL Ultrafiltrate + 600 μL 100 mM pH 8.9 borate buffer + 500 mg NaCl + 100 μL 1 mg/mL 4-methylpropranolol + 6 mL hexane:butanol 96:4, shake for 10 min, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 150 μL 5 mM sulfuric acid, shake for 10 min, centrifuge at 2500 g for 10 min, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: Permaphase ODS (DuPont)

Column: 110 × 4.7 5 μm Partisil 5 ODS 3
Mobile phase: MeCN:MeOH:40 mM ammonium chloride:triethylamine 20:40:40:0.08 containing 5 mM sodium 1-octanesulfonate, adjusted to pH 6.9 with 85% phosphoric acid
Flow rate: 1.4
Injection volume: 50-100
Detector: F ex 293 em 375

CHROMATOGRAM
Retention time: 3.7
Internal standard: 4-methylpropranolol (5.5)
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

REFERENCE
Panton,L.B.; Guillen,G.J.; Williams,L.; Graves,J.E.; Vivas,C.; Cediel,M.; Pollock,M.L.; Garzarella,L.; Krumerman,J.; Derendorf,H.; Lowenthal,D.T. The lack of effect of aerobic exercise training on propranolol pharmacokinetics in young and elderly adults, *J.Clin.Pharmacol.*, **1995**, *35*, 885–894.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum or plasma + 1 mL MeCN + 25 μL 2 μg/mL pronethalol hydrochloride, vortex for 15 s, centrifuge at 10000 rpm for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 50-60°, reconstitute the residue in 100 μL MeOH, vortex briefly, inject a 90 μL aliquot. (Silanize glassware with 5% dimethyldichlorosilane in toluene, rinse with toluene, rinse with MeOH, dry in air.)

HPLC VARIABLES
Guard column: 10 × 4.6 cyano (Alltech)
Column: 250 × 4.6 5 μm Hypersil CN
Mobile phase: MeCN:buffer 35:65 (Buffer was 1% acetic acid containing 0.2% triethylamine, pH 3.6.)
Flow rate: 1.5
Injection volume: 90
Detector: F ex 230 em 340

CHROMATOGRAM
Retention time: 9.5
Internal standard: pronethalol (7.5)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: aspirin, ibuprofen, procainamide, theophylline
Noninterfering: acetaminophen, diltiazem, dipyridamole, furosemide, hydrochlorothiazide, niacin, nifedipine, phenylpropanolamine
Interfering: hydralazine, isosorbide dinitrate, nitroglycerin, quinidine, verapamil

KEY WORDS
serum; plasma; pharmacokinetics

REFERENCE
Rekhi,G.S.; Jambhekar,S.S.; Souney,P.F.; Williams,D.A. A fluorimetric liquid chromatographic method for the determination of propranolol in human serum/plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1499–1505.

SAMPLE
Matrix: blood
Sample preparation: Condition an Styrosorb cross-linked polystyrene (Biochrom, Moscow) or Sep-Pak C18 SPE cartridge with two 3 mL portions of MeOH and two 3 mL portions of water.

Add 1 mL serum containing 2.5 μg metoprolol to the SPE cartridge, wash with two 3 mL portions of water, elute with 600 μL MeOH:diethylamine 99.7:0.3. Evaporate the eluate to dryness under a stream of air at 40°, reconstitute with 50 μL n-heptane:isopropanol:MeOH 83:13:4, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Silasorb-NH2 (Elsico, Moscow)
Mobile phase: n-Heptane:isopropanol:MeOH 83:13:4
Flow rate: 2.5
Injection volume: 20
Detector: F ex 220 em 320 (cut-off filter)

CHROMATOGRAM
Retention time: 3.4
Internal standard: metoprolol (4.1)
Limit of detection: 4 ng/mL

KEY WORDS
SPE: serum; silanize glassware

REFERENCE
Rumiantsev,D.O.; Ivanova,T.V. Solid-phase extraction of Styrosorb cartridges as a sample pretreatment method in the stereoselective analysis of propranolol in human serum, *J.Chromatogr.B*, **1995**, *674*, 301–305.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 100 mM KOH + 30 μL 2 μg/mL (R,S)-n-pentyl propranolol hydrochloride, vortex, add 7 mL n-hexane:n-butanol 99:1, extract. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 20% phosgene in toluene, add 1 mg 4-dimethylaminopyridine, heat at 40° for 3 h. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 LiChrosorb Si 100 modified with (R,R)-DACH-DNB (see J. Chromatogr. 1991, 539, 25)
Mobile phase: Dichloromethane:MeOH 99.75:0.25
Flow rate: 1
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM
Internal standard: (R,S)-n-pentyl propranolol hydrochloride
Limit of detection: 0.5-0.6 ng/mL

KEY WORDS
plasma; chiral; derivatization

REFERENCE
Stoschitzky,K.; Kahr,S.; Donnerer,J.; Schumacher,M.; Luha,O.; Maier,R.; Klein,W.; Lindner,W. Stereoselective increase of plasma concentrations of the enantiomers of propranolol and atenolol during exercise, *Clin.Pharmacol.Ther.*, **1995**, *57*, 543–551.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 290

CHROMATOGRAM

Retention time: 6.76
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, microsomal incubations
Sample preparation: Plasma. 100 μL Plasma + 500 μL 1250 U/mL β-glucuronidase/sulfatase (Helix pomatia, Sigma) in 500 mM pH 5.2 sodium acetate buffer, heat at 37° for 18 h. 500 μL Plasma or deconjugated plasma + 100 ng labetalol + 250 μL 1 M pH 10.3 sodium carbonate + 5 mL ether, shake for 10 min on a reciprocating shaker, centrifuge at 200 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, centrifuge for 4 min, inject an aliquot. Microsomal incu-

bations. 100 μL Microsomal incubation + 600 μL labetalol in MeCN at 4°, centrifuge, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 10 μm μBondapak C18 (Radial-Pak)
Mobile phase: MeCN:20 mM phosphoric acid 26:74 containing 2.7 mM dibutylamine
Injection volume: 10-100
Detector: F ex 240 em 350

CHROMATOGRAM
Internal standard: labetalol

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rabbit; liver

REFERENCE
Du Souich,P.; Maurice,H.; Héroux,L. Contribution of the small intestine to the first-pass uptake and systemic clearance of propranolol in rabbits, *Drug Metab.Dispos.*, **1995**, *23*, 279–284.

SAMPLE
Matrix: blood, perfusate
Sample preparation: 1 mL Serum, plasma, or liver perfusate + 100 μL 10% ascorbic acid + 100 μL 1.5 μg/mL 4-methylpropranolol + 2 mL pH 10 1 M sodium carbonate + 5 mL diethyl ether, vortex for 3 min, centrifuge at 1200 g for 10 min. Remove upper organic phase and evaporate to dryness under a stream of nitrogen at room temperature. Reconstitute in 2 mL mobile phase, inject a 100 μL aliquot. (Acidic metabolites can be determined by adding 250 μL 6 M HCl to the lower aqueous phase, inject 250 μL directly. It may be necessary to extract into ether first.)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChroSpher RP-18
Mobile phase: MeCN:MeOH:water 22:33:45 containing 0.033% triethylamine and 0.044% concentrated phosphoric acid, pH 3.2
Flow rate: 1
Injection volume: 100
Detector: F ex 300 em 375 (em 440 from 8 to 11.5 min)

CHROMATOGRAM
Retention time: 8.58
Internal standard: 4-methylpropranolol (12.98)
Limit of detection: 533 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, hydroxypropranolol, desisopropylpropranolol, propranolol glycol

KEY WORDS
serum; plasma; rat; dog

REFERENCE
Semple,H.A.; Xia,F. Simplified high-performance liquid chromatographic method for propranolol and five metabolites in liver perfusate, rat serum and dog plasma, *J.Chromatogr.B*, **1994**, *655*, 293–299.

SAMPLE
Matrix: blood, saliva
Sample preparation: 50 μL Plasma or saliva + 350 μL 1 μM oxprenolol hydrochloride in water + 100 μL 4 M NaOH, sonicate for 10 min, extract with 3 mL ethyl acetate, centrifuge at 3000 rpm for 3 min. Remove the organic layer and wash it with saturated NaCl solution, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, reconstitute the res-

idue in 100 μL MeCN:water:triethylamine 50:50:0.1, add 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject a 50 μL aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to

obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 56:44:0.1
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 24, 31 (enantiomers)
Internal standard: oxprenolol
Limit of detection: 25-29 fmole

KEY WORDS

derivatization; chiral; rat; plasma; pharmacokinetics

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 2.15
Internal standard: cianopramine

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS

serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 500 μL 1 M NaOH + 8 mL freshly distilled diethyl ether, shake on a rotating shaker at 60 rpm for 15 min, centrifuge at 1200 g for 15 min. Remove 6.5 mL of the organic layer and pass it through a 20 × 4 column filled with glass wool, evaporate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:buffer 48:52 containing 1 g/L sodium heptanesulfonate (Buffer was 100 mM citric acid-sodium citrate buffer adjusted to pH 2.85 with 1 M HCl.)
Column temperature: 28
Flow rate: 1.7
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM
Retention time: 4
Internal standard: propranolol

OTHER SUBSTANCES
Extracted: penbutolol (F ex 278 em 310)

KEY WORDS
plasma; propranolol is IS

REFERENCE
Bernard,N.; Cuisinaud,G.; Sassard,J. Determination of penbutolol and its hydroxylated metabolite in biological fluids by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *228*, 355–361.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum or urine + 20 μL 25 μg/mL naphthyzine nitrate + 20 μL 10 M KOH + 50 μL 25 mM tetrabutylammonium phosphate + 5 mL ethyl acetate, vortex for 30 s, centrifuge at 500 g for 10 min. Remove the organic layer and evaporate it to dryness at 40° under a stream of air. Reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4 Nucleosil 5 SA
Mobile phase: MeCN:water:diethylamine:orthophosphoric acid 156:300:1.9:1.55
Flow rate: 1.5
Detector: F ex 225 em 350 (cut-off filter)

CHROMATOGRAM
Retention time: 21.5
Internal standard: naphthyzine nitrate (17.8)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum

REFERENCE
Belolipetskaja,V.G.; Piotrovskii,V.K.; Metelitsa,V.I.; Pavlinov,S.A. Ion-exchange high-performance liquid chromatography in drug assay in biological fluids. V. Propranolol and metabolites, *J.Chromatogr.*, **1989**, *491*, 507–512.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or 1 mL urine + 50 μL 40 μg/mL pronethalol in MeOH + 1 mL 200 mM pH 9.8 carbonate buffer + 0.5 g NaCl (plasma samples only) + 5 mL toluene, shake horizontally for 30 min, centrifuge at 1500 g at 10° for 15 min. Remove 4 mL of the organic layer and evaporate it under reduced pressure. Reconstitute the residue in 200 μL MeOH, add 50 μL 1% triethylamine in MeOH, add 50 μL 2% R-(+)-phenylethylisocyanate in dichloromethane, vortex briefly, heat at 30° for 35 min, evaporate under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water:acetic acid 70:30:0.1
Column temperature: 28
Flow rate: 1.2
Injection volume: 20
Detector: F ex 295 em 345

CHROMATOGRAM
Retention time: 18 (R), 21 (S)
Internal standard: pronethalol (14 (R), 16 (S))
Limit of detection: 1 ng/mL

KEY WORDS
plasma; derivatization

REFERENCE
Spahn-Langguth,H.; Podkowik,B.; Stahl,E.; Martin,E.; Mutschler,E. Improved enantiospecific RP-HPLC assays for propranolol in plasma and urine with pronethalol as internal standard, *J.Anal.Toxicol.*, **1991**, *15*, 327–331.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 100 μL IS in MeOH + 100 μL 25% ammonium hydroxide + 2 mL MeOH:diethyl ether 10:90, vortex for 1.5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL 5 μL/mL R-(+)-1-phenylethylisocyanate (Fluka) in diethyl ether, vortex for 30 s, let stand at room temperature for 30 min, evaporate to dryness under a stream of nitrogen, add 100 μL mobile phase, vortex for 30 s, centrifuge at 3000 g for 7 min (plasma only), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm C18 (Brownlee)
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeOH:water 72.5:27.5
Flow rate: 1.6
Injection volume: 50
Detector: F ex 232 em 340

CHROMATOGRAM
Retention time: 5.5 (S), 6.2 (R)
Internal standard: 4-methylpropranolol (Cambridge Research Biochemicals) (8.8 (S), 10.1 (R))
Limit of detection: 1 ng/mL

KEY WORDS
derivatization; plasma; chiral; pharmacokinetics

REFERENCE
Pham-Huy,C.; Sahui-Gnassi,A.; Saada,V.; Gramond,J.P.; Galons,H.; Ellouk-Achard,S.; Levresse,V.; Fompeydie,D.; Claude,J.R. Microassay of propranolol enantiomers and conjugates in human plasma and urine by high-performance liquid chromatography after chiral derivatization for pharmacokinetic study, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1189–1198.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 200 μL pH 9.5 carbonate buffer + 600 μL 3 ng/mL 4-methylpropranolol in ethyl acetate, vortex for 1 min, centrifuge at 10000 rpm for 1.5 min. Remove 540 μL of the organic layer and add it to 40 μL pH 2.2 dilute sulfuric acid, vortex for 1 min, centrifuge, inject a 30 μL aliquot of the lower aqueous layer.

HPLC VARIABLES
Column: 300 mm long 10 μm μBondapak alkylphenyl
Mobile phase: MeCN:0.06% phosphoric acid 27:73
Flow rate: 1.4
Injection volume: 30
Detector: F ex 205 em 340

CHROMATOGRAM
Retention time: 6.8
Internal standard: 4-methylpropranolol (8.3)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Kim,E.J.; Yoon,W.H.; Lee,W.I.; Kim,O.N.; Lee,M.G. The effect of dehydration on the disposition kinetics of propranolol in rats, *Biopharm.Drug Dispos.*, **1995**, *16*, 251–257.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Condition a 3 mL Supelclean LC-18 SPE cartridge (Supelco) with MeOH and water. Hydrolyze 900 μL serum with β-glucuronidase (EC 3.2.1.31 type H-1 from Helix pomatia) at 60° for 1 h, add 500 μL (?) MeOH, centrifuge at 2000 g, add the supernatant to the SPE cartridge, wash with 1 mL water, dry under vacuum, elute with 2 mL MeOH:water 90:10, filter, inject an aliquot. Urine. 900 μL Urine + 500 μL MeOH, filter, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm HP C18
Column: 150 × 4.6 5 μm C8P-50 (Asahipak)
Mobile phase: Gradient. MeOH:buffer 30:70 for 4 min, to 45:55 over 6 min, to 50:50 over 2 min, to 60:40 over 2 min, re-equilibrate at initial conditions for 10 min. (Prepare buffer by mixing 100 mM NaH_2PO_4 and 100 mM Na_2HPO_4 to achieve a pH of 7.0 and adding 10 mM N-cetyl-N,N,N-trimethylammonium bromide.)
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: acebutolol, alprenolol, atenolol, metoprolol, oxprenolol

KEY WORDS
serum; comparison with CE; SPE

REFERENCE
Lukkari,P.; Sirén,H. Ion-pair chromatography and micellar electrokinetic capillary chromatography in analyzing β-adrenergic blocking agents from human biological fluids, *J.Chromatogr.A*, **1995**, *717*, 211–217.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 13.06

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Mix the compound with (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide in MeCN containing 0.1% quinuclidine, heat at 60° for 20 min, take up the reaction mixture in EtOH:water 90:10, add to an 18 × 6 column packed with 100 mg carboxymethyl Sephadex LH-200, elute with 100 mM methylamine in EtOH:water 90:10. Evaporate the eluate to dryness, reconstitute with ethyl acetate, inject an aliquot. (Derivatization occurs on the alcohol. Preparation of (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide is as follows. Treat 1-bromo-2-naphthol with sodium hydride in DMF, add iodomethane, stir at room temperature overnight to obtain 1-bromo-2-methoxynaphthalene (mp 85-86°). Add a solution of 37.7 g 1-bromo-2-methylnaphthalene in 200 mL ether over 1 h to a sonicated mixture of 7 g magnesium turnings in 50 mL ether, the mixture should reflux rapidly (Caution! There may be in an induction period!), sonicate for 2 h after addition is complete, add 200 mL benzene (Caution! Benzene is a carcinogen!), add this mixture dropwise to a stirred mixture of 100 mmoles 1-bromo-2-methoxynaphthalene and 655 mg bis(triphenylphosphine)nickel(II) chloride ($NiCl_2(PPh_3)_2$) in 150 mL benzene at room temperature over 1 h, stir at room temperature overnight, reflux for 3 h, remove the ether by distillation through a short Vigreux column, remove the solvent by evaporation under reduced pressure, remove excess 1-bromo-2-methylnaphthalene by heating at 150°/0.1 mm Hg, cool, dissolve the residue in hexane, pass through silica gel, evaporate to dryness, recrystallize from hexane to obtain 1-methoxy-2'-methylbinaphthalene (mp 118-121°). Reflux 10 mmoles 1-methoxy-2'-methyl-binaphthalene, 1.96 g N-bromosuccinimide, and 100 mg benzoyl peroxide in 70 mL carbon tetrachloride for 3 h, filter, evaporate the filtrate to obtain crude 1-bromomethyl-2'-methoxy-binaphthalene. Dissolve the crude 1-bromomethyl-2'-methoxy-binaphthalene in 60 mL DMSO under nitrogen, slowly add a

sodium ethoxide/nitropropane mixture, stir at room temperature for 3 h, stir at 60° for 3 h, pour into 300 mL ice-water, extract with dichloromethane, wash with 2 M HCl, wash with 1 M sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to obtain crude 2'-methoxy-1,1'-binaphthalene-2-carboxaldehyde. (Prepare the sodium ethoxide/nitropropane mixture by dissolving 580 mg sodium in 35 mL EtOH, add 3.25 g 2-nitropropane.) Reflux the crude 2'-methoxy-1,1'-binaphthalene-2-carboxaldehyde in 60 mL acetone, add a solution of 2.36 g potassium permanganate in 60 mL hot water dropwise over 1 h, heat for an additional hour, pass sulfur dioxide through the solution until it becomes clear (sodium metabisulfite may work). Filter off the precipitate and dissolve it in 200 mL hot toluene, add a small amount of activated charcoal, filter while hot, concentrate to about a third of the volume, recrystallize from EtOH:water 1:2 to obtain 2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (mp 258.5-260°) (Bull. Chem. Soc. Japan 1986, 59, 2044). Reflux 9.15 g racemic 2'-methoxy-1,1'-binaphthalene-2-carboxylic acid in 55 mL freshly distilled thionyl chloride for 5 h, evaporate under reduced pressure, add a little benzene, evaporate under reduced pressure, repeat the benzene evaporation twice more to obtain 2'-methoxy-1,1'-binaphthalene-2-carbonyl chloride as a brown solid. Dissolve the acid chloride in 70 mL benzene, add dropwise to 12.8 g (-)-menthol in 100 mL benzene containing 1 g 4-dimethylaminopyridine and 5 mL pyridine, stir overnight at room temperature, heat at 70° for 3 h, cool, dilute with benzene, wash with 2 M HCl, wash with 1 M sodium carbonate, wash with water, dry over anhydrous magnesium sulfate in the presence of activated charcoal, evaporate to dryness, remove as much menthol as possible by sublimation under vacuum, chromatograph twice on a column of silica gel with toluene to obtain the (aS,R) menthol ester (mp 145-146° from hexane) and the (aR,R) menthol ester (mp 126-129° from hexane) as well as a mixture of diastereomers. Reflux the (aS,R) menthol ester with KOH in aqueous EtOH for 8-10 h to obtain (aS)-2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (Bull. Chem. Soc. Japan 1989, 62, 1528). Add 1.5 mL oxalyl chloride to a solution of (aS)-2'-methoxy-1,1'-binaphthalene-2-carboxylic acid in 10 mL anhydrous benzene, reflux for 10 h, evaporate to dryness under reduced pressure. Take up the residue in 10 mL anhydrous benzene, add 1 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 5 h, evaporate to dryness, recrystallize from hexane/acetone to obtain (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide as orange-yellow needles (mp 143-146°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Kyoto)
Mobile phase: Hexane:ethyl acetate:triethylamine 66.6:33.3:0.1
Detector: F ex 330 em 420

CHROMATOGRAM

Retention time: 6 (+), 7.5 (-)
Limit of detection: 100 fmole

KEY WORDS

derivatization; chiral; normal phase

REFERENCE

Goto,J.; Shao,G.; Fukasawa,M.; Nambara,T.; Miyano,S. A chiral axis derivatization reagent for the resolution of β-adrenergic blockers by liquid chromatography with fluorescence detection, *Anal.Sci.*, **1991**, *7*, 645–647.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 1.5 mg compound in 1 mL reagent, add 3 μL triethylamine, sonicate for 20 min, add 3 μL diethylamine, let stand for 15 min, inject an aliquot. (Reagent was 2 mg/mL (R)-(-)-(naphth-1-yl)ethylisocyanate solution in dry chloroform:DMF 80:20.)

HPLC VARIABLES

Column: 200 × 4.6 Silica 100 RP 18
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM

Retention time: k' 9.41 (L), k' 11.31 (D)

OTHER SUBSTANCES
Simultaneous: propylhexedrine
Also analyzed: atenolol, methylphenidate, metipranolol, pindolol, propylhexedrine, talinolol

KEY WORDS
derivatization

REFERENCE
Jira,T.; Toll,C.; Vogt,C.; Beyrich,T. Zur Trennung einiger racemischer β-Blocker und α-Sympathikomimetika durch HPLC nach Derivatisierung [The separation of some racemic β-blockers and α-sympathomimetics with HPLC following derivatization], *Pharmazie*, **1991**, *46*, 432–434.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 90:10
Flow rate: 0.5
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: k' 2.65, k' 3.65 (enantiomers)

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45

Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.48, k' 12.17 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, xamoterol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: formulations
Sample preparation: Take up in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb C2
Mobile phase: MeCN:buffer 35:65 (1 mL 100 mM HCl + 1200 mL water + 5.84 g NaCl, mix to dissolve, add 700 mL MeOH, make up to 2 L, apparent pH 4.5.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 16.2

OTHER SUBSTANCES
Simultaneous: atenolol, nadolol, alprenolol, acebutolol, sotalol, metoprolol, practolol, pindolol, timolol
Interfering: oxprenolol

KEY WORDS
tablets

REFERENCE
Patel,B.R.; Kirschbaum,J.J.; Poet,R.B. High-pressure liquid chromatography of nadolol and other β-adrenergic blocking drugs, *J.Pharm.Sci.*, **1981**, *70*, 336–338.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL sample to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeCN:water:10 mM tetrabutylammonium hydrogen sulfate:500 mM KH_2PO_4 15:58:17:10, pH 7.0
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: amrinone

KEY WORDS
injections; stability-indicating; 5% dextrose; 0.45% NaCl

REFERENCE
Riley,C.M.; Junkin,P. Stability of amrinone and digoxin, procainamide hydrochloride, propranolol hydrochloride, sodium bicarbonate, potassium chloride, or verapamil hydrochloride in intravenous admixtures, *Am.J.Hosp.Pharm.*, **1991**, *48*, 1245–1252.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve 2 mg tablet or capsule in 10 mL pH 10 solution, extract twice with 2 mL ether, combine extracts, filter, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm β-cyclodextrin bonded C18 (Advanced Separation Technologies)
Mobile phase: MeCN:MeOH:acetic acid:triethylamine 95:5:0.3:0.2
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11, 12 (enantiomers)

OTHER SUBSTANCES
Simultaneous: atenolol, metoprolol

KEY WORDS
capsules; tablets; chiral

REFERENCE
Tran,C.D.; Dotlich,M. Enantiomeric separation of β-blockers by high performance liquid chromatography, *J.Chem.Educ.*, **1995**, *72*, 71–73.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 200 μL MeCN to 100 μL microsomal incubation, centrifuge at 3000 g for 5 min, inject a 120 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 3.2 Kromasil C18
Mobile phase: MeCN:MeOH:water:acetic acid 25:25:50:0.1
Flow rate: 1
Injection volume: 120
Detector: Radioactivity, Inus β-Ram using Inus Tru-Count scintillation fluid at a flow rate of 5 mL/min

CHROMATOGRAM
Retention time: 4.0

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE

Obach,R.S. Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol, *Drug Metab.Dispos.*, **1997**, *25*, 1359–1369.

SAMPLE

Matrix: microsomal incubations

Sample preparation: 500 μL Microsomal incubation + 50 μL 6% perchloric acid containing 2% ascorbic acid, centrifuge at 7500 g for 5 min. Removal a 400 μL aliquot of the supernatant and add it to 50 μL 50 μg/mL labetalol, mix, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 4 μm Novapak phenyl radial compression
Mobile phase: MeCN:water:triethylamine 23:77:1 adjusted to pH 3.5 with orthophosphoric acid
Flow rate: 3
Injection volume: 100
Detector: F ex 295 em 360

CHROMATOGRAM

Retention time: 7.2
Internal standard: labetalol (5.3)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

yeast

REFERENCE

Bichara,N.; Ching,M.S.; Blake,C.L.; Ghabrial,H.; Smallwood,R.A. Propranolol hydroxylation and N-desisopropylation by cytochrome P4502D6. Studies using the yeast-expressed enzyme and NADPH/O2 and cumene hydroperoxide-supported reactions, *Drug Metab.Dispos.*, **1996**, *24*, 112–118.

SAMPLE

Matrix: perfusate

Sample preparation: 2 mL Perfusate + 1 mL 100 mM pH 4 phosphate buffer saturated with NaCl, add 6 mL ether, shake for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL isopropanol, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Chiralcel OD
Mobile phase: n-Hexane:EtOH:diethylamine 85:15:0.6
Flow rate: 0.6
Injection volume: 20
Detector: F ex 285 em 340

KEY WORDS

chiral

REFERENCE

Ahmed,S.; Imai,T.; Otagiri,M. Stereoselective hydrolysis and penetration of propranolol prodrugs: In vitro evaluation using hairless mouse skin, *J.Pharm.Sci.*, **1995**, *84*, 877–883.

SAMPLE

Matrix: perfusate

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 10 mL water. 1 mL Perfusate + 2 mL water, add to the SPE cartridge, wash with 10 mL water, dry under vacuum, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of

nitrogen at 40°, reconstitute the residue in 50 μL mobile phase, centrifuge at 700 g for 5 min, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeOH:50 mM pH 5.8 phosphate buffer 55:45, final pH 5.1
Column temperature: 40
Flow rate: 1
Injection volume: 30
Detector: F ex 280 em 395

CHROMATOGRAM
Retention time: 7.1
Internal standard: propranolol
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: prazosin
Noninterfering: albuterol, alcuronium, aminophylline, atenolol, atropine, betamethasone, bupivacaine, cortisone, dexamethasone, diazepam, diltiazem, hydrocortisone, hyoscine, hyoscine-N-butylbromide, labetalol, lidocaine, methimazole, metoclopramide, norepinephrine, phenobarbital, L-phenylephrine, phenytoin, prednisolone, prednisone, promethazine, propylthiouracil, pyridoxine, ranitidine, verapamil

KEY WORDS
SPE; propranolol is IS

REFERENCE
Fletcher,A.J.; Addison,R.S.; Mortimer,R.H.; Cannell,G.R. Rapid determination of prazosin in perfusion media by HPLC with solid phase extraction, *J.Liq.Chromatogr.*, **1995**, *18*, 2911–2923.

SAMPLE
Matrix: saliva
Sample preparation: Condition a 100 mg 1 mL Bond-Elut C2 SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL pH 9.0 borate buffer. Centrifuge a cotton roll soaked with saliva at 1000 g for 5 min, remove the liquid supernatant. 1 mL Supernatant + 50 μL 10 μg/mL alprenolol, add to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL mobile phase, mix for 15 s, inject a 40 μL aliquot. (Acidified MeOH was 50 mL MeOH + 300 μL 96% acetic acid.)

HPLC VARIABLES
Guard column: RCSS silica guard-pack (Waters)
Column: 250 × 4.6 Chiralcel OD-H
Mobile phase: n-Hexane:EtOH:diethylamine 91:9:0.1
Flow rate: 1
Injection volume: 40
Detector: F ex 225 em 320 cut-off filter

CHROMATOGRAM
Retention time: 11 (R), 14 (S)
Internal standard: (S)-alprenolol (6.5)
Limit of detection: 1.33 ng
Limit of quantitation: 3 ng

KEY WORDS
pharmacokinetics; SPE; chiral

REFERENCE
Höld,K.M.; de Boer,D.; Zuidema,J.; Maes,R.A.A. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva, *J.Chromatogr.B*, **1995**, *663*, 103–110.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:10 mM pH 3.5 sodium phosphate buffer 60:40
Flow rate: 1
Detector: UV 215

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: μBondapak C18
Column: μBondapak C18
Mobile phase: MeCN:MeOH:water:triethylamine:85% phosphoric acid 33:22:45:0.03:0.04, pH 3.4
Flow rate: 1.0
Detector: UV 290

REFERENCE
Kunta,J.R.; Goskonda,V.R.; Brotherton,H.O.; Khan,M.A.; Reddy,I.K. Effect of menthol and related terpenes on the percutaneous absorption of propranolol across excised hairless mouse skin, *J.Pharm.Sci.*, **1997**, *86*, 1369–1373.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC GEL, ODS-AM coated with poly-(R)-1-(α-naphthyl)ethyl methacrylamide (Prepare (R)-1-(α-naphthyl)ethyl methacrylamide by reacting methacryl chloride with (R)-1-(α-naphthyl)ethylamine. Prepare poly-(R)-1-(α-naphthyl)ethyl methacrylamide by polymerizing this compound in anhydrous benzene/THF with 2,2'-azobis(isobutyronitrile) (Caution! Benzene is a carcinogen!). Average molecular weight = 2500. Coat 4 g 5 μm YMC GEL, ODS-AM with 0.8 g of this polymer using dichloromethane as a solvent.)
Mobile phase: MeCN:0.5M sodium perchlorate 40:60
Flow rate: 1

CHROMATOGRAM
Retention time: k' 6.90 (α = 1.11)

OTHER SUBSTANCES
Also analyzed: ketamine

KEY WORDS
chiral

REFERENCE
Oi,N.; Hashimoto,S.; Ishizuka,N.; Ohtake,J. Enantiomer separation with poly-(R)-1 (α-naphthyl)-ethyl-methacrylamide coated on ODS silica gel by reversed phase HPLC, *Biomed.Chromatogr.*, **1997**, *11*, 296–297.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Chiralpak AD (A) or Chiralcel OF (B)

Mobile phase: Hexane:EtOH:diethylamine 95:5:0.5 (A) or hexane:2-propanol:diethylamine 92:8:0.5 (B)
Flow rate: 0.8
Detector: UV 254

KEY WORDS
chiral; α = 1.50, Rs = 2.77 for Chiralpak AD; α = 1.35, Rs = 1.75 for Chiralpak OF

REFERENCE
Aboul-Enein,H.Y.; Bakr,S.A. Enantiomeric resolution of propranolol and analogs on two cellulose (Chiralcel OF and OC) and one amylose (Chiralpak AD) chiral stationary phases, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1137–1145.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL of an aqueous solution with 1 mL 100 mM nickel sulfate in water, 1 mL 20% aqueous ammonia, and 5 mL chloroform:carbon disulfide 98:2, shake vigorously for 1 min, wash the organic layer with three 2 mL portions of water, filter (phase-separation paper). Evaporate the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL mobile phase, inject a 10 μL aliquot. (Copper may also be used with electrochemical detection or UV detection at 270 nm.)

HPLC VARIABLES
Guard column: 30 × 4 40 μm LiChrosorb RP-18
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeOH:20 mM pH 5.8 sodium acetate buffer 80:20 containing 5 mM lithium perchlorate
Flow rate: 1.5
Injection volume: 10
Detector: UV 325, E, Merck-Clevenot E 230, Model LCC 231 thin-layer electrolytic cell with a glassy carbon electrode at +0.7 V, standard calomel reference electrode

CHROMATOGRAM
Retention time: k' 21.96
Limit of detection: 1 fmole (E), 1 nmole (UV)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, ephedrine, flecainide, methamphetamine

KEY WORDS
derivatization; complexation

REFERENCE
Leroy,P.; Nicolas,A. Determination of secondary amino drugs as their metal dithiocarbamate complexes by reversed-phase high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1984**, *317*, 513–521.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 500 μL aliquot of a 20-120 μg/mL solution in chloroform with 5 μL R-(+)-1-phenylethyl isocyanate, let stand at room temperature for 15 min, add 10 mL 100 mM HCl, shake on a reciprocating shaker for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm silica (Alltech)
Mobile phase: Chloroform:MeOH 100:1.2
Flow rate: 1
Injection volume: 15
Detector: UV 313

CHROMATOGRAM
Retention time: 4 (-), 4.5 (+)

OTHER SUBSTANCES
Simultaneous: 4-hydroxypropranolol

KEY WORDS
derivatization; normal phase; chiral

REFERENCE
Wilson,M.J.; Walle,T. Silica gel high-performance liquid chromatography for the simultaneous determination of propranolol and 4-hydroxypropranolol enantiomers after chiral derivatization, *J.Chromatogr.*, **1984**, *310*, 424–430.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine,

thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 cellulose tris(3,5-difluorophenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM

Retention time: 16 (+), 32 (-)

KEY WORDS

chiral

REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE

Matrix: solutions
Sample preparation: Evaporate an aliquot of a solution in MeCN containing 62.5 ng drug to dryness under a stream of nitrogen at room temperature, add 200 μL saturated sodium carbonate, add 200 μL 4% (-)-menthyl chloroformate in MeCN, vortex for 30 s, add an excess amount of 4-hydroxy-L-proline, vortex for 30 s, centrifuge for 3 min, inject a 10-25 μL aliquot of the upper layer.

HPLC VARIABLES

Guard column: 50 × 4.6 Pellicular ODS (Whatman)
Column: 100 × 4.6 5 μm Partisil 5 ODS3
Mobile phase: MeCN:water 60:40
Flow rate: 1
Injection volume: 10-25
Detector: F ex 200 em no emission filter

CHROMATOGRAM

Retention time: 32 (-), 35 (+)

OTHER SUBSTANCES

Simultaneous: metoprolol, toliprolol

KEY WORDS

derivatization; chiral

REFERENCE

Mehvar,R. Stereospecific liquid chromatographic analysis of racemic adrenergic drugs utilizing precolumn derivatization with (-)-menthyl chloroformate, *J.Chromatogr.*, **1989**, *493*, 402–408.

SAMPLE

Matrix: solutions

Sample preparation: Mix 20 μL of a solution of propranolol in buffer with 20 μL 4 mM (+)-1-(9-fluorenyl)ethyl chloroformate in dry acetone, let stand at room temperature for 10 min, inject a 10 μL aliquot. (Buffer was 100 mM boric acid/sodium bicarbonate buffer, adjusted to pH 8.5 with NaOH.)

HPLC VARIABLES
Column: 150 × 4 MicroPak SP C8
Mobile phase: MeCN:20 mM pH 4.0 sodium acetate 70:30
Flow rate: 2
Injection volume: 10
Detector: F ex 265 em 345

CHROMATOGRAM
Retention time: 6.7, 7.2 (enantiomers)
Limit of detection: 1 pmole

KEY WORDS
derivatization; chiral

REFERENCE
Lai,F.; Mayer,A.; Sheehan,T. Chiral separation and detection enhancement of propranolol using automated pre-column derivatization, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 117–120.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 210, 225, 286

CHROMATOGRAM
Retention time: 2.0
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES
Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE
Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 4 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 2.84

OTHER SUBSTANCES
Also analyzed: alprenolol, betaxolol, bopindolol, tertatolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thiorida-

zine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralpak AD (Daicel)
Mobile phase: Carbon dioxide:MeOH:diethylamine 69.5:30:0.5
Flow rate: 2
Detector: UV 210

CHROMATOGRAM

Retention time: 2.224, 2.596 (enantiomers)

KEY WORDS

SFC; chiral

REFERENCE

Kot,A.; Sandra,P.; Venema,A. Sub- and supercritical fluid chromatography on packed columns: A versatile tool for the enantioselective separation of basic and acidic drugs, *J.Chromatogr.Sci.*, **1994**, *32*, 439–448.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 25 μg/mL solution in MeCN:water 40:60, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 3 μm silica (Phenomenex)
Mobile phase: MeCN:6.25 mM pH 3.0 phosphate buffer 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 7.12

OTHER SUBSTANCES

Also analyzed: atenolol, clonidine, diltiazem, metoprolol, nifedipine, prazosin, verapamil

REFERENCE

Simmons,B.R.; Stewart,J.T. HPLC separation of selected cardiovascular agents on underivatized silica using an aqueous organic mobile phase, *J.Liq.Chromatogr.*, **1994**, *17*, 2675–2690.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 40 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil MOS C-8
Mobile phase: MeOH:water 70:30 containing 0.02% dimethyloctylamine, 25 mM sodium hexanesulfonate, and 20 mM acetic acid
Flow rate: 1
Injection volume: 40
Detector: UV 288

CHROMATOGRAM
Retention time: 6.4

OTHER SUBSTANCES
Simultaneous: alprenolol (F ex 275 em 305), atenolol (F ex 275 em 305), pindolol (F ex 275 em 305)

REFERENCE
Adson,A.; Burton,P.S.; Raub,T.J.; Barsuhn,C.L.; Audus,K.L.; Ho,N.F.H. Passive diffusion of weak organic electrolytes across Caco-2 cell monolayers: Uncoupling the contributions of hydrodynamic, transcellular, and paracellular barriers, *J.Pharm.Sci.*, **1995**, *84*, 1197–1204.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 ChyRoSine-A 2 (Sedere)
Mobile phase: Carbon dioxide:MeOH containing 1% n-propylamine 88:12
Flow rate: 4
Detector: UV 224

CHROMATOGRAM
Retention time: k' 19.8

KEY WORDS
SFC; pump head cooled at -5°; α = 2.07; chiral

REFERENCE
Bargmann-Leyder,N.; Sella,C.; Bauer,D.; Tambuté,A.; Caude,M. Supercritical fluid chromatographic separation of β-blockers on Chyrosine-A: Investigation of the chiral recognition mechanism using molecular modeling, *Anal.Chem.*, **1995**, *67*, 952–958.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol:diethylamine 90:10:0.1
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.30

KEY WORDS
narrow-bore; chiral; α 1.21

REFERENCE
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)

Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 70:25:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 292

KEY WORDS
chiral; α = 1.11 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Spherisorb S5SCX
Mobile phase: MeOH:MeCN:water 40:40:20 containing 25 mM perchloric acid
Flow rate: 2
Detector: F ex 215 no emission filter

CHROMATOGRAM
Retention time: 5.5
Internal standard: benzimidazole (7)

OTHER SUBSTANCES
Simultaneous: flecainide

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier, *J.Chromatogr.A*, **1995**, *693*, 289–306.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 60:40:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: k' 0.90, 1.52 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4 5 μm CHIRAL-AGP (ChromTech)

Mobile phase: Isopropanol:96 mM pH 4.1 acetate buffer 0.5:99.5
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: k' 7.04, 10.7 (enantiomers)

KEY WORDS
chiral

REFERENCE
Hermansson,J.; Grahn,A. Optimization of the separation of enantiomers of basic drugs. Retention mechanisms and dynamic modification of the chiral bonding properties on an α_1-acid glycoprotein column, *J.Chromatogr.A*, **1995**, *694*, 57–69.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 21.88

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 9.19 (A), 4.91 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 5 μm C8 Symmetry end-capped (prepared in the laboratory from Waters silica)
Mobile phase: MeOH:20 mM pH 7.00 potassium phosphate buffer 65:35
Column temperature: 23 ± 0.5
Flow rate: 1
Detector: UV

CHROMATOGRAM

Retention time: 1.5

OTHER SUBSTANCES

Simultaneous: amitriptyline

REFERENCE

O'Gara,J.E.; Alden,B.A.; Walter,T.H.; Petersen,J.S.; Niederländer,C.L.; Neue,U.D. Simple preparation of a C_8 HPLC stationary phase with an internal polar functional group, *Anal.Chem.*, **1995**, *67*, 3809–3813.

SAMPLE

Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Sumchiral CSP 10 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:1,2-dichloroethane:EtOH:trifluoroacetic acid 250:140:10:1
Flow rate: 1
Detector: UV 230-280

CHROMATOGRAM
Retention time: k' 10.54

KEY WORDS
chiral; α = 1.13

REFERENCE
Oi,N.; Kitahara,H.; Aoki,F. Direct enantiomer separations by high-performance liquid chromatography with chiral urea derivatives as stationary phases, *J.Chromatogr.A*, **1995**, *694*, 129–134.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica
Mobile phase: Heptane:isopropanol:diethylamine 80:20:0.1
Flow rate: 1
Injection volume: 1000
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.91

KEY WORDS
chiral; α 1.18

REFERENCE
Oliveros,L.; Lopez,P.; Minguillon,C.; Franco,P. Chiral chromatographic discrimination ability of a cellulose 3,5-dimethylphenylcarbamate/10-undecenoate mixed derivative fixed on several chromatographic matrices, *J.Liq.Chromatogr.*, **1995**, *18*, 1521–1532.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 μL of a 1 mM amine solution in MeCN:water:triethylamine 50:50:2 with 10 μL 5 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazolein MeCN, heat at 55° for 10 min, add 480 μL 1 M acetic acid in MeCN:water 50:50, dilute 10-fold with MeCN, inject a 5 μL aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole, (R)-(-)-NBD-PyNCS, is as follows. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation

under reduced pressure, dissolve the residue in 50 mL water, extract 4 times with 80 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro- 2,1,3-benzoxadiazole as dark red crystals (mp 178-181°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as red crystals (mp 165-170°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 40:60:0.05
Column temperature: 40
Flow rate: 1
Injection volume: 5
Detector: F ex 490 em 530

CHROMATOGRAM
Retention time: 10.5 ((R)-(+)-propranolol), 11.5 ((S)-(-)-propranolol)

OTHER SUBSTANCES
Simultaneous: alprenolol

KEY WORDS
derivatization; chiral

REFERENCE
Toyo'oka,T.; Liu,Y.-M. Development of optically active fluorescent Edman-type reagents, *Analyst*, **1995**, *120*, 385–390.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.81

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions

Sample preparation: Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmoles sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

HPLC VARIABLES

Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: MeCN:water 70:30
Flow rate: 0.8
Injection volume: 100
Detector: UV 230, F ex 270 em 350

CHROMATOGRAM

Retention time: k' 8.7 (S-(-)), 10.7 (R-(+))

OTHER SUBSTANCES

Simultaneous: alprenolol

KEY WORDS

derivatization; chiral; F not much more sensitive than UV; $\alpha = 1.23$

REFERENCE

Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.40

OTHER SUBSTANCES

Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil

Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE

Matrix: tissue

Sample preparation: Weigh out brain tissue and homogenize in 4 volumes 400 mM perchloric acid using a Tamson motor-driven PTFE/glass homogenizer at 1400 rpm to give a final tissue concentration of 25 mg/mL in the perchloric acid. For each 1 mL of homogenate add 40 μL 31.4 μg/mL clenbuterol in 200 mM sulfuric acid, centrifuge at 3000 g for 15 min. Remove 1 mL supernatant and add it to 10 μL 10 M NaOH and 350 μL buffer, vortex for 10 s, add 8 mL diethyl ether, shake mechanically for 45 min, centrifuge at 2000 g for 8 min. Remove the organic layer and add it to 200 μL 200 mM sulfuric acid, shake mechanically for 15 min, centrifuge at 2000 g for 8 min. Remove the aqueous layer and heat it at 45° for 1 h to remove traces of ether, inject a 50 μL aliquot. (Buffer was 90 g sodium carbonate and 32 g potassium carbonate in 1 L, pH 9.0.)

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm LC-18-DB (Supelchem)
Column: 250 × 4.6 5 μm LC-18-DB (Supelchem)
Mobile phase: MeCN:50 mM NaH_2PO_4:triethylamine 35:65:0.1, adjusted to pH 3.0 with orthophosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 7.3
Internal standard: clenbuterol (4.8)
Limit of detection: 22 ng/mL

OTHER SUBSTANCES

Simultaneous: labetalol

KEY WORDS

rat; brain

REFERENCE

Botterblom,M.H.A.; Feenstra,M.G.P.; Erdtsieck-Ernste,E.B.H.W. Determination of propranolol, labetalol and clenbuterol in rat brain by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 121–126.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste.

After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES
Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
Flow rate: A 1.25; B 1
Injection volume: 100
Detector: UV 220

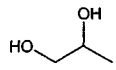
CHROMATOGRAM
Retention time: 15.3
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, timolol

KEY WORDS
column-switching

REFERENCE
Saarinen,M.T.; Sirén,H.; Riekkola,M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J.Chromatogr.B*, **1995**, *664*, 341–346.

Propylene glycol



Molecular formula: $C_3H_8O_2$
Molecular weight: 76.10
CAS Registry No.: 57-55-6
Merck Index: 8040

SAMPLE
Matrix: formulations
Sample preparation: Remove the water from 10 μL syrup under reduced pressure for 10 min, reconstitute with 2 mL pyridine. Remove a 25 μL aliquot and add it to 75 μL reagent, shake well, let stand at room temperature for 10 min, evaporate to dryness under reduced pressure at room temperature, flush the tube with a stream of air or nitrogen, add 2 mL 5% sodium carbonate solution containing 2.5 mg/mL 4-dimethylaminopyridine, shake or sonicate for 5 min, extract with 2 mL chloroform. Wash the extract with 2 mL 5% sodium bicarbonate solution, wash twice with 3 mL portions of 50 mM HCl containing 5% NaCl, inject an aliquot. (Prepare reagent by dissolving 100 mg 4-nitrobenzoyl chloride in pyridine with gentle warming.)

HPLC VARIABLES
Column: 150 × 3 5 μm LiChrosorb SI 60
Mobile phase: n-Hexane:chloroform:MeCN 10:3:1.9 containing 0.1% water
Flow rate: 1.4
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 1.5

OTHER SUBSTANCES
Simultaneous: dextrose, fructose, glycerin, saccharose, sorbitol

KEY WORDS
syrup; derivatization; normal phase

REFERENCE
Nachtmann,F.; Budna,K.W. Sensitive determination of derivatized carbohydrates by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, *136*, 279–287.

SAMPLE
Matrix: formulations
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 2 mL MeOH and 20 mL water. Dilute formulation ten-fold with water, add a 0.5 mL aliquot to the SPE cartridge, elute with three 1 mL portions of water, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 8 mm i.d. C18 radial compression (Waters)
Mobile phase: Water
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 4.75

OTHER SUBSTANCES
Simultaneous: dihydroxyacetone, dioxane, ethylene glycol, formic acid, glyceraldehyde, glycerol, methylglyoxal

KEY WORDS
SPE

REFERENCE
Bobin,M.F.; Martini,M.C.; Gudefin,A.; Cotte,J. Dosage de la dihydroxyacétone dans les émulsions [Assay of dihydroxyacetone in emulsions], *Farmaco.[Prat].*, **1983**, *38*, 403–414.

SAMPLE
Matrix: solutions
Sample preparation: Add 0.5 mmole propylene glycol to a solution of 0.5 g 3,5-dinitrobenzoyl chloride in 30 mL pyridine, mix well, heat at 60° for 15 min, adjust pH to 2.5 with 2 M HCl (Caution! Exothermic reaction!), cool to room temperature, add 25 mL butyl acetate, shake for 3 min. Remove the organic layer and add it to 50 mL 1% sodium carbonate solution, shake for 2 min. Remove the organic layer and add it to 25 mL 0.25 M sulfuric acid, shake for 2 min. Remove the organic layer and add it to 10 mL water, shake for 1 min, inject a 10 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 2000 × 2.1 Corasil II
Mobile phase: Heptane:ethyl acetate 75:25
Flow rate: 1.7
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: diethylene glycol, ethylene glycol

KEY WORDS
derivatization; normal phase

REFERENCE
Carey,M.A.; Persinger,H.E. Liquid chromatographic determination of traces of aliphatic carbonyl compounds and glycols as derivatives that contain the dinitrophenyl group, *J.Chromatogr.Sci.*, **1972**, *10*, 537–543.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in water containing 5 mg/mL galactose, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Aminex carbohydrate HPX-87 (BioRad)
Mobile phase: water
Column temperature: 80
Flow rate: 0.2
Injection volume: 20
Detector: RI

CHROMATOGRAM
Retention time: 8.5
Internal standard: galactose (7)

OTHER SUBSTANCES
Simultaneous: arabinose, arabitol, dextran, dextrose, erythritol, fructose, fucose, galactan, galactitol, galactomannan, gentibiose, iditol, lyxose, maltose, maltotriose, mannose, melezitose, melibiose, pentaerythritol, pullulan, raffinose, rhamnose, ribitol, sorbitol, sorbose, sucrose, trehalose, turanose, xylitol, xylose
Noninterfering: xylan
Interfering: glycol, mannitol

KEY WORDS
detector temp 30°

REFERENCE
Dokladalova,J.; Barton,A.Y.; Mackenzie,E.A. High pressure liquid chromatographic determination of sorbitol in bulk sorbitol, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 664–666.

SAMPLE
Matrix: solutions

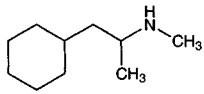
HPLC VARIABLES
Column: 300 × 7.8 Sarasep CAR-Ca (MetaChem)
Mobile phase: 50 μg/mL dicalcium EDTA
Column temperature: 80
Flow rate: 0.7
Detector: RI

CHROMATOGRAM
Retention time: 14.9

OTHER SUBSTANCES
Simultaneous: acetone, acetonitrile, adonitol, arabinose, arabitol, cellobiose, dextrose, erythritol, ethanol, ethylene glycol, fructose, fucose, galactinol, galactitol, galactopinitol, galactose, gentianose, gentiobiose, glucoheptose, glycerol, isomaltose, ketose, lactitol, lactose, lactulose, maltitol, maltose, maltotetraose, maltotriose, mannoheptulose, mannose, melezitose, melibiose, methanol, myo-inositol, nystose, palatinol, palatinose, perseitol, pinitol, raffinose, rhamnose, ribose, sedoheptulosan, sedoheptulose, sorbitol, sorbose, styachyose, sucrose, tagatose, trehalose, turanose, volemitol, xylitol, xylose
Interfering: mannitol

REFERENCE
MetaChem Catalog, **1994**, p. 65.

Propylhexedrine



Molecular formula: $C_{10}H_{21}N$
Molecular weight: 155.28
CAS Registry No.: 3595-11-7, 101-40-6
Merck Index: 8045
Lednicer No.: 1 37

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 1.5 mg compound in 1 mL reagent, add 3 μL triethylamine, sonicate for 20 min, add 3 μL diethylamine, let stand for 15 min, inject an aliquot. (Reagent was 2 mg/mL (R)-(-)-(naphth-1-yl)ethylisocyanate solution in dry chloroform:DMF 80:20.)

HPLC VARIABLES
Column: 200 × 4.6 Silica 100 RP 18
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: k' 9.26 , k' 10.19 (enantiomers)

OTHER SUBSTANCES
Also analyzed: atenolol, methylphenidate, metipranolol, pindolol, propranolol, talinolol

KEY WORDS
derivatization; chiral

REFERENCE
Jira,T.; Toll,C.; Vogt,C.; Beyrich,T. Zur Trennung einiger racemischer β-Blocker und α-Sympathikomimetika durch HPLC nach Derivatisierung [The separation of some racemic β-blockers and α-sympathomimetics with HPLC following derivatization], *Pharmazie*, **1991**, *46*, 432–434.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 1.5 mg compound in 1 mL reagent, add 3 μL triethylamine, sonicate for 20 min, add 3 μL diethylamine, let stand for 15 min, inject an aliquot. (Reagent was 2 mg/mL (R)-(+)-1-phenylethylisocyanate ((R)-(+)-α-methylbenzylisocyanate) solution in dry chloroform:DMF 80:20.)

HPLC VARIABLES
Column: 200 × 4.6 Silica 100 RP 18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Detector: UV 254

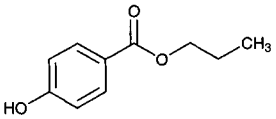
CHROMATOGRAM
Retention time: k' 4.36, k' 4.73 (enantiomers)

OTHER SUBSTANCES
Simultaneous: propranolol

KEY WORDS
derivatization; chiral

REFERENCE
Jira,T.; Toll,C.; Vogt,C.; Beyrich,T. Zur Trennung einiger racemischer β-Blocker und α-Sympathikomimetika durch HPLC nach Derivatisierung [The separation of some racemic β-blockers and α-sympathomimetics with HPLC following derivatization], *Pharmazie*, **1991**, *46*, 432–434.

Propylparaben



Molecular formula: $C_{10}H_{12}O_3$
Molecular weight: 180.20
CAS Registry No.: 94-13-3
Merck Index: 8051

SAMPLE
Matrix: solutions

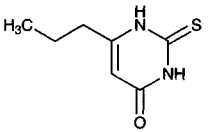
HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendi-

metrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Propylthiouracil



Molecular formula: $C_7H_{10}N_2OS$
Molecular weight: 170.24
CAS Registry No.: 51-52-5
Merck Index: 8054
Lednicer No.: 1 265

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M NaOH, shake, add 4 mL chloroform, vortex, centrifuge at 300 g for 10 min, discard the chloroform layer, add 300 μL 10% HCl to the aqueous layer, vortex, add 2 mL chloroform, vortex, centrifuge at 300 g for 10 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 30 μL MeOH, add 20 μL mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Newguard RP-18
Column: 250 × 4.6 Spheri-5 RP-18
Mobile phase: MeOH:50 mM pH 7.4 phosphate buffer 30:70
Column temperature: 37
Flow rate: 1.5
Injection volume: 20
Detector: UV 214

CHROMATOGRAM

Retention time: 7.25
Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: acetaminophen, albuterol, atenolol, bupivacaine, caffeine, carbamazepine, clonazepam, cortisone, diazepam, estriol, hydrocortisone, hyoscine, methimazole, metoprolol, phenobarbital, prednisolone, prednisone, progesterone, propranolol, ritodrine, verapamil

KEY WORDS

plasma; pharmacokinetics

REFERENCE
Cannell,G.R.; Williams,J.P.; Yap,A.S.; Mortimer,R.H. Selective liquid chromatographic assay for propylthiouracil in plasma, *J.Chromatogr.*, **1991**, *564*, 310–314.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 mg disodium EDTA + 3 mL ethyl acetate, vortex for 3 min, centrifuge at 5000 *g* for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 3 LiChroprep RP-18 (Merck)
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: Gradient. MeOH:25 mM pH 3 phosphate buffer from 10:90 to 70:30 over 30 min
Flow rate: 1
Injection volume: 10
Detector: UV 276

CHROMATOGRAM
Retention time: 17.30
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: methimazole (UV 258), methylthiouracil, phenylthiouracil, thiouracil

KEY WORDS
cow; plasma

REFERENCE
Moretti,G.; Betto,P.; Cammarata,P.; Fracassi,F.; Giambenedetti,M.; Borghese,A. Determination of thyreostatic residues in cattle plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *616*, 291–296.

SAMPLE
Matrix: blood
Sample preparation: 450 μL Plasma + 60 μL 100 mM HCl + 50 μL 25 μg/mL methylthiouracil in water, vortex for 10 s, add 6 mL dichloromethane:acetone 75:25, vortex for 2 min, centrifuge at 1500 *g* for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 μL water, vortex for 20 s, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Lichrospher RP-18
Column: 125 × 4 5 μm Lichrospher RP-18
Mobile phase: THF:buffer 0.9:99.1, pH 6.0 ± 0.1 (Buffer was 34 g KH_2PO_4 and 170 mL 200 mM NaOH, make up to 5 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 275

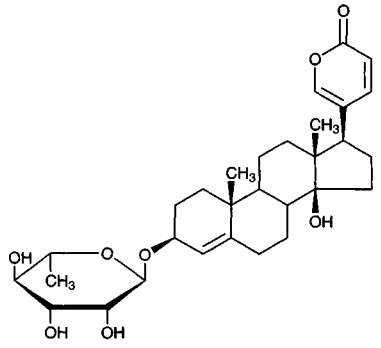
CHROMATOGRAM
Retention time: 15.20
Internal standard: methylthiouracil (2.60)
Limit of detection: 5 ng/mL
Limit of quantitation: 40 ng/mL

KEY WORDS
dog; plasma

REFERENCE
Kabanda,L.; De Muynck,C.; Lefebvre,R.A.; Remon,J.P. Validation of a HPLC method for the determination of propylthiouracil in plasma, *J.Liq.Chromatogr.*, **1994**, *17*, 2069–2083.

Proscillaridin

Molecular formula: $C_{30}H_{42}O_8$
Molecular weight: 530.66
CAS Registry No.: 466-06-8
Merck Index: 8060



SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.585

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Protamine sulfate

CAS Registry No.: 9009-65-8

SAMPLE
Matrix: formulations
Sample preparation: Centrifuge 10 mL of an insulin formulation at 5° at 2000 rpm for 20 min, discard the supernatant, dissolve the pellet in 500 μL 100 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: MeCN:buffer 2.5:97.5 (Buffer was 100 mM NaH_2PO_4 adjusted to pH 2 with phosphoric acid.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 17.5, 21.5, 27, 42.5

KEY WORDS
salmon; fish

REFERENCE
Hoffmann,J.A.; Chance,R.E.; Johnson,M.G. Purification and analysis of the major components of chum salmon protamine contained in insulin formulations using high-performance liquid chromatography, *Protein Expr.Purif.*, **1990**, *1*, 127–133.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 SynChropak RP C18 (Baxter)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.05% trifluoroacetic acid in isopropanol. A:B from 95:5 to 0:100 over 30 min.
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: angiotensin, aprotinin, insulin B

REFERENCE
Baxter Scientific Products Catalog, 1992-3, p. 187.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 10 Vydac C18
Mobile phase: Gradient. A was MeOH:water 10:90 containing 0.1% heptafluorobutyric acid. B was MeOH containing 0.1% heptafluorobutyric acid. A:B from 100:0 to 10:90 over 60 min, maintain at 10:90 for 5 min.
Detector: UV 230

CHROMATOGRAM
Retention time: 57

KEY WORDS
fish; yellow perch; testes; semi-preparative

REFERENCE
Chao,H.; Davies,P.L. Amino acid sequence of the unique protamine from yellow perch, *FEBS Lett.*, **1992**, *299*, 166–168.

SAMPLE
Matrix: solutions

Sample preparation: Centrifuge at 14000 rpm for 10 min, filter (0.22 μm), dilute with 10 mM HCl (if necessary), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: MeCN:100 mM pH 2 sodium phosphate buffer 2:98
Flow rate: 1
Detector: UV 215

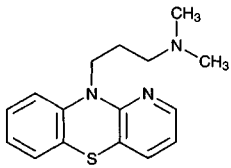
CHROMATOGRAM
Retention time: 6, 8, 10, 18

OTHER SUBSTANCES
Simultaneous: insulinotropin

KEY WORDS
salmon

REFERENCE
Kim,Y.; Rose,C.A. Precipitation of insulinotropin in the presence of protamine: Effect of phenol and zinc on the isophane ratio and the insulinotropin concentration in the supernatant, *Pharm.Res.*, **1995**, *12*, 1284–1288.

Prothipendyl



Molecular formula: $C_{16}H_{19}N_3S$
Molecular weight: 285.41
CAS Registry No.: 303-69-5, 1225-65-6 (HCl)
Merck Index: 8073
Lednicer No.: 1 430

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.95

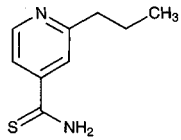
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine,

droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Protionamide



Molecular formula: $C_9H_{12}N_2S$
Molecular weight: 180.27
CAS Registry No.: 14222-60-7
Merck Index: 8076

SAMPLE

Matrix: blood
Sample preparation: Add 60 μL 30% trichloracetic acid to 300 μL serum. Immediately vortex and centrifuge at 4° at 23000 g for 10 min. Mix 200 μL supernatant with 45 μL 1 M sodium bicarbonate and centrifuge. Inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 5 × 3 5 μm Kromasil 100 C4
Column: 100 × 3 5 μm Kromasil 100 C4
Mobile phase: MeCN:buffer 25:75 (Buffer was 9.534 g sodium tetraborate and 6.4625 g dibutylamine in 1 liter water, adjusted to pH 8 with concentrated HCl.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 291

CHROMATOGRAM

Retention time: 4.7
Limit of quantitation: 27 ng/mL

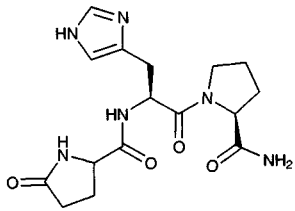
OTHER SUBSTANCES

Noninterfering: clofazimine, ofloxacin, rifabutin, thiacetazone

KEY WORDS
serum

REFERENCE
Bartels,H.; Bartels,R. Simple, rapid and sensitive determination of protionamide in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *707*, 338–341.

Protirelin



Molecular formula: $C_{16}H_{22}N_6O_4$
Molecular weight: 362.39
CAS Registry No.: 24305-27-9
Merck Index: 9720

SAMPLE
Matrix: formulations
Sample preparation: Dilute 4 mL injection to 200 mL with water. Remove a 5 mL aliquot and add it to 10 mL 1 mg/mL p-aminobenzoic acid in water, make up to 200 mL with water, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer:triethylamine 4:4:92:0.01 (Buffer was 0.05% sodium octanesulfonate adjusted to pH 2.2 with 3 M phosphoric acid.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 16
Internal standard: p-aminobenzoic acid (9.5)

OTHER SUBSTANCES
Simultaneous: benzaldehyde, benzoic acid, benzyl alcohol, degradation products.

KEY WORDS
stability-indicating; injections

REFERENCE
Rao,G.N.; Sutherland,J.W.; Menon,G.N. High-performance liquid chromatographic assay for thyrotropin releasing hormone and benzyl alcohol in injectable formulation, *Pharm.Res.*, **1987**, *4*, 38–41.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 mm Superspher 100 RP-18
Mobile phase: MeCN:0.1% pH 2.0 trifluoroacetic acid 5:95
Flow rate: 1
Detector: UV 215

CHROMATOGRAM
Retention time: 2.4
Limit of detection: 500 nM

REFERENCE
Werner,U.; Kisel,T.; Stüber,W. Effects of peptide structure on transport properties of seven thyrotropin releasing hormone (TRH) analogues in a human intestinal cell line (Caco-2), *Pharm.Res.*, **1997**, *14*, 246–250.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 0.1% phosphoric acid. B was MeCN:0.1% phosphoric acid 70:30. A:B from 95:5 to 30:70 over 20 min.
Flow rate: 1
Detector: UV 206 or RIA

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: gonadorelin, somatostatin, substance P

REFERENCE
McDermott,J.R.; Smith,A.I.; Biggins,J.A.; Al-Noaemi,M.C.; Edwardson,J.A. Characterization and determination of neuropeptides by high-performance liquid chromatography and radioimmunoassay, *J.Chromatogr.*, **1981**, *222*, 371–379.

SAMPLE
Matrix: solutions

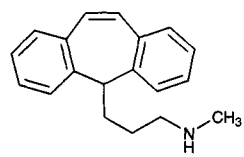
HPLC VARIABLES
Column: 250 × 4.6 TSKgel ODS-120T
Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, α-endorphin, β-endorphin, calcitonin (human), gonadorelin (LH-RH), somatostatin

REFERENCE
Varian Catalog, **1993**, p. 182.

Protriptyline



Molecular formula: $C_{19}H_{21}N$
Molecular weight: 263.38
CAS Registry No.: 438-60-8, 1225-55-4 (HCl)
Merck Index: 8088
Lednicer No.: 1 152

SAMPLE
Matrix: blood

REFERENCE
Werner,U.; Kisel,T.; Stüber,W. Effects of peptide structure on transport properties of seven thyrotropin releasing hormone (TRH) analogues in a human intestinal cell line (Caco-2), *Pharm.Res.*, **1997**, *14*, 246–250.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 0.1% phosphoric acid. B was MeCN:0.1% phosphoric acid 70:30. A:B from 95:5 to 30:70 over 20 min.
Flow rate: 1
Detector: UV 206 or RIA

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: gonadorelin, somatostatin, substance P

REFERENCE
McDermott,J.R.; Smith,A.I.; Biggins,J.A.; Al-Noaemi,M.C.; Edwardson,J.A. Characterization and determination of neuropeptides by high-performance liquid chromatography and radioimmunoassay, *J.Chromatogr.*, **1981**, *222*, 371–379.

SAMPLE
Matrix: solutions

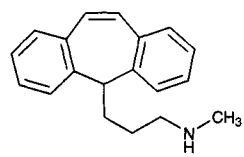
HPLC VARIABLES
Column: 250 × 4.6 TSKgel ODS-120T
Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, α-endorphin, β-endorphin, calcitonin (human), gonadorelin (LH-RH), somatostatin

REFERENCE
Varian Catalog, **1993**, p. 182.

Protriptyline



Molecular formula: $C_{19}H_{21}N$
Molecular weight: 263.38
CAS Registry No.: 438-60-8, 1225-55-4 (HCl)
Merck Index: 8088
Lednicer No.: 1 152

SAMPLE
Matrix: blood

Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Add 1 mL plasma or serum to the SPE cartridge. Wash twice with 2 mL water. Acidify the cartridge with 1 mL MeOH:250 mM HCl 10:90, wash with 500 μL MeCN, elute twice with 500 μL 10 mM acetic acid and twice with 500 μL 5 mM diethylamine in MeOH. Evaporate the eluate to dryness with a gentle stream of air at 37°. Reconstitute the residue with 100 μL mobile phase. Inject a 40 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm Select-B C8 (Merck)
Mobile phase: MeCN:MeOH:10 mM pH 3.7 dipotassium hydrogen phosphate 30:2:100
Flow rate: 1.5
Injection volume: 40
Detector: UV 220

CHROMATOGRAM

Retention time: 8.5
Internal standard: protriptyline

OTHER SUBSTANCES

Extracted: clozapine
Simultaneous: amitriptyline, clomipramine, chlorpromazine, chlorprothixene, citalopram, desipramine, desmethylcitalopram diazepam, doxepin, fluoxetine, haloperidol, imipramine, levomepromazine, maprotiline, medazepam, mianserin, midazolam, nitrazepam, norclomipramine, nordoxepin, norfluoxetine, normaprotiline, nortrimipramine, nortryptiline, thioridazine, thiothixene, trazodone, trimipramine
Noninterfering: carbamazepine, carbamazepine-10-epoxide, carbamazepine-11-epoxide, clobazam, ethosuximide, flunitrazepam, 10-hydroxycarbazepine, norclobazam, oxazepam, oxcarbazepine, pentobarbital, phenobarbital, primidone, temazepam

KEY WORDS

plasma; serum; SPE; protriptyline is IS

REFERENCE

Åkerman,K.A. Analysis of clozapine and norclozapine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *696*, 253–259.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 200 μL 80 g/L $NaHCO_3$ + 5 mL hexane, vortex for 15 s, centrifuge for 5 min. Remove the hexane layer and evaporate it in a stream of nitrogen at 60°. Reconstitute in 100 μL mobile phase, vortex for 15 s, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak CN
Mobile phase: MeCN:MeOH:5 mM phosphate buffer 60:15:25, adjusted to pH 7.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 12.20
Internal standard: protriptyline

OTHER SUBSTANCES

Simultaneous: imipramine, trimipramine, doxepin, desmethyldoxepin, amitriptyline, nortriptyline, desipramine, chlorpromazine, thioridazine, propranolol, propoxyphene, maprotiline, procainamide, disopyramide
Noninterfering: caffeine, theophylline, salicylic acid, chlordiazepoxide, methaqualone, diazepam, acetaminophen, trifluoperazine

KEY WORDS

serum; protriptyline is IS

REFERENCE

Koteel,P.; Mullins,R.E.; Gadsden,R.H. Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum, *Clin.Chem.*, **1982**, *28*, 462–466.

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond-Elut C18 column with 2 volumes MeOH then 2 volumes water. Add 1 mL serum then 200 μL 700 ng/mL promazine in MeOH:0.1 M HCl 13:87 to each column, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with MeOH/water, add 200 μL 10 mM ammonium acetate in MeOH, wait for 30 s, elute with vacuum, repeat elution process two more times. Combine eluates and evaporate them to dryness at 56-8° under compressed air. Reconstitute with 200 μL mobile phase, vortex 10 s, inject 75-100 μL aliquot. (MeOH/water was 500 mL MeOH:water 65:35 plus 25 μL concentrated HCl.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelco silica

Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Mix 1 gallon EtOH with 77 mL MeCN and 1.9 mL t-butylamine.)

Flow rate: 2

Injection volume: 75-100

Detector: UV 229

CHROMATOGRAM

Retention time: 12.1

Internal standard: promazine (5.2)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, desipramine, desmethyldoxepin, doxepin, imipramine, nortriptyline

Simultaneous: thioridazine, hydroxyamoxapine, meperidine, chlorpromazine, disopyramide, amphetamine, 2-hydroxyimipramine, iprindole, pyrilamine, promethazine, prolixin, amoxapine, N-acetylprocainamide, procainamide, zimeldine, morphine, codeine, trifluoperazine, desmethyldisopyramide, 10-hydroxynortriptyline, prochlorperazine, oxaprotiline, 2-hydroxydesipramine, chlorpheniramine, maprotiline, norzimeldine, iminostilbene, desmethylchlordiazepoxide, buprion, diazepam, demoxepam, chlordiazepoxide, propoxyphene, dextropropoxyphene, cocaine, oxapam, trimipramine, mianserin, trimeprazine, loxepin, fluphenazine, methadone, trifluopromazine, phenteramine, chlorimipramine, perphenazine, quinidine

Noninterfering: thiopropazine

KEY WORDS

serum; normal phase

REFERENCE

Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Serum or plasma + 0.6 mL water, vortex, extract with 10 mL toluene:isoamyl alcohol 99:1 for 10 min on a rotator, centrifuge for 5 min. Remove upper organic layer, evaporate under a stream of nitrogen at 37°, take up in 150 μL mobile phase, vortex for 2 min, add 0.5 mL hexane, vortex briefly, centrifuge for 5 min, discard upper hexane layer, inject a 100 μL aliquot of the lower layer.

HPLC VARIABLES

Column: 250 × 4 Bio-Sil ODS-10 (Bio-Rad)

Mobile phase: MeCN:pH 4.5 50 mM phosphate buffer 30:70 (Buffer was 6.9 g KH_2PO_4 in 1 L adjusted to pH 4.5 with orthophosphoric acid.)

Column temperature: 45

Flow rate: 2.5

Injection volume: 100

Detector: UV 202

CHROMATOGRAM
Retention time: 6.9

OTHER SUBSTANCES
Extracted: alprazolam, imipramine, nortriptyline, triazolam
Noninterfering: N-acetylprocainamide, amitriptyline, caffeine, chlordiazepoxide, chlorpromazine, diazepam, flurazepam, lorazepam, oxazepam, prazepam, procainamide, propranolol, thioridazine
Interfering: desipramine

KEY WORDS
plasma; serum

REFERENCE
McCormick,S.R.; Nielsen,J.; Jatlow,P. Quantification of alprazolam in serum or plasma by liquid chromatography, *Clin.Chem.*, **1984**, *30*, 1652–1655.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 37 μL 2 μg/mL IS in MeOH + 500 μL pH 10 borate buffer + 1.5 mL hexane:isoamyl alcohol 95:5, shake for 10 min. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in 100 μL MeOH, inject a 50 μL aliquot. (The borate buffer was prepared as follows. Prepare a solution of 61.8 g boric acid and 74.6 g KCl in 1 L water. Add 630 mL of this solution to 370 mL 106 g/L sodium carbonate solution. Adjust pH to 10.0 with 6 M NaOH and store at 35-37°.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeOH:ammonium hydroxide 998:2
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 16
Internal standard: desipramine hydrochloride (12)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: desmethylclomipramine, clomipramine, maprotiline, metabolites
Also analyzed: doxepin, desmethyldoxepin, amitriptyline, nortriptyline, imipramine, 2-hydroxyimipramine, 2-hydroxydesipramine
Noninterfering: chlordiazepoxide, diazepam, flurazepam, oxazepam, thioridazine

KEY WORDS
plasma

REFERENCE
Sutfin,T.A.; D'Ambrosio,R.; Jusko,W.J. Liquid-chromatographic determination of eight tri- and tetracyclic antidepressants and their major active metabolites, *Clin.Chem.*, **1984**, *30*, 471–474.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500 μL Serum + 50 μL 1 μg/mL N-propionylprocainamide in 2.5 mM HCl, add to SPE cartridge, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with 1 volume MeOH:2.5 mM HCl 10:90. Add 200 μL 10 mM acetic acid and 5 mM diethylamine in MeOH to column, let stand 1 min, elute under vacuum, repeat, evaporate eluents to dryness under nitrogen at room temperature, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: 15.8
Internal standard: N-propionylprocainamide (6)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, doxepin, imipramine, nortriptyline, trimipramine
Simultaneous: atropine, butalbital, chlorpromazine, maprotiline, methadone, norpropoxyphene, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, quinidine, trifluoperazine, trimeprazine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil

KEY WORDS
serum; SPE

REFERENCE
Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 μL 5 μg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 μL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 μL 1 M pH 10.3 carbonate buffer and 25 μL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 μL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 μL/L orthophosphoric acid + 600 μL/L n-butylamine
Flow rate: 2
Injection volume: 25-40
Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM
Retention time: 11.88
Internal standard: maprotiline (12.8)

OTHER SUBSTANCES
Simultaneous: fluoxetine, propranolol, clovoxamine, fluvoxamine, fenfluramine, amoxapine, nortriptyline, sertraline, norfluoxetine

Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine
Interfering: desipramine

KEY WORDS
plasma

REFERENCE
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL trimipramine in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 6.6
Internal standard: trimipramine (9.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, doxepin, fluoxetine, imipramine, maprotiline, nortriptyline
Interfering: desmethylmaprotiline, desipramine, fluvoxamine

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)

Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 4.0

OTHER SUBSTANCES
Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, imipramine, lidocaine, maprotiline, methadone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, pentazocine, propoxyphene, propranolol, quinidine, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: desipramine, methaqualone, norverapamil, ibuprofen, promazine, propafenone

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 292

CHROMATOGRAM
Retention time: 7.97
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 1 mL 500 mM NaOH + 4 mL toluene:n-hexane:isoamyl alcohol 77:22:3, mix for 10 min, centrifuge at 3000 rpm for 5 min. Remove the upper organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.7 5 μm Supelcosil LC-PCN cyanopropyl

Mobile phase: MeCN:MeOH:10 mM pH 7.2 potassium phosphate buffer 60:15:25 (Prepare buffer by mixing 194 mL 1.36 g/L KH_2PO_4 with 274 mL 1.74 g/L K_2HPO_4.)

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 8.1

Internal standard: protriptyline

OTHER SUBSTANCES

Extracted: amitriptyline, cyclobenzaprine, norcyclobenzaprine, nortriptyline

KEY WORDS
serum; protriptyline is IS

REFERENCE
Wong,E.C.C.; Koenig,J.; Turk,J. Potential interference of cyclobenzaprine and norcyclobenzaprine with HPLC measurement of amitriptyline and nortriptyline: resolution by GC-MS analysis, *J.Anal.Toxicol.*, **1995**, *19*, 218–224.

SAMPLE
Matrix: blood, milk
Sample preparation: Centrifuge milk at 1200 g, remove the middle aqueous layer. 1 mL Plasma or milk + 100 μL MeOH:water 50:50, mix, inject a 250 μL aliquot of this mixture on to column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 37-50 μm 10 × 1.5 Corasil RP C18; B 100 × 4 Techsphere 3CN (HPLC Technology)
Mobile phase: A water; B MeCN:50 mM pH 7.2 acetate buffer 70:30
Flow rate: A 0.8; B 0.9
Injection volume: 250
Detector: UV 246

CHROMATOGRAM
Retention time: 6.8
Internal standard: protriptyline

OTHER SUBSTANCES
Extracted: tripelennamine

KEY WORDS
column-switching; cow; plasma; protriptyline is IS

REFERENCE
Dadgar,D.; Power,A. Applications of column-switching techniques in biopharmaceutical analysis. II. High-performance liquid chromatographic determination of tripelennamine in bovine plasma and milk, *J.Chromatogr.*, **1987**, *421*, 216–222.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 5.50
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: norpropoxyphene, desipramine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.6

OTHER SUBSTANCES
Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, pentazocine, promethazine, pyrilamine, pyrimethamine, tripelennamine

KEY WORDS
tablets; syrups; elixirs; injections

REFERENCE
Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane:butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18

Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Retention time: 4
Internal standard: protriptyline

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, dothiepin, doxepin, haloperidol, imipramine, mianserin, nortriptyline

KEY WORDS
may be interferences; protriptyline is IS

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine,

phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Econosil C8
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Injection volume: 20
Detector: UV 210

CHROMATOGRAM

Retention time: 8.8
Limit of quantitation: < 1000 ng/mL

OTHER SUBSTANCES

Simultaneous: doxepin, desipramine, cyclobenzaprine, maprotiline
Also analyzed: amitriptyline, amoxapine, carbamazepine, imipramine, nortriptyline

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KEY WORDS

UV spectra given

REFERENCE

Ryan,T.W. Identification and quantification of tricyclic antidepressants by UV-photodiode array detection with multicomponent analysis, *J.Liq.Chromatogr.*, **1993**, *16*, 1545–1560.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM

Retention time: 5.85

OTHER SUBSTANCES

Simultaneous: benactyzine, buclizine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

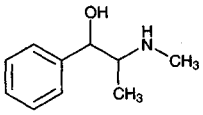
CHROMATOGRAM
Retention time: 13.20 (A), 6.33 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Pseudoephedrine



Molecular formula: $C_{10}H_{15}NO$
Molecular weight: 165.24
CAS Registry No.: 90-82-4, 345-78-8 (HCl), 7460-12-0 (sulfate)
Merck Index: 3645

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 0.1-1 μg IS + 500 μL 25% potassium carbonate + 10 mL diethyl ether, shake for 10 min, centrifuge at 1000 g for 5 min, freeze in MeOH/dry ice. Remove the organic layer and add it to 1 mL 50 mM HCl, shake for 5 min, centrifuge at 1000 g for 2 min, freeze in MeOH/dry ice, discard the organic layer, remove traces of ether with a stream of nitrogen. Thaw the aqueous layer and add 40 μL 1 M NaOH and 500 μL 200 mM pH 8.5 borate buffer, add 400 μL 0.5% 4-chloro-7-nitrobenzo-2,1,3-oxadiazole (NBD-Cl) in methylisobutylketone, heat at 79° for 1 h (mix at 5 min intervals), cool on ice, add 3 mL cyclohexane, vortex, centrifuge at 1000 g for 3 min, inject a 200 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B, after 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A Guard Pak CN (Waters); B 250 × 4.6 silica (Alltech)
Mobile phase: A Cyclohexane; B Cyclohexane:toluene:MeOH:butanol 50:47:1:2
Flow rate: 2
Injection volume: 200
Detector: F ex 460-500 (bandpass filter) em >500 (cutoff filter)

CHROMATOGRAM

Retention time: 12.0
Internal standard: 2-phenylglycinol (2-amino-2-phenylethanol) (9.7)
Limit of detection: 2 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: dimethoxyphenethylamine, ephedrine, norpsdeudoephedrine, phenylephrine, phenylethanolamine, phenylethylamine, phenylpropanolamine, tyramine
Noninterfering: acetaminophen, aspirin, caffeine, salicylamide, theophylline

KEY WORDS

column-switching; normal phase; plasma; pharmacokinetics; derivatization

REFERENCE

Veals,J.; Kim,H.; Korduba,C.; Curtis,D.; Durante,E.; Lin,C. Determination of plasma pseudoephedrine by fluorescence detection and high performance liquid chromatography, *J.Liq.Chromatogr.*, **1988**, *11*, 417–433.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 SPE cartridge (J.T. Baker) with 5 mL MeOH, 7.5 mL 300 mM HCl in MeOH, and 10 mL water. Plasma + 1 mL water + 50 μL 10 μg/mL IS + 30 μL 25% ammonium hydroxide, vortex briefly, add to the SPE cartridge, rinse sample tube with 2 mL MeCN:30 mM pH 3.0 HCl 40:60, add rinse to the SPE cartridge, dry under vacuum, elute with 300 μL 100 mM HCl in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:buffer 30:70 (Buffer was 30 mM sodium heptane sulfonate adjusted to pH 3.0 with 100 mM HCl.)

Flow rate: 1
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 5.4
Internal standard: (±)α-methylaminomethyl benzyl alcohol (4.7)
Limit of detection: 20 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Noninterfering: guaifenesin

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Pade,V.; Aluri,J.; Manning,L.; Stavchansky,S. Bioavailability of pseudoephedrine from controlled release formulations in the presence of guaifenesin in human volunteers, *Biopharm.Drug Dispos.*, **1995**, *16*, 381–391.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-pak C18 SPE cartridge with EtOH, 5% aqueous bovine serum albumin, and water. 100-500 μL Plasma + 100 ng (l)-norephedrine + 2 mL 500 mM pH 7.0 phosphate buffer, add to the SPE cartridge, wash with 5 mL water, wash with 3 mL EtOH:water 20:80, elute with 8 mL EtOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL MeCN, add 100 μL 6 mg/mL dansyl chloride in MeCN containing 0.03% triethylamine, heat at 50° for 20 min, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL EtOH:water 90:10, add to an 18 × 6 column containing 80 mg carboxymethyl Sephadex LH-20 (0.95 meq/g), wash with EtOH:water 90:10, elute with 6 mL 50 mM methylamine in EtOH:water 90:10 at 0.1 mL/min. Evaporate the eluate to dryness, reconstitute with 50-100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm Shimpack G-ODS guard column (Shimadzu)
Column: 150 × 6 5 μm Shimpack CLC-ODS (Shimadzu)
Mobile phase: MeOH:0.6% pH 6.5 phosphate buffer 80:30
Flow rate: 1.3
Detector: F ex 316 em 486

CHROMATOGRAM
Retention time: 15
Internal standard: (l)-norephedrine (10)

OTHER SUBSTANCES
Extracted: ephedrine

KEY WORDS
plasma; SPE; guinea pig; human; derivatization; pharmacokinetics

REFERENCE
Shao,G.; Wang,D.-S.; Wu,F.; Chen,S.-J.; Luo,X. Separation and determination of (l)-ephedrine and (d)-pseudoephedrine in plasma by high-performance liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.*, **1995**, *18*, 2133–2145.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 10 mg/mL solution in 500 mM sodium bicarbonate solutions, extract a 10 mL aliquot twice with 15 mL portions of dichloromethane. Combine the extracts and add 10 μL phenylisothiocyanate, evaporate to dryness under a stream of air, reconstitute with 10 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:PELL ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water:acetic acid 45:54:1
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: ephedrine, lidocaine, phenylpropanolamine

KEY WORDS
derivatization

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic analysis of samples containing cocaine, local anesthetics, and other amines, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 151–157.

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: amphetamine, ephedrine, methamphetamine, phenmetrazine, phentermine, phenylpropanolamine

KEY WORDS
derivatization

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water and filter (0.45 μm)

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water:glacial acetic acid 45:55:2 containing 5 mM octanesulfonic acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: acetaminophen, guaifenesin, pholcodine, methyl paraben, ethyl paraben, propyl paraben, butyl paraben

KEY WORDS
cough mixture

REFERENCE
Carnevale,L. Simultaneous determination of acetaminophen, guaifenesin, pseudoephedrine, pholcodine, and paraben preservatives in cough mixture by high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 196–198.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water:glacial acetic acid 45:55:2 containing 5 mM octanesulfonic acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Simultaneous: acetaminophen, guaifenesin, pholcodine, methyl paraben, ethyl paraben, propyl paraben, butyl paraben

KEY WORDS
cough mixture

REFERENCE
Carnevale,L. Simultaneous determination of acetaminophen, guaifenesin, pseudoephedrine, pholcodine, and paraben preservatives in cough mixture by high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 196–198.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, dissolve in 100 mL 100 mM pH 5.0 acetate buffer, let sit for 1 h with occasional mixing, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax SCX
Mobile phase: MeCN:30 mM KH_2PO_4 50:50
Flow rate: 2
Injection volume: 50
Detector: UV 263

CHROMATOGRAM
Retention time: 10.0

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, dextromethorphan

KEY WORDS
tablets

REFERENCE
Murtha,J.L.; Julian,T.N.; Radebaugh,G.W. Simultaneous determination of pseudoephedrine hydrochloride, chlorpheniramine maleate, and dextromethorphan hydrobromide by second-derivative photodiode array spectroscopy, *J.Pharm.Sci.*, **1988**, *77*, 715–718.

SAMPLE
Matrix: solutions
Sample preparation: Mix 40 mL of a 325-375 mM amine solution in THF with 150 mL 10% potassium carbonate, add dropwise 40 mL 275-325 mM reagent in THF, heat at 50° while maintaining at pH 8 or above for 3 h, cool, extract with chloroform. Evaporate the extracts to dryness, reconstitute, inject an aliquot. (Prepare reagent (1-[(4-nitrophenyl)sulfonyl]prolyl chloride) as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles 4-nitrobenzenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(4-nitrophenyl)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!). Stir 15 mmoles 1-[(4-nitrophenyl)sulfonyl]proline in 100 mL benzene and add 75 mmoles thionyl chloride in 50 mL benzene dropwise, heat at 35-40° until the reaction is complete (about 48 h; monitor by IR), evaporate to dryness, recrystallize from n-heptane to give 1-[(4-nitrophenyl)sulfonyl]prolyl chloride (mp 110-110.5°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 60:40
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 7, 8.5 (enantiomers)

OTHER SUBSTANCES
Simultaneous: ephedrine

KEY WORDS
derivatization; chiral

REFERENCE
Clark,C.R.; Barksdale,J.M. Synthesis and liquid chromatographic evaluation of some chiral derivatizing agents for resolution of amine enantiomers, *Anal.Chem.*, **1984**, *56*, 958–962.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.65

OTHER SUBSTANCES

Simultaneous: norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

Interfering: phenylephrine, ephedrine, methylephedrine, dimethylamphetamine, pholcodine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.9

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperido-

late, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 5:1.5:0.5:93
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Simultaneous: phenylpropanolamine, ephedrine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1.5 mg/mL solution of pseudoephedrine hydrochloride, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: Supelguard LC-8-DB (Supelco)
Column: 50 × 4.6 Supelcosil LC-8-DB
Mobile phase: MeCN:buffer 10:90 containing 0.02% triethylamine (Buffer was KH_2PO_4 adjusted to pH 2.0 with phosphoric acid.)
Column temperature: 35
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 1.3

OTHER SUBSTANCES

Simultaneous: chlorpheniramine, methscopolamine, phenylpropanolamine, triprolidine

REFERENCE

Supelco Catalog, **1992**, p. 179.

SAMPLE

Matrix: solutions

Sample preparation: Mix 50 μL of a 1 mg/mL solution in MeCN:500 mM pH 9.0 borate buffer 50:50 with 25 mg reagent, after 1 min elute with 1 mL dichloromethane, inject a 5 μL aliquot. (Reagent was dinitrophenyl carbamate benzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF, with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55).Dissolve 3,5-dinitrobenzoyl chloride in the minimum amount of glacial acetic acid, add an equimolar amount of sodium azide, stir for 30 min, dilute with water, filter to obtain 3,5-dinitrobenzoyl azide (Caution! Azides are toxic and potentially explosive!) (J. Liq. Chromatogr. 1986, 9, 443). Heat 71 mg 3,5-dinitrobenzoyl azide in 15 mL toluene (dried over calcium hydride) at ??? for 30 min, cool using an ice bath, add 200 mg polymer, allow to warm to room temperature with stirring for 1 h, filter, wash the polymer with four 10 mL portions of warm (40°) dichloromethane, dry under high vacuum for 1 h.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-(R)-naphthylurea (Supelco)
Mobile phase: Hexane:isopropanol:MeOH 83:12:5
Flow rate: 2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 7 (D), 8 (L)

KEY WORDS

derivatization; chiral

REFERENCE

Bourque,A.J.; Krull,I.S. Immobilized isocyanates for derivatization of amines for chiral recognition in liquid chromatography with UV detection, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 495–503.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine,

chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 25 µL 1 mg/mL phenylpropanolamine in mobile phase + 100 µL 10 M NaOH + 2 mL diethyl ether + 3 g sodium sulfate, shake for 20 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 µm LiChrospher 60 RP Select B

Mobile phase: 200 mM pH 5.5 phosphate buffer containing 150 mM triethylamine (Wash column with MeOH for 15 min and with water for 15 min at the end of each day.)

Column temperature: 40

Flow rate: 1.3

Injection volume: 20

Detector: UV 215

CHROMATOGRAM

Retention time: 9

Internal standard: phenylpropanolamine (11.5)

Limit of detection: 500 ng/mL

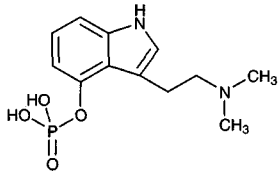
OTHER SUBSTANCES

Extracted: norephedrine, norpseudoephedrine, ephedrine, N-methylephedrine, ethylephedrine
Noninterfering: amfepramone, amphetamine, caffeine, chlorphentermine, cocaine, codeine, cropropamide, crotethamide, dimethylamphetamine, etamivan, fencamfamine, heptaminol, leptazol, lidocaine, methoxamine, methylamphetamine, methylphenidate, nicotine, niketamine, meperidine, phendimetrazine, phenmetrazine, pipradol, procaine, prolintane, strychnine

REFERENCE

Imaz,C.; Carreras,D.; Navajas,R.; Rodriguez,C.; Rodriguez,A.F.; Maynar,J.; Cortes,R. Determination of ephedrines in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *631*, 201–205.

Psilocybin



Molecular formula: $C_{12}H_{17}N_2O_4P$
Molecular weight: 284.25
CAS Registry No.: 520-52-5
Merck Index: 8111

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM

Retention time: 3.343

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pumactant

Merck Index: 8126

SAMPLE

Matrix: formulations

Sample preparation: Dilute liposome dispersions 10-fold with chloroform:MeOH 60:40, centrifuge at 2700 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax amino

Mobile phase: MeCN:MeOH:buffer 64:28:8 (Buffer was 10 mM phosphoric acid adjusted to pH 4.8 with dilute ammonium hydroxide solution. To prepare mobile phase mix MeCN and MeOH and then add buffer.)

Flow rate: 1.5

Injection volume: 5-20

Detector: RI

CHROMATOGRAM

Retention time: 6 (phosphatidylglycerol) [Determination of dipalmitoylphosphatidylcholine is implied but not described.]

Limit of detection: 29 μg/mL (phosphatidylglycerol)

OTHER SUBSTANCES

Simultaneous: acyl lysophosphatidylcholine, acyl lysophosphatidylglycerol, phosphatidylcholine

KEY WORDS

liposome dispersions

REFERENCE

Grit,M.; Crommelin,D.J.A.; Lang,J. Determination of phosphatidylcholine, phosphatidylglycerol and their lyso forms from liposome dispersions by high-performance liquid chromatography using high-sensitivity refractive index detection, *J.Chromatogr.*, **1991**, *585*, 239–246.

SAMPLE

Matrix: lung lavage fluid

Sample preparation: Centrifuge lung lavage fluid at 4° at 450 g for 10 min. Shake 10 mL supernatant and 40 mL chloroform:MeOH 2:1 at 4° for 3 min. Remove the lower organic phase and wash it with 2 mL 50 mM NaCl, centrifuge, dry under a stream of nitrogen at 45°, reconstitute with 500 μL mobile phase, vortex at 4° for 1 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2.1 5 μm Encapharm 100 spherical silica gel (Molnar, Berlin)

Column: 120 × 4.6 5 μm Encapharm 100 spherical silica gel (Molnar, Berlin)

Mobile phase: Gradient. A was chloroform:MeOH:ammonium hydroxide 80:19.5:0.5. B was chloroform:MeOH:water:ammonium hydroxide 60:34:5.5:0.5. A:B from 100:0 to 0:100 over 14 min, return to initial conditions over 7 min, re-equilibrate for 10 min.

Column temperature: 30

Flow rate: 1

Injection volume: 100

Detector: Evaporative light-scattering, SEDERE Sedex-45, evaporation temperature 50°, nebulization gas nitrogen, pressure 200 kPa, flow 6 L/min, response is non-linear but proportional to the power 1.7 of the mass and must be calibrated for each compound

CHROMATOGRAM

Retention time: 5.37 (phosphatidylglycerol), 13.14 (dipalmitoylphosphatidylcholine)

Limit of detection: 100 ng (phosphatidylglycerol)

OTHER SUBSTANCES
Extracted: diarachidoylphosphatidylcholine, dilinoleylphosphatidylcholine, diphosphatidylglycerol, lysophosphatidylcholine, phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin

KEY WORDS
normal phase

REFERENCE
Bünger,H.; Pison,U. Quantitative analysis of pulmonary surfactant phospholipids by high-performance liquid chromatography and light-scattering detection, *J.Chromatogr.B*, **1995**, *672*, 25–31.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge 10 mL rabbit lung or Eustachian tube washing saline at 1000 g for 5 min, lyophilize the supernatant, reconstitute with 5 mL chloroform, filter (0.45 μm). Evaporate the filtrate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm ODS Hypersil
Mobile phase: MeOH:THF 97:3 containing 40 mM choline chloride
Flow rate: 1
Injection volume: 100
Detector: F 340 ex 460 following post-column derivatization. The column effluent mixed with the reagent pumped at 1.2 mL/min and the mixture flowed through a 300 × 5 PTFE column filled with 250 μm glass beads and a 3 m × 0.5 mm i.d. coil of PTFE tubing at 50° to the detector. The reagent was 150 μL 3 μM 1,6-diphenyl-1,3,5-hexatriene in THF made up to 1 L with water containing 0.001% Tween 20.

CHROMATOGRAM
Retention time: 5 (dipalmitoylphosphatidylcholine)
Limit of detection: 1.5 μg/mL

KEY WORDS
post-column reaction; rabbit

REFERENCE
Kitsos,M.; Gandini,C.; Massolini,G.; De Lorenzi,E.; Caccialanza,G. High-performance liquid chromatography post-column derivatization with fluorescence detection to study the influence of ambroxol on dipalmitoylphosphatidylcholine levels in rabbit eustachian tube washings, *J.Chromatogr.*, **1991**, *553*, 1–6.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Potter-Elvehjem) liver or lung with 20 volumes chloroform:MeOH 2:1, filter (paper), wash with a volume of 50 mM NaCl equal to one-fifth the volume of extract, centrifuge (J.Biol.Chem. 1957, 226, 497). Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute with chloroform:MeOH 25:75, inject an aliquot.

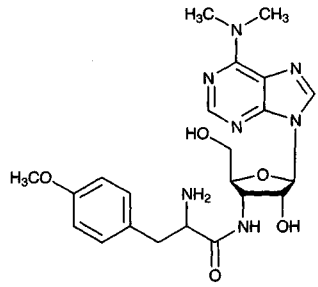
HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Nucleosil 5 NH2
Column: 50 × 4.6 5 μm Nucleosil 5 NH2 + 175 × 4.6 5 μm Nucleosil 5 NH2
Mobile phase: MeCN:MeOH:water:50% methylphosphonic acid in water 73:25:1.5:0.03, adjusted to pH 3 with 25% ammonium hydroxide in water
Flow rate: 1
Detector: F ex 340 em 460 following post-column derivatization. The column effluent mixed with the reagent pumped at 4.5 mL/min and the mixture flowed through a 2 m × 0.8 mm i.d. coil of PTFE tubing at 50° to the detector.

CHROMATOGRAM
Retention time: 22 (phosphatidylglycerol) (dipalmitoylphosphatidylcoline is separated but retention time is not given)

KEY WORDS
pig; lung; liver; gastric mucosa; post-column reaction

REFERENCE
Bernhard,W.; Linck,M.; Creutzburg,H.; Postle,A.D.; Arning,A.; Martin-Carrera,I.; Sewing,K.-F. High-performance liquid chromatographic analysis of phospholipids from different sources with combined fluorescence and ultraviolet detection, *Anal.Biochem.*, **1994**, *220*, 172–180.

Puromycin



Molecular formula: $C_{22}H_{29}N_7O_5$
Molecular weight: 471.52
CAS Registry No.: 53-79-2
Merck Index: 8130

SAMPLE
Matrix: solutions

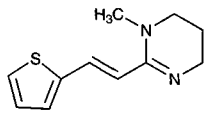
HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic

acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Pyrantel



Molecular formula: $C_{11}H_{14}N_2S$
Molecular weight: 206.31
CAS Registry No.: 15686-83-6, 22204-24-6 (pamoate), 33401-94-4 (tartrate)
Merck Index: 8139
Lednicer No.: 1 266

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH, 1 mL water, and 5 mL 100 mM sodium carbonate. Mix 500 μL plasma with 500 μL 100 mM sodium carbonate, add to the SPE cartridge, wash with 5 mL 100 mM sodium carbonate, dry the cartridge in air for 30 s, elute with 750 μL MeCN:1% phosphoric acid 18:82, mix the eluate with 250 μL 20% ammonium acetate (pH adjusted to 4.6 with phosphoric acid), inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 5 μm Symmetry C18 (Waters)
Column: 150 × 2.1 5 μm Symmetry C18 (Waters)
Mobile phase: MeOH:THF:buffer 2.5:2:95.5 (The buffer was 50 mM ammonium acetate adjusted to pH 4.6 with 20% phosphoric acid.)
Flow rate: 0.4
Injection volume: 50
Detector: UV 317

CHROMATOGRAM
Retention time: 3.8 (pyrantel pamoate)
Limit of detection: 4 ng/mL
Limit of quantitation: 15 ng/mL

KEY WORDS
dog; pharmacokinetics; plasma; SPE

REFERENCE
Morovján,G.; Csokán,P.; Makranaszki,L.; Abdellah-Nagy,E.A.; Tóth,K. Determination of fenbendazole, praziquantel and pyrantel pamoate in dog plasma by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *797*, 237–244.

SAMPLE
Matrix: bulk, formulations, bulk
Sample preparation: Add a powdered tablet or 100 mg bulk pyrantel pamoate to 50 mL DMF, sonicate for 2 min, cool to room temperature, make up to 100 mL with DMF. Add a 10 mL aliquot to 10 mL 100 μg/mL carbaryl in MeCN, make up to 100 mL with mobile phase, filter (tablets only), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb octyl
Mobile phase: MeCN:water 42:58 containing 100 mM n-butylamine, pH adjusted to 3.0 with perchloric acid
Flow rate: 1
Detector: UV 313

CHROMATOGRAM
Retention time: 7.80
Internal standard: carbaryl (11.40)

OTHER SUBSTANCES
Simultaneous: oxantel

KEY WORDS
tablets; protect from light

REFERENCE
Allender,W.J. High-performance liquid chromatographic determination of oxantel and pyrantel pamoate, *J.Chromatogr.Sci.*, **1988**, *26*, 470–472.

SAMPLE
Matrix: feed
Sample preparation: 10 g Ground feed + 100 mL methanolic NaCl, sonicate for 1 h, cool to room temperature, filter through a glass wool pad, wash with three 20 mL portions of methanolic NaCl, make up filtrate to 200 mL with methanolic NaCl, mix. Add a 25 mL aliquot of the filtrate to 10 mL 550 mg/mL KI in water, mix, add 50 mL chloroform, shake vigorously for 10 s, repeat extraction. Combine the chloroform layers and add them to 25 mL 100 mM NaOH, shake vigorously for 10 s. Remove the organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 10 mL mobile phase, inject a 25 μL aliquot. (Prepare methanolic NaCl by mixing 1 L 100 g/L NaCl in water and 1 L MeOH, prepare fresh daily.)

HPLC VARIABLES
Guard column: 400 mg Corasil II
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeCN:acetic acid:water:diethylamine 94:2.5:2.5:1
Flow rate: 1.6
Injection volume: 25
Detector: UV 313

CHROMATOGRAM
Retention time: 7.3

OTHER SUBSTANCES
Simultaneous: cis-isomer, degradation products, tylosin
Noninterfering: carbadox, lincomycin

KEY WORDS
protect from light; normal phase

REFERENCE
Goras,J.T. High performance liquid chromatographic method for pyrantel tartrate in swine feeds and supplements, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 1291–1296.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20 mesh sieve. 10 g Ground feed + 15 mL water, mix well, let stand for 5 min, add 25 mL MeCN:MeOH 50:50, shake vigorously for 30 min, centrifuge or filter (Whatman glass fiber GFA), pass 15 mL supernatant or filtrate through 4 g alumina in a 10 mm dia column, collect the first 4 mL eluate, inject a 25 μL aliquot. (Prepare alumina as follows. Stir 200 g Fisher neutral alumina (A-950) in 1 L water for 30 min, pour off fines, resuspend, filter (Whatman glass fiber GFA), dry with vacuum, wash 3 times with MeOH, dry at 80° overnight, store in desiccator.)

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Waters)
Column: 100 × 5 C18 radial compression (Waters)
Mobile phase: MeCN:buffer 18:82 (Buffer was 25 mL dibutylamine acetate made up to 1 L with water, pH 3.7. Dibutylamine acetate was prepared by titrating 100 mL dibutylamine to pH 2.5 with acetic acid (ca. 270 mL).)
Flow rate: 2
Injection volume: 25
Detector: UV 313

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: carbadox (UV 365)

KEY WORDS
protect from light

REFERENCE
Lowie,D.M.,Jr.; Teague,R.T.,Jr.; Quick,F.E.; Foster,C.L. High pressure liquid chromatographic determination of carbadox and pyrantel tartrate in swine feed and supplements, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 602–605.

SAMPLE
Matrix: feed
Sample preparation: 5 g Ground feed + 100 mL methanolic HCl, shake on a reciprocating shaker for 30 min. Remove a 40 mL aliquot and centrifuge at 2000 rpm for 10 min. Remove 5 mL of the supernatant and make up to 100 mL with MeCN, mix, centrifuge at 2000 rpm for 10 min, inject an aliquot of the supernatant. (Prepare methanolic HCl by slowly adding 8.5 mL concentrated HCl to 992 mL MeOH:water 50:50.)

HPLC VARIABLES
Guard column: 400 mg Corasil II
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeCN:acetic acid:water:diethylamine 95:2:2:1
Flow rate: 1.6
Injection volume: 25
Detector: UV 313

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: morantel

KEY WORDS
protect from light; normal phase

REFERENCE
Goras,J.T.; Gauthier,A.R. Liquid chromatographic determination of morantel tartrate in cattle feed, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 598–601.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20-mesh sieve. Add 10 g feed to 10 mL water with swirling, let stand for 5 min (make sure all particles are wet), add 50 mL DMF:water 95:5, shake vigorously for 15 s, shake on a wrist action shaker for 30 min, filter (paper), pass 15 mL through 8 g alumina in a 300 × 10 glass column, if necessary dilute eluate with DMF:water 95:5, inject a 20 μL aliquot. (The alumina was Alcoa F-20, 80-200 mesh, Sargeant-Welch No. SC 10492-005LB, do not substitute.)

HPLC VARIABLES
Guard column: 100 × 2 μBondapak C18/Corasil
Column: Partisil 10 ODS-3
Mobile phase: DMF:buffer 23.5:76.5 (22.3 g $Na_4P_2O_7.H_2O$ and 50 mL phosphoric acid in 700 mL water, add 235 mL DMF, adjust pH to 1.9 ± 0.1 with phosphoric acid , make up to 1 L. Shake DMF with activated carbon and filter before use.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 313

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: morantel, carbadox
Noninterfering: lincomycin, tylosin, furazolidone, nitrofurazone, ethopabate, amprolium, sulfa drugs, chlortetracycline, oxytetracycline

REFERENCE
Thorpe,V.A. A collaborative study: high-pressure liquid chromatographic determination of carbadox and pyrantel tartrate in animal feeds, *J.Chromatogr.Sci.*, **1988**, *26*, 545–550.

SAMPLE
Matrix: milk
Sample preparation: 20 mL Milk + 1.5 mL HCl, heat at 95° with stirring for 2 h, cool in an ice bath, basify with 2.5 mL 12 M KOH, add 25 mL toluene, shake, centrifuge, repeat extraction. Combine the toluene layers and extract them vigorously twice with 4 mL portions of 100 mM HCl. Combine the aqueous layers and basify them with 2.5 mL 12 M KOH, heat at 95° for 5 h, acidify with 3 mL HCl, cool in an ice bath, extract twice with 5 mL portions of chloroform. Combine the organic layers, add 500 μL 100 mM NaOH, mix, centrifuge. Remove the aqueous layer and place it on a vortex evaporator for 5 min to remove residual chloroform, inject an aliquot. (Prepare 12 M KOH by cautiously dissolving 790 g KOH pellets in enough water to make 1 L in a fume hood, use an ice bath.)

HPLC VARIABLES
Guard column: 30 mm long μC18 Corasil Bondapak
Column: 100 × 5 Radial-Pak A (Waters)
Mobile phase: MeOH:MeCN:water:acetic acid 40:10:49:1
Flow rate: 1
Injection volume: 100
Detector: UV 313

CHROMATOGRAM
Retention time: 4.2 (as 3-(2-thienyl)-acrylic acid, the hydrolysis product)
Internal standard: pyrantel

OTHER SUBSTANCES
Extracted: morantel (as 3-(3-methyl-2-thienyl)acrylic acid, the hydrolysis product)

KEY WORDS
protect from light; pyrantel is IS

REFERENCE
Lynch,M.J.; Mosher,F.R.; Brunner,L.A.; Bartolucci,S.R. Liquid chromatographic determination and identification of morantel-related residues as precursors of 3-(3-methyl-2-thienyl) acrylic acid (CP-20,107) in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 931–935.

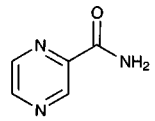
SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Pyrazinamide



Molecular formula: $C_5H_5N_3O$
Molecular weight: 123.11
CAS Registry No.: 98-96-4
Merck Index: 8140
Lednicer No.: 1 277

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 50 μL 50 μg/mL N-butarylaminophenol + 400 μL 10% acetic acid + 7 mL diethyl ether:dichloromethane 2:1, shake, centrifuge at 2059 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 30 μm C8
Column: 250 × 4.6 5 μm Spherisorb C8
Mobile phase: Gradient. MeCN:5 mM pH 3.5 phosphate buffer from 6:94 to 90:10 over 5 min, maintain at 90:10 for 12 min.
Flow rate: 2
Injection volume: 20
Detector: UV 248

CHROMATOGRAM
Retention time: 2.91
Internal standard: N-butarylaminophenol (4.22)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: rifampin

KEY WORDS
plasma

REFERENCE
Walubo,A.; Smith,P.; Folb,P.I. Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 391–396.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM
Retention time: 3.49
Internal standard: heptanophenone (19.2)
Limit of quantitation: 4000 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, rifampin, trimeprazine, trimethoprim

KEY WORDS
plasma; serum; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 3 mL CN-bonded maxi-clean cartridge (Alltech) with 5 mL MeOH and 2 mL 1% aqueous acetic acid. 2 mL Plasma or urine + 1 mL isopropanol:chloroform 50:50 + 1 μg rifampin, shake for 30 s, add to the SPE cartridge with rinses, collect the eluate, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Excalibar C18-CN (Alltech)
Mobile phase: MeOH:5 mM tetra-n-butylammonium hydroxide 80:20 adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 1.4
Internal standard: rifampin (4.3)
Limit of detection: 225 ng/mL (urine), 250 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: isoniazid

KEY WORDS
plasma; SPE

REFERENCE
Gaitonde,C.D.; Pathak,P.V. Rapid liquid chromatographic method for the estimation of isoniazid and pyrazinamide in plasma and urine, *J.Chromatogr.*, **1990**, *532*, 418–423.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM
Retention time: 3.823

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 10-fold with MeOH. Remove a 20 μL aliquot and add it to 1 mL mobile phase, add 20 μL 1.5 mg/mL rifampin, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 160 × 4 5 μm Zorbax Rx-C8
Mobile phase: MeOH:buffer 70:30 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.7
Injection volume: 10
Detector: UV 265

CHROMATOGRAM
Retention time: 2.1
Internal standard: rifampin (3.8)

KEY WORDS
suspensions; syrup; stability-indicating

REFERENCE
Nahata,M.C.; Morosco,R.S.; Peritore,S.P. Stability of pyrazinamide in two suspensions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1558–1560.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 50-350 times with water, ultrafilter, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 11 × 4 5 µm Nucleosil 100 C18
Column: 250 × 4 5 µm Nucleosil 100 C18
Mobile phase: 10 mM KH_2PO_4 adjusted to pH 5.2 with K_2HPO_4
Flow rate: 0.9
Injection volume: 20
Detector: UV

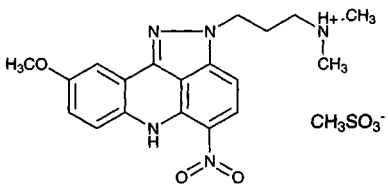
CHROMATOGRAM
Retention time: 26.19
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat; ultrafiltrate

REFERENCE
Mehmedagic,A.; Vérité,P.; Ménager,S.; Tharasse,C.; Chabenat,C.; André,D.; Lafont,O. Determination of pyrazinamide and its main metabolites in rat urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *695*, 365–372.

Pyrazoloacridine



Molecular formula: $C_{20}H_{25}N_5O_6S$
Molecular weight: 463.50
CAS Registry No.: 99009-20-8

SAMPLE
Matrix: blood
Sample preparation: Vortex 250 µL plasma with 40 µL 4 µg/mL IS for 10 s, add 600 µL MeOH. Vortex for 20 s, place on ice for 15 min, centrifuge at 1000 g for 10 min, inject a 20-40 µL aliquot of the supernatant.

HPLC VARIABLES
Guard column: CN
Column: 150 × 4.6 5 µm Ultrasphere CN (Beckman)
Mobile phase: MeCN:125 mM pH 4.75 ammonium acetate buffer 24:76 (Prepare buffer by dissolving 38.7 g ammonium acetate in 4.0 L water, adjust pH to 4.75 with 25% HCl.)
Flow rate: 0.9
Injection volume: 20-40
Detector: UV 460

KEY WORDS
suspensions; syrup; stability-indicating

REFERENCE
Nahata,M.C.; Morosco,R.S.; Peritore,S.P. Stability of pyrazinamide in two suspensions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1558–1560.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 50-350 times with water, ultrafilter, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 11 × 4 5 µm Nucleosil 100 C18
Column: 250 × 4 5 µm Nucleosil 100 C18
Mobile phase: 10 mM KH_2PO_4 adjusted to pH 5.2 with K_2HPO_4
Flow rate: 0.9
Injection volume: 20
Detector: UV

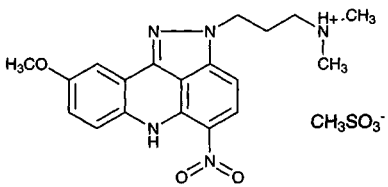
CHROMATOGRAM
Retention time: 26.19
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat; ultrafiltrate

REFERENCE
Mehmedagic,A.; Vérité,P.; Ménager,S.; Tharasse,C.; Chabenat,C.; André,D.; Lafont,O. Determination of pyrazinamide and its main metabolites in rat urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *695*, 365–372.

Pyrazoloacridine



Molecular formula: $C_{20}H_{25}N_5O_6S$
Molecular weight: 463.50
CAS Registry No.: 99009-20-8

SAMPLE
Matrix: blood
Sample preparation: Vortex 250 µL plasma with 40 µL 4 µg/mL IS for 10 s, add 600 µL MeOH. Vortex for 20 s, place on ice for 15 min, centrifuge at 1000 g for 10 min, inject a 20-40 µL aliquot of the supernatant.

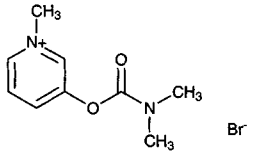
HPLC VARIABLES
Guard column: CN
Column: 150 × 4.6 5 µm Ultrasphere CN (Beckman)
Mobile phase: MeCN:125 mM pH 4.75 ammonium acetate buffer 24:76 (Prepare buffer by dissolving 38.7 g ammonium acetate in 4.0 L water, adjust pH to 4.75 with 25% HCl.)
Flow rate: 0.9
Injection volume: 20-40
Detector: UV 460

CHROMATOGRAM
Retention time: 13.7
Internal standard: ethyl orange (9.5)
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jayewardene,A.L.; Santoro,J.E.; Gambertoglio,J.G. High-performance liquid chromatographic determination of pyrazoloacridine, a nitro-9-methoxyacridine anticancer agent, in human plasma, *J.Chromatogr.B*, **1997**, *702*, 203–210.

Pyridostigmine bromide



Molecular formula: $C_9H_{13}BrN_2O_2$
Molecular weight: 261.12
CAS Registry No.: 101-26-8
Merck Index: 8161

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 µL 500 ng/mL edrophonium in water + 500 µL 100 mM picric acid in 100 mM NaOH (pH adjusted to 7) + 400 µL 100 mM NaH_2PO_4 + 12 mL water saturated dichloromethane, shake vigorously for 5 min, centrifuge at 2000 g for 10 min. Remove 10 mL of the organic phase and add it to 200 µL 1 mM tetrabutylammonium hydrogen sulfate, shake vigorously for 30 s, centrifuge at 2000 g for 2 min, discard most of the organic layer, centrifuge at 2000 g for 1 min, inject a 50 µL aliquot of the aqueous layer. (Store glassware in 100 mM tetramethylammonium chloride solution and wash 5 times with water before use.)

HPLC VARIABLES
Guard column: 50 × 3.2 30-40 µm Perisorb RP-2 (Merck)
Column: 150 × 4.6 5 µm Ultrasphere octyl
Mobile phase: MeCN:water 17:83, pH adjusted to 3 with concentrated sulfuric acid
Flow rate: 2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 2.5
Internal standard: edrophonium (4.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: neostigmine

KEY WORDS
serum

REFERENCE
De Ruyter,M.G.M.; Cronnelly,R.; Castagnoli,N.,Jr. Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds: determination of pyridostigmine, neostigmine and edrophionium in biological fluids, *J.Chromatogr.*, **1980**, *183*, 193–201.

SAMPLE
Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. 2 mL Plasma + 4 mL 500 mM pH 10.6 phosphate buffer, add to the SPE cartridge, wash with 5 mL 50 mM pH 10.6 phosphate buffer, wash with 5 mL MeOH, elute with 3 mL 1% acetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute in 60 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm CPS Hypersil NC-04
Mobile phase: MeCN:buffer 70:30 (Buffer was 0.1% triethylamine in water adjusted to pH 3.2 with acetic acid.)
Column temperature: 22
Flow rate: 1
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 13.5
Limit of detection: 1-2 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Michaelis,H.C. Determination of pyridostigmine plasma concentrations by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 291–294.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a C18 Sep Pak SPE cartridge with 5 mL MeOH and 5 mL water. Adjust the pH of 5 mL urine or plasma to 10.2-10.5 with NaOH, centrifuge for 1 min. Filter (0.45 μm) (urine only) the supernatant and add it to the SPE cartridge, wash with 5 mL water. wash with 5 mL MeOH, push 3 mL air through the SPE cartridge, elute with two 1 mL aliquots of 100 mM acetic acid in MeOH, add 25 μL 25-50 μg/mL methylparaben in water, evaporate the eluate to dryness under nitrogen at 40°, reconstitute in MeOH:water 50:50, inject a 5-100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 20:80:0.5 containing 5 mM 1-octanesulfonic acid (Pic B-8)
Flow rate: 2.5
Injection volume: 5-100
Detector: UV 270

CHROMATOGRAM
Retention time: 3.68
Internal standard: methylparaben (5.93)
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
SPE; plasma; rat

REFERENCE
Ellin,R.I.; Zvirblis,P.; Wilson,M.R. Method for isolation and determination of pyridostigmine and metabolites in urine and blood, *J.Chromatogr.*, **1982**, *228*, 235–244.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma. Mix 4 volumes of plasma with 1 volume of 100 mM NaH_2PO_4. 1-2 mL Plasma + 0.5-1 mL buffer, add to a 100 mg Bond Elut CBA cation-exchange SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH, elute with 1 mL acidified MeOH. Evaporate the eluate to dryness under nitrogen at 35°, reconstitute in 120 μL 50 mM sulfuric acid, inject a 100 μL aliquot. Urine. Add 0.2-2 mL urine to a 100 mg Bond Elut CBA cation-exchange SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH, elute with 1.5 mL acidified MeOH. Evaporate the eluate to dryness under nitrogen at 35°, reconstitute in 120 μL 50 mM sulfuric acid, inject a 100 μL aliquot. (Buffer contained 5% sodium carbonate and 5% sodium bicarbonate. Acidified MeOH was 11.4 mL 10.2 M aqueous HCl and 88.6 mL MeOH.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:isopropanol:buffer 11.2:1:87.8 (Buffer was 10 mM NaH_2PO_4, 2 mM tetramethylammonium bromide, and 5 mM sodium 1-heptanesulfonate, pH adjusted to 2.8 with sulfuric acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM

Retention time: 8
Limit of detection: 1 ng/mL
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Biermann,B.; Sommer,N.; Winne,D.; Schumm,F.; Maier,U.; Breyer-Pfaff,U. Renal clearance of pyridostigmine in myasthenic patients and volunteers under the influence of ranitidine and pirenzepine, *Xenobiotica*, **1993**, *23*, 1263–1275.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

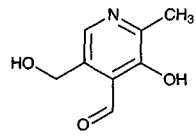
Retention time: 3.228

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pyridoxal



Molecular formula: $C_8H_9NO_3$
Molecular weight: 167.16
CAS Registry No.: 66-72-8
Merck Index: 8162

SAMPLE
Matrix: baker's yeast, eggs, milk
Sample preparation: Baker's yeast. Mix 50 mg yeast, 50 μL 1 mM isopyridoxal in water, and 3 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the supernatant, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate. Egg yolk. Mix 2 g egg yolk with 100 μL 1 mM isopyridoxal water and 6 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the clear middle part of the extract, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate. Milk. Mix 2 g egg yolk with 10 μL 1 mM isopyridoxal water and 1 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the clear middle part of the extract, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 30 mm long 5 μm Phenosphere ODS 2 (Phenomenex)
Column: 250 × 4.6 5 μm Phenosphere ODS 2 (Phenomenex)
Mobile phase: 150 mM NaH_2PO_4 adjusted to pH 2.5 with 70% perchloric acid (A 50 mm long column filled with ODS material was placed before the injector.)
Flow rate: 1
Injection volume: 20
Detector: F ex 290 em 389 following post-column derivatization. The column effluent mixed with 1 g/L sodium bisulfite in water pumped at 0.1 mL/min and the mixture flowed through a 2 m × 0.5 mm I.D. PTFE mixing coil to the detector. (The post-column derivatization is only required for pyridoxal phosphate. For the other compounds it is not necessary.)

CHROMATOGRAM
Retention time: 6 (pyridoxal phosphate), 9.5 (pyridoxal)
Internal standard: isopyridoxal (11)

OTHER SUBSTANCES
Extracted: pyridoxamine, pyridoxamine phosphate, 4-pyridoxic acid, 4-pyridoxic acid phosphate, pyridoxine, pyridoxine phosphate

KEY WORDS
post-column reaction

REFERENCE
Argoudelis,C.J. Simple high-performance liquid chromatographic method for the determination of all seven vitamin B6-related compounds, *J.Chromatogr.A*, **1997**, *790*, 83–91.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Serum + 100 μL 1 μM IS + 100 (200 ?) μL 4 M perchloric acid, mix, centrifuge at 1500 g for 5 min. Filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2 (12% C, endcapped)
Mobile phase: 67 mM KH_2PO_4 containing 125 μM sodium hexanesulfonate, adjusted to pH 2.5 with concentrated orthophosphoric acid (As column ages it may be necessary to increase concentration of sodium hexanesulfonate to 250 μM to maintain separation.)
Flow rate: 1
Injection volume: 200
Detector: F ex 325 em 400 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and flowed through a 300 mm tube to the detector. (The reagent was 67 mM KH_2PO_4 containing 1 g/L sodium sulfite adjusted to pH 7.5 with Na_2HPO_4.)

CHROMATOGRAM

Retention time: 8.1 (pyridoxal-5'-phosphate), 13.4 (pyridoxal)
Internal standard: pyridoxamine-5'-phosphate (4.6)
Limit of detection: 12.5 nM (pyridoxal), 5 nM (pyridoxal-5'-phosphate)

OTHER SUBSTANCES

Extracted: pyridoxamine, pyridoxine

KEY WORDS

post-column reaction; serum

REFERENCE

Reynolds,T.M.; Brain,A. A simple internally-standardised isocratic HPLC assay for vitamin B_6 in human serum, *J.Liq.Chromatogr.*, **1992**, *15*, 897–914.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 150 μL 3 M perchloric acid, vortex, centrifuge at >1500 g. Remove a 500 μL aliquot of the supernatant and add it to 300 μL buffer, mix, add 100 μL 10 mg/mL acid phosphatase (2 U/mg, grade II, Boehringer-Mannheim) in water, heat at 40° for 16 h, add 150 μL 3 M perchloric acid, vortex, centrifuge at >1500 g, inject a 20 μL aliquot of the supernatant. (Buffer was 1 M sodium acetate/acetic acid containing 24 g/L NaOH, pH 4.6.)

HPLC VARIABLES

Column: 125 × 4 Nucleosil 120 5 C18
Mobile phase: 50 mM Perchloric acid containing 20 mM triethylamine
Flow rate: 2
Injection volume: 20
Detector: F ex 365 em 480 following post-column reaction. The column effluent mixed with the 3.35 g/L semicarbazide hydrochloride in 1.5 M NaOH pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.3 mm ID crocheted coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 1.75
Limit of detection: 2 ng/mL

KEY WORDS

post-column reaction; plasma; pharmacokinetics

REFERENCE

Mascher,H. Determination of total pyridoxal in human plasma following oral administration of vitamin B6 by high-performance liquid chromatography with post-column derivatization, *J.Pharm.Sci.*, **1993**, *82*, 972–974.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 500 μL 800 mM perchloric acid, vortex vigorously, centrifuge at 35000 g for 5 min, inject a 50-500 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 AQ-302 ODS (YMC)
Mobile phase: 100 mM pH 3.0 KH_2PO_4 containing 100 mM sodium perchlorate and 0.5 g/L sodium bisulfite
Flow rate: 1
Injection volume: 50-500
Detector: F ex 300 em 400

CHROMATOGRAM
Retention time: 3 (pyridoxal phosphate), 5 (pyridoxal)
Limit of detection: 1 pmole (pyridoxal), 0.5 pmole (pyridoxal phosphate)

OTHER SUBSTANCES
Extracted: 4-pyridoxic acid
Simultaneous: pyridoxamine, pyridoxamine phosphate, pyridoxine

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KEY WORDS
plasma; rat; human; protect from light; derivatization

REFERENCE
Kimura,M.; Kanehira,K.; Yokoi,K. Highly sensitive and simple liquid chromatographic determination in plasma of B_6 vitamers, especially pyridoxal 5'-phosphate, *J.Chromatogr.A*, **1996**, *722*, 295–301.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

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CHROMATOGRAM
Retention time: 7
Limit of quantitation: ≤50 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, pyridoxine, vitamin B12, niacinamide, folic acid, pyridoxamine

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REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: 5 mM Sodium perchlorate containing 10 mM sodium 1-hexanesulfonate, pH adjusted to 2.5 with perchloric acid
Column temperature: 45
Flow rate: 0.8
Injection volume: 10
Detector: UV 650 following post-column reaction. The column effluent mixed with 0.5 mg/mL 2,6-dibromo-N-chloro-p-benzoquinoneimine (2,6-dibromoquinone-4-chlorimide) (Eastman) at 1.4 mL/min and flowed through a 2 m × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with 2.5% ammonia solution pumped at 1 mL/min and this mixture flowed through a 2 m × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM
Retention time: 6
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: pyridoxamine, 4-pyridoxic acid, pyridoxine

KEY WORDS
post-column reaction

REFERENCE
Kawamoto,T.; Okada,E.; Fujita,T. Post-column derivatization of vitamin B6 using 2,6-dibromoquinone-4-chlorimide, *J.Chromatogr.*, **1983**, *267*, 414–419.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxamine, pyridoxine, riboflavin, thiamine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize tissue with 10 volumes 10 mM pH 7.4 potassium phosphate buffer. Remove a 50 μL aliquot and add it to 50 μL 10% trichloroacetic acid, mix, heat at 50° for 15 min, add 35 μL 3.3 M K_2HPO_4, add 1 μL 80 mM KCN, heat at 50° for 25 min, add 12.5 μL 28% phosphoric acid, centrifuge at 13000 g for 10 min, filter (0.45 μm) the supernatant, inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4 5 μm STR ODS-H (Shimadzu)
Mobile phase: 2 M Acetic acid containing 1 mM sodium 1-heptanesulfonate, adjusted to pH 3.75 with solid KOH
Flow rate: 0.8
Injection volume: 5

Detector: F ex 318 em 418

CHROMATOGRAM
Retention time: 3 (pyridoxal-5-phosphate)
Limit of detection: 50 fmole

KEY WORDS
derivatization; brain

REFERENCE
Naoi,M.; Ichinose,H.; Takahashi,T.; Nagatsu,T. Sensitive assay for determination of pyridoxal-5-phosphate in enzymes using high-performance liquid chromatography after derivatization with cyanide, *J.Chromatogr.*, **1988**, *434*, 209–214.

SAMPLE
Matrix: tissue, CSF, blood
Sample preparation: Dilute rat plasma 1:10. Dilute human plasma 1:25. Homogenize 10 mg liver, 20 mg brain, or 250 μL CSF or diluted plasma with 250 μL 5 or 10% metaphosphoric acid by sonication at 300 W for 30 s, centrifuge at 0-4° at 10000 g for 20 min. Remove the supernatant and add it to 250 μL dichloromethane, vortex, centrifuge at 0-4° at for 15 min, filter (0.22 μm) the aqueous layer, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 3 μm Ultramex C18
Column: 150 × 4.6 3 μm Ultramex C18
Mobile phase: Gradient. A was 33 mM phosphoric acid containing 10 mM 1-octanesulfonic acid adjusted to pH 2.2 with 6 M KOH. B was isopropanol:330 mM phosphoric acid adjusted to pH 2.2 with 6 M KOH. A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 15 min, return to initial conditions over 4.5 min, re-equilibrate for 5.5 min. (Every 30 samples flush with water at 0.5 mL/min for 1 h and with isopropanol at 0.2 mL/min for 1 h. Every morning flush with water at 0.5 mL/min for 1 h.)
Flow rate: 1.2
Injection volume: 25
Detector: F ex 328 em 393 following post-column reaction with the reagent. (Reagent was 1 mg/mL sodium bisulfite in 100 mM potassium phosphate buffer adjusted to pH 7.4 with 6 M KOH.)

CHROMATOGRAM
Retention time: 13.6 (pyridoxal), 2.04 (pyridoxal phosphate)
Limit of detection: 4.1 pmole
Limit of quantitation: 10.5 pmole

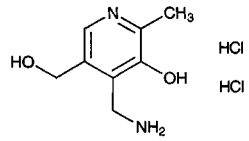
OTHER SUBSTANCES
Extracted: 4-deoxypyridoxine, pyridoxamine, pyridoxamine phosphate, 4-pyridoxic acid, pyridoxine

KEY WORDS
protect from light; rat; human; liver; brain; plasma; CSF; post-column reaction

REFERENCE
Sharma,S.K.; Dakshinamurti,K. Determination of vitamin B_6 vitamers and pyridoxic acid in biological samples, *J.Chromatogr.*, **1992**, *578*, 45–51.

Pyridoxamine dihydrochloride

Molecular formula: $C_8H_{14}Cl_2N_2O_2$
Molecular weight: 241.12
CAS Registry No.: 524-36-7 (di HCl)
Merck Index: 8164



SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 100 μL 1 μM IS + 100 (200 ?) μL 4 M perchloric acid, mix, centrifuge at 1500 g for 5 min. Filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2 (12% C, endcapped)
Mobile phase: 67 mM KH_2PO_4 containing 125 μM sodium hexanesulfonate, adjusted to pH 2.5 with concentrated orthophosphoric acid (As column ages it may be necessary to increase concentration of sodium hexanesulfonate to 250 μM to maintain separation.)
Flow rate: 1
Injection volume: 200
Detector: F ex 325 em 400 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and flowed through a 300 mm tube to the detector. (The reagent was 67 mM KH_2PO_4 containing 1 g/L sodium sulfite adjusted to pH 7.5 with Na_2HPO_4.)

CHROMATOGRAM

Retention time: 5.5
Internal standard: pyridoxamine-5'-phosphate (4.6)
Limit of detection: 1.5 nM

OTHER SUBSTANCES

Extracted: pyridoxal, pyridoxal-5'-phosphate, pyridoxine

KEY WORDS

post-column reaction; serum

REFERENCE

Reynolds,T.M.; Brain,A. A simple internally-standardised isocratic HPLC assay for vitamin B_6 in human serum, *J.Liq.Chromatogr.*, **1992**, *15*, 897–914.

SAMPLE

Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES

Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 19

OTHER SUBSTANCES
Simultaneous: folic acid, niacin (UV 254), niacinamide (UV 254), thiamine (UV 254), riboflavin (UV 254), pyridoxine, ascorbic acid

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: 5 mM Sodium perchlorate containing 10 mM sodium 1-hexanesulfonate, pH adjusted to 2.5 with perchloric acid
Column temperature: 45
Flow rate: 0.8
Injection volume: 10
Detector: UV 650 following post-column reaction. The column effluent mixed with 0.5 mg/mL 2,6-dibromo-N-chloro-p-benzoquinoneimine (2,6-dibromoquinone-4-chlorimide) (Eastman) at 1.4 mL/min and flowed through a 2 m × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with 2.5% ammonia solution pumped at 1 mL/min and this mixture flowed through a 2 m × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM
Retention time: 12
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: pyridoxal, 4-pyridoxic acid, pyridoxine

KEY WORDS
post-column reaction

REFERENCE
Kawamoto,T.; Okada,E.; Fujita,T. Post-column derivatization of vitamin B6 using 2,6-dibromoquinone-4-chlorimide, *J.Chromatogr.*, **1983**, *267*, 414–419.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.3

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, thiamine, riboflavin, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE

Matrix: tissue, CSF, blood

Sample preparation: Dilute rat plasma 1:10. Dilute human plasma 1:25. Homogenize 10 mg liver, 20 mg brain, or 250 μL CSF or diluted plasma with 250 μL 5 or 10% metaphosphoric acid by sonication at 300 W for 30 s, centrifuge at 0-4° at 10000 g for 20 min. Remove the supernatant and add it to 250 μL dichloromethane, vortex, centrifuge at 0-4° at for 15 min, filter (0.22 μm) the aqueous layer, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 3 μm Ultramex C18

Column: 150 × 4.6 3 μm Ultramex C18

Mobile phase: Gradient. A was 33 mM phosphoric acid containing 10 mM 1-octanesulfonic acid adjusted to pH 2.2 with 6 M KOH. B was isopropanol:330 mM phosphoric acid adjusted to pH 2.2 with 6 M KOH. A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 15 min, return to initial conditions over 4.5 min, re-equilibrate for 5.5 min. (Every 30 samples flush with water at 0.5 mL/min for 1 h and with isopropanol at 0.2 mL/min for 1 h. Every morning flush with water at 0.5 mL/min for 1 h.)

Flow rate: 1.2

Injection volume: 25

Detector: F ex 328 em 393 following post-column reaction with the reagent. (Reagent was 1 mg/mL sodium bisulfite in 100 mM potassium phosphate buffer adjusted to pH 7.4 with 6 M KOH.)

CHROMATOGRAM

Retention time: 25.2 (pyridoxamine), 10 (pyridoxamine phosphate)

Limit of detection: 1.3 pmole

Limit of quantitation: 2.6 pmole

OTHER SUBSTANCES

Extracted: 4-deoxypyridoxine, pyridoxal, pyridoxal phosphate, 4-pyridoxic acid, pyridoxine

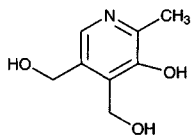
KEY WORDS

protect from light; rat; human; liver; brain; plasma; CSF; post-column reaction

REFERENCE

Sharma,S.K.; Dakshinamurti,K. Determination of vitamin B_6 vitamers and pyridoxic acid in biological samples, *J.Chromatogr.*, **1992**, *578*, 45–51.

Pyridoxine



Molecular formula: $C_8H_{11}NO_3$

Molecular weight: 169.18

CAS Registry No.: 65-23-6, 58-56-0 (HCl)

Merck Index: 8166

SAMPLE

Matrix: baker's yeast, eggs, milk

Sample preparation: Baker's yeast. Mix 50 mg yeast, 50 μL 1 mM isopyridoxal in water, and 3 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the supernatant, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate. Egg yolk. Mix 2 g egg yolk with 100 μL 1 mM isopyridoxal water and 6 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the clear middle part of the extract, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate. Milk. Mix 2 g egg yolk with 10 μL 1 mM isopyridoxal water and 1 mL 1 M perchloric acid, vortex for a few s, let stand for 30 min, vortex, centrifuge at 600 g for 15 min. Remove the clear middle part of the extract, adjust pH to 3-4 with a 10 M KOH, let stand in the refrigerator

for a few h. Centrifuge and remove 500 μL of the supernatant and filter (0.45 μm nylon-66) it. Inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 30 mm long 5 μm Phenosphere ODS 2 (Phenomenex)
Column: 250 × 4.6 5 μm Phenosphere ODS 2 (Phenomenex)
Mobile phase: 150 mM NaH_2PO_4 adjusted to pH 2.5 with 70% perchloric acid (A 50 mm long column filled with ODS material was placed before the injector.)
Flow rate: 1
Injection volume: 20
Detector: F ex 290 em 389 following post-column derivatization. The column effluent mixed with 1 g/L sodium bisulfite in water pumped at 0.1 mL/min and the mixture flowed through a 2 m × 0.5 mm I.D. PTFE mixing coil to the detector. (The post-column derivatization is only required for pyridoxal phosphate. For the other compounds it is not necessary.)

CHROMATOGRAM

Retention time: 6.5 (pyridoxine phosphate), 13.5 (pyridoxine)
Internal standard: isopyridoxal (11)

OTHER SUBSTANCES

Extracted: pyridoxal, pyridoxal phosphate, pyridoxamine, pyridoxamine phosphate, 4-pyridoxic acid, 4-pyridoxic acid phosphate

KEY WORDS

post-column reaction

REFERENCE

Argoudelis,C.J. Simple high-performance liquid chromatographic method for the determination of all seven vitamin B6-related compounds, *J.Chromatogr.A*, **1997**, *790*, 83–91.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 100 μL 1 μM IS + 100 (200 ?) μL 4 M perchloric acid, mix, centrifuge at 1500 g for 5 min. Filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2 (12% C, endcapped)
Mobile phase: 67 mM KH_2PO_4 containing 125 μM sodium hexanesulfonate, adjusted to pH 2.5 with concentrated orthophosphoric acid (As column ages it may be necessary to increase concentration of sodium hexanesulfonate to 250 μM to maintain separation.)
Flow rate: 1
Injection volume: 200
Detector: F ex 325 em 400 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and flowed through a 300 mm tube to the detector. (The reagent was 67 mM KH_2PO_4 containing 1 g/L sodium sulfite adjusted to pH 7.5 with Na_2HPO_4.)

CHROMATOGRAM

Retention time: 18.1
Internal standard: pyridoxamine-5'-phosphate (4.6)
Limit of detection: 12.5 nM

OTHER SUBSTANCES

Extracted: pyridoxal, pyridoxal-5'-phosphate, pyridoxamine

KEY WORDS

post-column reaction; serum

REFERENCE

Reynolds,T.M.; Brain,A. A simple internally-standardised isocratic HPLC assay for vitamin B_6 in human serum, *J.Liq.Chromatogr.*, **1992**, *15*, 897–914.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 2.895

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 10
Limit of quantitation: ≤50 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, vitamin B12, folic acid, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 6 5 μm Capcell Pak C8 (Shiseido, Japan)
Mobile phase: MeOH:50 mM KH_2PO_4 containing 5 mM tetra-n-butylammonium phosphate 15:85, adjusted to pH 2.6 with 5% orthophosphoric acid (After one week of use, wash the column with water and MeOH:water 70:30 at 1 mL/min for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10-20
Detector: UV 215

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, dipotassium glycyrrizate, fumaric acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, maleic acid, neostigmine methylsulfate, tetrahydrozoline, vitamin B12
Noninterfering: chondroitin sulfate, lysozyme

KEY WORDS
ophthalmic solutions; ion-pair agents

REFERENCE
Yamato,S.; Nakajima,M.; Shimada,K. Simultaneous determination of chlorpheniramine and maleate by high-performance liquid chromatography using tetra-n-butylammonium phosphate as an ion-pair reagent, *J.Chromatogr.A*, **1996**, *731*, 346–350.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: pantothenic acid, thiamine, riboflavin, niacinamide

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE

Matrix: formulations

Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1 L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES

Column: 100 × 8 Radial Pak A C18 (Waters)

Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)

Flow rate: 2

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: niacinamide, thiamine, riboflavin, ascorbic acid (UV 254)

KEY WORDS

multi-vitamin; protect from light; tablets; capsules

REFERENCE

Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE

Matrix: formulations

Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES

Guard column: pellicular Corasil

Column: 10 μm μBondapak C18

Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.

Flow rate: 1.5

Injection volume: 50

Detector: UV 280

CHROMATOGRAM

Retention time: 17 (step gradient), 16 (curve gradient)

OTHER SUBSTANCES
Simultaneous: folic acid, niacin (UV 254), niacinamide (UV 254), pyridoxamine, thiamine (UV 254), riboflavin (UV 254), ascorbic acid

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, niacinamide, riboflavin, thiamine
Interfering: ascorbic acid

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 4.2

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, riboflavin, saccharin, thiamine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1
Injection volume: 5
Detector: UV 290

CHROMATOGRAM
Retention time: 7.65
Internal standard: theobromine (8)
Limit of detection: 0.260 ng

OTHER SUBSTANCES
Simultaneous: folic acid (UV 272), niacin (UV 272), niacinamide (UV 272), thiamine (UV 272), riboflavin (UV 272)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 2.1 3 μm Spherisorb ODS-2
Mobile phase: MeOH:buffer:triethylamine 20:80:0.1, pH 2.8 (Buffer was 5 mM sodium hexanesulfonate in 10 mM KH_2PO_4 adjusted to pH 2.8 with phosphoric acid.)
Flow rate: 0.2 for 5 min, to 0.3 over 0.5 min, maintain at 0.3 for 12.5 min
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: folic acid, niacin (UV 254), riboflavin (UV 254), thiamine (UV 254), niacinamide (UV 254)

KEY WORDS
multivitamin; narrow bore

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: 5 mM Sodium perchlorate containing 10 mM sodium 1-hexanesulfonate, pH adjusted to 2.5 with perchloric acid
Column temperature: 45
Flow rate: 0.8
Injection volume: 10
Detector: UV 650 following post-column reaction. The column effluent mixed with 0.5 mg/mL 2,6-dibromo-N-chloro-p-benzoquinoneimine (2,6-dibromoquinone-4-chlorimide) (Eastman) at 1.4 mL/min and flowed through a 2 m × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with 2.5% ammonia solution pumped at 1 mL/min and this mixture flowed through a 2 m × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM
Retention time: 8
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: pyridoxal, pyridoxamine, 4-pyridoxic acid

KEY WORDS
post-column reaction

REFERENCE
Kawamoto,T.; Okada,E.; Fujita,T. Post-column derivatization of vitamin B6 using 2,6-dibromoquinone-4-chlorimide, *J.Chromatogr.*, **1983**, *267*, 414–419.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, pyridoxamine, thiamine, riboflavin, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 1.5

OTHER SUBSTANCES
Simultaneous: niacin, riboflavin, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 1.1

OTHER SUBSTANCES
Simultaneous: niacin, pantothenic acid, riboflavin, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 780.

SAMPLE
Matrix: tissue, CSF, blood
Sample preparation: Dilute rat plasma 1:10. Dilute human plasma 1:25. Homogenize 10 mg liver, 20 mg brain, or 250 μL CSF or diluted plasma with 250 μL 5 or 10% metaphosphoric acid by sonication at 300 W for 30 s, centrifuge at 0-4° at 10000 g for 20 min. Remove the supernatant and add it to 250 μL dichloromethane, vortex, centrifuge at 0-4° at for 15 min, filter (0.22 μm) the aqueous layer, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 3 μm Ultramex C18
Column: 150 × 4.6 3 μm Ultramex C18
Mobile phase: Gradient. A was 33 mM phosphoric acid containing 10 mM 1-octanesulfonic acid adjusted to pH 2.2 with 6 M KOH. B was isopropanol:330 mM phosphoric acid adjusted to pH 2.2 with 6 M KOH. A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 15 min, return to initial conditions over 4.5 min, re-equilibrate for 5.5 min. (Every 30 samples flush with water at 0.5 mL/min for 1 h and with isopropanol at 0.2 mL/min for 1 h. Every morning flush with water at 0.5 mL/min for 1 h.)
Flow rate: 1.2
Injection volume: 25
Detector: F ex 328 em 393 following post-column reaction with the reagent. (Reagent was 1 mg/mL sodium bisulfite in 100 mM potassium phosphate buffer adjusted to pH 7.4 with 6 M KOH.)

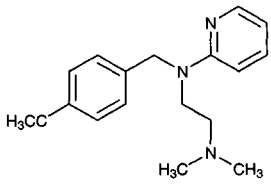
CHROMATOGRAM
Retention time: 18.5
Limit of detection: 2.6 pmole
Limit of quantitation: 7.5 pmole

OTHER SUBSTANCES
Extracted: 4-deoxypyridoxine, pyridoxal, pyridoxal phosphate, pyridoxamine, pyridoxamine phosphate, 4-pyridoxic acid

KEY WORDS
protect from light; rat; human; liver; brain; plasma; CSF; post-column reaction

REFERENCE
Sharma,S.K.; Dakshinamurti,K. Determination of vitamin B_6 vitamers and pyridoxic acid in biological samples, *J.Chromatogr.*, **1992**, *578*, 45–51.

Pyrilamine



Molecular formula: $C_{17}H_{23}N_3O$
Molecular weight: 285.39
CAS Registry No.: 91-84-9, 59-33-6 (maleate)
Merck Index: 8168
Lednicer No.: 1 51

SAMPLE
Matrix: blood
Sample preparation: 4 mL Whole blood + 10 mL pH 10.0 phosphate buffer (μ = 0.4), vortex, add 5 mL chloroform:hexane 40:60, shake gently horizontally for 30 min, centrifuge. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micropak CN-10
Mobile phase: n-Hexane:dichloromethane:MeCN:propylamine 50:25:25:0.1
Column temperature: 30
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 4.0
Internal standard: pyrilamine maleate (mepyramine maleate)

OTHER SUBSTANCES
Extracted: papaverine
Simultaneous: carbetapentane, cocaine, dioxyline, ethaverine, fluphenazine, imipramine, methapyrilene, papaveraldine, procaine, promethazine, strychnine, yohimbine
Interfering: diamorphine, thonzylamine

KEY WORDS
whole blood; pyrilamine (mepyramine) is IS

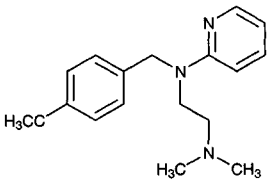
REFERENCE
Hoogewijs,G.; Michotte,Y.; Lambrecht,J.; Massart,D.L. High-performance liquid chromatographic determination of papaverine in whole blood, *J.Chromatogr.*, **1981**, *226*, 423–430.

SAMPLE
Matrix: formulations
Sample preparation: Crush 10 tablets, add 250 mL 50 mM HCl in EtOH:water 50:50, heat for 15 min on a steam bath, shake mechanically for 2 h, filter (glass fiber GF/A, Whatman), inject a 30 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil-10-ODS
Mobile phase: MeCN:buffer 50:50 (Buffer was 2.85 mM ethylenediamine sulfate adjusted to pH 7.44 ± 0.02 with 1 M ammonium hydroxide.)
Flow rate: 3.8
Injection volume: 30
Detector: UV 216.5

REFERENCE
Sharma,S.K.; Dakshinamurti,K. Determination of vitamin B_6 vitamers and pyridoxic acid in biological samples, *J.Chromatogr.*, **1992**, *578*, 45–51.

Pyrilamine



Molecular formula: $C_{17}H_{23}N_3O$
Molecular weight: 285.39
CAS Registry No.: 91-84-9, 59-33-6 (maleate)
Merck Index: 8168
Lednicer No.: 1 51

SAMPLE
Matrix: blood
Sample preparation: 4 mL Whole blood + 10 mL pH 10.0 phosphate buffer (μ = 0.4), vortex, add 5 mL chloroform:hexane 40:60, shake gently horizontally for 30 min, centrifuge. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micropak CN-10
Mobile phase: n-Hexane:dichloromethane:MeCN:propylamine 50:25:25:0.1
Column temperature: 30
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 4.0
Internal standard: pyrilamine maleate (mepyramine maleate)

OTHER SUBSTANCES
Extracted: papaverine
Simultaneous: carbetapentane, cocaine, dioxyline, ethaverine, fluphenazine, imipramine, methapyrilene, papaveraldine, procaine, promethazine, strychnine, yohimbine
Interfering: diamorphine, thonzylamine

KEY WORDS
whole blood; pyrilamine (mepyramine) is IS

REFERENCE
Hoogewijs,G.; Michotte,Y.; Lambrecht,J.; Massart,D.L. High-performance liquid chromatographic determination of papaverine in whole blood, *J.Chromatogr.*, **1981**, *226*, 423–430.

SAMPLE
Matrix: formulations
Sample preparation: Crush 10 tablets, add 250 mL 50 mM HCl in EtOH:water 50:50, heat for 15 min on a steam bath, shake mechanically for 2 h, filter (glass fiber GF/A, Whatman), inject a 30 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil-10-ODS
Mobile phase: MeCN:buffer 50:50 (Buffer was 2.85 mM ethylenediamine sulfate adjusted to pH 7.44 ± 0.02 with 1 M ammonium hydroxide.)
Flow rate: 3.8
Injection volume: 30
Detector: UV 216.5

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: aposcopolamine, methscopolamine, pheniramine, phenylpropanolamine, tropic acid

KEY WORDS
tablets

REFERENCE
Heidemann,D.R. High-pressure liquid chromatographic determination of methscopolamine nitrate, phenylpropanolamine hydrochloride, pyrilamine maleate, and pheniramine maleate in tablets, *J.Pharm.Sci.*, **1981**, *70*, 820–822.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.0

OTHER SUBSTANCES
Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrimethamine, tripelennamine

KEY WORDS
tablets; syrups; elixirs; injections

REFERENCE
Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. One tablet + 50 mL MeOH, sonicate, make up to 100 mL with MeOH, centrifuge for 15 min. Remove 1 mL supernatant, make up to 10 mL with mobile phase, inject a 50 μL aliquot. Drops. Dilute drops with the mobile phase so that the concentration of pyrilamine maleate is 25 μg/mL, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Cyclobond I (Advanced Separation Technologies)
Mobile phase: MeOH:50 mM NaH_2PO_4 adjusted to pH 7.0 with 0.1 M NaOH 30:70
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: pheniramine, phenylpropanolamine

KEY WORDS
tablets; drops

REFERENCE
el-Gizawy,S.M.; Ahmed,A. High-performance liquid chromatographic determination of mepyramine maleate, pheniramine maleate and phenylpropanolamine hydrochloride in tablets and drops, *Analyst*, **1987**, *112*, 867–869.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, add 100 mL water and 30-40 mL MeCN, dissolve, add N,N-dimethylbenzylamine, make up to 250 or 500 mL with water, centrifuge an aliquot, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Asahipak ODP-50 C18
Mobile phase: MeCN:200 mM pH 7.0 phosphate buffer 27:73
Flow rate: 0.8
Injection volume: 20-100
Detector: Chemiluminescence following post-column reaction. Oxidize a 1 mM tris(2,2'-bipyridine) ruthenium(II) hexachloride solution in 50 mM pH 5.5 acetate buffer to Ru(III) using a Princeton Applied Research polarographic analyzer with a platinum gauze working electrode, platinum wire auxiliary electrode, and a silver wire reference electrode, +950 mV. Pump the reagent solution at 0.28 mL/min and mix with the column effluent, allow to flow through detector. The chemiluminescence detector was a fluorescence detector with the light source removed.

CHROMATOGRAM
Retention time: 10
Internal standard: N,N-dimethylbenzylamine

OTHER SUBSTANCES
Simultaneous: brompheniramine, diphenhydramine, chlorpheniramine, pheniramine

KEY WORDS
tablets

REFERENCE
Holeman,J.A.; Danielson,N.D. Liquid chromatography of antihistamines using post-column tris(2, 2'-bipyridine) ruthenium(III) chemiluminescence detection, *J.Chromatogr.A*, **1994**, *679*, 277–284.

SAMPLE
Matrix: fungal incubations
Sample preparation: Cedntrifuge 30 mL fungal incubation, wash pellet with water then MeOH. Combine the supernatant and the washes and add 40 mL 1 M K_2HPO_4, extract five times with 100 mL portions of dichloromethane, filter the extracts through a plug of anhydrous sodium sulfate, evaporate the filtrate to dryness under reduced pressure at 40°, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 containing 20 mM trimethylamine, adjusted to pH 7.0 with aqueous KOH.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Hansen,E.B.,Jr.; Cerniglia,C.E.; Korfmacher,W.A.; Miller,D.W.; Heflich,R.H. Microbial transformation of the antihistamine pyrilamine maleate. Formation of potential mammalian metabolites, *Drug Metab.Dispos.*, **1987**, *15*, 97–106.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 2.9
Internal standard: promazine (5.2)

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, amitriptyline, buprion, chlordiazepoxide, chlorimipramine, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, fluphenazine, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, loxepin, maprotiline, meperidine, methadone, mianserin, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, perphenazine, phentermine, procainamide, prochlorperazine, propoxyphene, protriptyline, quinidine, thioridazine, trifluoperazine, triflupromazine, trimeprazine, trimipramine, zimeldine
Noninterfering: thiopropazine
Interfering: amoxapine, amphetamine, disopyramide, doxepin, 2-hydroxyimipramine, iprindole, prolixin, promethazine

KEY WORDS
normal phase

REFERENCE
Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 305 × 7 PRP-1 (Hamilton)
Mobile phase: Gradient. A was water:triethylamine 99.9:0.1. B was MeCN:triethylamine 99.9:0.1. A:B 60:40 for 7 min, to 20:80 over 5 min, maintain at 20:80 for 5 min, to 60:40 over 6 min, re-equilibrate at 60:40 for 2 min.
Column temperature: 40
Flow rate: 3.5
Injection volume: 500
Detector: UV 254

CHROMATOGRAM

Retention time: 16.0

OTHER SUBSTANCES

Simultaneous: diphenylpyraline, doxylamine, guaifenesin, hydrocodone, phenylephrine, phenylpropanolamine, pheniramine
Interfering: etafedrine

REFERENCE

Black,D.B.; By,A.W.; Lodge,B.A. Isolation and identification of hydrocodone in narcotic cough syrups by high-performance liquid chromatography with infrared spectrometric identification, *J.Chromatogr.*, **1986**, *358*, 438–443.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 1.60

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-CN (Supelco)
Mobile phase: MeCN:50 mM pH 7 ammonium acetate 30:70
Flow rate: 1.2-2
Injection volume: 50
Detector: E, Bioanalytical Systems LC-4B, TL-5 glassy carbon electrode, +0.9 V or UV 254

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: pyrilamine N-oxide

REFERENCE

Billedeau,S.M.; Holder,C.L.; Getek,T.A. High-performance liquid chromatography of the antihistamine pyrilamine and its N-oxide using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 151–159.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Supelguard (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH_2PO_4 containing 0.2% triethylamine, pH 2.5.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, chlorpheniramine, clonidine, diphenhydramine, promethazine, triprolidine

REFERENCE

Supelco Catalog, **1994**, p. 768.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.61

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

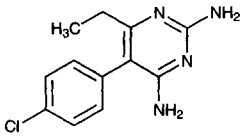
CHROMATOGRAM
Retention time: k' 2.19

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Pyrimethamine



Molecular formula: $C_{12}H_{13}ClN_4$
Molecular weight: 248.71
CAS Registry No.: 58-14-0
Merck Index: 8169
Lednicer No.: 1 262

SAMPLE
Matrix: amniotic fluid, blood, tissue
Sample preparation: Homogenize (Ultraturrax) tissue with four volumes of physiological saline, centrifuge at 1000 g for 20 min. 200 μL Serum, tissue supernatant, or amniotic fluid + 200 μL MeCN, vortex, centrifuge at 4000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb 5 ODS
Mobile phase: MeCN:water 50 :50 containing 1 g/L 85% phosphoric acid and 0.3 g/L tetramethylammonium chloride
Flow rate: 1.5
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 50 ng/mL

KEY WORDS
serum; monkey; pharmacokinetics; placenta; brain; heart; liver; spleen; lung

REFERENCE
Schoondermark-Van de Ven,E.; Galama,J.; Vree,T.; Camps,W.; Baars,I.; Eskes,T.; Meuwissen,J.; Melchers,W. Study of treatment of congenital *Toxoplasma gondii* infection in rhesus monkeys with pyrimethamine and sulfadiazine, *Antimicrob.Agents Chemother.*, **1995**, *39*, 137–144.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 3 mL MeOH and 3 mL 50 mM pH 3.4 oxalate buffer. 1 mL Plasma + 1 mL 50 mM pH 3.4 oxalate buffer + 50 μL 200 μg/mL IS in MeOH:water 50:50, mix, add to the SPE cartridge. Wash with 3 mL 50 mM pH 3.4 oxalate buffer, 1 mL MeOH:water 20:80, and 2 mL hexane:ether 80:20. Elute with two 1 mL portions of MeOH:25% ammonia solution 99:1. Evaporate the eluate to dryness under a gentle stream of nitrogen at 30°. Dissolve the residue in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 5 μm Symmetry C18 (Waters)
Column: 250 × 4.6 5 μm Symmetry C18 (Waters)
Mobile phase: MeCN:MeOH:water 25:10:65 containing 1% triethylamine, adjusted to pH 5.6 with phosphoric acid
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Internal standard: sulfadimethoxine
Limit of detection: 7.01 ng/mL
Limit of quantitation: 9.56 ng/mL

OTHER SUBSTANCES
Extracted: sulfadoxine
Simultaneous: acetaminophen, 4-chlorophenylbiguanide, cycloguanil, proguanil, quinine, sulfadiazine

KEY WORDS
plasma; SPE

REFERENCE
Astier,H.; Renard,C.; Cheminel,V.; Soares,O.; Mounier,C.; Peyron,F.; Chaulet,J.F. Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction, *J.Chromatogr.B*, **1997**, *698*, 217–223.

SAMPLE
Matrix: blood
Sample preparation: Add 150 μL 100 mM zinc sulfate to 600 μL plasma while vortexing over 15 s, add 700 μL MeCN containing 4 μm WR 184806 and 75 μM sulfadimethoxine while vortexing over 15 s, , let stand for 15 min, centrifuge at 10000 g for 10 min. Remove the supernatant and add it to 2 mL pH 9.0 phosphate buffer, add 2 mL 60 mM tetrabutylammonium hydroxide, add 5 mL MTBE, shake for 10 min, centrifuge at 1200 g for 5 min. Remove the upper organic layer and evaporate it to dryness at 50°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 3 μm Spherisorb S3-ODS-1
Mobile phase: MeCN:100 mM phosphate buffer 48:52, adjusted to pH 3.5
Flow rate: 0.5
Injection volume: 100
Detector: UV 229

CHROMATOGRAM
Retention time: 8.4
Internal standard: sulfadimethoxine (6.22), 2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-tert-butylamino)propyl]quinoline phosphate (WR 184806) (Walter Reed) (21.00)
Limit of detection: 50 nM

OTHER SUBSTANCES
Extracted: mefloquine, sulfadoxine

KEY WORDS
plasma

REFERENCE
Bergqvist,Y.; Eckerbom,S.; Larsson,H.; Malekzadeh,M. Reversed-phase liquid chromatographic method for the simultaneous determination of the antimalarial drugs sulfadoxine, pyrimethamine, mefloquine and its major carboxylic metabolite in plasma, *J.Chromatogr.*, **1991**, *571*, 169–177.

SAMPLE
Matrix: blood
Sample preparation: 150 μL Whole blood or plasma + 25 μL monopropionyldapsone in EtOH, mix at 2200 vibrations/min for 10 min, add 100 μL 2 M NaOH, add 3 mL MTBE, shake mechanically for 15 min, centrifuge at 1200 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, vortex for 20 s, centrifuge at 1200 g for 2 min, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:MeOH:25 mM pH 2.3 phosphate buffer 20:10:70
Flow rate: 1.2
Injection volume: 80
Detector: UV 286

CHROMATOGRAM
Retention time: 2.5
Internal standard: monopropionyldapsone (7.5) (Reflux dapsone with propionic anhydride in ethyl acetate for 10 min, purify by preparative TLC.)

OTHER SUBSTANCES
Extracted: monoacetyldapsone, dapsone
Noninterfering: chloroquine, quinine, sulfamethoxazole, trimethoprim, acetaminophen
Interfering: proguanil

KEY WORDS
whole blood; plasma

REFERENCE
Lemnge,M.M.; Ronn,A.; Flachs,H.; Bygbjerg,I.C. Simultaneous determination of dapsone, monoacetyldapsone and pyrimethamine in whole blood and plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 340–346.

SAMPLE
Matrix: blood
Sample preparation: Whole blood. 150 μL Whole blood + 25 μL 800 ng/mL monopropionyldapsone + 1.2 mL 200 mM NaOH + 5 mL MTBE, mix for 25 min, centrifuge at 1600 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot. Dried blood. Let 150 μL blood dry on filter paper. Cut paper into small pieces, add 25 μL 800 ng/mL monopropionyldapsone, add 1.2 mL 200 mM NaOH, mix gently for 30 min, add 5 mL MTBE, mix for 25 min, centrifuge at 1600 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-ABZ (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:MeOH:buffer 14:7:49 (Buffer was 25 mM phosphate adjusted to pH 2.3 with orthophosphoric acid.)
Flow rate: 1.2
Injection volume: 80
Detector: UV 286

CHROMATOGRAM
Retention time: 2.3
Internal standard: monopropionyldapsone (7.1)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: monoacetyldapsone, metabolites, dapsone
Noninterfering: acetaminophen, chloroquine, quinine, sulfadoxine, sulfamethoxazole, trimethoprim

KEY WORDS
whole blood; dried blood

REFERENCE

Ronn,A.M.; Lemnge,M.M.; Angelo,H.R.; Bygbjerg,I.C. High-performance liquid chromatography determination of dapsone, monoacetyldapsone, and pyrimethamine in filter paper blood spots, *Ther.Drug Monit.*, **1995**, *17*, 79–83.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 273

CHROMATOGRAM

Retention time: 5.00

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM
Retention time: 12.497

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrilamine, tripelennamine

KEY WORDS
tablets; syrups; elixirs; injections

REFERENCE
Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, add 40 mg pyrimethamine, dissolve in 20 mL MeCN, add 40 mL mobile phase, filter (paper), wash filter with mobile phase, make up filtrate to 100 mL with mobile phase. Dilute a 5 mL aliquot to 50 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:MeCN:water:triethylamine 55:5:40:0.1, pH adjusted to 4.0 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5
Internal standard: pyrimethamine

OTHER SUBSTANCES
Simultaneous: aspirin, dipyridamole

KEY WORDS
tablets; pyrimethamine is IS

REFERENCE
Sane,R.T.; Ghadge,J.K.; Jani,A.B.; Vaidya,A.J.; Kotwal,S.S. Simultaneous high-performance liquid chromatographic determination of haloperidol with propantheline bromide, nalidixic acid with phenazopyridine hydrochloride, and dipyridamole with aspirin in combined dosage (forms), *Indian Drugs*, **1992**, *29*, 240–244.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, er-

gosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax-Sil
Mobile phase: Dichloromethane:MeOH:1 M perchloric acid 100:9:0.4
Flow rate: 0.8
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Retention time: 9.6

OTHER SUBSTANCES

Simultaneous: amodiaquine, chloroquine, dapsone, desethylchloroquine, dihydroquinine, mefloquine, primaquine, proguanil, quinidine, quinine, sulfadoxine, sulfalene, sulfamethoxazole
Interfering: dihydroquinidine

KEY WORDS

normal phase

REFERENCE

Dua,V.K.; Sarin,R.; Prakash,A. Determination of quinine in serum, plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 87–93.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:MeOH:1 M perchloric acid:water 30:9:0.8:95
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 7.70

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, dapsone, primaquine, quinidine, quinine, sulfadoxine, sulfalene, sulfamethoxazole

REFERENCE
Dua,V.K.; Sarin,R.; Sharma,V.P. Sulphadoxine concentrations in plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases after treatment with Fansidar using high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1317–1323.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 48

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

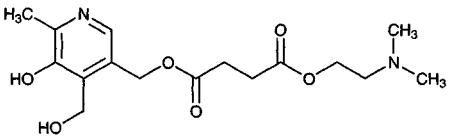
CHROMATOGRAM
Retention time: 57

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

Pyrisuccideanol



Molecular formula: $C_{16}H_{24}N_2O_6$
Molecular weight: 340.38
CAS Registry No.: 33605-94-6, 53659-00-0 (dimaleate)
Merck Index: 8175

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

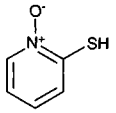
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 3.462

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Pyrithione



Molecular formula: C_5H_5NOS
Molecular weight: 127.17
CAS Registry No.: 1121-30-8, 13463-41-7 (zinc derivative)
Merck Index: 8178

SAMPLE

Matrix: formulations
Sample preparation: Weigh out shampoo containing 10 mg zinc pyrithione and make up to 100 mL with buffer saturated with chloroform (if phases separate use water saturated with chloroform), shake well for a few min, sonicate for a few min. Remove a 10 mL aliquot and add it to 10 mL chloroform saturated with water, add 2 mL 1 M copper(II) sulfate, shake vigorously for 5 min, centrifuge at 1500 g for 5 min, inject a 5 μL aliquot of the lower organic layer. (Buffer was 100 mM citric acid:200 mM Na_2HPO_4 97:103, pH 5.0.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil 5C18
Mobile phase: MeOH:water 60:40 (Before use flush system with 0.1% EDTA at 0.5 mL/min, then water.)
Column temperature: 25
Flow rate: 1
Injection volume: 5
Detector: UV 320

CHROMATOGRAM

Retention time: 15
Limit of quantitation: 100 ng

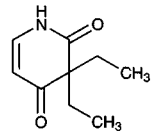
KEY WORDS

shampoo; complexation; copper complexes

REFERENCE

Nakajima,K.; Yasuda,T.; Nakazawa,H. High-performance liquid chromatographic determination of zinc pyrithione in antidandruff preparations based on copper chelate formation, *J.Chromatogr.*, **1990**, *502*, 379–384.

Pyrithyldione



Molecular formula: $C_9H_{13}NO_2$
Molecular weight: 167.21
CAS Registry No.: 77-04-3
Merck Index: 8179

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2

Detector: UV 210

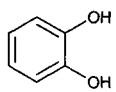
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Pyrocatechol



Molecular formula: $C_6H_6O_2$
Molecular weight: 110.11
CAS Registry No.: 120-80-9
Merck Index: 8183

SAMPLE

Matrix: solutions

Sample preparation: Aqueous food simulants. Pipette 1.0 mL 200 mg/L IS in MeOH into a 25 mL volumetric flask and dilute to the mark with the food simulant obtained from migration experiment, shake. Repeat the procedure to obtain a duplicate sample, filter a portion through a 0.2 μm membrane filter, inject a 20 μL aliquot. Olive oil simulants. Weigh 25 g olive oil food simulant obtained from migration experiment into a beaker, pour oil into a separating funnel, allow beaker to drain for 30 s. Rinse it with 25 mL hexane and add washes to separating funnel. Add 1.0 mL 200 mg/L IS in MeOH into funnel and mix. Add 10 mL water, shake vigorously by hand for 30 s, allow to stand for 5 min. Collect aqueous phase and reextract oil with a 10 mL water. Combine aqueous extracts, make up to 25 mL with water, filter the extracts through a small cotton plug to remove any entrained oil. Repeat the procedure to obtain a duplicate sample. Inject a 20 μL aliquot. (Aqueous food simulants were: distilled water, 3% acetic acid in water; EtOH:water 15:85.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 15:85 (Prepare mobile phase as follows. Dissolve 7.5 g sodium dihydrogen orthophosphate in 800 mL water, add 150 mL MeCN and adjust to pH 3.6 with glacial acetic acid. Make up to 1000 mL with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 8.7
Internal standard: 2-methyl-1,3-dihydroxybenzene (7.4)
Limit of detection: 300-400 ng/g

OTHER SUBSTANCES

Extracted: hydroquinone, resorcinol

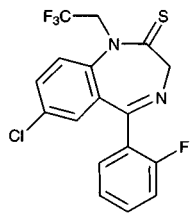
KEY WORDS

aqueous food simulants; olive oil simulants

REFERENCE

Philo,M.R.; Jickells,S.M.; Castle,L. Testing for compliance with migration limits: Determination of 1,2-, 1,3-, and 1,4-dihydroxybenzenes in food-simulating solvents by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 746–750.

Quazepam



Molecular formula: $C_{17}H_{11}ClF_4N_2S$
Molecular weight: 386.80
CAS Registry No.: 36735-22-5
Merck Index: 8211
Lednicer No.: 3 196

SAMPLE

Matrix: blood
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL water, do not allow to dry. Add 100 μL 1 μg/mL diazepam in 1 M pH 10.5 glycine buffer then 500 μL plasma to the column, wash with 2 mL water, wash with 50 μL MeOH, elute with three 200 μL portions of MeOH, combine the eluates and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 50-80 μL aliquot.

HPLC VARIABLES

Column: 75 × 3.9 Nova-Pak C18
Mobile phase: MeOH:2 mM pH 7.2 phosphate buffer 60:40

Sample preparation: Aqueous food simulants. Pipette 1.0 mL 200 mg/L IS in MeOH into a 25 mL volumetric flask and dilute to the mark with the food simulant obtained from migration experiment, shake. Repeat the procedure to obtain a duplicate sample, filter a portion through a 0.2 μm membrane filter, inject a 20 μL aliquot. Olive oil simulants. Weigh 25 g olive oil food simulant obtained from migration experiment into a beaker, pour oil into a separating funnel, allow beaker to drain for 30 s. Rinse it with 25 mL hexane and add washes to separating funnel. Add 1.0 mL 200 mg/L IS in MeOH into funnel and mix. Add 10 mL water, shake vigorously by hand for 30 s, allow to stand for 5 min. Collect aqueous phase and reextract oil with a 10 mL water. Combine aqueous extracts, make up to 25 mL with water, filter the extracts through a small cotton plug to remove any entrained oil. Repeat the procedure to obtain a duplicate sample. Inject a 20 μL aliquot. (Aqueous food simulants were: distilled water, 3% acetic acid in water; EtOH:water 15:85.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 15:85 (Prepare mobile phase as follows. Dissolve 7.5 g sodium dihydrogen orthophosphate in 800 mL water, add 150 mL MeCN and adjust to pH 3.6 with glacial acetic acid. Make up to 1000 mL with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 8.7
Internal standard: 2-methyl-1,3-dihydroxybenzene (7.4)
Limit of detection: 300-400 ng/g

OTHER SUBSTANCES

Extracted: hydroquinone, resorcinol

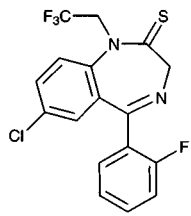
KEY WORDS

aqueous food simulants; olive oil simulants

REFERENCE

Philo,M.R.; Jickells,S.M.; Castle,L. Testing for compliance with migration limits: Determination of 1,2-, 1,3-, and 1,4-dihydroxybenzenes in food-simulating solvents by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 746–750.

Quazepam



Molecular formula: $C_{17}H_{11}ClF_4N_2S$
Molecular weight: 386.80
CAS Registry No.: 36735-22-5
Merck Index: 8211
Lednicer No.: 3 196

SAMPLE

Matrix: blood
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL water, do not allow to dry. Add 100 μL 1 μg/mL diazepam in 1 M pH 10.5 glycine buffer then 500 μL plasma to the column, wash with 2 mL water, wash with 50 μL MeOH, elute with three 200 μL portions of MeOH, combine the eluates and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 50-80 μL aliquot.

HPLC VARIABLES

Column: 75 × 3.9 Nova-Pak C18
Mobile phase: MeOH:2 mM pH 7.2 phosphate buffer 60:40

Flow rate: 1
Injection volume: 50-80
Detector: UV 265

CHROMATOGRAM
Retention time: 17.56
Internal standard: diazepam (8.12)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Gupta,S.K.; Ellinwood,E.H.,Jr. Liquid chromatographic assay and pharmacokinetics of quazepam and its metabolites following sublingual administration of quazepam, *Pharm.Res.*, **1988**, *5*, 365–368.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendi-

metrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 11.94 (A), 17.69 (B)

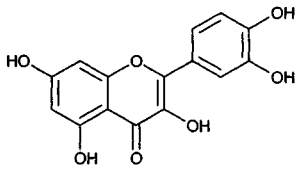
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Quercetin



Molecular formula: $C_{15}H_{10}O_7$
Molecular weight: 302.24
CAS Registry No.: 117-39-5, 6151-25-3 (2.H2O)
Merck Index: 8216

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 14.168

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

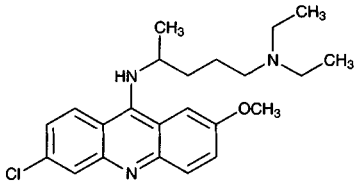
HPLC VARIABLES
Column: 250 × 2.1 Alltima C18 (Alltech)
Mobile phase: MeCN:water 48:52 containing 0.1% trifluoroacetic acid
Flow rate: 0.3
Injection volume: 5
Detector: UV 337

CHROMATOGRAM
Retention time: 3.06

OTHER SUBSTANCES
Simultaneous: apigenin

REFERENCE
Li,B.; Robinson,D.H.; Birt,D.F. Evaluation of properties of apigenin and [G-3H]apigenin and analytic method development, *J.Pharm.Sci.*, **1997**, *86*, 721–725.

Quinacrine



Molecular formula: $C_{23}H_{30}ClN_3O$
Molecular weight: 399.96
CAS Registry No.: 83-89-6, 69-05-6 (di HCl), 6151-30-0 (di HCl dihydrate)
Merck Index: 8225
Lednicer No.: 1 396

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 300 µL 200 mM pH 8 Na_2HPO_4 containing 0.2 mM decylamine + 4 mL dichloromethane, extract (rotamixer), centrifuge at 1200 g. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 200 µL mobile phase, inject a 40 µL aliquot.

HPLC VARIABLES
Column: 250 × 4 7 µm LiChroCart RP-18 (Merck)
Mobile phase: MeOH:10 mM phosphate buffer 65:35 containing 0.1 mM decylamine and 8 mM ethanolamine, adjust pH to 3.0 with HCl
Flow rate: 1
Injection volume: 40
Detector: F ex 270 em 495 following post-column reaction. The column effluent mixed with MeOH:500 mM NaOH 65:35 pumped at 0.3 mL/min and flowed to the detector. (Without post-column reaction use F 285 em 500, sensitivity 3 times less.)

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.2 ng/mL

KEY WORDS
plasma; protect from light; post-column reaction; rinse glassware with mobile phase; decylamine prevents glass adsorption

REFERENCE
Bjorkman,S.; Elisson,L.O. Determination of quinacrine (mepacrine) in plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1987**, *420*, 341–348.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

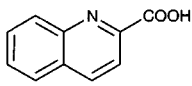
Column: 250 × 4.6 Chirex 3022 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 40:35:25 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 282

CHROMATOGRAM
Retention time: 14, 17.5 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Quinaldic acid



Molecular formula: $C_{10}H_7NO_2$
Molecular weight: 173.17
CAS Registry No.: 93-10-7
Merck Index: 8227

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

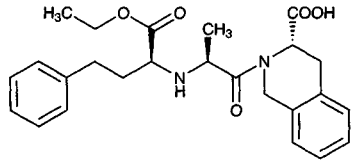
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, ox-

ymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Quinapril



Molecular formula: $C_{25}H_{30}N_2O_5$

Molecular weight: 438.52

CAS Registry No.: 85441-61-8, 90243-99-5 (HCl monohydrate), 82586-55-8 (HCl)

Merck Index: 8233

Lednicer No.: 4 146

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.782

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Add MeOH to powdered capsules or tablets so as to give a quinapril concentration of ca. 130 μg/mL, stir for 15 min, inject a 20 μL aliquot.

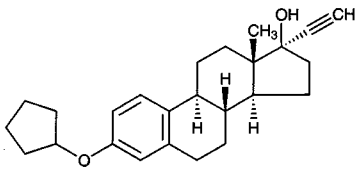
HPLC VARIABLES
Column: 250 × 4.5 5 μm Hypersil ODS
Mobile phase: MeCN:THF:20 mM pH 2.5 sodium heptanesulfonate 45.6:2.4:52
Flow rate: 1
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 23.0

KEY WORDS
capsules; tablets

REFERENCE
Bonazzi,D.; Gotti,R.; Andrisano,V.; Cavrini,V. Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1997**, *16*, 431–438.

Quinestrol



Molecular formula: $C_{25}H_{32}O_2$
Molecular weight: 364.53
CAS Registry No.: 152-43-2
Merck Index: 8239

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets, weigh out amount equivalent to 100 μg quinestrol, suspend in 1 mL water, heat on a steam bath for 5 min, make up to 5 mL with MeCN, vortex for 2 min

HPLC VARIABLES
Column: 250 × 4.6 5-6 μm Zorbax ODS
Mobile phase: MeCN:water 80:20
Flow rate: 2
Injection volume: 50
Detector: UV 281

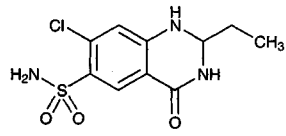
CHROMATOGRAM
Retention time: 8.5

OTHER SUBSTANCES
Simultaneous: degradation products, estrone, estrone 3-cyclopentyl ether, ethinyl estradiol

KEY WORDS
tablets; stability-indicating

REFERENCE
Tsilifonis,D.C.; Chafetz,L. High-pressure liquid chromatographic assay of quinestrol tablets, *J.Pharm.Sci.*, **1980**, *69*, 1461–1463.

Quinethazone



Molecular formula: $C_{10}H_{12}ClN_3O_3S$
Molecular weight: 289.74
CAS Registry No.: 73-49-4
Merck Index: 8240
Lednicer No.: 1 354

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100 mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiCHrosorb RP-18
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 5
Detector: UV 271

CHROMATOGRAM
Retention time: 3.4
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: chlorothiazide, hydrochlorothiazide, chlorthalidone, methyclothiazide, clopamide, furosemide, metolazone, mefruside, bendroflumethiazide, cyclopenthiazide, bumetanide
Simultaneous: indapamide, clorexolone, ethacrynic acid
Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCE
Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 7.6 (A), 7.8 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 1500 ng/mL

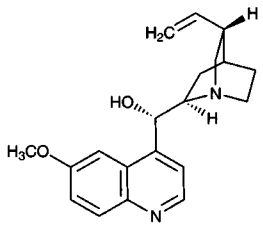
OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, triamterene, flumethiazide, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

Quinidine



Molecular formula: $C_{20}H_{24}N_2O_2$
Molecular weight: 324.42
CAS Registry No.: 56-54-2, 7054-25-3 (gluconate), 7681-28-9 (polygalacturonate), 50-54-4 (sulfate), 6591-63-5 (sulfate dihydrate)
Merck Index: 8244

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M NaOH + 5 mL chlorobutane:MeCN 90:10, shake thoroughly for 1 min, centrifuge at 2500 g for 5 min. Remove the organic phase and add it to 100 μL 100 mM HCl, mix for 1 min, centrifuge at 2500 g for 5 min, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4.55 5 μm Spherisorb CN
Mobile phase: MeCN:20 mM pH 3.0 KH_2PO_4 40:60
Flow rate: 2
Injection volume: 20
Detector: UV 275

CHROMATOGRAM

Retention time: 5.8
Internal standard: quinidine sulfate

OTHER SUBSTANCES

Simultaneous: metoclopramide

KEY WORDS
plasma; quinidine is IS

REFERENCE
Buss,D.C.; Hutchings,A.D.; Scott,S.; Routledge,P.A. A rapid liquid chromatographic method for the determination of metoclopramide in human plasma, *Ther.Drug Monit.*, **1990**, *12*, 293–296.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 500 mM NaOH + 100 μL 5 μg/mL quinine in water, vortex briefly, add 5 mL dichloromethane:MeOH 85:15, shake for 10 min, centrifuge at 1000 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Altex ODS-C18 (Prior to analysis make a conditioning injection of 5 μg each of quinidine, quinine, and quinidine metabolites.)
Mobile phase: MeCN:buffer 4:96 (Buffer was 10 mM K_2HPO_4 adjusted to pH 2.4 with phosphoric acid containing 375 μL/L nonylamine.)
Flow rate: 2
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 3.1
Internal standard: quinine (3.8)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amiodarone, amitriptyline, carbamazepine, chloramphenicol, desipramine, digoxin, ethosuximide, gentamicin, lidocaine, methotrexate, mexiletine, netilmicin, nortriptyline, phenobarbital, phenytoin, pirmenol, primidone, procainamide, propafenone, salicylic acid, sotalol, theophylline, tobramycin, vancomycin

KEY WORDS
serum

REFERENCE
Hoyer,G.L.; Clawson,D.C.; Brookshier,L.A.; Nolan,P.E.; Marcus,F.I. High-performance liquid chromatographic method for the quantitation of quinidine and selected quinidine metabolites, *J.Chromatogr.*, **1991**, *572*, 159–169.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 20% Na_3PO_4 + 2 mL MTBE, vortex for 30 s, centrifuge at 2000 g for 10 min, freeze in dry ice/acetone. Remove the organic phase, repeat the extraction with 2 mL MTBE. Combine the organic phases and evaporate them under a stream of nitrogen, reconstitute the residue in 70 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil cyanopropyl
Mobile phase: Hexane:isopropanol:MeOH 82:4:14 modified with 125 μL triethylamine in MeOH (1:40)
Flow rate: 2
Injection volume: 50
Detector: UV 285

CHROMATOGRAM
Retention time: k' 13.65

Internal standard: quinidine

OTHER SUBSTANCES
Simultaneous: mefloquine

KEY WORDS
plasma; quinidine is IS

REFERENCE
Gimenez,F.; Dumartin,C.; Wainer,I.W.; Farinotti,R. Improved column-switching liquid chromatographic method for the determination of the enantiomers of mefloquine, *J.Chromatogr.*, **1993**, *619*, 161–166.

SAMPLE
Matrix: blood
Sample preparation: Inject a 2 μL aliquot directly.

HPLC VARIABLES
Guard column: 15 × 3.2 10 μm New Guard ODS
Column: 250 × 4.6 10 μm 100 Å Selectosil ODS C18 (Phenomenex)
Mobile phase: MeCN:water:triethylamine:orthophosphoric acid 13:85.4:1:0.6, pH 3.0
Flow rate: 1
Injection volume: 2
Detector: F ex 250 em 430

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; horse; pharmacokinetics; direct injection

REFERENCE
Huang,J.L.; Morgan,D.J. Simple direct injection high-performance liquid chromatographic method to determine quinidine in plasma, *J.Chromatogr.*, **1993**, *620*, 278–280.

SAMPLE
Matrix: blood
Sample preparation: Condition a 10 × 3 Bakerbond C18 SPE cartridge with 4 mL MeOH and 4 mL water at 2 mL/min. Add 100 μL plasma to the SPE cartridge, wash with 2 mL water at 1 mL/min, elute the contents of the SPE cartridge with the mobile phase onto the analytical column for 1 min.

HPLC VARIABLES
Column: Ultrasep C8
Mobile phase: MeCN:water 15:85 containing 0.3% triethylamine, pH adjusted to 2.5 with phosphoric acid
Flow rate: 0.6
Detector: UV 250

CHROMATOGRAM
Retention time: 9
Internal standard: quinine
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: dihydroquinidine

KEY WORDS
SPE; plasma

REFERENCE
Brandsteterová,E.; Romanová,D.; Králiková,D.; Bozeková,L.; Kriska,M. Automatic solid-phase extraction and high-performance liquid chromatographic determination of quinidine in plasma, *J.Chromatogr.A*, **1994**, *665*, 101–104.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 1.5
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, maprotiline, methadone, methaqualone, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: encainide, lidocaine, mexiletine

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood

Sample preparation: 200 μL Serum + 50 μL 40 μg/mL cinchonidine in water + 100 μL 100 mM NaOH + 1.5 mL chloroform, vortex for 30 s, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 60 RP select B
Column: 250 × 4 7 μm Nucleosil 120 C_6H_5
Mobile phase: MeCN:THF:50 mM KH_2PO_4 buffer 15:5:80
Flow rate: 1
Injection volume: 50
Detector: F ex 245 em 340 (cut-off filter) (J. Chromatogr. 1980, 183, 514)

CHROMATOGRAM

Retention time: 14.62
Internal standard: cinchonidine (13.62)
Limit of quantitation: 25 ng/mL

KEY WORDS

serum

REFERENCE

Brandsteterova,E.; Kubalec,P.; Rády,A.; Krcméry,V. Pharmacokinetic interactions of nifedipine and quinidine, *Pharmazie*, **1995**, *50*, 613–616.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 500 ng/mL 4-methylpropranolol in solvent + 1 mL buffer, mix briefly, add 5 mL MTBE, shake vigorously for 10 min, centrifuge at 2500 g for 10 min. Remove the 4 mL of the organic layer and evaporate it to dryness using a vortex evaporator at 30°, reconstitute the residue in 2 mL hexane and 200 μL solvent, vortex for 2 min, discard the upper hexane layer, wash again with 2 mL hexane, inject a 100 μL aliquot of the aqueous phase. (Buffer was 200 mM K_2HPO_4 adjusted to pH 10 with 5 M KOH. Solvent was MeCN:MeOH:10 mM pH 2 sulfuric acid 10:45:45 containing 56 mM sodium octanesulfonate. MTBE was stored over activated charcoal and filtered (Whatman No. 2v) immediately before use. Hexane was purified by stirring 4 volumes hexane with 1 volume concentrated sulfuric acid overnight then washing twice with 1 volume water.)

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Suplex pKb-100 (Supelco)
Column: 150 × 4.6 5 μm Suplex pKb-100 (Supelco)
Mobile phase: MeCN:MeOH:10 mM pH 2 sulfuric acid 10:45:45 containing 10 mM sodium octanesulfonate
Flow rate: 1
Injection volume: 200
Detector: F ex 247 em 270

CHROMATOGRAM

Retention time: 7.5
Internal standard: 4-methylpropranolol (Wyeth-Ayerst) (17.1)
Limit of quantitation: 4 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, diltiazem (UV 237)

KEY WORDS

plasma

REFERENCE

Carignan,G.; Carrier,K.; Laganière,S.; Lessard,M. Simultaneous determination of diltiazem and quinidine in human plasma by liquid chromatography, *J.Chromatogr.B*, **1995**, *672*, 261–269.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 20 μL 10 μg/mL quinine + 200 μL 500 mM pH 8.0 phosphate buffer, mix, add 2 mL MTBE, shake on a reciprocating shaker for 10 min, centrifuge at 4000 rpm for 5 min. Remove 1.8 mL of the organic phase and add it to 100 μL 100 mM HCl, centrifuge at 4000 rpm for 5 min, remove 80 μL of the aqueous phase, vortex, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher RP 18
Mobile phase: MeCN:buffer 16:84 (Buffer was 0.1% triethylamine in water adjusted to pH 2.5 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 40
Detector: UV 250

CHROMATOGRAM
Retention time: 4.38
Internal standard: quinine (4.91)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Meineke,I.; Rohde,S.; Gundert-Remy,U. An inexpensive and sensitive method for the determination of quinidine in plasma by high-performance liquid chromatography with ultraviolet detection, *Ther.Drug Monit.*, **1995**, *17*, 75–78.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 233

CHROMATOGRAM
Retention time: 4.34
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride;

naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF, perilymph

Sample preparation: To 100 μL CSF or plasma or 10 μL perilymph (made up to 20 μL with saline) add 25 μL (100 μL for plasma) ammonia solution. Extract with 250 μL (1 mL for plasma) hexane:ethyl acetate 90:10 by vortexing for 60 s, centrifuge at 1000 g for 10 min, evaporate the organic phase using a nitrogen evaporator at 35°, redissolve the residue in 20 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 8 10 μm μBondapak C18

Column: 100 × 8 10 μm Rad-Pak μBondapak 18

Mobile phase: MeCN:water 9:91 containing 1% triethylamine (Add 30 mL triethylamine to 3 L mobile phase, adjust to pH 2.5 with concentrated orthophosphoric acid.)

Flow rate: 3.5

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 6

Internal standard: quinidine

OTHER SUBSTANCES

Extracted: quinine

KEY WORDS

rat; quinidine is IS

REFERENCE

Chmurzynski,L. High-performance liquid chromatographic determination of quinine in rat biological fluids, *J.Chromatogr.B*, **1997**, *693*, 423–429.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 3.55
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, sulforidazine, thioridazine, thiothixene, tranylcypromine, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: nomifensine, trazodone, quinine, nordoxepin, norfluoxetine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine

Sample preparation: 100 μl Plasma or urine + 1 mL 100 mM NaOH, vortex for 10 s, add 2 mL toluene:butanol 75:25, shake for 10 min, centrifuge at 3000 g for 10 min. Remove the organic layer, add it to 100 μL mobile phase, shake for 10 min, centrifuge at 3000 g for 10 min, discard the organic layer, inject a 40 μl aliquot of the aqueous layer.

HPLC VARIABLES

Column: 100 × 4.6 3 μm CT-sil C18 (Chrom Tech, Hägersten, Sweden)
Mobile phase: MeCN:THF:buffer:1 M phosphoric acid:triethylamine 6:2:92:4:1.6 adjusted to pH 2.7 with phosphoric acid (Buffer was 1 M phosphoric acid:1 M disodium hydrogen phosphate: water 10:10:80, adjusted to pH 2.2.)
Flow rate: 1
Injection volume: 40

Detector: F ex 350 em 450

CHROMATOGRAM
Retention time: 6
Internal standard: quinidine

OTHER SUBSTANCES
Extracted: quinine
Noninterfering: acetaminophen, chloroquine, clomipramine, imipramine, proguanil, salicylate

KEY WORDS
plasma; quinidine is IS

REFERENCE
Mirghani,R.A.; Ericsson,.; Gustafsson,L.L. High-performance liquid chromatographic method for the determination of the major quinine metabolite, 3-hydroxyquinine, in plasma and urine, *J.Chromatogr.B*, **1998**, *708*, 209–216.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 200 μL 200μg/mL pronethalol in water + 1 mL 600 mM pH 9.0 borate buffer + 10 mL dichloromethane:isopropanol 80:20, vortex for 1 min, centrifuge at 540-1200 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot. Urine. 200 μL Urine + 400 μL 200μg/mL pronethalol in water + 200 μL 600 mM pH 9.0 borate buffer + 10 mL dichloromethane:isopropanol 80:20, vortex for 1 min, centrifuge at 540-1200 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak alkyl phenyl
Mobile phase: MeCN:THF:50 mM pH 4.75 phosphate buffer 15:5:80
Flow rate: 1.5
Injection volume: 50
Detector: UV 230 (urine) or F ex 245 em 340 (cut-off filter) (plasma)

CHROMATOGRAM
Retention time: 18.1
Internal standard: pronethalol (12.1)
Limit of detection: 10 ng/mL (F)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Guentert,T.W.; Rakhit,A.; Upton,R.A.; Riegelman,S. An integrated approach to measurements of quinidine and metabolites in biological fluids, *J.Chromatogr.*, **1980**, *183*, 514–518.

SAMPLE
Matrix: blood, urine
Sample preparation: 250 μL Plasma or 100 μL urine + 25 (plasma) or 10 (urine) μL 1 M NaOH + 100 μL 10 μM quinine in MeOH + 3 mL dichloromethane, shake vigorously at 210 rpm for 5 min, centrifuge at 1400 g for 10 min, remove the aqueous phase, freeze at -50° for 1-2 min. Remove the organic layer and evaporate it to dryness at 50°, reconstitute the residue in 0.1-1 mL mobile phase, vortex for 10 s, centrifuge at 1400 g for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 60 RP-select B

Column: 125 × 4 5 μm Lichrospher 60 RP-select B
Mobile phase: MeCN:MeOH:buffer 6.2:17.3:76.5 (Buffer was 14.05 g sodium perchlorate and 1.6 mL 60% perchloric acid in 5 L water, pH 2.5.)
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 415

CHROMATOGRAM
Retention time: 8.87
Internal standard: quinine (10.51)
Limit of detection: 25 nM (urine), 10 nM (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Nielsen,F.; Nielsen,K.K.; Brosen,K. Determination of quinidine, dihydroquinidine, (3S)-3-hydroxyquinidine and quinidine N-oxide in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *660*, 103–110.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM
Retention time: 11.025

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Sample + 9 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:500 mM KH_2PO_4:100 mM tetrabutylammonium hydrogen sulfate:water 22.5:10:25:42.5 adjusted to pH 5.9 with 10 M NaOH
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: milrinone

KEY WORDS
stability-indicating; 5% dextrose; injections

REFERENCE
Riley,C.M. Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1988**, *45*, 2079–2091.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine,

phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: cinchonidine, cinchonine, dihydrocinchonine, dihydrocinchonidine, quinine, dihydroquinidine, dihydroquinine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clen-

buterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 8.72 (A), 4.55 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonaze-

pam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 10-20 μg/mL solution.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultracarb 5 ODS (Phenomenex)
Mobile phase: MeCN:MeOH:50 mM pH 5.2 ammonium acetate 4:35:61
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: cinchonidine, cinchonine, dihydroquinidine, dihydroquinine, quinine

REFERENCE
Theodoridis,G.; Papadoyannis,I.; Hermans-Lokkerbol,A.; Verpoorte,R. A study of the behaviour of some new column materials in the chromatographic analysis of Cinchona alkaloids, *Chromatographia*, **1995**, *41*, 153–160.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)

Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

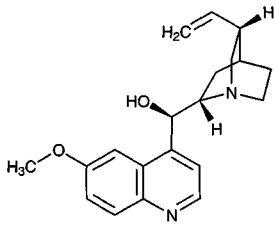
CHROMATOGRAM
Retention time: k' 3.89

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Quinine



Molecular formula: $C_{20}H_{24}N_2O_2$
Molecular weight: 324.42
CAS Registry No.: 130-95-0, 146-40-7 (ascorbate), 549-56-4 (bisulfate), 60-93-5 (di HCl), 83-75-0 (ethylcarbonate), 750-90-3 (monosalicylate), 6119-70-6 (sulfate dihydrate), 804-63-7 (sulfate), 1407-83-6 (tannate), 146-06-5 (carbonate), 549-47-3 (di HBr), 130-90-5 (formate), 4325-25-1 (gluconate), 549-50-8 (HI), 549-49-5 (HBr), 130-89- 2 (HCl), 7631-46-1 (iodosulfate), 10486-11-0 (oleate), 549-52-0 (urea HCl)
Merck Index: 8245
Lednicer No.: 1 337

SAMPLE
Matrix: beverage
Sample preparation: Sonicate 25 mL beverage for 15-20 min, filter (0.45 μm) if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 10:20:70:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: benzoic acid, hydroquinine, saccharin

Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

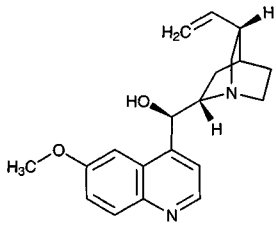
CHROMATOGRAM
Retention time: k' 3.89

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Quinine



Molecular formula: $C_{20}H_{24}N_2O_2$
Molecular weight: 324.42
CAS Registry No.: 130-95-0, 146-40-7 (ascorbate), 549-56-4 (bisulfate), 60-93-5 (di HCl), 83-75-0 (ethylcarbonate), 750-90-3 (monosalicylate), 6119-70-6 (sulfate dihydrate), 804-63-7 (sulfate), 1407-83-6 (tannate), 146-06-5 (carbonate), 549-47-3 (di HBr), 130-90-5 (formate), 4325-25-1 (gluconate), 549-50-8 (HI), 549-49-5 (HBr), 130-89- 2 (HCl), 7631-46-1 (iodosulfate), 10486-11-0 (oleate), 549-52-0 (urea HCl)
Merck Index: 8245
Lednicer No.: 1 337

SAMPLE
Matrix: beverage
Sample preparation: Sonicate 25 mL beverage for 15-20 min, filter (0.45 μm) if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 10:20:70:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: benzoic acid, hydroquinine, saccharin

KEY WORDS
tonic water; soft drinks

REFERENCE
Valenti,L.P. Liquid chromatographic determination of quinine, hydroquinine, saccharin, and sodium benzoate in quinine beverages, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 782–784.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL 40 μg/mL quinine hemisulfate in water, vortex 30 s, add 400 μL MeCN, vortex 1 min, centrifuge at 4000 rpm for 10 min. Remove organic layer and evaporate to 200 μL under a stream of dry nitrogen at 45°. Inject 10-20 μL aliquot.

HPLC VARIABLES
Column: 10 μm μBondapak C18
Mobile phase: MeCN:100 mM NaH_2PO_4 adjusted to pH 3.9 with phosphoric acid 20:80
Flow rate: 2.5
Injection volume: 10-20
Detector: F ex 280 em 455

CHROMATOGRAM
Retention time: 5.2
Internal standard: quinine
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, ofloxacin, pefloxacin, norfloxacin, lomefloxacin, acebutolol
Noninterfering: nadolol, atenolol, thiamine, verapamil, digoxin, metoclopramide, prednisolone, hyoscine, midodrine, deoxyprenaline, metronidazole, ranitidine, gentamicin, netilmicin.

KEY WORDS
serum; quinine is IS

REFERENCE
Jim,L.K.; el-Sayed,N.; al-Khamis,K.I. A simple high-performance liquid chromatographic assay for ciprofloxacin in human serum, *J.Clin.Pharm.Ther.*, **1992**, *17*, 111–115.

SAMPLE
Matrix: blood
Sample preparation: Let 100 μL whole blood dry on filter paper, cut into small pieces. Add dried blood sample or 500 μL plasma, serum, or red blood cells to 150 μL 2 M NaOH, add 6 mL ethylene dichloride, shake for 20 min on an orbital mixer, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness on a vortex evaporator at 60°, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax-Sil
Mobile phase: Dichloromethane:MeOH:1 M perchloric acid 100:9:0.4
Flow rate: 0.8
Injection volume: 25
Detector: UV 254 or F ex 350 em 418 (cut-off filter)

CHROMATOGRAM
Retention time: 11
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, dapsone, desethylchloroquine, dihydroquinidine, dihydroquinine, mefloquine, primaquine, proguanil, pyrimethamine, quinidine, sulfadoxine, sulfalene, sulfamethoxazole

KEY WORDS
plasma; serum; whole blood; red blood cells; normal phase; dried blood; pharmacokinetics

REFERENCE
Dua,V.K.; Sarin,R.; Prakash,A. Determination of quinine in serum, plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 87–93.

SAMPLE
Matrix: blood
Sample preparation: Serum. 50 μL Serum + 50 μL 10 μg/mL IS in water + 50 μL 4 M NaOH + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject 100 μL of the organic layer. Whole blood. 50 μL Whole blood + 500 μL water + 50 μL 10 μg/mL IS in water + 50 μL 4 M NaOH + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject 100 μL of the organic layer. Dried blood. Spread 100 μL whole blood on a 70 × 30 mm piece of filter paper, allow to dry, cut paper into 10 × 5 mm strips, add 50 μL 10 μg/mL IS in water, add 1.5 mL 0.5 M NaOH, vortex for 30 s, let stand for 30 min at room temperature, add 300 μL MTBE, vortex for 30 s, centrifuge at 2000 g for 5 min, inject a 100 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 5 5 μm Spherisorb S5SCX sulfophenylpropyl-modified silica
Mobile phase: MeOH:water 98.5:1.5 containing 9.41 g/L ammonium perchlorate, adjust apparent pH to 8.0 with 220 mL/L 50 mM NaOH in MeOH
Flow rate: 1.5
Injection volume: 100
Detector: F ex 215 em no filter

CHROMATOGRAM
Retention time: 3.5
Internal standard: hydroxychloroquine (8)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: chloroquine, metabolites
Simultaneous: acebutolol, N-acetylprocainamide, atenolol, butriptyline, chlorpromazine, desipramine, flecainide, fluoxetine, imipramine, labetalol, maprotiline, mepacrine, metoprolol, mexiletine, norbutriptyline, normaprotiline, procainamide, propranolol, sotalol
Noninterfering: amitriptyline, amodiaquin, carbamazepine, clomipramine, dapsone, diazepam, dothiepin, doxepin, fluvoxamine, lorazepam, mefloquine, nitrazepam, norclomipramine, nordiazepam, nordothiepin, nordoxepin, nortriptyline, primaquine, proguanil, pyrimethamine

KEY WORDS
serum; whole blood; dried blood

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. Simple and rapid HPLC of quinine, hydroxychloroquine, chloroquine, and desethylchloroquine in serum, whole blood, and filter paper-adsorbed dry blood, *J.Anal.Toxicol.*, **1994**, *18*, 255–260.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 200 μL 500 mM pH 8.0 phosphate buffer, mix, add 2 mL MTBE, shake on a reciprocating shaker for 10 min, centrifuge at 4000 rpm for 5 min. Remove 1.8 mL of the organic phase and add it to 100 μL 100 mM HCl, centrifuge at 4000 rpm for 5 min, remove 80 μL of the aqueous phase, vortex, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher RP 18
Mobile phase: MeCN:buffer 16:84 (Buffer was 0.1% triethylamine in water adjusted to pH 2.5 with 85% phosphoric acid.)
Flow rate: 1.5

Injection volume: 40
Detector: UV 250

CHROMATOGRAM
Retention time: 4.91
Internal standard: quinine

OTHER SUBSTANCES
Extracted: quinidine

KEY WORDS
plasma; quinine is IS

REFERENCE
Meineke,I.; Rohde,S.; Gundert-Remy,U. An inexpensive and sensitive method for the determination of quinidine in plasma by high-performance liquid chromatography with ultraviolet detection, *Ther.Drug Monit.*, **1995**, *17*, 75–78.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 233

CHROMATOGRAM
Retention time: 4.28
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazo-

lam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 220

CHROMATOGRAM

Retention time: 9.8

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, CSF, perilymph

Sample preparation: To 100 μL CSF or plasma or 10 μL perilymph (made up to 20 μL with saline) add 20 μL 2 μg/mL quinidine in water and 25 μL (100 μL for plasma) ammonia solution.

Extract with 250 μL (1 mL for plasma) hexane:ethyl acetate 90:10 by vortexing for 60 s, centrifuge at 1000 g for 10 min, evaporate the organic phase using a nitrogen evaporator at 35°, redissolve the residue in 20 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 8 10 μm μBondapak C18
Column: 100 × 8 10 μm Rad-Pak μBondapak 18
Mobile phase: MeCN:water 9:91 containing 1% triethylamine (Add 30 mL triethylamine to 3 L mobile phase, adjust to pH 2.5 with concentrated orthophosphoric acid.)
Flow rate: 3.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: quinidine (6)
Limit of detection: 10 ng/mL (serum)

KEY WORDS
rat

REFERENCE
Chmurzynski,L. High-performance liquid chromatographic determination of quinine in rat biological fluids, *J.Chromatogr.B*, **1997**, *693*, 423–429.

SAMPLE
Matrix: blood, erythrocytes, urine
Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL buffer. Hemolyze erythrocytes in water 1:3. Dilute urine with water 1:99. 1 mL Plasma, hemolyzed erythrocytes, or diluted urine + 100 μL 5 μg/mL hydroxychloroquine sulfate in MeOH:water 50:50, mix, add to the SPE cartridge, wash with 4 mL buffer, wash with 2 mL MeOH:buffer 50:50, elute with 3 mL MeOH:ammonia 99:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in the initial mobile phase, vortex, inject a 50 μL aliquot. (Prepare buffer by mixing equal volumes of 100 mM ammonium formate and 100 mM ammonia solution, pH 9.2.)

HPLC VARIABLES
Guard column: 10 × 4 Inertsil
Column: 250 × 4 5 μm Inertsil
Mobile phase: Gradient. A was MeCN. B was MeOH:25% ammonia solution 92.5:7.5. A:B 78:22 for 3 min, then to 65:35 over 2 min (Waters curve no. 3), maintain at 65:35 for 20 min, return to 78:22 over 5 min (Waters curve no. 3).
Flow rate: 0.85
Injection volume: 50
Detector: F ex 325 em 375

CHROMATOGRAM
Retention time: 8.53
Internal standard: hydroxychloroquine sulfate (11.5)
Limit of detection: 23.5 ng/mL
Limit of quantitation: 35.5 ng/mL

OTHER SUBSTANCES
Extracted: chloroquine, monodesethylchloroquine, bidesethylchloroquine
Simultaneous: halofantrine, quinidine
Noninterfering: proguanil, cycloguanil, 4-chlorophenylbiguanide, amodiaquine, mefloquine, pyrimethamine, sulfadoxine, cinchonine, cinchonidine

KEY WORDS
plasma; SPE

REFERENCE

Chaulet,J.-F.; Robet,Y.; Prevosto,J.-M.; Soares,O.; Brazier,J.-L. Simultaneous determination of chloroquine and quinine in human biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 303–310.

SAMPLE

Matrix: blood, microsomal incubations, urine

Sample preparation: Plasma. Mix 100 μL plasma with 200 μL cold MeOH, vortex for 10 s, centrifuge at 1500 g for 10 min, inject a 30 μL aliquot. Urine. Dilute 100 μL urine with 900 μL water. Mix 100 μL diluted urine with 200 μL cold MeOH, vortex for 10 s, centrifuge at 1500 g for 10 min, inject a 30 μL aliquot. Microsomal incubation. Mix 500 μL microsomal incubation with 1 mL cold MeOH, vortex for 10 s, centrifuge at 1500 g for 10 min, dilute the supernatant 1 in 4 with water, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 5 μm Hypersil ODS

Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM Na_2HPO_4 containing 10 mM sodium dodecyl sulfate and 0.1 mM tetrabutylammonium bromide, adjusted to pH 2.1 with orthophosphoric acid.)

Flow rate: 0.5

Injection volume: 30

Detector: F ex 350 em 450

CHROMATOGRAM

Retention time: 47.7

Limit of detection: 160 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: piroxicam

Noninterfering: arteether, artemether, chloroquine, cycloguanil, debrisoquinine, 4-hydroxy debrisoquinine, ethambutol, isoniazid, mefloquine, primaquine, proguanil, propranolol, pyrazinamide, rifampicin, streptomycin

Interfering: cinchocaine, cinchonine

KEY WORDS

plasma; liver; microbore

REFERENCE

Wanwimolruk,S.; Wong,S.M.; Zhang,H.; Coville,P.F. Simultaneous determination of quinine and a major metabolite 3-hydroxyquinine in biological fluids by HPLC without extraction, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 293–305.

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Dilute 1 mL urine to 10 mL with water. 1 mL Plasma, saliva, or diluted urine + 20 μL 100 μg/mL primaquine in 100 mM HCl + 200 μL perchloric acid, mix for 5 s, add 1 mL 5 M NaOH, add 4 mL diethyl ether, mix for 1 min, centrifuge at 3000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, mix for 1 min, centrifuge for 5 min, inject a 10 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:MeOH:20 mM KH_2PO_4 10:15:75 containing 74 mM perchloric acid, pH 2.8

Flow rate: 1.2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 7.8

Internal standard: primaquine (10.8)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, amodiaquine, chloroquine, chlorpheniramine, proguanil, promethazine, pyrimethamine

KEY WORDS
plasma

REFERENCE
Babalola,C.P.; Bolaji,O.O.; Dixon,P.A.F.; Ogunbona,F.A. Column liquid chromatographic analysis of quinine in human plasma, saliva and urine, *J.Chromatogr.*, **1993**, *616*, 151–154.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 3.53
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, sulforidazine, thioridazine, thiothixene, tranylcypromine, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: nomifensine, trazodone, quinidine, nordoxepin, norfluoxetine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine

Sample preparation: 100 μL Plasma or urine + 25 μL 292 ng/mL IS in phosphate buffer + 1 mL 100 mM NaOH, vortex for 10 s, add 2 mL toluene:butanol 75:25, shake for 10 min, centrifuge at 3000 g for 10 min. Remove the organic layer, add to 100 μL mobile phase, shake for 10 min, centrifuge at 3000 g for 10 min, discard the organic layer, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 100 × 4.6 3 μm CT-sil C18 (Chrom Tech, Hägersten, Sweden)
Mobile phase: MeCN:THF:phosphate buffer:1 M phosphoric acid:triethylamine 6:2:92:4:1.6, adjusted to pH 2.7 with phosphoric acid (Phosphate buffer was 1 M phosphoric acid:1 M disodium hydrogen phosphate:water 10:10:80 adjusted to pH 2.2.)
Flow rate: 1
Injection volume: 40
Detector: F ex 350 em 450

CHROMATOGRAM
Retention time: 11.6
Internal standard: quinidine (6)
Limit of quantitation: 4.5 nM (plasma), 204.6 nM (urine)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, chloroquine, clomipramine, imipramine, proguanil, salicylic acid

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KEY WORDS
plasma; pharmacokinetics

REFERENCE
Mirghani,R.A.; Ericsson,.; Gustafsson,L.L. High-performance liquid chromatographic method for the determination of the major quinine metabolite, 3-hydroxyquinine, in plasma and urine, *J.Chromatogr.B*, **1998**, *708*, 209–216.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208

CHROMATOGRAM
Retention time: 11.253

KEY WORDS
whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: cinchonidine, cinchonine, dihydrocinchonine, dihydrocinchonidine, quinidine, dihydroquinidine, dihydroquinine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, cocaine, codeine, desipramine, desmethyldoxepin, dextromoramide, diphenhydramine, doxepin, ephedrine, imipramine, methadone, norpropoxyphene, nortriptyline, oxazepam, oxycodone, pentazocine, pericyazine, pheniramine, propoxyphene, propranolol, thiopropazate, thioridazine
Interfering: meperidine, normeperidine

REFERENCE
Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 5 Spherisorb S5SCX
Mobile phase: MeOH:water 98.5:1.5 containing 80 mM ammonium perchlorate, adjusted to pH 8.0 with 50 mM NaOH in MeOH
Flow rate: 1.5
Detector: F ex 215 no emission filter

CHROMATOGRAM

Retention time: 3
Internal standard: hydroxychloroquine (8)

OTHER SUBSTANCES
Simultaneous: hydroquinine, chloroquine

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier, *J.Chromatogr.A*, **1995**, *693*, 289–306.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.34 (A), 4.47 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 10-20 μg/mL solution.

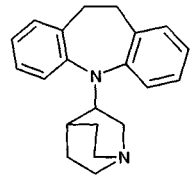
HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultracarb 5 ODS (Phenomenex)
Mobile phase: MeCN:MeOH:50 mM pH 5.2 ammonium acetate 4:35:61
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 19.5

OTHER SUBSTANCES
Simultaneous: cinchonidine, cinchonine, dihydroquinidine, dihydroquinine, quinidine

REFERENCE
Theodoridis,G.; Papadoyannis,I.; Hermans-Lokkerbol,A.; Verpoorte,R. A study of the behaviour of some new column materials in the chromatographic analysis of Cinchona alkaloids, *Chromatographia*, **1995**, *41*, 153–160.

Quinupramine



Molecular formula: $C_{21}H_{24}N_2$
Molecular weight: 304.44
CAS Registry No.: 31721-17-2
Merck Index: 8267

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 7.62
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

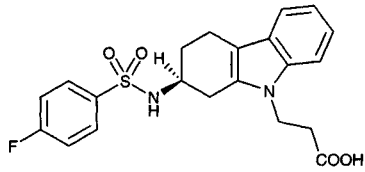
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.168

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ramatroban



Molecular formula: $C_{21}H_{21}FN_2O_4S$
Molecular weight: 416.48
CAS Registry No.: 116649-85-5

SAMPLE
Matrix: blood
Sample preparation: Extract plasma with diethyl ether at pH 6.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Hypersil ODS
Column: 125 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:5mM pH 7.4 tetrabutyl ammonium phosphate buffer 38:62
Column temperature: 40
Flow rate: 1
Detector: UV 284

CHROMATOGRAM
Retention time: 5

KEY WORDS
dog; pharmacokinetics; plasma

REFERENCE
Boberg,M.; Ahi,H.-J.; Beckermann,B.; Bühner,K.; Siefert,H.-M.; Steinke,W.; Wünsche,C.; Hirayama,M. Pharmacokinetics and metabolism of the new thromboxane A2 receptor antagonist ramatroban in animals. 1st Communication: Absorption, concentrations in plasma, metabolism, and excretion after single administration to rats and dogs, *Arzneimittelforschung*, **1997**, *47*, 928–938.

SAMPLE
Matrix: blood
Sample preparation: Mix 200 μL plasma with IS and extract with diethyl ether at pH 6. Evaporate the ether layer to dryness, reconstitute, inject sample onto column A, elute to waste with mobile phase A, switch the eluate containing ramatroban and IS onto column B between 6.4 and 8.6 min. Backflush the contents of column B onto column C with mobile phase B, elute with mobile phase B, monitor the effluent from column C.

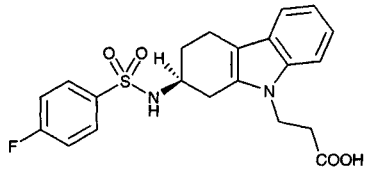
HPLC VARIABLES
Column: A 250 × 4.6 5 μm Hypersil CPS; B 20 × 4.6 5 μm Hypersil ODS; C 125 × 4.6 5 μm Hypersil ODS
Mobile phase: A MeOH:100 mM pH 6 acetate buffer 30:70; B MeOH:THF:pH 6 acetate buffer 33:5:62 (C)
Column temperature: 45
Flow rate: 1.5
Detector: UV 284

CHROMATOGRAM
Retention time: 15.168

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ramatroban



Molecular formula: $C_{21}H_{21}FN_2O_4S$
Molecular weight: 416.48
CAS Registry No.: 116649-85-5

SAMPLE
Matrix: blood
Sample preparation: Extract plasma with diethyl ether at pH 6.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Hypersil ODS
Column: 125 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:5mM pH 7.4 tetrabutyl ammonium phosphate buffer 38:62
Column temperature: 40
Flow rate: 1
Detector: UV 284

CHROMATOGRAM
Retention time: 5

KEY WORDS
dog; pharmacokinetics; plasma

REFERENCE
Boberg,M.; Ahi,H.-J.; Beckermann,B.; Bühner,K.; Siefert,H.-M.; Steinke,W.; Wünsche,C.; Hirayama,M. Pharmacokinetics and metabolism of the new thromboxane A2 receptor antagonist ramatroban in animals. 1st Communication: Absorption, concentrations in plasma, metabolism, and excretion after single administration to rats and dogs, *Arzneimittelforschung*, **1997**, *47*, 928–938.

SAMPLE
Matrix: blood
Sample preparation: Mix 200 μL plasma with IS and extract with diethyl ether at pH 6. Evaporate the ether layer to dryness, reconstitute, inject sample onto column A, elute to waste with mobile phase A, switch the eluate containing ramatroban and IS onto column B between 6.4 and 8.6 min. Backflush the contents of column B onto column C with mobile phase B, elute with mobile phase B, monitor the effluent from column C.

HPLC VARIABLES
Column: A 250 × 4.6 5 μm Hypersil CPS; B 20 × 4.6 5 μm Hypersil ODS; C 125 × 4.6 5 μm Hypersil ODS
Mobile phase: A MeOH:100 mM pH 6 acetate buffer 30:70; B MeOH:THF:pH 6 acetate buffer 33:5:62 (C)
Column temperature: 45
Flow rate: 1.5
Detector: UV 284

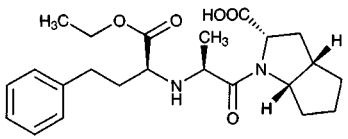
CHROMATOGRAM
Retention time: 17
Internal standard: present but not named
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
column-switching; heart cut; pharmacokinetics; rat; plasma

REFERENCE
Boberg,M.; Ahi,H.-J.; Beckermann,B.; Bühner,K.; Siefert,H.-M.; Steinke,W.; Wünsche,C.; Hirayama,M. Pharmacokinetics and metabolism of the new thromboxane A2 receptor antagonist ramatroban in animals. 1st Communication: Absorption, concentrations in plasma, metabolism, and excretion after single administration to rats and dogs, *Arzneimittelforschung*, **1997**, *47*, 928–938.

Ramipril



Molecular formula: $C_{23}H_{32}N_2O_5$
Molecular weight: 416.52
CAS Registry No.: 87333-19-5
Merck Index: 8283

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 15.678

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Add MeOH:water 50:50 to powdered capsules or tablets so as to give a ramipril concentration of ca. 40 μg/mL, stir for 15 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Hypersil ODS
Mobile phase: MeCN:THF:20 mM pH 2.5 sodium heptanesulfonate 45.6:2.4:52
Flow rate: 1
Injection volume: 20
Detector: UV 215

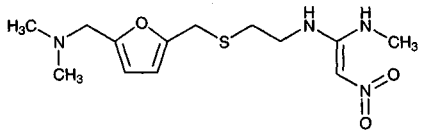
CHROMATOGRAM
Retention time: 19.0

OTHER SUBSTANCES
Simultaneous: benazepril

KEY WORDS
capsules; tablets

REFERENCE
Bonazzi,D.; Gotti,R.; Andrisano,V.; Cavrini,V. Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1997**, *16*, 431–438.

Ranitidine



Molecular formula: $C_{13}H_{22}N_4O_3S$
Molecular weight: 314.41
CAS Registry No.: 66357-35-5, 66357-59-3 (HCl)
Merck Index: 8286
Lednicer No.: 3 131; 4 89, 112, 114

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 10 μg/mL IS in water + 50 μL 1 M NaOH, vortex carefully. Add 1 mL dichloromethane and shake for 1 min. Centrifuge at 700 g for 10 min. Add 100 μL 0.1% phosphoric acid to the organic layer, vortex, let stand at room temperature for 5 min. Inject a 50 μL aliquot of the phosphoric acid layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:10 mM pH 3.75 phosphate buffer 15:85
Flow rate: 1.0
Injection volume: 50
Detector: UV 313

CHROMATOGRAM
Retention time: 4.9
Internal standard: AH 20480, N-[3-[5-[[(dimethylamino)methyl]phenoxy]propyl]]-N'-methyl-2-nitro-1,1'-ethenediamine (6.5)
Limit of detection: 2 ng/mL
Limit of quantitation: 7 ng/mL

OTHER SUBSTANCES
Noninterfering: amikacin, cefotaxime, metamizole, metronidazole

KEY WORDS
serum; pharmacokinetics

REFERENCE
Lopez-Calull,C.; Garcia-Capdevila,L.; Arroyo,C.; Bonal,J. Simple and robust high-performance liquid chromatographic method for the determination of ranitidine in microvolumes of human serum, *J.Chromatogr.B*, **1997**, *693*, 228–232.

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Bond Elut cyano SPE cartridge (Varian) with 1 mL MeCN and two 1 mL portions of extraction buffer. Add 25 μL N-propionylprocainamide to 500 μL plasma, add 500 μL extraction buffer, vortex for 10s. Add to the SPE cartridge, wash with two 500 μL portions of extraction buffer, elute with 250 μL MeCN:water 50:50, vortex the eluate. Inject a 25 μL aliquot. (The extraction buffer was 100 mM K_2HPO_4 adjusted to pH 10.0 with 5 M KOH.)

HPLC VARIABLES
Guard column: 30 × 4.6 40-50 μm C18 (Alltech)
Column: 150 × 3.2 3 μm Hypersil phenyl (Phenomenex)
Mobile phase: MeCN:buffer:triethylamine 12:87.9:0.1 (Buffer was 20 mM K_2HPO_4 adjusted to pH 6.0 with concentrated phosphoric acid.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 228

CHROMATOGRAM
Retention time: 5.6
Internal standard: N-propionylprocainamide (7.2)
Limit of detection: 10 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Farthing,D.; Brouwer,K.L.R.; Fakhry,I.; Sica,D. Solid-phase extraction and determination of ranitidine in human plasma by a high-performance liquid chromatographic method utilizing midbore chromatography, *J.Chromatogr.B*, **1997**, *688*, 350–353.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Plasma. 25 μL Plasma + 50 μL 100 μg/mL cimetidine + 100 μL 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 1650 g for 10 min. Evaporate a 4 mL aliquot of the organic phase. Dissolve the residue in 100 μL mobile phase. Inject a 25 μL aliquot. Tissue. Homogenize brain tissue with 100 μL 25 μg/mL cimetidine and 1 mL saline on ice for 1 min. Add 100 μL 1 M NaOH, extract with 5 mL dichloromethane. Evaporate a 3 mL aliquot of the organic phase. Dissolve the residue in 100 μL mobile phase, centrifuge at 10000 g. Inject a 25 μL aliquot. CSF. Inject a 25 μL aliquot of the CSF directly.

HPLC VARIABLES
Column: 250 × 4 Senshu gel 5C18H (Senshu, Japan)
Mobile phase: MeCN:5 mM NaH_2PO_4 containing 5 mM tetramethylammonium chloride 5:95
Column temperature: 40
Flow rate: 2
Injection volume: 25
Detector: UV 320

CHROMATOGRAM
Internal standard: cimetidine

KEY WORDS
plasma; brain; rat

REFERENCE

Nakada,Y.; Yamamoto,K.; Kawakami,J.; Sawada,Y.; Iga,T. Effect of renal failure on neurotoxicity of ranitidine in rats, *Biol.Pharm.Bull.*, **1996**, *19*, 323–325.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 228.7

CHROMATOGRAM

Retention time: 3.74

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μL Plasma + 100 μL 100 mM NaOH, mix, add 3 mL dichloromethane, shake for 10 min, centrifuge at 2000 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of argon, reconstitute with 500 μL mobile phase, inject a 100 μL aliquot. Urine. Dilute 1 mL urine with 25 mL water, filter (0.2 μm), inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm Spherisorb ODS-2

Column: 150 × 4 5 μm Spherisorb ODS-2

Mobile phase: Gradient. MeCN:7.5 mM pH 6 phosphate buffer 7:93 for 8 min, to 25:75 over 1 min, maintain at 25:75 for 6 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (At the end of each day wash column with MeCN then water then re-equilibrate with mobile phase.)

Flow rate: 1

Injection volume: 100

Detector: F ex 350 em 450 following post-column reaction. The column effluent mixed with 5 mM sodium hypochlorite in 50 mM pH 4.5 sodium acetate buffer pumped at 1 mL/min and this mixture flowed through a 0.8 m × 0.5 mm ID PTFE coil at 25°. The effluent from this coil mixed with 20 mM o-phthalaldehyde in EtOH:500 mM pH 10.5 borate buffer 2:98 pumped at 1 mL/min and 100 mM 2-mercaptoethanol in 500 mM pH 10.5 borate buffer pumped at 1 mL/min and this mixture flowed through a 2.5 m × 0.5 mm ID PTFE coil at 25° to the detector.

(The hypochlorite oxidizes the secondary to a primary amine which then reacts with the o-phthalaldehyde and 2-mercaptoethanol.)

CHROMATOGRAM
Retention time: 13
Limit of detection: 32 ng/mL
Limit of quantitation: 106 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
post-column reaction; plasma

REFERENCE
Vinas,P.; Campillo,N.; Lopez-Erroz,C.; Hernandez-Cordoba,M. Use of post-column fluorescence derivatization to develop a liquid chromatographic assay for ranitidine and its metabolites in biological fluids, *J.Chromatogr.B*, **1997**, *693*, 443–449.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:THF:20 mM pH 6.0 sodium phosphate buffer 30:67.5:2.5
Flow rate: 1
Detector: UV 318

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

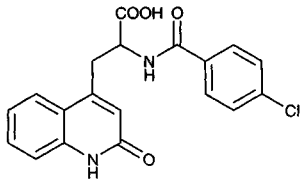
HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS1
Mobile phase: MeOH:50 mM pH 3.0 phosphoric acid 10:90
Column temperature: 30
Flow rate: 1.5
Detector: radioactivity detection

OTHER SUBSTANCES
Also analyzed: atenolol, cimetidine, hydrochlorothiazide

KEY WORDS
14C labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

Rebamipide



Molecular formula: $C_{19}H_{15}ClN_2O_4$
Molecular weight: 370.79
CAS Registry No.: 111911-87-6
Merck Index: 8296

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 500 μL Plasma + 100 μL 10% metaphosphoric acid + 3 mL toluene, shake for 5 min, centrifuge at 1800 g for 5 min, discard the toluene layer. Add 10 μL 40 μg/mL IS in MeOH, 100 μL 10% metaphosphoric acid, and 500 μL ethyl acetate to the aqueous layer, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 100 μL MeOH, inject a 40 μL aliquot. Urine. 500 μL Urine + 500 μL 500 mM NaOH + 3 mL toluene, shake for 5 min, centrifuge at 1800 g for 5 min, discard the toluene layer. Add 10 μL 100 μg/mL IS in MeOH, 1 mL 10% metaphosphoric acid, and 500 μL ethyl acetate to the aqueous layer, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 200 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm YMC Pack A-303 ODS (Yamamura)
Mobile phase: MeCN:THF:acetic acid:water 32:3:1:64
Flow rate: 1.2
Injection volume: 10-40
Detector: UV 280 or F ex 330 em 375

CHROMATOGRAM

Retention time: 6.2
Internal standard: α-(p-chlorobenzamido)-1,2-dihydro-1-methyl-2-oxo-4-quinolinepropionic acid (rebamipide methylated on N of quinoline) (OPC-12 823, Otsuka) (10.0)
Limit of quantitation: 500 ng/mL (urine), 10 ng/mL (plasma)

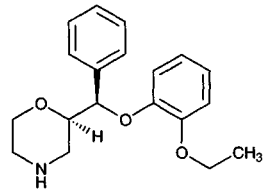
KEY WORDS

plasma; pharmacokinetics

REFERENCE

Shioya,Y.; Shimizu,T. High-performance liquid chromatographic procedure for the determination of a new antigastric ulcer agent, 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propioni c acid, in human plasma and urine, *J.Chromatogr.*, **1988**, *434*, 283–287.

Reboxetine



Molecular formula: $C_{19}H_{23}NO_3$
Molecular weight: 313.40
CAS Registry No.: 98769-81-4

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL Tris buffer + 7 mL diethyl ether, vortex for 1 min, centrifuge at 1200 g for 5 min. Remove the organic phase and add it to 200 μL 10 mM phosphoric acid, vortex for 1 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 200 μL borate buffer and 200 μL reagent, shake, let stand at room temperature for 5 min, add 100 μL 100 mM L-proline in water, add 3 mL n-hexane, vortex for 1 min. Remove

the organic layer and add it to 1 mL MeCN, extract, discard the upper n-hexane layer, wash the MeCN layer with 1 mL n-hexane. Evaporate the MeCN layer to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 200 μL aliquot. (Tris buffer was 25 mL 200 mM Tris solution and 5 mL 100 mM HCl made up to 100 mL with water, pH 9.1. Borate buffer was 61.8 g boric acid in 900 mL water, adjust pH to 8.0 with 20% NaOH, make up to 1 L with water. Prepare reagent by diluting 1 mL 18 mM (+)-1-(9-fluorenyl)ethyl chloroformate in acetone to 50 mL with MeCN.)

HPLC VARIABLES
Guard column: 30-38 μm Survival pellicular ODS (Whatman)
Column: 250 × 4.6 4 μm Supersphere 60 RP-8 (end-capped) (Merck)
Mobile phase: THF:buffer 46.5:53.5 (Prepare buffer by dissolving 13.2 g $(NH_4)_2HPO_4$ in 900 mL water, adjust pH to 7.5 with 85% phosphoric acid, make up to 1 L with water.)
Flow rate: 0.5
Injection volume: 200
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 57 (-), 59 (+)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma; chiral; pharmacokinetics; derivatization

REFERENCE
Frigerio,E.; Pianezzola,E.; Strolin Benedetti,M. Sensitive procedure for the determination of reboxetine enantiomers in human plasma by reversed-phase high-performance liquid chromatography with fluorimetric detection after derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate, *J.Chromatogr.A*, **1994**, *660*, 351–358.

SAMPLE
Matrix: blood
Sample preparation: Buffer 1 mL plasma to 9.1 with 50 mM Tris buffer, extract with diethyl ether. Extract the diethyl ether layer with 10 mM phosphoric acid, wash the aqueous layer with n-hexane, inject an aliquot of the aqueous layer.

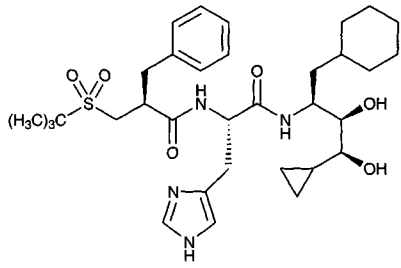
HPLC VARIABLES
Guard column: CO:PELL:ODS
Column: 110 × 4.6 5 μm Partisphere C8 (Whatman)
Mobile phase: MeCN:10 mM pH 2.3 phosphate buffer 64:36
Flow rate: 0.45
Detector: UV 210

CHROMATOGRAM
Internal standard: phenmetrazine
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Edwards,D.M.F.; Pellizzoni,C.; Breuel,H.P.; Berardi,A.; Castelli,M.G.; Frigerio,E.; Poggesi,I.; Rocchetti,M.; Dubini,A.; Strolin Benedetti,M. Pharmacokinetics of reboxetine in healthy volunteers. Single oral doses, linearity and plasma protein binding, *Biopharm.Drug Dispos.*, **1995**, *16*, 443–460.

Remikiren



Molecular formula: $C_{33}H_{50}N_4O_6S$
Molecular weight: 630.85
CAS Registry No.: 126222-34-2

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 5 μL 2 μg/mL IS in MeOH + 1 mL butyl acetate, extract for 2 min, centrifuge at 2000 g for 20 min. Remove the organic layer and evaporate it to dryness under a stream of helium at 60°, reconstitute the residue in 40 μL MeCN and 25 μL 1 M pH 6.3 borate buffer, vortex for 2 min, add 10 μL 25.85 mg/mL 9-fluorenylmethyl chloroformate in MeCN, vortex for 1 min, let stand for 1 h, add 20 μL 225 mM L-proline in water, inject an aliquot.

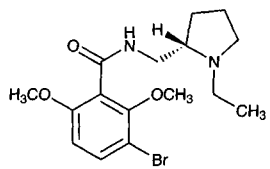
HPLC VARIABLES
Column: 125 × 4 3 μm Nucleosil C18
Mobile phase: A:B 92:8, after 15 min wash with MeCN for 5 min, re-equilibrate at initial conditions for 10 min. A was MeCN containing 350 μg/mL N-hexylmethylamine and 15 mM trifluoroacetic acid, adjusted to pH 3.0. B was 5 mM trifluoroacetic acid in water.
Flow rate: 0.75
Detector: F ex 261 em 308

CHROMATOGRAM
Retention time: 8.6
Internal standard: (S)-α-[(S)-α-[(tertbutylsulfonyl)methyl]hydrocinnamido]-N-[1S,2R,3S-1-(cyclohexylmethyl)-2,3-dihydroxy-4-methylpentyl]imidazole-4-propionamide (Ro 42-4661) (10.8)
Limit of quantitation: 2 ng/mL

KEY WORDS
derivatization; plasma; rat; dog; monkey; pharmacokinetics

REFERENCE
Coassolo,P.; Fischli,W.; Clozel,J.-P.; Chou,R.C. Pharmacokinetics of remikiren, a potent orally active inhibitor of human renin, in rat, dog and primates, *Xenobiotica*, **1996**, *26*, 333–345.

Remoxipride



Molecular formula: $C_{16}H_{23}BrN_2O_3$
Molecular weight: 371.27
CAS Registry No.: 80125-14-0, 82935-42-0 (HCl)
Merck Index: 8301
Lednicer No.: 4 42

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL MeCN, mix for 5 s, add 1 mL saturated sodium carbonate, mix for 5 s, add 7 mL pentane:dichloromethane 75:25, shake gently for 10 min, centrifuge at 18° at 1735 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 140 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:MeOH:40 mM pH 6.8 ammonium acetate 82:8:10
Column temperature: 40
Flow rate: 1.5
Injection volume: 150
Detector: E, ESA Coulochem model 5100A, model 5011 analytical cell, screening electrode 0.6 V, detection electrode 0.92 V, model 5020 guard cell 1 V

CHROMATOGRAM

Retention time: 17
Internal standard: remoxipride
Limit of detection: <2.5 ng/mL

OTHER SUBSTANCES

Extracted: risperidone
Simultaneous: pseudoephedrine
Noninterfering: acetaminophen, benztropine, clonazepam, clozapine, fluphenazine, haloperidol, ibuprofen, lorazepam, trihexyphenidyl

KEY WORDS

plasma; remoxipride is IS

REFERENCE

Aravagiri,M.; Marder,S.R.; Van Putten,T.; Midha,K.K. Determination of risperidone in plasma by high-performance liquid chromatograhy with electrochemical detection: application to therapeutic drug monitoring in schizophrenic patients, *J.Pharm.Sci.*, **1993**, *82*, 447–449.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + IS in 50 mM pH 2 NaH_2PO_4 + 500 μL 1 M NaOH + 4 mL diethyl ether:n-heptane 30:70, rotate for 10 min (if necessary break emulsion by freezing at -20° for 1 h), centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 300 μL pH 2 phosphate buffer, add 750 μL diethyl ether:n-heptane 30:70, vortex for 10 s, inject a 200 μL aliquot of the aqueous phase. Urine. 500 μL Urine + IS in 50 mM pH 2 NaH_2PO_4 + 500 μL 0.2 M NaOH + 4 mL diethyl ether:n-heptane 80:20, rotate for 10 min (if necessary break emulsion by freezing at -20° for 1 h), centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 0.5-3 mL pH 2 phosphate buffer, add 750 μL diethyl ether:n-heptane 30:70, vortex for 10 s, inject a 75 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Nucleosil 120-3C18
Mobile phase: MeCN:pH 2 phosphate buffer 30:70 containing 0.4 mM N,N-dimethyloctylamine and 0.5 mM decyl sulfate (plasma) or MeCN:pH 2 phosphate buffer 25:75 containing 0.2 mM N,N-dimethyloctylamine (urine)
Flow rate: 1.3
Injection volume: 75-200
Detector: UV 206

CHROMATOGRAM

Retention time: 2.2 (urine), 3 (plasma)
Internal standard: 3-bromo-N-[(1-propyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide (3.5 (urine), 5 (plasma))
Limit of quantitation: 2 nM

OTHER SUBSTANCES

Noninterfering: metabolites

KEY WORDS

plasma

REFERENCE
Nilsson,L.B. Determination of remoxipride in plasma and urine by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1990**, *526*, 139–150.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or 50 μL urine + 50 μL 5 μg/mL IS in 100 mM phosphoric acid + 50 μL 100 mM phosphoric acid + 200 μL 1 M NaOH + 5 mL hexane:MTBE 20:80, vortex for 2 min, let stand until the phases separate, freeze in dry ice/acetone. Remove the organic layer and it wash twice with 250 μL portions of 1 M NaOH. Add 500 μL 100 mM phosphoric acid to the organic layer, vortex, let stand for 5 min, discard the organic layer, evaporate any residual organic solvent with a stream of nitrogen, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 50 × 4.6 3 μm Sepralyte C18 (Analytichem)
Mobile phase: MeCN:buffer 25:75 (plasma) or 31:69 (urine) (Buffer was 200 mM sodium perchlorate containing 100 mM phosphoric acid, pH 1.7.)
Column temperature: 40
Flow rate: 1.3
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 1.8 (urine), 2.8 (plasma)
Internal standard: 3-bromo-N-[(1-propyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide (2.6 (urine), 4.5 (plasma))
Limit of quantitation: 50 ng/mL (urine), 12.5 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Chiou,R.H.-Y.; Lo,M.-W. Determination of remoxipride in human plasma and urine by reversed-phase ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *581*, 300–305.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 7 μm Hypercarb porous graphitic carbon (Shandon)
Mobile phase: MeCN:0.1% trifluoroacetic acid 50:50
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3

REFERENCE
Gu,G.; Lim,C.K. Separation of anionic and cationic compounds of biomedical interest by high-performance liquid chromatography on porous graphitic carbon, *J.Chromatogr.*, **1990**, *515*, 183–192.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6

Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.84 (A), 4.64 (B)

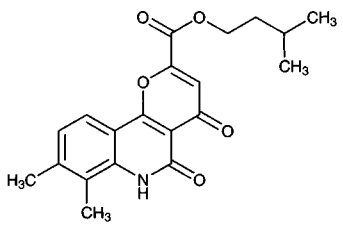
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Repirinast

Molecular formula: $C_{20}H_{21}NO_5$
Molecular weight: 355.39
CAS Registry No.: 73080-51-0
Merck Index: 8305
Lednicer No.: 5 175



SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Adjust pH of 1 mL plasma to 3-4 with 100 µL 1 M HCl, add 1 mL 1 M HCl, add 50 µL 10 µg/mL IS in water, add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat the extraction. Combine the organic phases and add them to 2 mL 100 mM pH 7.5 phosphate buffer, extract. Remove the aqueous layer and add it to 100 µL PIC A low

UV reagent (tetrabutylammonium bromide, Waters), add the mixture to a Bond Elut C8 SPE cartridge, elute with 7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 500 μL mobile phase, inject an aliquot. Urine. Dilute 100 μL urine to 10 mL with pH 2 citrate/HCl buffer, inject an aliquot.

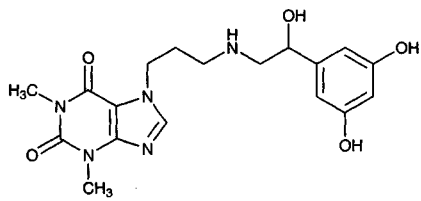
HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:buffer 5:30:65 to 5:45:50 (Buffer was 5 mM pH 3 tetrabutylammonium bromide.)
Column temperature: 65
Flow rate: 1.2
Detector: UV 345

CHROMATOGRAM
Retention time: 9.3 (as the active metabolite, the free acid, BAY w 8199)
Internal standard: BAY × 1453 (10.1)
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Beermann,D.; Schaefer,H.G.; Wargenau,M.; Heibel,B.; Sturm,Y.; Kuhlmann,J. Pharmacokinetics of the active metabolite of the prodrug repirinast in healthy Caucasian volunteers after a single oral dose, *Eur.J.Clin.Pharmacol.*, **1992**, *42*, 307–312.

Reproterol



Molecular formula: $C_{18}H_{23}N_5O_5$
Molecular weight: 389.41
CAS Registry No.: 54063-54-6, 13055-82-8 (HCl)
Merck Index: 8307

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL C18 Bond Elut SPE cartridge with 1mL MeCN and 1 mL 100 mM pH 6.5 ammonium acetate buffer. Mix 250 μL plasma with 10 ng IS, 100 μL 10 mM HCl, and 500 μL 100 mM pH 6.5 ammonium acetate buffer. Make up to 1 mL with 100 mM pH 6.5 ammonium acetate buffer, vortex briefly. Add the mixture to the SPE cartridge. Wash with 400 μL MeCN:2.5 mM pH 6.5 ammonium acetate buffer 20:80, elute with 400 μL mobile phase (also defined as MeCN:2.5 mM pH 6.5 ammonium acetate buffer 80:20). Use the automatic sample processor (ASPEC XL, Gilson, France) to transfer a 100 μL aliquot into autosampler vial. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 30 × 4.6 5 μm. Guard column ODS (30) Phenomenex-Ultracarb (Phenomenex, USA)
Mobile phase: MeCN:2.5 mM pH 6.5 aqueous ammonium acetate 20:80
Flow rate: 1.0
Injection volume: 100
Detector: MS, Finnigan MAT TSQ 7000 triple-stage-quadrupole, positive mode, collision gas xenon, 133.32 Pa, 20 eV, heated vaporiser temperature 475°, sheath gas nitrogen 344.74 KPa, auxiliary gas nitrogen, exact flow rate not measured, 5 μA, heated capillary temperature 180°., m/z 390

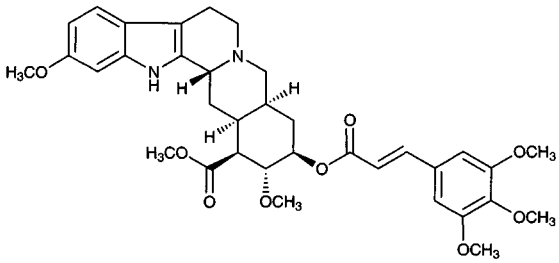
CHROMATOGRAM
Retention time: 0.3-0.5
Internal standard: D-4908 (7-{3-[2-(2,5-dihydroxyphenyl)-2-hydroxyethylamino]-(1-methylpropyl)}theophylline; ASTA Medica, Germany; m/z 404) (0.3-0.5)

Limit of quantitation: 400 pg/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Knebel,N.G.; Winkler,M. Rapid and automated determination of the β2-agonist reproterol in human plasma by atmospheric pressure chemical ionisation high-performance liquid chromatography--tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *702*, 119–129.

Rescinnamine



Molecular formula: $C_{35}H_{42}N_2O_9$
Molecular weight: 634.73
CAS Registry No.: 24815-24-5
Merck Index: 8311
Lednicer No.: 1 319

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaver-

ine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

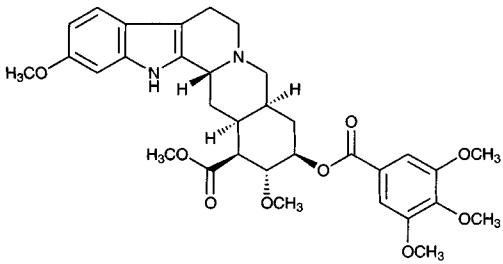
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendi-

metrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Reserpine

Molecular formula: $C_{33}H_{40}N_2O_9$
Molecular weight: 608.69
CAS Registry No.: 50-55-5
Merck Index: 8314



SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 2 mL saturated sodium borate in water + 3 mL benzene (Caution! Benzene is a carcinogen!), rotate at slow speed for 5 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 30 µL reagent, mix well, let stand for at least 10 min, inject a 20 µL aliquot. (Prepare reagent by adding 1 mL saturated vanadium pentoxide in concentrated phosphoric acid to 9 mL MeOH.)

HPLC VARIABLES
Column: 300 × 4 µBondapak C18
Mobile phase: MeOH:10 mM sodium heptanesulfonate 65:35
Flow rate: 2.5
Injection volume: 20
Detector: F ex 390 em 470 (cut-off filter)

CHROMATOGRAM
Retention time: 6
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Interfering: rescinnamine

KEY WORDS
plasma; horse; comparison with TLC method

REFERENCE
Sams,R. Determination of reserpine in plasma using high-performance liquid chromatography with fluorescence detection, *Anal.Lett.*, **1978**, *B11*, 697–707.

SAMPLE
Matrix: blood
Sample preparation: 3 mL Plasma + 45 μL 100 ng/mL IS in 10 mM HCl + 1 mL 600 mM pH 9.5 carbonate buffer + 10 mL n-heptane:isoamyl alcohol 98.5:1.5, shake for 10 min, centrifuge for 10 min. remove the organic layer and add it to 1.2 mL 100 mM HCl, shake for 10 min, centrifuge for 10 min. Remove the aqueous layer and add it to 500 μL 600 mM pH 9.5 carbonate buffer, add 500 μL MTBE, mix, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, add 40 μL reagent, let stand for at least 10 min, inject the whole amount. (Reagent was a 1 in 10 mixture of a saturated solution of vanadium pentoxide in MeOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-1 trimethylsilyl (Supelco)
Mobile phase: MeCN:100 mM pH 4.2 acetate buffer containing 5 mM heptanesulfonate and 10 mM triethylamine
Flow rate: 1.6
Injection volume: 40
Detector: F ex 390 em 480

CHROMATOGRAM
Retention time: 5.2
Internal standard: methyl-18-triethoxybenzoylreserpate (7.8)
Limit of detection: 0.1 ng/mL
Limit of quantitation: 0.3 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Suckow,R.F.; Cooper,T.B.; Asnis,G.M. An improved method for the determination of reserpine in plasma using liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.*, **1983**, *6*, 1111–1122.

SAMPLE
Matrix: blood
Sample preparation: 3 mL Plasma + 3 mL saturated sodium borate in water + 4.5 mL n-hexane:dichloromethane 50:50, mix for 20 min on a rotating tumbler, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 μL reagent, let stand for 25 min, inject a 20 μL aliquot. (Reagent was 1 mL of a saturated solution of vanadium pentoxide in concentrated phosphoric acid and 9 mL MeOH.)

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:10 mM sodium heptanesulfonate in water 65:35
Flow rate: 2
Injection volume: 20
Detector: F ex 390 em 470

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 70 pg/mL

KEY WORDS
plasma; horse; pharmacokinetics; derivatization

REFERENCE
Chapman,C.B.; Courage,P.; Huntington,P.J. Detection of reserpine in horses by high-performance liquid chromatography, *Aust.Vet.J.*, **1991**, *68*, 296–298.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 268

CHROMATOGRAM

Retention time: 5.94

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 218.1

CHROMATOGRAM
Retention time: 16.433

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 25 mg reserpine in 500 μL chloroform, make up to 50 mL with MeOH. Dilute a 20 mL aliquot to 100 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μPorasil
Mobile phase: MeOH or MeOH:20 mg/mL sodium 1-pentanesulfonate in water 100:0.05
Flow rate: 0.7
Injection volume: 20
Detector: F ex 280 em 360

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: ajmalicine

KEY WORDS
normal phase

REFERENCE
Cieri,U.R. Determination of ajmalicine in reserpine raw materials by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1995**, *78*, 944–945.

SAMPLE
Matrix: cell cultures

Sample preparation: Extract 5 g cell culture with 20 mL MeOH with sonication for 10 min, repeat extraction twice. Evaporate extracts to dryness under reduced pressure, reconstitute in 100 mL 10 mM HCl, filter, adjust pH to 6 with 10 mM NaOH, inject a 5-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Armsorb-300-C8 (Armchrom, Yerevan, Armenia)
Mobile phase: Gradient. A was MeCN:water 10:90 containing 0.1% trifluoroacetic acid. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 50:50 over 50 min.
Flow rate: 0.8
Injection volume: 5-100
Detector: UV 280

CHROMATOGRAM
Retention time: 41

OTHER SUBSTANCES
Extracted: ajmaline, ajmalicine, raucaffricine, serpentine, yohimbine

REFERENCE
Klyushnichenko,V.E.; Yakimov,S.A.; Tuzova,T.P.; Syagailo,Y.V.; Kuzovkina,I.N.; Wulfson,A.N.; Miroshnikov,A.I. Determination of indole alkaloids from *R. serpentina* and *R. vomitoria* by high-performance liquid chromatography and high-performance thin-layer chromatography, *J.Chromatogr.A*, **1995**, *704*, 357–362.

SAMPLE
Matrix: formulations
Sample preparation: Add one crushed tablet or 100 mg Rauwolfia serpentina powder to 6 mL MeOH, swirl to disperse, add 60 mL 250 mM sulfuric acid, mix well, extract five times with 30 mL portions of chloroform, pass extracts through column, collect eluates in 50 mL MeOH, evaporate to 25 mL on a steam bath with an air current (in a hood), add 25 mL MeOH, evaporate to 25 mL, make up to 50 mL with MeOH, mix, inject a 100 μL aliquot. (Column was 3 g Celite 545 and 2 mL 100 mM NaOH in a 20 mm dia glass column.)

HPLC VARIABLES
Column: 300 × 3.9 μPorasil
Mobile phase: MeOH
Flow rate: 1.5
Injection volume: 100
Detector: F ex 280 em 360

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: rescinnamine (F 330 em 435)

KEY WORDS
normal phase; reserpine and rescinnamine have same retention time but are discriminated by different detector settings; tablets; powder; rauwolfia serpentina

REFERENCE
Cieri,U.R. Determination of reserpine and rescinnamine in *Rauwolfia serpentina* preparations by liquid chromatography with fluorescence detection, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 540–546.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to one tablet, add 40 mL MeOH, warm on a steam bath for 5 min, cool to room temperature, make up to 100 mL with MeOH, filter through paper, inject 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μPorasil

Mobile phase: MeOH
Flow rate: 1.5
Injection volume: 100
Detector: F ex 280 em 360

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide

KEY WORDS
tablets; normal phase

REFERENCE
Cieri,U.R. Determination of reserpine and hydrochlorothiazide in commercial tablets by liquid chromatography with fluorescence and UV absorption detectors in series, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 515–518.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, add 10 mL DMSO, shake vigorously for 5 min, make up to 100 mL with MeOH, mix, filter (paper), discard the first 5 mL filtrate. Dilute 10 mL of the filtrate to 100 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 75 × 3.9 Novapak silica
Mobile phase: MeOH:20 g/L sodium 1-pentanesulfonate in water 100:1
Flow rate: 1
Injection volume: 20
Detector: F ex 280 em 360

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: chlorothiazide (UV 300)

KEY WORDS
tablets

REFERENCE
Cieri,U.R. Determination of reserpine and chlorothiazide in commercial tablets by liquid chromatography with fluorescence and UV absorbance detectors in series, *J.AOAC Int.*, **1995**, *78*, 1384–1387.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: cimetidine, clomipramine, halofantrine, haloperidol, minoxidil, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 400 × 3 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:acetic acid:water 30:10:0.4:15
Flow rate: 1.2
Injection volume: 40
Detector: F ex 313 em 420

CHROMATOGRAM
Retention time: 14.7

OTHER SUBSTANCES
Simultaneous: tilisolol

REFERENCE
Yonezawa,K.; Sato,K.; Kobayashi,A. High-performance liquid chromatography of a new β-blocker, 4-[3-(tert.-butylamino)-2-hydroxypropoxy]-N-methylisocarbostyril hydrochloride, in plasma using fluorometric detection, *J.Chromatogr.*, **1985**, *339*, 219–222.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 10 μm PRP-1 (Hamilton)
Mobile phase: Gradient. MeCN:20 mM ammonium hydroxide from 15:85 to 100:0 over 17 min
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: cocaine, codeine, methadone, thebaine, yohimbine

REFERENCE
Keystone Scientific Catalog, 1993-4, p. 22.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 9.2

OTHER SUBSTANCES
Simultaneous: trifluprom azine, carphenazine, methotrimeprazine, promazine, perphenazine, chlorprothixene, deserpidine, thiothixene
Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine,

quinine, ranitidine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Develosil ODS-5

Mobile phase: MeOH:water 70:30

Flow rate: 1

Detector: MS, JEOL JMS-SX102A reversed geometry (BE), accelerating voltage +5 kV, air pressure chemical ionization APCI, nebulizer 300°, ion source chamber 400°, discharge electrode, skimmer 1 aperture 300 μm, skimmer 2 aperture 400 μm, no nebulizer gas, m/z 608

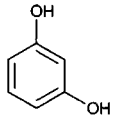
CHROMATOGRAM

Limit of quantitation: 10 pg

REFERENCE

Nojima,K.; Fujimaki,S.; Hertsens,R.C.; Morita,T. Application of liquid chromatography-atmospheric pressure chemical ionization mass spectrometry to a sector mass spectrometer, *J.Chromatogr.A*, **1995**, *712*, 17–19.

Resorcinol



Molecular formula: $C_6H_6O_2$

Molecular weight: 110.11

CAS Registry No.: 108-46-3

Merck Index: 8323

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200

CHROMATOGRAM
Retention time: 8.027

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot of a solution in MeOH:50 mM pH 3.0 triethylamine phosphate 40:60.

HPLC VARIABLES
Column: 150 × 3.2 5 μm Hypersil ODS
Mobile phase: THF:50 mM pH 3.0 triethylamine phosphate 12:88
Flow rate: 0.6
Injection volume: 20
Detector: UV 275 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID crocheted PTFE coil irradiated with an 8 W low-pressure mercury lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: acetaminophen (post-column irradiation gives little increase in peak height), aspirin, caffeine (post-column irradiation gives little increase in peak height), propyphenazone (post-column irradiation gives a decrease in peak height)

KEY WORDS
post-column photochemical derivatization

REFERENCE
Di Pietra,A.M.; Gatti,R.; Andrisano,V.; Cavrini,V. Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics, *J.Chromatogr.A*, **1996**, *729*, 355–361.

SAMPLE
Matrix: solutions
Sample preparation: Aqueous food simulants. Pipette 1.0 mL 200 mg/L IS in MeOH into a 25 mL volumetric flask and dilute to the mark with the food simulant obtained from migration experiment, shake. Repeat the procedure to obtain a duplicate sample, filter a portion through a 0.2 μm membrane filter, inject a 20 μL aliquot. Olive oil simulants. Weigh 25 g olive oil food simulant obtained from migration experiment into a beaker, pour oil into a separating funnel, allow beaker to drain for 30 s. Rinse it with 25 mL hexane and add washes to separating funnel. Add 1.0 mL 200 mg/L IS in MeOH into funnel and mix. Add 10 mL water, shake vigorously by hand for 30 s, allow to stand for 5 min. Collect aqueous phase and reextract oil with a 10 mL water. Combine aqueous extracts, make up to25 mL with water, filter the extracts through a small cotton plug to remove any entrained oil. Repeat the procedure to obtain a duplicate sample. Inject a 20 μL aliquot. (Aqueous food simulants were: distilled water, 3% acetic acid in water; EtOH:water 15:85.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 15:85 (Prepare mobile phase as follows. Dissolve 7.5 g sodium dihydrogen orthophosphate in 800 mL water, add 150 mL MeCN and adjust to pH 3.6 with glacial acetic acid. Make up to 1000 mL with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 6.5
Internal standard: 2-methyl-1,3-dihydroxybenzene (7.4)
Limit of detection: 100 ng/g

OTHER SUBSTANCES
Extracted: hydroquinone, pyrocatechol

KEY WORDS
aqueous food simulants; olive oil simulants

REFERENCE
Philo,M.R.; Jickells,S.M.; Castle,L. Testing for compliance with migration limits: Determination of 1,2-, 1,3-, and 1,4-dihydroxybenzenes in food-simulating solvents by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 746–750.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeOH:10 mM pH 5.5 potassium phosphate buffer 3.5:96.5
Flow rate: 2-3
Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: catechol, hydroquinone (quinol), phenol, phenyl glucuronide, phenyl glucoside, phenyl galactopyranoside, phenyl sulfate

REFERENCE
Beyer,J.; Frank,G. Hydroxylation and conjugation of phenol by the frog *Rana temporaria*, *Xenobiotica*, **1985**, *15*, 277–280.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: Gradient. MeOH:1% acetic acid in water from 0:100 to 75:25 over 25 min, to 100:0 over 0.5 min maintain at 100:0 for 5.5 min, return to initial conditions over 1 min, re-equilibrate for 7 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: phthalic acid, 2-(2',4'-dihydroxybenzoyl)benzoic acid

REFERENCE
Calvey,R.J.; Goldberg,A.L. Liquid chromatographic determination of intermediates in D&C Yellow No. 7 and D&C Yellow No. 8, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 471–473.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Spheri 10 RP-18
Mobile phase: MeOH:water 36:64 containing 20 mM KH_2PO_4
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 4.25

OTHER SUBSTANCES
Simultaneous: phthalic acid

REFERENCE
Lancaster,F.E.; Lawrence,J.F. High-performance liquid chromatographic determination of subsidiary dyes, intermediates and side reaction products in erythrosine, *J.Chromatogr.*, **1987**, *388*, 248–252.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in 1% acetic acid.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere-ODS C18
Mobile phase: Gradient. A was MeCN:acetic acid 99:1. B was 1% acetic acid in water. A:B from 0:100 to 10:90 over 10 min, to 20:80 over 25 min, wash with A for 6 min, re-equilibrate for 14 min.
Flow rate: 2
Injection volume: 20
Detector: F ex 284 em 313

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: catechol (F ex 280 em 325), hydroquinone (F ex 304 em 338), phenol (ex 274 em 298)

REFERENCE
Risner,C.H.; Cash,S.L. A high-performance liquid chromatographic determination of major phenolic compounds in tobacco smoke, *J.Chromatogr.Sci.*, **1990**, *28*, 239–244.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

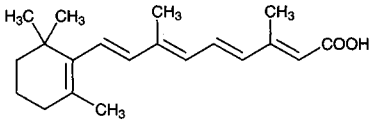
CHROMATOGRAM
Retention time: k' 0.38

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

Retinoic acid



Molecular formula: $C_{20}H_{28}O_2$
Molecular weight: 300.44
CAS Registry No.: 302-79-4 (tretinoin (all-trans)), 4759-48-2 (isotretinoin (13-cis)), 5300-03-8 (alitretinoin (9-cis))
Merck Index: 8333
Lednicer No.: 3 12 (isotretinoin)

SAMPLE
Matrix: blood
Sample preparation: Evaporate 25-100 μL IS in MeOH to near dryness under a stream of nitrogen. Add 0.2-1 mL plasma and 100 μL buffer, extract with 2 mL diethyl ether:ethyl acetate 50:50 for 5 min. Centrifuge at 2000 g for 10 min at 4°, evaporate the organic phase to dryness. Dissolve the residue in 30-100 μL MeOH, inject an aliquot. (Solution was prepared in yellow amber glass and all handling was performed in a room with dim yellow light! Buffer was 25 mM KH_2PO_4 containing 40 mM Na_2HPO_4, pH 7.)

HPLC VARIABLES
Column: 250 × 4.6 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeCN:MeOH:THF 33.25:61.75:5. B was 2% acetic acid. A:B from 75:25 to 88:12 over 11 min, maintain at 88:12 for 19 min, return to initial condition at 30 min, equilibrate for 10 min.
Flow rate: 1
Injection volume: 25
Detector: UV 350

CHROMATOGRAM
Retention time: 24.9 (isotretinoin), 26.7 (tretinoin), 26.2 (alitretinoin (9-cis))
Internal standard: acitretin (21), 13-cis-acitretin (19.8)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, acyclovir, alprazolam, amikacin, amitriptyline, amphotericin B, aspirin, atenolol, bromazepam, caffeine, carbamazepine, ceftriaxone, chlorpromazine, cimetidine, clonazepam, dextromethorphan, diazepam, erythromycin, flunitrazepam, haloperidol, ketoconazole, lorazepam, meprobamate, metronidazole, methylprednisolone, miconazole, midazolam, nifedipine, nitrazepam, netilmicin, nordiazepam, nystatin, oxazepam, phenytoin, prednisolone, prednisone, sulconazole, theophylline, thiopental, zidovudine

KEY WORDS
plasma; rabbit; rat

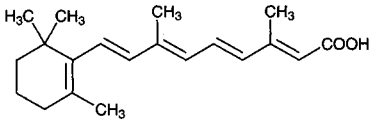
CHROMATOGRAM
Retention time: k' 0.38

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

Retinoic acid



Molecular formula: $C_{20}H_{28}O_2$
Molecular weight: 300.44
CAS Registry No.: 302-79-4 (tretinoin (all-trans)), 4759-48-2 (isotretinoin (13-cis)), 5300-03-8 (alitretinoin (9-cis))
Merck Index: 8333
Lednicer No.: 3 12 (isotretinoin)

SAMPLE
Matrix: blood
Sample preparation: Evaporate 25-100 μL IS in MeOH to near dryness under a stream of nitrogen. Add 0.2-1 mL plasma and 100 μL buffer, extract with 2 mL diethyl ether:ethyl acetate 50:50 for 5 min. Centrifuge at 2000 g for 10 min at 4°, evaporate the organic phase to dryness. Dissolve the residue in 30-100 μL MeOH, inject an aliquot. (Solution was prepared in yellow amber glass and all handling was performed in a room with dim yellow light! Buffer was 25 mM KH_2PO_4 containing 40 mM Na_2HPO_4, pH 7.)

HPLC VARIABLES
Column: 250 × 4.6 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeCN:MeOH:THF 33.25:61.75:5. B was 2% acetic acid. A:B from 75:25 to 88:12 over 11 min, maintain at 88:12 for 19 min, return to initial condition at 30 min, equilibrate for 10 min.
Flow rate: 1
Injection volume: 25
Detector: UV 350

CHROMATOGRAM
Retention time: 24.9 (isotretinoin), 26.7 (tretinoin), 26.2 (alitretinoin (9-cis))
Internal standard: acitretin (21), 13-cis-acitretin (19.8)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, acyclovir, alprazolam, amikacin, amitriptyline, amphotericin B, aspirin, atenolol, bromazepam, caffeine, carbamazepine, ceftriaxone, chlorpromazine, cimetidine, clonazepam, dextromethorphan, diazepam, erythromycin, flunitrazepam, haloperidol, ketoconazole, lorazepam, meprobamate, metronidazole, methylprednisolone, miconazole, midazolam, nifedipine, nitrazepam, netilmicin, nordiazepam, nystatin, oxazepam, phenytoin, prednisolone, prednisone, sulconazole, theophylline, thiopental, zidovudine

KEY WORDS
plasma; rabbit; rat

REFERENCE
Disdier,B.; Bun,H.; Catalin,J.; Durand,A. Simultaneous determination of all-trans-, 13-cis, 9-cis-retinoic acid and their 4-oxometabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *683*, 143–154.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 1.5 mL EtOH containing IS, freeze at -20° for 30 min, centrifuge. Inject a 1.4 mL aliquot of the supernatant onto column A and elute to waste with mobile phase A (time not given). Elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A C18-Corasil or C18-Lichrospher; B two 250 × 4 Supersphere 100 RP-18 endcapped columns in series
Mobile phase: A. MeCN:acetic acid (ratio not given) containing 1% ammonium acetate; B. Gradient. MeCN:acetic acid:10% ammonium acetate:water 60:1:6:30, 95:2:0.5:2, and 99:0.5:0:0.5 (times not given).
Detector: UV 360

CHROMATOGRAM
Internal standard: acitretin
Limit of quantitation: 300 pg/mL

OTHER SUBSTANCES
Extracted: metabolites, 4-oxo-isotretinoin, 4-oxo-tretinoin

KEY WORDS
plasma; column-switching; pharmacokinetics; for isotretinoin and tretinoin

REFERENCE
Chen,C.; Mistry,G.; Jensen,B.; Heizmann,P.; Timm,U.; van Brummelen,P.; Rakhit,A.K. Pharmacokinetics of retinoids in women after meal consumption or vitamin A supplementation, *J.Clin.Pharmacol.*, **1996**, *36*, 799–808.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL serum with 200 μL MeCN and 10 μL 20 mM ascorbic acid, centrifuge at 16000 g for 5 min. Mix the supernatant with 200 μL water, inject a 2 μL aliquot. (Protect all solutions from light.)

HPLC VARIABLES
Column: 50 × 0.18 3 μm ODS-AQ (YMC, Wilmington, NC) (The separation capillary column was formed from fused-silica capillaries (Polymicro Technologies, Phoenix) by inserting a small 50 μm I.D. capillary ca. 15 mm into a larger 180 μm I.D. capillary and fixed by applying epoxy (No. 353ND, Epoxy Technology, Billerica MA). A glass filter paper frit (Whatman GF/A) was inserted into the larger capillary and forced against the smaller capillary with a stream of isopropanol. The stationary phase was suspended in 3 mL isopropanol and pumped into the larger capillary until a 50 mm bed was formed. The larger and smaller diameter capillaries extended no more than 100 and 16 mm from the frit, respectively.)
Mobile phase: MeCN:MeOH:water 65:2.5:32.5 containing 1% tetrabutylammonium perchlorate, adjusted to pH 5.0 with acetic acid and 174 mM sodium acetate
Flow rate: 0.004
Injection volume: 2
Detector: E, carbon-fiber working electrode +900 mV, Ag/AgCl reference electrode (details of preparation in paper)

CHROMATOGRAM
Retention time: 9.0 (isotretinoin), 9.5 (tretinoin)
Limit of detection: 410 pg/mL (isotretinoin), 64 pg/mL (tretinoin)
Limit of quantitation: 49.6 fmol (isotretinoin), 87.6 fmol (tretinoin)

OTHER SUBSTANCES
Extracted: retinaldehyde, retinol

KEY WORDS
cow; serum; capillary HPLC

REFERENCE
Hagen,J.J.; Washco,K.A.; Monnig,C.A. Determination of retinoids by reversed-phase capillary liquid chromatography with amperometric electrochemical detection, *J.Chromatogr.B*, **1996**, *677*, 225–231.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 750 μL 100 ng/mL acitretin in MeCN:9 mM NaOH 20:80, centrifuge at 1500 g for 3 min, inject a 500 μL aliquot onto column A with mobile phase A and elute for 7 min, elute column A in backflush mode with mobile phase A for 3 min, backflush contents of column A onto column B with mobile phase B and start the gradient for mobile phase B. At the end of the process flush the lines with component B of mobile phase B, re-equilibrate columns for 4 min. (Keep sample at 10° in the autosampler.)

HPLC VARIABLES
Column: A 14 × 4.6 37-50 μm Bondapak C18 Corasil (column fitted with 3 μm sieves not glass fiber filters); B 30 × 4 5 μm Spherisorb ODS 1 + 125 × 4 5 μm Spherisorb ODS 1 + 125 × 4 5 μm Spherisorb ODS 1
Mobile phase: A MeCN:1% ammonium acetate 10:90; B Gradient. A was MeCN:water:10% ammonium acetate:acetic acid 600:400:4:30. B was MeCN:water:10% ammonium acetate:acetic acid 850:146:4:10. A:B 100:0 to 70:30 over 6 min, then to 0:100 over 5 min, stay at 0:100 for 11 min.
Flow rate: A 1.5; B 1
Injection volume: 500
Detector: UV 360

CHROMATOGRAM
Retention time: 25 (isotretinoin), 27 (tretinoin)
Internal standard: acitretin (23)
Limit of detection: 0.5-1 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: tretinoin, 4-oxoisotretinoin, 4-oxotretinoin, metabolites

KEY WORDS
plasma; column-switching

REFERENCE
Wyss,R. Determination of retinoids in plasma by high-performance liquid chromatography and automated column switching, *Methods Enzymol.*, **1990**, *189*, 146–155.

SAMPLE
Matrix: culture media
Sample preparation: 100 μL Culture media + 200 μL ice-cold EtOH, mix thoroughly, let stand for 15 min, centrifuge at 12000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: Whatman CO:PELL ODS guard column
Column: 100 × 8 5 μm Nova-Pak C18 (radial-packed)
Mobile phase: MeOH:100 mM pH 7.0 ammonium acetate 90:10
Flow rate: 1
Detector: UV 340

CHROMATOGRAM
Retention time: 9.44 (tretinoin)

OTHER SUBSTANCES
Extracted: isotretin, motretinid, acitretin, Vitamin A (retinol), retinal, etretinate

REFERENCE
Kochhar,D.M.; Penner,J.D.; Minutella,L.M. Biotransformation of etretinate and developmental toxicity of etretin and other aromatic retinoids in teratogenesis bioassays, *Drug Metab.Dispos.*, **1989**, *17*, 618–624.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 1.25 g 0.1% isotretinoin cream or 0.05% isotretinoin gel, make up to 10 mL with MeOH, vortex for 15 min. Cut open two 10 mg isotretinoin capsules, add about 40 mL MeOH, sonicate for 10 min, filter, make up to 100 mL with MeOH. Grind 10% isotretinoin beadlets to powder, weigh out a 1.25 mg aliquot. Add 100 μL cream, gel, or capsule solution to 0.5 mL stainless steel extraction cartridge partially filled with Celite, complete filling with Celite, load the cartridge into the extraction chamber. Alternatively, directly add 1.25 mg beadlets powder to the extraction chamber (Supercritical Fluid Extractor, Isco, model SFX 2-10). Extract using the following conditions: chamber and restrictor temperature 45°, pressure 325 atm, static extraction time 2.5 min, dynamic extraction time 5 min, mobile phase MeOH:CO_2 5:95, solvent trap 17 mL MeOH. After extraction cool the MeOH extract to room temperature for 10 min, make up to 25 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:MeOH:0.05% glacial acid 42.5:32.5:25
Flow rate: 1.2
Injection volume: 20
Detector: UV 360

CHROMATOGRAM
Retention time: 25.0 (isotretinoin), 30.3 (alitretinoin (9-cis)), 33.2 (tretinoin)

OTHER SUBSTANCES
Extracted: 11,13-di-*cis* retinoic acid, 9,13-di-*cis* retinoic acid, 9-*cis* retinoic acid, tretinoin

KEY WORDS
beadlets; capsule; cream; gel; SFE

REFERENCE
Simmons,B.R.; Chukwumerije,O.; Stewart,J.T. Supercritical fluid extraction of 13-cis retinoic acid and its photoisomers from selected pharmaceutical dosage forms, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 395–403.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20 min. Extract twice with 10 mL n-hexane. Evaporate the extracts to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 2.97 (isotretinoin), 3.38 (tretinoin)

OTHER SUBSTANCES
Extracted: retinal, vitamin A, vitamin D2, vitamin D3, vitamin E, vitamin K1, vitamin K2

REFERENCE
Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: silicone oils
Sample preparation: Condition a 1 g Si Bond-Elut SPE cartridge with 5 mL n-hexane. Mix 1 g silicone oil with 2 mL dichloromethane, vortex for 2 min, centrifuge at 3000 g. Withdrawn the supernatant, repeat this procedure twice, filter (0.45 μm), heat the filtrate at 50°, expose to a stream of helium for 30 min. Add 2.5 μg retinol acetate, 2.5 μg α-tocopherol acetate, and 25 μg BHT. Add the mixture to the SPE cartridge, elute with 500 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. A was MeCN:200 mM ammonium acetate 72:25. B was MeOH:water 95:5. A:B 100:0 for 10 min, to 0:100 over 1 min, maintain at 0:100 for 14 min
Flow rate: 2 for 10 min then 1.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 2
Internal standard: retinol acetate (9.5)
Limit of detection: 79.2 ng/mL
Limit of quantitation: 264.1 ng/mL

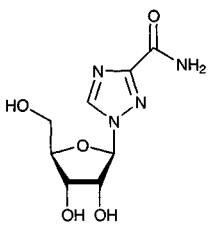
OTHER SUBSTANCES
Extracted: cholesterol (UV 210), retinal (UV 350), α-tocopherol acetate (UV 210), vitamin A (UV 350), vitamin E (UV 210)

KEY WORDS
ophthalmic silicone oils; SPE

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Marinero,P. Simultaneous HPLC determination of cholesterol, α-tocopherol, retinol, retinal and retinoic acid in silicone oils used as vitreous substitutes in eye surgery, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1151–1167.

Ribavirin

Molecular formula: $C_8H_{12}N_4O_5$
Molecular weight: 244.21
CAS Registry No.: 36791-04-5
Merck Index: 8365



SAMPLE
Matrix: blood, CSF
Sample preparation: Prepare a boronate affinity gel SPE column by packing 100 mg Affi-Gel 601 (Bio-Rad) into a 65 × 6 1.5 mL polypropylene column, condition with 10 mL buffer, store at 4°, immediately before use condition further with two 1 mL aliquots of buffer. 500 μL Serum, plasma, or CSF + 500 μL buffer + 25 μL 100 μg/mL 3-methylcytidine methosulfate in water, mix, add to the SPE column, wash with five 1 mL portions of buffer, elute with two 1 mL portions of 100 mM formic acid. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject the whole amount. Buffer was 250 mM pH 8.8 ammonium acetate.)

HPLC VARIABLES
Column: two 300 × 4 μBondapak C18 columns in series
Mobile phase: 10 mM Ammonium phosphate adjusted to pH 2.5 with 85% phosphoric acid
Flow rate: 1.5
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 8.0
Internal standard: 3-methylcytidine methosulfate (11.0)
Limit of detection: 100 nM

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, acyclovir, amikacin, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, amitriptyline, azidothymidine, caffeine, carbamazepine, chloramphenicol, cyclosporin A , cytidine, desipramine, diazepam, digoxin, disopyramide, ethosuximide, gentamicin, imipramine, kanamycin, lidocaine, lithium, methotrexate, 1-methyladenosine, 5-methylcytidine, 7-methylguanosine, 7-methylinosine, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, 2-thiocytidine, tobramycin, uridine, valproic acid, vancomycin, zalcitabine

KEY WORDS
serum; plasma; SPE

REFERENCE
Granich,G.G.; Krogstad,D.J.; Connor,J.D.; Desrochers,K.L.; Sherwood,C. High-performance liquid chromatography (HPLC) assay for ribavirin and comparison of the HPLC assay with radioimmunoassay, *Antimicrob.Agents Chemother.*, **1989**, *33*, 311–315.

SAMPLE
Matrix: blood, tissue, tracheal aspirate
Sample preparation: Dilute tracheal aspirates 10-100 fold with 250 mM pH 8.8 ammonium acetate buffer. Homogenize (glass/PTFE homogenizer) mouse lung with 1 mL water, centrifuge at 13000 g. Filter (Amicon CF25) 1 mL plasma, serum, diluted tracheal aspirate, or lung homogenate while centrifuging at 500 relative centrifugal force (RCF) for 30 min, dilute ultrafiltrate with a volume of 2.5 M pH 8.8 ammonium acetate buffer equal to 10% of the volume of ultrafiltrate, add the mixture to the SPE cartridge, wash with 7 mL 250 mM pH 8.8 ammonium acetate buffer, elute with 6 mL 100 mM formic acid. Lyophilize the eluate, reconstitute with a volume of mobile phase equal to the original volume of ultrafiltrate, inject a 50 μL aliquot. (Soak the CF25 filters in water for 1 h before use. Prepare SPE cartridges by slurrying Matrex PBA-60 gel (88.87 μmole boron per mL, Amicon) in 250 mM pH 8.8 ammonium acetate and packing it into a 40 × 7 column to a bed volume of 1 mL, pass 50 mL 250 mM pH 8.8 ammonium acetate through the column.)

HPLC VARIABLES
Guard column: Guard Pak C18 (Waters)
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: MeOH:buffer 1:99 adjusted to pH 5.10 with 10% ammonium hydroxide or 8% phosphoric acid (Buffer was 20 mM ammonium phosphate. Following each analysis flush column with a gradient of MeCN:water from 0:100 to 80:20.)
Flow rate: 1
Injection volume: 50
Detector: UV 207

CHROMATOGRAM
Retention time: 5.3
Limit of detection: 100 ng/mL

KEY WORDS
mouse; lung; ultrafiltrate; SPE

REFERENCE
Smith,R.H.A.; Gilbert,B.E. Quantification of ribavirin in biological fluids and tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *414*, 202–210.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 1 mL Serum + 5 μg uridine, vortex, add 1 mL water, vortex, filter (Centricon 30 membrane, 30 000 exclusion limit) while centrifuging at 2750 g for 2 h, wash the filtrate with 1.5 mL dichloromethane, pass the aqueous layer through a 10 mm i.d. column containing 2.3 mL wet 50-100 mesh Dowex 1X4-100 (chloride form) (Sigma) on top of 0.8 mL wet 60-150 mesh Lewatit S-1080 (hydrogen form) (Merck), elute with 3 mL water. Collect all the eluate and evaporate it to dryness under vacuum at 30°. reconstitute in 600 μL water, inject a 20-100 μL aliquot. Urine. 200 μL Urine + 5 μg uridine, vortex for a few s, add the urine mixture dropwise with stirring to 3 mL MeOH, centrifuge at 10° at 10000 g for 20 min. Remove the supernatant and add it to 2 mL n-hexane, mix, discard the hexane layer. Evaporate the MeOH layer to dryness under a nitrogen and vacuum, reconstitute the residue in 3 mL water, inject a 100 μL aliquot (J. Chromatogr. 1978, 160 169).

HPLC VARIABLES
Guard column: Guard Pak RP-18 (Waters)
Column: 250 × 5 7 μm LiChrosorb C18
Mobile phase: Water
Flow rate: 1
Injection volume: 20-100
Detector: UV 207

CHROMATOGRAM
Retention time: 6.9
Internal standard: uridine (13)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; ultrafiltrate

REFERENCE
Paroni,R.; Sirtori,C.R.; Borghi,C.; Kienle,M.G. High-performance liquid chromatographic determination of ribavirin in serum and urine and of its urinary metabolite 1,2,4-triazole-3-carboxamide, *J.Chromatogr.*, **1987**, *420*, 189–196.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize hamster brain with phosphate-buffered saline, centrifuge at 1600 g for 10 min. Remove the supernatant and add it to EtOH, heat at 90° to remove EtOH, suspend the protein-free sample in mobile phase, centrifuge at 8300 g for 5 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: TSKgel ODS-120T (Tosoh)
Mobile phase: MeCN:25 mM pH 2.5 buffer 2:98
Flow rate: 1
Injection volume: 10
Detector: UV 226

CHROMATOGRAM
Retention time: 4.7
Limit of detection: 10 μg/g

KEY WORDS
hamster; brain; some interference from endogenous substances; pharmacokinetics

REFERENCE
Ishii,T.; Hosoya,M.; Mori,S.; Shigeta,S.; Suzuki,H. Effective ribavirin concentration in hamster brains for antiviral chemotherapy for subacute sclerosing panencephalitis, *Antimicrob.Agents Chemother.*, **1996**, *40*, 241–243.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine ten-fold with water, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

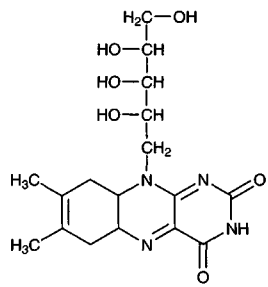
HPLC VARIABLES
Column: 250 × 4.6 Lichrosorb RP18
Mobile phase: Water
Flow rate: 1
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 5 μg/mL

KEY WORDS
mouse

REFERENCE
Ryan,D.M.; Ticehurst,J.; Dempsey,M.H.; Penn,C.R. Inhibition of influenza virus replication in mice by GG167 (4-guanidino-2,4-dideoxy-2,3-dehydro-*N*-acetylneuramic acid) is consistent with extracellular activity of viral neuraminidase (sialidase), *Antimicrob.Agents Chemother.*, **1994**, *38*, 2270–2275.

Riboflavin



Molecular formula: $C_{17}H_{20}N_4O_6$
Molecular weight: 376.37
CAS Registry No.: 83-88-5, 130-40-5 (phosphate sodium)
Merck Index: 8367

SAMPLE
Matrix: blood
Sample preparation: Filter (Amicon 25000 molecular-weight cut-off) serum, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 25 μm pellicular reversed-phase (Whatman)
Column: 300 × 3.9 μBondapak
Mobile phase: Gradient. A was 20 mM pH 5.6 KH_2PO_4. B was MeOH:water 60:40. A:B from 100:0 to 60:40 over 35 min.
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Extracted: creatinine, cytidine, hypoxanthine, inosine, kynurenine, 5-methylcytidine, tryptophan, tyrosine, uric acid, xanthine

KEY WORDS
dog; human; serum

REFERENCE
Assenza,S.P.; Brown,P.R. Comparison of high-performance liquid chromatographic serum profiles of humans and dogs, *J.Chromatogr.*, **1980**, *181*, 169–176.

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 19.67
Internal standard: xanthine (4.65)
Limit of detection: 2.5 ng

OTHER SUBSTANCES
Extracted: ascorbic acid, folic acid, niacin, niacinamide, vitamin B12

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 267.7

CHROMATOGRAM
Retention time: 7.182

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 17
Limit of quantitation: ≤50 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, pyridoxine, vitamin B12, folic acid, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: pantothenic acid, thiamine, niacinamide, pyridoxine

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE
Matrix: formulations
Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1 L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES
Column: 100 × 8 Radial Pak A C18 (Waters)
Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)
Flow rate: 2
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: niacinamide, thiamine, pyridoxine, ascorbic acid (UV 254)

KEY WORDS
multi-vitamin; protect from light; tablets; capsules

REFERENCE
Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil

Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4 (step gradient), 8 (curve gradient), 2 (or riboflavin-5'-phosphate (either system))

OTHER SUBSTANCES
Simultaneous: folic acid (UV 280), niacin, niacinamide, pyridoxamine (UV 280), thiamine, pyridoxine (UV 280), ascorbic acid (UV 280)

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, niacinamide, pyridoxine, thiamine, ascorbic acid

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5

Detector: UV 220

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 100 × 2.1 3 μm Spherisorb ODS-2
Mobile phase: MeOH:buffer 20:80 containing 0.1% triethylamine (Buffer was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid.)
Flow rate: 0.2 for 5 min, to 0.3 over 0.5 min, 0.3 for 12.5 min
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 16
Limit of detection: 1.80 ng

OTHER SUBSTANCES
Simultaneous: folic acid (UV 280), pyridoxine (UV 280), niacin, niacinamide, thiamine

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light; narrow bore

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1
Injection volume: 5
Detector: UV 272

CHROMATOGRAM
Retention time: 11.12
Internal standard: theobromine (8)
Limit of detection: 0.465 ng

OTHER SUBSTANCES
Simultaneous: folic acid, niacin, niacinamide, thiamine, pyridoxine (UV 290)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, pyridoxamine, thiamine, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Simultaneous: niacin, pantothenic acid, pyridoxine, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 780.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4

Detector: UV 254

CHROMATOGRAM
Retention time: 4.2

OTHER SUBSTANCES
Simultaneous: niacin, pyridoxine, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: biotin, folic acid, niacin, pantothenic acid, niacinamide

REFERENCE
MetaChem Catalog, **1995,** p. 21.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Nucleosil C18 SPE cartridge with 2 mL MeOH, 2 mL MeOH containing 5 mM sodium heptanesulfonate, and two 2 mL portions of water. Suspend 5 g homogenized tissue with 35 mL 10 mM HCl, autoclave at 121° for 30 min, add 2 mL 25 mg/mL taka-distase (Fluka) in 2.5 M sodium acetate, add 2 mL 10 (muscle) or 20 (liver) mg/mL clara-distase (Fluka) in water, add 2 mL 50 mg/mL papain (Merck) in water, adjust pH to 4.5, heat at 37° for 16-18 h, filter (paper), adjust pH to 6.5, filter again, make up to 50 mL with water, add 4 mL to the SPE cartridge, wash with 2 mL MeOH:water 20:80 containing 5 mM sodium heptanesulfonate, elute with 2 mL MeOH:water 50:50 containing 5 mM sodium heptanesulfonate, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Nucleosil C18
Column: 150 × 4.6 3 μm Nucleosil C18
Mobile phase: MeCN:10 mM pH 3.0 KH_2PO_4 16:84 (muscle) or 15:85 (liver) containing 5 mM sodium heptane sulfonate (Wash with MeCN:water 20:80 at the end of the day, store column in MeCN.)
Column temperature: 45
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5 (muscle), 5 (liver)
Limit of detection: 160 ng/g

OTHER SUBSTANCES
Extracted: thiamine

KEY WORDS
pig; muscle; liver; protect from light; SPE

REFERENCE

Barna,I.; Dworschák,E. Determination of thiamine (vitamin B1) and riboflavin (vitamin B2) in meat and liver by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *668*, 359–363.

SAMPLE

Matrix: urine
Sample preparation: Centrifuge urine at 1400 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:buffer 35:65 (Buffer was 10 mM KH_2PO_4 adjusted to pH 5.0 with 1 M NaOH.)
Flow rate: 2
Injection volume: 50
Detector: F ex 320-400 em 400-700 (filter)

CHROMATOGRAM

Retention time: 4.5 (riboflavin), 3 (riboflavin-5-phosphate)
Limit of detection: 50 ng/mL

KEY WORDS

protect from light

REFERENCE

Smith,M.D. Rapid method for determination of riboflavin in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 285–291.

SAMPLE

Matrix: urine
Sample preparation: Preserve urine with oxalic acid, centrifuge at 2000 g for 10 min, inject a 25-100 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 34:66
Flow rate: 1
Injection volume: 25-100
Detector: F ex 450 em 530 (long-pass filter)

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: lumichrome, lumiflavin
Noninterfering: acriflavine, apresoline, corticosterone, dipyridamole, estradiol, estriol, estrone, ethylenediamine, hydrocortisone, prednisolone, succinic acid, tetrahydrocortisone, testosterone, urobilin

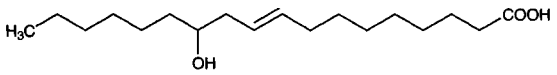
KEY WORDS

protect from light

REFERENCE

Gatautis,V.J.; Naito,H.K. Liquid-chromatographic determination of urinary riboflavin, *Clin.Chem.*, **1981**, *27*, 1672–1675.

Ricinoleic acid



Molecular formula: $C_{18}H_{34}O_3$
Molecular weight: 298.47
CAS Registry No.: 141-22-0
Merck Index: 8378

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.17-1.7 mg/mL solution in MeOH, inject a 30 μL aliquot.

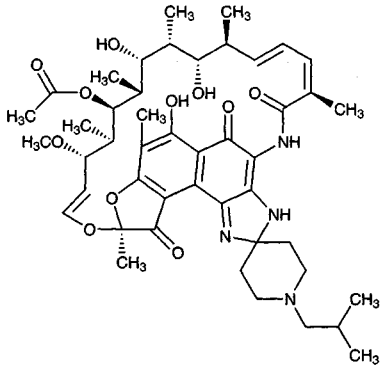
HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeOH containing 0.05% acetic acid. B was water containing 0.05% acetic acid. A:B 85:15 to 100:0 over 40 min, maintain at 100:0.
Flow rate: 1
Injection volume: 30
Detector: evaporative light scattering (ELSD, MK VIII, Varex), drift tube 75°, nitrogen flow 1 L/min, nitrogen pressure 22 psi or UV 205

CHROMATOGRAM
Retention time: 8.95

OTHER SUBSTANCES
Simultaneous: linoleic acid, fatty acids

REFERENCE
Lin,J.-T.; McKeon,T.A.; Stafford,A.E. Gradient reversed-phase high-performance liquid chromatography of saturated, unsaturated and oxygenated free fatty acids and their methyl esters, *J.Chromatogr.A*, **1995**, *699*, 85–91.

Rifabutin



Molecular formula: $C_{46}H_{62}N_4O_{11}$
Molecular weight: 847.02
CAS Registry No.: 72559-06-9
Merck Index: 8380

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C8 SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Plasma + 25 μL 2 μg/mL sulindac in water, vortex briefly, centrifuge at 630 rpm for 5 min, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 RP-8 (Brownlee, ABI)

Column: 250 × 4.6 5 μm Zorbax RX C8
Mobile phase: MeCN:buffer 47:53 (Buffer was 50 mM KH_2PO_4 containing 50 mM sodium acetate, pH adjusted to 4.0 with acetic acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM
Retention time: 10.8
Internal standard: sulindac (6.9)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: atevirdine, delavirdine, U-89,255, U-96,183

KEY WORDS
plasma; protect from light; pharmacokinetics; rugged; SPE

REFERENCE
Lau,Y.Y.; Hanson,G.D.; Carel,B.J. Determination of rifabutin in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1996**, *676*, 125–130.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 500 μL 50 mM pH 7.4 KH_2PO_4, mix, add 2 mL dichloromethane:isooctane 40:60, vortex for 10 min, centrifuge at 1200 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 300 μL mobile phase, add 1 mL n-hexane, wash, centrifuge at 1200 g for 5 min, discard hexane layer, repeat wash, inject a 200 μL aliquot of the aqueous phase. Urine. Inject an aliquot directly.

HPLC VARIABLES
Guard column: 30-38 μm CO:PELL ODS
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:50 mM KH_2PO_4 40:60
Flow rate: 1
Injection volume: 200
Detector: UV 275

CHROMATOGRAM
Retention time: 24.0
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; human; rabbit; monkey

REFERENCE
Battaglia,R.; Pianezzola,E.; Salgarollo,G.; Zini,G.; Strolin Benedetti,M. Absorption, disposition and preliminary metabolic pathway of ^{14}C-rifabutin in animals and man, *J.Antimicrob.Chemother.*, **1990**, *26*, 813–822.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 20 μL 5 μg/mL medazepam in MeOH + 1 mL buffer + 7 mL hexane:ethyl acetate 80:20, vortex for 10 s, centrifuge at 1200 rpm for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under reduced pressure at 40°, reconstitute with 250 μL hexane:ethyl acetate 80:20, add 200 μL 50 mM phosphoric acid, vortex for 10 s, centrifuge at 1200 rpm for 1-2 min, freeze in dry ice/isopro-

panol, discard the organic layer, wash the frozen aqueous layer with 1 mL hexane, thaw, place under reduced pressure at room temperature for 10 min, add 100 μL 250 mM ammonium acetate in MeOH, mix, inject a 100 μL aliquot. Urine. 1 mL Urine + 20 μL 50 μg/mL medazepam in MeOH + 50 μL 3.6 M aqueous sulfuric acid, mix. Remove a 200 μL aliquot and add it to 800 μL 100 mM ammonium acetate in MeOH:water 40:60, mix, inject a 100 μL aliquot. (Buffer was 250 mM KH_2PO_4 containing 50 mM sodium 1-heptanesulfonate adjusted to pH 7.4.)

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP-18 (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:buffer 38:62 (Buffer was 50 mM KH_2PO_4:triethylamine 61.5:0.5 adjusted to pH 4.2 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM
Retention time: 22
Internal standard: medazepam (19)
Limit of quantitation: 100 ng/mL (urine), 5 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: clofazimine
Noninterfering: amikacin, isoniazid, streptomycin, zidovudine

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KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lewis,R.C.; Hatfield,N.Z.; Narang,P.K. A sensitive method for quantitation of rifabutin and its desacetyl metabolite in human biological fluids by high-performance liquid chromatography (HPLC), *Pharm.Res.*, **1991**, *8*, 1434–1440.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM
Retention time: 17.583

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations, tissue
Sample preparation: Tissue. Homogenize liver slices with 200 μL water and about 250 mg 1 mm glass beads, using a Mini Bead Beater (Biospec Products), extract with 1 mL MeCN. Extract a 250 μL aliquot of the slice incubation medium with 1 mL MeCN. Mix, centrifuge at 11000 g for 4 min at 4°. Dry the supernatant under vacuum, reconstitute the residue with 150 μL mobile phase, inject an aliquot. Microsomal incubations. Add 1 mL MeCN to 1 mL microsomal incubation, mix, centrifuge at 1900 g for 15 min at 4°. Extract a 200 μL aliquot of the supernatant with 1 mL MeCN, centrifuge at 11000 g for 4 min at 4°. Dry the supernatant under vacuum, reconstitute the residue with 150 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18 Sentry Guard (Waters)
Column: 250 × 4.6 5 μm Symmetry C18 (Waters)
Mobile phase: MeCN:10 mM pH 4.0 ammonium phosphate triethylamine50:49.9:0.1
Flow rate: 1
Detector: UV 278

CHROMATOGRAM
Retention time: 31.6

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Jamis-Dow,C.A.; Katki,A.G.; Collins,J.M.; Klecker,R.W. Rifampin and rifabutin and their metabolism by human liver esterases, *Xenobiotica*, **1997**, *27*, 1015–1024.

SAMPLE
Matrix: tissue
Sample preparation: Vortex 200 μL incubation mixture and 1 mL MeOH containing 0.1% acetic acid, immerse in ice for 15 min, centrifuge at 6500 g for 5 min, evaporate the supernatant to dryness under nitrogen, reconstitute the residue in 50 μL mobile phase, combine two aliquots, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: Gradient. A was 3% triethylamine containing 0.3% trifluoroacetic acid adjusted to pH 2 with phosphoric acid. B was MeCN. A:B from 73:27 to 58:42 over 45 min
Flow rate: 1.5
Injection volume: 75
Detector: Radioactivity, Radiomatic A-500 (Flow Scintillation Analyzer)

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver; enterocytes; intestine

REFERENCE
Iatsimirskaia,E.; Tulebaev,S.; Storozhuk,E.; Utkin,I.; Smith,D.; Gerber,N.; Koudriakova,T. Metabolism of rifabutin in human enterocyte and liver microsomes: kinetic parameters, identification of enzyme systems, and drug interactions with macrolides and antifungal agents, *Clin.Pharmacol.Ther.*, **1997**, *61*, 554–562.

SAMPLE
Matrix: urine
Sample preparation: Inject a 50-200 μL aliquot of urine directly.

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:50 mM pH 4.5 KH_2PO_4 40:60 for 10 min, to 60:40 over 5 min, maintain at 60:40 for 5 min.
Flow rate: 1 for 10 min then 1.5
Injection volume: 50-200
Detector: UV 275 or radioactivity

CHROMATOGRAM
Retention time: 16.5

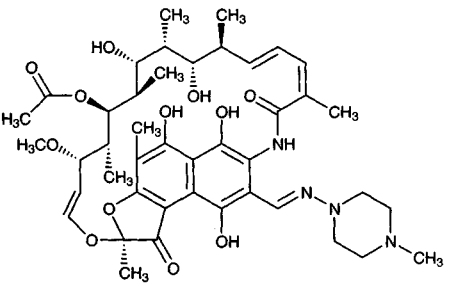
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat

REFERENCE
Battaglia,R.; Salgarollo,G.; Zini,G.; Montesanti,L.; Strolin Benedetti,M. Absorption, disposition, and urinary metabolism of ^{14}C-rifabutin in rats, *Antimicrob.Agents Chemother.*, **1991**, *35*, 1391–1396.

Rifampin

Molecular formula: $C_{43}H_{58}N_4O_{12}$
Molecular weight: 822.95
CAS Registry No.: 13292-46-1
Merck Index: 8382



SAMPLE
Matrix: blood
Sample preparation: Add 400 μL 1 M pH 4.0 phosphate buffer and 40 μL 10 mg/mL ascorbic acid solution to 1 mL plasma. Mix for 10 s, add 4 mL ethyl acetate. Vortex for 5 min, centrifuge at 1800 g for 10 min. Remove a 3 mL aliquot of the aqueous layer and evaporate it to dryness under nitrogen at 100°. Reconstitute the residue with 500 μL MeOH, vortex for 1 min. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb
Mobile phase: MeOH:10 mM pH 5.5 phosphate buffer 68:32
Column temperature: 28
Flow rate: 1

Injection volume: 20
Detector: UV 336

CHROMATOGRAM
Retention time: 4.33
Internal standard: rifampin

OTHER SUBSTANCES
Extracted: rifapentine

KEY WORDS
serum; rifampin is IS

REFERENCE
He,X.; Wang,J.; Liu,X.; Chen,X. High-performance liquid chromatography assay of rifapentine in human serum, *J.Chromatogr.B*, **1996**, *681*, 412–415.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL MeCN to 100 μL plasma, vortex for 10 s, centrifuge for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 4 μm Nova-Pak C8
Mobile phase: MeCN:MeOH:10 mM potassium dihydrogen phosphate 35:5:60
Flow rate: 1
Injection volume: 20
Detector: UV 334

CHROMATOGRAM
Retention time: 6.5
Limit of quantitation: 200 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Le Guellec,C.; Gaudet,M.-L.; Lamanetre,S.; Breteau,M. Stability of rifampin in plasma: Consequences for therapeutic monitoring and pharmacokinetic studies, *Ther.Drug Monit.*, **1997**, *19*, 669–674.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C2 SPE cartridge with 1 mL MeOH and 1 mL 100 mM HCl just before use. 500 μL Plasma + 500 μL 100 mM HCl + 50 μL 19 μg/mL sulindac in MeOH, vortex briefly, add to SPE cartridge, wash with 1 mL 100 mM HCl, elute with 400 μL MeOH:MeCN 60:40, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 RCSS Guard Pak
Column: 100 × 8 4 μm Nova Pak C18 Radial Pak
Mobile phase: MeCN:buffer 42:58 (Buffer was 50 mM sodium citrate adjusted to pH 4.3 with 50 mM HCl.)
Flow rate: 2.3
Injection volume: 20
Detector: UV 342

CHROMATOGRAM
Retention time: 4.65
Internal standard: sulindac (3.07)
Limit of quantitation: 160 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Swart,K.J.; Papgis,M. Automated high-performance liquid chromatographic method for the determination of rifampicin in plasma, *J.Chromatogr.*, **1992**, *593*, 21–24.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 50 μL 50 μg/mL N-butarylaminophenol + 400 μL 10% acetic acid + 7 mL diethyl ether:dichloromethane 2:1, shake, centrifuge at 2059 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 30 μm C8
Column: 250 × 4.6 5 μm Spherisorb C8
Mobile phase: Gradient. MeCN:5 mM pH 3.5 phosphate buffer from 6:94 to 90:10 over 5 min, maintain at 90:10 for 12 min.
Flow rate: 2
Injection volume: 20
Detector: UV 248

CHROMATOGRAM
Retention time: 11.9
Internal standard: N-butarylaminophenol (4.22)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: pyrazinamide

KEY WORDS
plasma

REFERENCE
Walubo,A.; Smith,P.; Folb,P.I. Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 391–396.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 15 mg SPEC.C8 SPE disc (ANSYS) or a Bond-Elut C8 SPE cartridge with two 1 mL portions of MeCN and two 1 mL portions of 100 mM HCl. 500 μL Serum + 200 μL 5% ascorbic acid in 100 mM HCl + 100 μL 50 μg/mL papaverine hydrochloride in water, mix well, add to the SPE disc or cartridge, apply a vacuum for 20 min, elute with five 100 μL portions of MeCN:MeOH 60:40. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL 100 mM HCl:water 67:33, inject a 50 μL aliquot. (Disc gave higher recoveries than cartridge.)

HPLC VARIABLES
Guard column: ODS
Column: 250 × 4.6 Spherisorb ODS-1
Mobile phase: MeCN:100 mM pH 4.7 KH_2PO_4 35:65
Column temperature: 40
Flow rate: 1.2
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 11.5
Internal standard: papaverine (14.9)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE

REFERENCE
Ye,L.; Stewart,J.T.; Zhang,H. A comparison of disc and cartridge solid-phase extraction for the LC determination of rifampin and 25-desacetylrifampin in human serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1185–1188.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C2 SPE cartridge with 1 mL MeOH and 1 mL water. 500 µL Plasma + 25 µL 2 µg/mL sulindac in water + 100 µL 100 mM HCl, vortex briefly, centrifuge at 630 rpm for 5 min, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeOH, add the eluate to 500 µL 3 mg/mL ascorbic acid, inject a 150 µL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 µm RP-8 (Brownlee, ABI)
Column: 250 × 4.6 5 µm Zorbax RX C8
Mobile phase: MeCN:50 mM KH_2PO_4 45:55
Flow rate: 1
Injection volume: 150
Detector: UV 340

CHROMATOGRAM
Retention time: 4.4
Internal standard: sulindac (7.8)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: atevirdine, delavirdine, U-89,255, U-96,183

KEY WORDS
plasma; protect from light; pharmacokinetics; rugged; SPE

REFERENCE
Lau,Y.Y.; Hanson,G.D.; Carel,B.J. Determination of rifampin in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1996**, *676*, 147–152.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 µL CSF or plasma + dimethylaminobenzoic acid + 200 µL MeOH, mix, add 1 mL buffer, add 7 mL dichloromethane:diethyl ether 40:60, shake for 15 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness AT 45°, reconstitute the residue in 50 µl MeOH, vortex for 10 s, inject a 25 µL aliquot. (Buffer was 1 M KH_2PO_4 containing 0.2% ascorbic acid, pH adjusted to 4.2 with 1 M HCl.)

HPLC VARIABLES
Guard column: 50 × 4.6 30 µm C8
Column: 250 × 4.6 5 µm Lichrosorb RP-8
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 48:52
Flow rate: 1.5
Injection volume: 25
Detector: UV 215

CHROMATOGRAM
Retention time: 5.8
Internal standard: dimethylaminobenzoic acid (3.8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: p-aminosalicylic acid, pyrazinamide, isoniazid

KEY WORDS
plasma; rabbit

REFERENCE
Chan,K. Rifampicin concentrations in cerebrospinal fluid and plasma of the rabbit by high performance liquid chromatography, *Methods Find.Exp.Clin.Pharmacol.*, **1986**, *8*, 721–726.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM
Retention time: 12.93, 16.28, 17.16 (compound undergoes decomposition)
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES
Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, trimeprazine, trimethoprim

KEY WORDS
plasma; serum; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, saliva, urine
Sample preparation: 1 mL Plasma, urine, or saliva + 50 μL MeOH + 1 mL buffer + 1 mL isooctane:dichloromethane 60:40, shake mechanically at 350 rpm for 10 min, centrifuge at 2000 g for 5 min, inject a 5-200 μL aliquot of the supernatant. (Buffer was 2 g ascorbic acid and 10 g anhydrous sodium sulfate made up to 50 mL with concentrated pH 6 buffer solution (Merck Cat. No. 9886), stir continuously, prepare fresh each day.)

HPLC VARIABLES
Column: 100 × 7.5 5 μm LiChrosorb Si 60
Mobile phase: Isooctane:dichloromethane:EtOH:water:acetic acid 45:36.6:16.8:1.65:0.002

Flow rate: 3
Injection volume: 5-200
Detector: UV 254

CHROMATOGRAM
Retention time: 3
Limit of detection: 20 ng/mL (Clin.Pharmacol.Ther. 1991, 50, 682)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lecaillon,J.B.; Febvre,N.; Metayer,J.P.; Souppart,C. Quantitative assay of rifampicin and three of its metabolites in human plasma, urine and saliva by high-performance liquid chromatography, *J.Chromatogr.*, **1978**, *145*, 319–324.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 236.9

CHROMATOGRAM
Retention time: 16.167

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of

maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 225.2

CHROMATOGRAM
Retention time: 20.85

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:50 mM pH 4.7 KH_2PO_4 40:60
Flow rate: 1
Injection volume: 75
Detector: UV 340

CHROMATOGRAM
Retention time: 6.2
Internal standard: papaverine
Limit of detection: 100-1000 ng/mL

REFERENCE
Darouiche,R.O.; Hamill,R.J. Antibiotic penetration of and bactericidal activity within endothelial cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1059–1064.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 208TP54 C8
Mobile phase: MeCN:buffer 32.8:67.2 (Buffer was 50 mM K_2HPO_4 adjusted to pH 6.5 with phosphoric acid.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 6.8

OTHER SUBSTANCES
Simultaneous: minocycline, degradation products

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Pearson,S.D.; Trissel,L.A. Stability and compatibility of minocycline hydrochloride and rifampin in intravenous solutions at various temperatures, *Am.J.Hosp.Pharm.*, **1993**, *50*, 698–702.

SAMPLE
Matrix: microsomal incubations, tissue
Sample preparation: Tissue. Homogenize liver slices with 200 μL water and about 250 mg 1 mm glass beads, using a Mini Bead Beater (Biospec Products), extract with 1 mL MeCN. Extract a 250 μL aliquot of the slice incubation medium with 1 mL MeCN. Mix, centrifuge at 11000 g for 4 min at 4°. Dry the supernatant under vacuum, reconstitute the residue with 150 μL mobile phase, inject an aliquot. Microsomal incubations. Add 1 mL MeCN to 1 mL microsomal incubation, mix, centrifuge at 1900 g for 15 min at 4°. Extract a 200 μL aliquot of the supernatant with 1 mL MeCN, centrifuge at 11000 g for 4 min at 4°. Dry the supernatant under vacuum, reconstitute the residue with 150 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18 Sentry Guard (Waters)
Column: 250 × 4.6 5 μm Symmetry C18 (Waters)
Mobile phase: MeCN:10 mM pH 4.0 ammonium phosphate:triethylamine 30:69.9:0.1
Flow rate: 1
Detector: UV 240

CHROMATOGRAM
Retention time: 34.4

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Jamis-Dow,C.A.; Katki,A.G.; Collins,J.M.; Klecker,R.W. Rifampin and rifabutin and their metabolism by human liver esterases, *Xenobiotica*, **1997**, *27*, 1015–1024.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge and filter cell solutions (0.22 μm), inject an aliquot.

HPLC VARIABLES
Guard column: Guard-PAK C18 (Waters)
Column: 150 × 3.9 5 μm NOVA PAK C18
Mobile phase: MeCN:50 mM pH 6.0 KH_2PO_4 40:60
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.3

REFERENCE
Koga,H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes, *Antimicrob.Agents Chemother.*, **1987**, *31*, 1904–1908.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 5 mg/mL solution in MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 4.6 Hypersil C18
Mobile phase: MeOH
Flow rate: 1
Injection volume: 10
Detector: MS, HP 5985B quadrupole MS, direct liquid introduction interface, 5 μm orifice, water-cooled stainless steel diaphragm, ion source pressure 1 Torr, ion source 250°, LC eluent used as reagent gas

OTHER SUBSTANCES
Also analyzed: rifapentine, rifamycin SV

REFERENCE
Vékey,K.; Edwards,D.M.F.; Zerilli,L.F. Liquid chromatographic-mass spectrometric studies on rifamycin antibiotics, *J.Chromatogr.*, **1989**, *474*, 317–327.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Excalibar C18-CN (Alltech)
Mobile phase: MeOH:5 mM tetra-n-butylammonium hydroxide 80:20 adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 4.3
Internal standard: rifampin

OTHER SUBSTANCES
Simultaneous: isoniazid, pyrazinamide

KEY WORDS
rifampin is IS

REFERENCE
Gaitonde,C.D.; Pathak,P.V. Rapid liquid chromatographic method for the estimation of isoniazid and pyrazinamide in plasma and urine, *J.Chromatogr.*, **1990**, *532*, 418–423.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in MeOH:water 50:50 (electrospray) or a 400 μg/mL solution in MeOH:200 mM ammonium acetate 50:50 (thermospray), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeOH:200 mM ammonium acetate 60:40
Flow rate: 1.2
Injection volume: 20
Detector: MS, Delsi/Nermag R3010 triple quadrupole, Delsi/Nermag electrospray interface, flow rate 1 μL/min into source or Finnigan MAT TSQ 70 triple quadrupole, Finnigan TSPI interface and source, positive ion, discharge off, filament off, vaporizer 75°, block (jet) 330

CHROMATOGRAM
Retention time: 13.5

OTHER SUBSTANCES
Simultaneous: rifamycin SV, rifamycin B

KEY WORDS
electrospray; thermospray

REFERENCE
Korfmacher,W.A.; Bloom,J.; Churchwell,M.I.; Getek,T.A.; Hansen,E.B.,Jr.; Holder,C.L.; McManus,K.T. Characterization of three rifamycins via electrospray mass spectrometry and HPLC-thermospray mass spectrometry, *J.Chromatogr.Sci.*, **1993**, *31*, 498–501.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 160 × 4 5 μm Zorbax Rx-C8
Mobile phase: MeOH:buffer 70:30 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.7
Injection volume: 10
Detector: UV 265

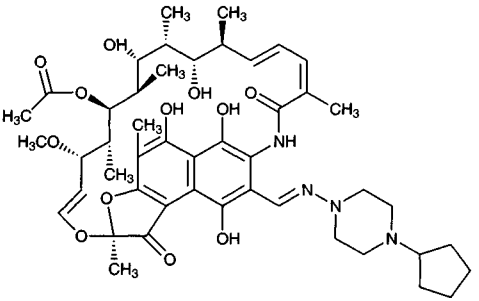
CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Simultaneous: pyrazinamide

REFERENCE
Nahata,M.C.; Morosco,R.S.; Peritore,S.P. Stability of pyrazinamide in two suspensions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1558–1560.

Rifapentine

Molecular formula: $C_{47}H_{64}N_4O_{12}$
Molecular weight: 877.04
CAS Registry No.: 61379-65-5
Merck Index: 8385



SAMPLE
Matrix: blood
Sample preparation: Add 400 μL 1 M pH 4.0 phosphate buffer, 40 μL 10 mg/mL ascorbic acid solution, and 50 μL 100 μg/mL rifampin solution to 1 mL plasma. Mix for 10 s, add 4 mL ethyl acetate. Vortex for 5 min, centrifuge at 1800 g for 10 min. Remove a 3 mL aliquot of the aqueous layer and evaporate it to dryness under nitrogen at 100°. Reconstitute the residue with 500 μL MeOH, vortex for 1 min. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18

Mobile phase: MeOH:10 mM pH 5.5 phosphate buffer 68:32
Column temperature: 28
Flow rate: 1
Injection volume: 20
Detector: UV 336

CHROMATOGRAM
Retention time: 6.83
Internal standard: rifampin (4.33)
Limit of quantitation: 5 ng

KEY WORDS
serum

REFERENCE
He,X.; Wang,J.; Liu,X.; Chen,X. High-performance liquid chromatography assay of rifapentine in human serum, *J.Chromatogr.B*, **1996**, *681*, 412–415.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.45 μm) plasma, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelcosil LC HISEP
Column: 150 × 4.6 Supelcosil LC HISEP (Invert column between runs to prevent clogging.)
Mobile phase: Gradient. A was MeCN:2.6 mM 2-(N-morpholino)ethanesulfonic acid 5:95 containing 1% trifluoroacetic acid, adjusted to pH 6.5 with ethanolamine. B was MeCN:THF:2.6 mM 2-(N-morpholino)ethanesulfonic acid 20:10:70 containing 1% trifluoroacetic acid, adjusted to pH 6.5 with ethanolamine. A:B 70:30 for 4 min, to 30:70 over 24 min, re-equilibrate for 6 min.
Flow rate: 1.4
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 21
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; direct injection

REFERENCE
Riva,E.; Merati,R.; Cavenaghi,L. High-performance liquid chromatographic determination of rifapentine and its metabolite in human plasma by direct injection into a shielded hydrophobic phase column, *J.Chromatogr.*, **1991**, *553*, 35–40.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 300 μL 10 μg/mL rifampin in 1% ascorbic acid solution, mix, keep at 4°, inject a 100 μL aliquot on column A with mobile phase A and elute to waste, after 6 min backflush the contents of column A onto column B with mobile phase B, after 4 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 15 min.

HPLC VARIABLES
Column: A 40 × 20 37-50 μm Corasil RP C18; B 20 × 4.6 25-40 μm LiChrosorb RP-8 + 300 × 3.9 10 μm μBondapak C18

Mobile phase: A 50 mM pH 7.0 phosphate buffer; B MeCN:THF:50 mM pH 7.0 phosphate buffer 42:5:53
Flow rate: 1
Injection volume: 100
Detector: UV 332

CHROMATOGRAM
Retention time: 12.6
Internal standard: rifampin (7.2)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, p-aminosalicylic acid, aspirin, caffeine, cefuroxime, ciprofloxacin, ibuprofen, isonicotinic acid, theophylline, vitamin A, thiamine, riboflavin, pyridoxine, ascorbic acid

KEY WORDS
serum; column-switching; dog; pharmacokinetics

REFERENCE
Lee,H.S.; Shin,H.C.; Han,S.S.; Roh,J.K. High-performance liquid chromatographic determination of rifapentine in serum using column switching, *J.Chromatogr.*, **1992**, *574*, 175–178.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 5 mg/mL solution in MeOH, inject a 10 μL aliquot.

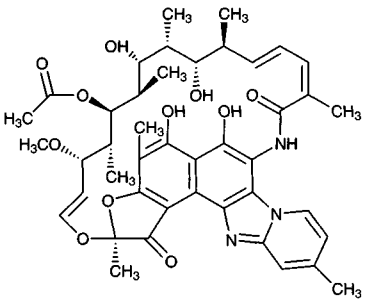
HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 4.6 Hypersil C18
Mobile phase: MeOH
Flow rate: 1
Injection volume: 10
Detector: MS, HP 5985B quadrupole MS, direct liquid introduction interface, 5 μm orifice, water-cooled stainless steel diaphragm, ion source pressure 1 Torr, ion source 250°, LC eluent used as reagent gas

OTHER SUBSTANCES
Also analyzed: rifampin, rifamycin SV

REFERENCE
Vékey,K.; Edwards,D.M.F.; Zerilli,L.F. Liquid chromatographic-mass spectrometric studies on rifamycin antibiotics, *J.Chromatogr.*, **1989**, *474*, 317–327.

Rifaximin

Molecular formula: $C_{43}H_{51}N_3O_{11}$
Molecular weight: 785.89
CAS Registry No.: 80621-81-4
Merck Index: 8386



SAMPLE
Matrix: bile

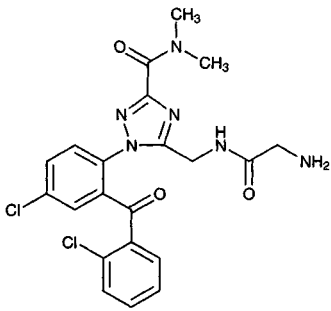
Sample preparation: 1 mL Bile + 4 mL pH 4.5 buffer + 200 µL 50 µg/mL IS in MeOH, extract twice with chloroform. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness, reconstitute the residue in 100 µL MeOH, inject an aliquot.

HPLC VARIABLES
Column: HSC18
Mobile phase: MeOH:1 mM pH 3 phosphate buffer 65:35
Column temperature: 35
Flow rate: 1
Detector: UV 276

CHROMATOGRAM
Internal standard: M 302

REFERENCE
Verardi,S.; Verardi,V. Bile rifaximin concentration after oral administration in patients undergoing cholecystectomy, *Farmaco*, **1990**, *45*, 131–135.

Rilmazafone



Molecular formula: $C_{21}H_{20}Cl_2N_6O_3$
Molecular weight: 475.33
CAS Registry No.: 99593-25-6
Merck Index: 8387

SAMPLE
Matrix: feed
Sample preparation: 1 g Feed + 10 mL MeOH, shake for 45 min, centrifuge at 2000 rpm for 10 min. Remove 5 mL of the supernatant and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 1 mL MeCN, add 100 µL 1 M tartaric acid containing 50 mM sodium 1-dodecanesulfonate, vortex for 10 min, add 5 mL n-hexane, vortex for 10 min, filter (0.45 µm) the aqueous layer, inject a 10 µL aliquot of the filtrate.

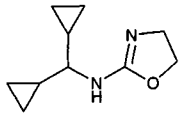
HPLC VARIABLES
Column: two 300 × 4 10 µm Nucleosil 10C18 columns in series
Mobile phase: MeCN:EtOH:buffer 35:12.5:52.5 (Buffer was 100 mM tartaric acid containing 5 mM sodium 1-dodecanesulfonate and 0.29 mM triethylamine.)
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Ikenishi,R.; Kitagawa,T. Analytical studies on 1-(2-o-chlorobenzoyl-4-chlorophenyl)-3-dimethylcarbamoyl-5-glycyl-aminomethyl-1H-1,2,4-triazole hydrochloride dihydrate. III. High-performance liquid chromatographic method applicable to animal feed, *Chem.Pharm.Bull.*, **1987**, *35*, 4544–4551.

Rilmenidine



Molecular formula: $C_{10}H_{16}N_2O$
Molecular weight: 180.25
CAS Registry No.: 54187-04-1
Merck Index: 8388

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 9.8

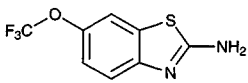
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Riluzole



Molecular formula: $C_8H_5F_3N_2OS$
Molecular weight: 234.20
CAS Registry No.: 1744-22-5
Merck Index: 8389

SAMPLE

Matrix: blood
Sample preparation: Add 100-200 ng IS to 1 mL plasma adjusted to pH 6.0. Vortex, centrifuge at 2000 g for 5 min. Add to a 1 mL C2 Bond Elut SPE cartridge or CBA Bond Elut ion exchange SPE cartridge. Wash with 1 mL 10 mM dipotassium hydrogen phosphate, 500 µL water, and MeOH:water 10:90. Elute with 500 µL mobile phase, dry under a stream of nitrogen. Inject a 300 µL aliquot.

HPLC VARIABLES
Guard column: 39 × 4.6 μBondapak C18
Column: 300 × 4.6 μBondapak C18
Mobile phase: MeCN:MeOH:acetic acid:dipotassium hydrogen phosphate 10:55:1:35
Flow rate: 1
Injection volume: 300
Detector: UV 265

CHROMATOGRAM
Retention time: 9.2
Internal standard: RP 61307 (riluzole N-methyl derivative) (12.4)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Le Liboux,A.; Lefebvre,P.; Le Roux,Y.; Truffinet,P.; Aubeneau,M.; Kirkesseli,S.; Montay,G. Single- and multiple-dose pharmacokinetics of riluzole in white subjects, *J.Clin.Pharmacol.*, **1997**, *37*, 820–827.

SAMPLE
Matrix: urine
Sample preparation: Add 20 μg IS to 1 mL urine adjusted to pH 6.0. Vortex, centrifuge at 2000 g for 5 min. Add to a 1 mL C2 Bond Elut SPE cartridge or CBA Bond Elut ion exchange cartridge. Wash with 1 mL 10 mM dipotassium hydrogen phosphate, 500 μL water, MeOH: water 10:90. Elute with 500 μL mobile phase, inject a 300 μL aliquot.

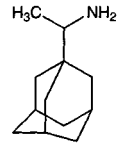
HPLC VARIABLES
Guard column: 39 × 4.6 μBondapak C18
Column: 300 × 4.6 μBondapak C18
Mobile phase: MeCN:MeOH:acetic acid:dipotassium hydrogen phosphate 10:55:1:35
Flow rate: 0.8-1
Injection volume: 300
Detector: UV 265

CHROMATOGRAM
Retention time: 9.2
Internal standard: RP 61 307 (riluzole N-methyl derivative) (12.4)
Limit of detection: 10 ng/mL

KEY WORDS
pharmacokinetics; SPE

REFERENCE
Le Liboux,A.; Lefebvre,P.; Le Roux,Y.; Truffinet,P.; Aubeneau,M.; Kirkesseli,S.; Montay,G. Single- and multiple-dose pharmacokinetics of riluzole in white subjects, *J.Clin.Pharmacol.*, **1997**, *37*, 820–827.

Rimantadine



Molecular formula: $C_{12}H_{21}N$
Molecular weight: 179.31
CAS Registry No.: 13392-28-4, 1501-84-4 (HCl)
Merck Index: 8390
Lednicer No.: 2 19

SAMPLE
Matrix: solutions

Sample preparation: 50 μL 5 mg/mL Rimantadine in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer was 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 36.4 mg 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside in 1 mL MeOH, protect from light, keep on ice.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:buffer 85:15 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 420 or UV 254

CHROMATOGRAM

Retention time: 5.88, 7.11 (enantiomers)
Limit of detection: 6 ng (UV)

KEY WORDS

derivatization; protect from light; chiral

REFERENCE

Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

SAMPLE

Matrix: urine
Sample preparation: Adjust pH of 8 mL urine to 5.5 with 2 M HCl, add 80 μL Glusulase (DuPont), heat at 37° for 18 h. Remove a 2 mL aliquot and add it to 500 μL 5 M NaOH, add 8 mL cyclohexane saturated with triethanolamine:chloroform 2:1, add 100 μL 2% pentafluorobenzoyl chloride in cyclohexane, shake at 30 strokes/min for 20 min, centrifuge at 1500 g. Remove 7.5 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 160 μL heptane:isopropanol 95:5, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax silica
Mobile phase: Gradient. Hexane:isopropanol 95:5 for 10 min, to 90:10 over 20 min, re-equilibrate for 5 min.
Flow rate: 1.5
Detector: radioactivity

CHROMATOGRAM

Retention time: 6.4

OTHER SUBSTANCES

Extracted: metabolites

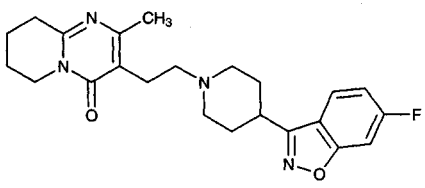
KEY WORDS

14C-labeled; derivatization; normal phase

REFERENCE

Rubio,F.R.; Fukuda,E.K.; Garland,W.A. Urinary metabolites of rimantadine in humans, *Drug Metab.Dispos.*, **1988**, *16*, 773–777.

Risperidone



Molecular formula: $C_{23}H_{27}FN_4O_2$
Molecular weight: 410.49
CAS Registry No.: 106266-06-2
Merck Index: 8397
Lednicer No.: 5 150

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 500 μL 600 mM pH 10 sodium carbonate/bicarbonate buffer + 50 μL 3.76 mg/mL IS in MeOH + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min. Freeze the aqueous layer, evaporate the heptane layer to dryness under a gentle stream of nitrogen at 60°. Dissolve the residue in 75 μL mobile phase, inject a 65 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 LiChroCart
Mobile phase: MeOH:40 mM pH 7.0 ammonium acetate buffer 90:10
Flow rate: 1
Injection volume: 65
Detector: UV 280

CHROMATOGRAM

Retention time: 4.42
Internal standard: haloperidol (5.10)
Limit of quantitation: 1.2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites IN clozapine, diltiazem, fluphenazine, hydroxyzine, mianserine, perphenazine, zuclopenthixol
Simultaneous: amitriptyline, citalopram, chlorprothixene, clomipramine, desipramine, desmethylcitalopram, desmethylclomipramine, desmethylsertraline, fluoxetine, 10-hydroxyamitriptyline, 8-hydroxyclomipramine, 8-hydroxydesmethylclomipramine, 10-hydroxynortriptyline, imipramine, methotrimeprazine sulfoxide, norfluoxetine, nortriptyline, paroxetine, sertraline
Noninterfering: carbamazepine, clonazepam, flunitrazepam, nitrazepam, oxazepam, oxcarbazepine

KEY WORDS

serum

REFERENCE

Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for determination of risperidone and 9-hydroxyrisperidone in serum from patients comedicated with other psychotropic drugs, *J.Chromatogr.B*, **1997**, *698*, 209–216.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 25 ng/mL remoxipride in MeCN, mix for 5 s, add 1 mL saturated sodium carbonate, mix for 5 s, add 7 mL pentane:dichloromethane 75:25, shake gently for 10 min, centrifuge at 18° at 1735 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 140 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:MeOH:40 mM pH 6.8 ammonium acetate 82:8:10
Column temperature: 40
Flow rate: 1.5

Injection volume: 150
Detector: E, ESA Coulochem model 5100A, model 5011 analytical cell, screening electrode 0.6 V, detection electrode 0.92 V, model 5020 guard cell 1 V

CHROMATOGRAM
Retention time: 14
Internal standard: remoxipride (17)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Simultaneous: pseudoephedrine
Noninterfering: metabolites, acetaminophen, benztropine, clonazepam, clozapine, fluphenazine, haloperidol, ibuprofen, lorazepam, trihexyphenidyl

KEY WORDS
plasma

REFERENCE
Aravagiri,M.; Marder,S.R.; Van Putten,T.; Midha,K.K. Determination of risperidone in plasma by high-performance liquid chromatograhy with electrochemical detection: application to therapeutic drug monitoring in schizophrenic patients, *J.Pharm.Sci.*, **1993**, *82*, 447–449.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane: dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)
Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM
Retention time: 7.8
Internal standard: methylrisperidone (R68808) (14.3)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, chlorpromazine, clomipramine, cyamemazine, desipramine, droperidol, flunitrazepam, haloperidol, imipramine, trihexyphenidyl
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid
Interfering: pipamperone

KEY WORDS
plasma; SPE

REFERENCE
Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Urine. Inject up to 1.95 mL urine directly. Feces. Homogenize (Ultra-Turrax TP-25) feces with MeOH, centrifuge, repeat extraction twice more. Evaporate a 10 mL aliquot of the extracts to dryness under a stream of nitrogen, reconstitute in DMSO, inject a 200 μL aliquot. Plasma. Add an equal volume of MeCN to plasma, centrifuge. Remove the supernatant and reduce the volume under a stream of nitrogen at 40°, inject a 2 mL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Hypersil C18
Mobile phase: Gradient. A was 100 mM ammonium acetate containing 0.2% diethylamine, pH 6.0. B was MeCN:MeOH:1 M ammonium acetate containing 2% diethylamine, pH 6.0 80:10:10. A:B 100:0 to 50:50 over 1 h, maintain at 50:50 for 5 min, to 0:100 over 5 min.
Injection volume: 200-2000
Detector: UV 280 or radioactivity

CHROMATOGRAM
Retention time: 49

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Mannens,G.; Huang,M.-L.; Meuldermans,W.; Hendrickx,J.; Woestenborghs,R.; Heykants,J. Absorption, metabolism, and excretion of risperidone in humans, *Drug Metab.Dispos.*, **1993**, *21*, 1134–1141.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Plasma, urine. 1 mL Plasma or urine + 200 μL MeOH + 100 μL 2 μg/mL IS in MeOH + 1 mL 50 mM sodium borate, add 4 mL ethyl acetate, rotate at 10 rpm for 10 min, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and add them to 3 mL 50 mM sulfuric acid, extract. Add the aqueous layer to 150 μL concentrated ammonia, extract twice with 2.5 mL portions of heptane:isoamyl alcohol 90:10. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 80 μL mobile phase, inject a 30 μL aliquot. Tissue. Grind (Waring blender) tissue, homogenize (Ultra-Turrax) with 4 volumes of water. 1 mL Homogenate + 100 μL 2 μg/mL IS in MeOH + 1 mL 100 mM sodium borate, add 4 mL ethyl acetate, rotate at 10 rpm for 10 min, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and add them to 3 mL 50 mM sulfuric acid, extract. Add the aqueous layer to 150 μL concentrated ammonia, extract twice with 2.5 mL portions of heptane:isoamyl alcohol 90:10. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 80 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 5 μm ODS-Hypersil
Mobile phase: MeCN:water:diethylamine 35:65:0.02
Flow rate: 0.8
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 2.9
Internal standard: 3-[2-[4-([6-fluoro-1,2-benzisoxazol-3-yl]methyl)-1-piperidinyl]ethyl]-2,7-dimethyl-4H-pyrido[1,2-a]pyrimidin-4-one (Janssen R 68808) (5.4)
Limit of quantitation: 10 ng/g (tissue), 2 ng/mL (plasma, tissue)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: diazepam, oxazepam
Interfering: desmethyldiazepam

KEY WORDS
plasma; dog; human; muscle; pharmacokinetics

REFERENCE
Woestenborghs,R.; Lorreyne,W.; Van Rompaey,F.; Heykants,J. Determination of risperidone and 9-hydroxyrisperidone in plasma, urine and animal tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *583*, 223–230.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

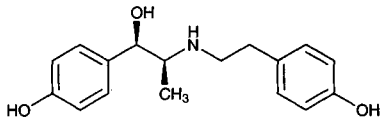
CHROMATOGRAM
Retention time: 9.12 (A), 4.63 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Ritodrine



Molecular formula: $C_{17}H_{21}NO_3$
Molecular weight: 287.36
CAS Registry No.: 26652-09-5, 23239-51-2 (HCl)
Merck Index: 8401
Lednicer No.: 2 39

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL water + 1 mL 600 mM potassium carbonate + 8 mL ethyl acetate, shake mechanically for 5 min, centrifuge at 2000 rpm for 15 min. Remove the organic layer and add it to 1.2 mL 100 mM HCl, shake for 5 min, centrifuge at 2000 rpm for 10 min. Remove the aqueous layer and add it to 500 μL 600 mM potassium carbonate and 1 mL ethyl acetate, shake for 5 min, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 5 μm Biophase ODS
Mobile phase: MeCN:buffer:water 20:10:70 containing 3 mM sodium 1-heptanesulfonate, pH 3.7 (Buffer was 2.1 M acetic acid containing 400 mM ammonium acetate.)
Flow rate: 1
Injection volume: 50
Detector: E, Bioanalytical Systems Model 4B, glassy carbon electrode 0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 9.33
Internal standard: nalbuphine (13.58)
Limit of detection: 0.6 ng/mL

KEY WORDS
plasma

REFERENCE
Kuhnert,P.; Erhard,P.; Dixon,A.; Kuhnert,B.; Gross,T. Determination of ritodrine in plasma using HPLC, *J.Liq.Chromatogr.*, **1983**, *6*, 2775–2783.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of 1 mL serum to 9.4 with 2 M sodium carbonate buffer, add 200 ng IS, vortex, add 6 mL ethyl acetate, shake for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 400 μL mobile phase, vortex vigorously, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 71 × 1.2 HC Pellosil (Whatman)
Column: 260 × 4.6 μBondapak C18
Mobile phase: MeOH:buffer 25:75 (Buffer was 10 mM KH_2PO_4 containing 0.3 mM sodium octanesulfonate and 0.1 mM EDTA, pH 4.5.)
Flow rate: 1.5
Injection volume: 40
Detector: E, Bioanalytical Systems Model LC-4B, glassy carbon electrode +0.90 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7.8
Internal standard: 1-(3,5-dihydroxyphenyl)-2-(1,1-dimethylbutylamino)ethanol (12.0)

Limit of detection: 0.2 ng

KEY WORDS
serum

REFERENCE
Lin,L.S.; Caritis,S.N.; Wong,L.K. Analysis of ritodrine in serum by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Sci.*, **1984**, *73*, 131–133.

SAMPLE
Matrix: blood
Sample preparation: Dilute blood with an equal volume of water. 1 mL Plasma or 900 μL diluted blood + 50 μL 1.6 μg/mL isoxsuprine hydrochloride + 0.9-1 mL buffer + 5 mL freshly distilled ethyl acetate, vortex for 1 min, centrifuge at 1750 g for 7 min. Remove the organic layer and evaporate it almost to dryness under a stream of nitrogen at 57°, evaporate the final 500 μL at room temperature, reconstitute the residue in 100 μL MeCN, vortex for 15 s, inject the whole amount. (Buffer was 26.5 g sodium carbonate and 21 g sodium bicarbonate in 500 mL water, pH 9.48.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl (plasma) or 200 × 4.6 5 μm Spheri-5 RP-18 (blood)
Mobile phase: MeCN:0.05% orthophosphoric acid 17:83 (plasma) or 63:37 (blood)
Flow rate: 2
Injection volume: 100
Detector: F ex 200 no emission filter or UV 254

CHROMATOGRAM
Retention time: 3.7 (plasma), 9.3 (blood)
Internal standard: isoxsuprine hydrochloride (15.1 (plasma), 16.3 (blood))
Limit of detection: 1 ng/mL (F)

OTHER SUBSTANCES
Simultaneous: fenoterol
Noninterfering: acetaminophen, albuterol, betamethasone, bupivacaine, caffeine, chloral hydrate, dexamethasone, diazepam, lignocaine, meperidine, metoclopramide, morphine, nitrazepam, terbutaline

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Gross,A.S.; Brown,K.F.; Baird-Lambert,J.A.; Nation,R.L. Determination of ritodrine in blood and plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *416*, 400–408.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 225

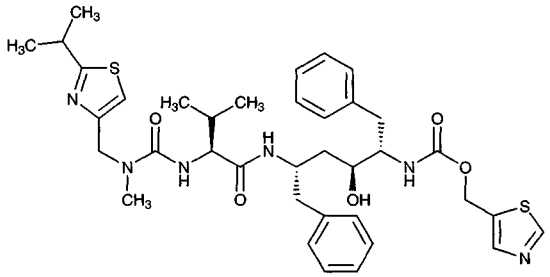
CHROMATOGRAM
Retention time: 3.43
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Ritonavir

Molecular formula: $C_{37}H_{48}N_6O_5S_2$
Molecular weight: 720.96
CAS Registry No.: 155213-67-5
Merck Index: 8402



SAMPLE
Matrix: bile, blood, feces, urine

Sample preparation: Rat, dog plasma. Add 3 volumes MeCN to plasma, vortex, centrifuge, evaporate the supernatant to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, filter (0.45 μm) while centrifuging, inject an aliquot. Human plasma. Add 3 volumes MeCN to plasma, vortex, centrifuge, rinse the protein pellet with MeCN and MeOH. Evaporate the combined supernatants to dryness under a stream of nitrogen, reconstitute the residue with 200 μL MeCN:MeOH:25 mM ammonium acetate 15:15:70 adjusted to pH 4.8 with trifluoroacetic acid, inject an aliquot. Feces. Centrifuge fecal homogenate, rinse the pellet twice with EtOH, filter the supernatants (0.45 μm), inject an aliquot. Bile. Filter bile and inject an aliquot. Urine. Concentrate urine under a stream of nitrogen or via lyophilization, reconstitute in mobile phase, filter while centrifuging, inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm Nucleosil C18
Column: 250 × 4.6 5 μm Beckman Ultrasphere C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid adjusted to pH 4.8 with ammonium acetate. B was MeCN. A:B from 75:25 to 40:60 over 50 min
Flow rate: 1
Detector: A UV 220; B Radioactivity, Flo-One/Beta Model A-500 radioactivity with 0.5 mL flow cell

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CHROMATOGRAM
Retention time: 43
Limit of detection: 150 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; rat; dog; human; radiolabeled

REFERENCE
Denissen,J.F.; Grabowski,B.A.; Johnson,M.K.; Buko,A.M.; Kempf,D.J.; Thomas,S.B.; Surber,B.W. Metabolism and disposition of the HIV-1 protease inhibitor ritonavir (ABT-538) in rats, dogs, and humans, *Drug Metab.Dispos.*, **1997**, *25*, 489–501.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL of 500 mM sodium carbonate and 100μL IS to 1 mL of plasma, extract with 6 mL of MTBE. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve in 300 μL MeCN:10 mM perchloric acid 45:55, add 4 mL of hexane, vortex, centrifuge to separate the layers, freeze in a dry ice/acetone bath, discard the organic layer, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 23 × 4 5 μm YMC ODS-AQ
Column: 50 × 4 3 μm YMC ODS-AQ
Mobile phase: MeCN:MeOH:10 mM tetramethylammonium perchlorate in water:trifluoroacetic acid 40:5:55:0.1
Flow rate: 1.5
Injection volume: 100
Detector: UV 205

CHROMATOGRAM
Limit of quantitation: 5.6 ng/mL

OTHER SUBSTANCES
Extracted: ABT-378

KEY WORDS
plasma

REFERENCE
Bryan,P.; el-Shourbagy,T.; Emry,M.; Marsh,K.; McDonald,E.; Brooks,R.; Sapochak,L.; Hsu-Beischer,R.; Chu,S. A sensitive and specific HPLC method for the simultaneous determination of ABT-378 and ritonavir in human plasma using uv detection (Abstract 2646), *Pharm.Res.*, **1997**, *14*, S427.

SAMPLE
Matrix: blood, CSF, saliva
Sample preparation: Add 400 μL MeCN to 100 μL plasma, CSF, or saliva, vortex for 30 s, centrifuge at 10500 g for 3 min, evaporate 400 μL supernatant to dryness under a gentle stream of nitrogen at 40°, redissolve the residue in 150 μL mobile phase, vortex for 60 s, centrifuge at 10500 g for 3 min, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Chromguard C18 (Chrompack, Netherlands)
Column: 75 × 4.6 3.5 μm Zorbax SB-C18
Mobile phase: MeCN:buffer 44:56 (Buffer was 25 mM sodium acetate with 25 mM hexane-1-sulfonic acid adjusted to pH 4.0 with 37% HCl.)
Flow rate: 1
Injection volume: 100
Detector: UV 239

CHROMATOGRAM
Retention time: 9
Limit of detection: 20 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: didanosine, fluconazole, folinic acid, ganciclovir, indinavir, lamivudine, methadone, methotrexate, nelfinavir, oxazepam, pyrazinamide, ranitidine, rifampin, saquinavir, stavudine, sulfamethoxazole, trimethoprim, zalcitabine, zidovudine, zidovudine glucuronide

KEY WORDS
plasma

REFERENCE
Hoetelmans,R.M.W.; van Essenberg,M.; Profijt,M.; Meenhorst,P.L.; Mulder,J.W.; Beijnen,J.H. High-performance liquid chromatographic determination of ritonavir in human plasma, cerebrospinal fluid and saliva, *J.Chromatogr.B*, **1998**, *705*, 119–126.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add four volumes of MeCN to microsomal incubation, centrifuge at 1500 g for 10 min, evaporate supernatant to dryness under nitrogen at 40°, resuspend the residue in 100-120 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil 5C18 (Phenomenex)
Mobile phase: Gradient. A was MeCN. B was 0.1% triethylamine in water adjusted to pH 4.8 with ammonium acetate. A:B from 25:75 to 75:25 over 15 min
Flow rate: 1.5
Injection volume: 80
Detector: UV 210

CHROMATOGRAM
Retention time: 12.9

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE

Koudriakova,T.; Iatsimirskaia,E.; Utkin,I.; Gangl,E.; Vouros,P.; Storozhuk,E.; Orza,D.; Marinina,J.; Gerber,N. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: Mechanism-based inactivation of cytochrome P4503A by ritonavir, *Drug Metab.Dispos.*, **1998**, *26*, 552–561.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 5 μm Delta-pak C4 (Waters)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM ammonium dihydrogen phosphate and 1 mM 1-heptanesulfonic acid sodium salt, pH adjusted to 4.8 with ammonium hydroxide.)
Flow rate: 0.6
Injection volume: 35
Detector: UV 210

CHROMATOGRAM

Retention time: 22-27

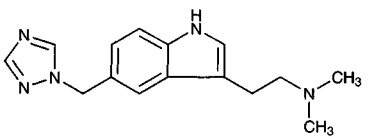
OTHER SUBSTANCES

Simultaneous: indinavir, nelfinavir, saquinavir
Noninterfering: didanosine, lamivudine, stavudine, zalcitabine, zidovudine

REFERENCE

Iayewardene,A.L.; Zhu,F.; Aweeka,F.T.; Gambertoglio,J.G. Simple high-performance liquid chromatographic determination of the protease inhibitor indinavir in human plasma, *J.Chromatogr.B*, **1998**, *707*, 203–211.

Rizatriptan



Molecular formula: $C_{15}H_{19}N_5$
Molecular weight: 269.35
CAS Registry No.: 144034-80-0, 145202-66-0 (benzoate), 59776-67-7 (sulfate)

SAMPLE

Matrix: bulk
Sample preparation: Prepare an 1 mg/mL solution of the free base in MeCN:water 10:90. Inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax SB-phenyl
Mobile phase: MeCN:0.1% trifluoroacetic acid in water 16: 84 (A) or MeCN:0.1% phosphoric acid in water 12:88 (B)
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM

Retention time: 6.3 (A), 6.7 (B)

OTHER SUBSTANCES

Extracted: impurities

REFERENCE

Antonucci,V.; Wright,L.; Toma,P. The reversed-phase liquid chromatographic behavior of the new 5-HT1D receptor agonist rizatriptan benzoate and its potential process impurities, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1649–1670.

SAMPLE
Matrix: bulk
Sample preparation: Prepare an 1 mg/mL solution of the free base in MeCN:water 10:90. Inject an aliquot.

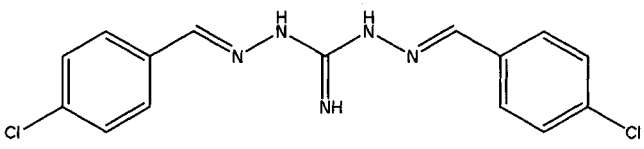
HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8
Mobile phase: MeCN:0.1% trifluoroacetic acid in water 16: 84 (A) or MeCN:0.1% phosphoric acid in water 12:88 (B)
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: 5.5 (A), 6.7 (B)

OTHER SUBSTANCES
Extracted: impurities

REFERENCE
Antonucci,V.; Wright,L.; Toma,P. The reversed-phase liquid chromatographic behavior of the new 5-HT1D receptor agonist rizatriptan benzoate and its potential process impurities, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1649–1670.

Robenidine



Molecular formula: $C_{15}H_{13}Cl_2N_5$
Molecular weight: 334.21
CAS Registry No.: 25875-51-8, 25875-50-7 (HCl)
Merck Index: 8403

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 200 μg/mL solution in dichloromethane:MeOH 90:10, evaporate an aliquot to dryness under a stream of nitrogen at 60°, reconstitute with 1 mL 4 mg/mL 4-dimethylaminopyridine in dichloromethane, add 1 mL 2 mg/mL dansyl chloride in dichloromethane, heat in a capped tube at 80° for 1 h, cool on ice, add to a 3 mL Sep-Pak silica SPE cartridge, rinse the tube with 5 mL dichloromethane, add the rinse to the SPE cartridge, elute with 5 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 150 × 3.2 7 μm silica (Brownlee)
Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: Hexane:chloroform:THF:MeOH 50:50:2:1
Flow rate: 2
Injection volume: 20
Detector: F ex 320 em 485

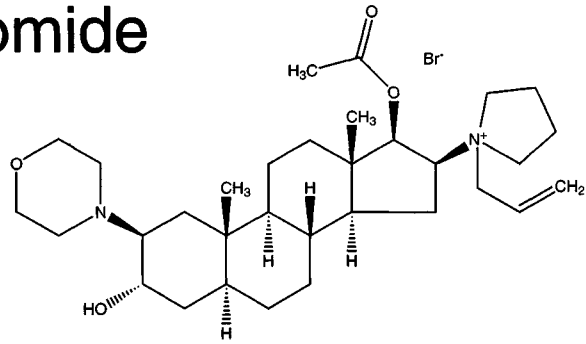
CHROMATOGRAM
Retention time: 4
Limit of detection: 400 ng/mL

KEY WORDS
derivatization; normal phase; protect from light; SPE

REFERENCE
Cohen,H.; Armstrong,F.; Campbell,H. Sensitive fluorescence detection of robenidine by derivatization with dansyl chloride and high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *694*, 407–413.

Rocuronium bromide

Molecular formula: $C_{32}H_{53}BrN_2O_4$
Molecular weight: 609.69
CAS Registry No.: 119302-91-9
Merck Index: 8407



SAMPLE
Matrix: bile, blood, stoma fluid, tissue, urine
Sample preparation: Homogenize (Ultra-Turrax) 1 g tissue with 9 mL 1 M NaH_2PO_4 for 10 min. Acidify 1 mL plasma, urine, or bile with 200 µL 1 M NaH_2PO_4. Homogenize 1 mL stoma fluid with 200 µL 1 M NaH_2PO_4. Make up 50-1000 µL plasma, 200-1000 µL urine, 5-200 µL bile, 1000 µL stoma fluid, or 100-1000 µL tissue homogenate to 2 mL with water, add 1 mL buffer, add 150 ng IS, add 7 mL dichloromethane, vortex for 15 s, centrifuge at 740 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 200 µL mobile phase, inject a 100 µL aliquot (or less). (Buffer was prepared by mixing 6 mL of an aqueous solution containing 7.505 mg/mL glycine and 5.85 mg/mL NaCl, 4 mL 100 mM NaOH and 6.2 g KI.)

HPLC VARIABLES
Guard column: 4 × 6 µBondapak C18
Column: 150 × 3.9 5 µm Lichrospher 100-RP18
Mobile phase: Dioxane:buffer 16:84 (Caution! Dioxane is a carcinogen!) (Buffer was 100 mM NaH_2PO_4 containing 0.11 mM 9,10-dimethoxyanthracene-2-sulfonate and 0.11 mM 1-heptanesulfonic acid, pH adjusted to 3.0 with orthophosphoric acid. After each series of analyses flush column with 15 mL water and 75 mL MeOH.)
Flow rate: 1
Injection volume: 100
Detector: F ex 385 em 452 following post-column extraction. The column effluent mixed with dichloroethane pumped at 1 mL/min and the mixture flowed through a 1 m × 0.25 mm i.d. stainless steel coil to a phase separator (Organon International) then the organic phase flowed through the detector (J. Chromatogr. 1987, 421, 327; Anal. Chim. Acta 1987, 192, 267).

CHROMATOGRAM
Retention time: 9
Internal standard: 1-(3α,17β-dihydroxy-2β-morpholino-5α-androstan-16β-yl)-1-methylpiperidinium bromide (Org 7402, Organon) (21)
Limit of detection: 5 ng tissue, 4 ng (urine, bile), 3 ng (plasma)
Limit of quantitation: 20 ng/mL (stoma fluid), 100 ng/mL (bile), 25 ng/mL (urine), 10 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cephamandole, cephradine, dixyrazine, meperidine, metocurine, metoprolol, sulfamethoxazole, trimethoprim
Noninterfering: alfentanil, aprotinin, atropine, bupivacaine, chlorpromazine, daltaparin, dexamethasone, diazepam, dopamine, droperidol, etomidate, fentanyl, furosemide, gallamine, haloperidol, midazolam, morphine, neostigmine, nitroglycerin, nitroprusside, oxytocin, pancuron-

ium, pentobarbital, phenylephrine, phenytoin, pipecuronium, piperacillin, promethazine, propofol, ranitidine, succinylcholine, sufentanil, terbutaline, thiopental, vecuronium, verapamil
Interfering: alizapride, atracurium, ketamine, ketogan, lidocaine, metoclopramide, nimodipine, prochlorperazine, tubocurarine

KEY WORDS
human; dog; plasma; liver; lung

REFERENCE
Kleef,U.W.; Proost,J.H.; Roggeveld,J.; Wierda,J.M.K.H. Determination of rocuronium and its putative metabolites in body fluids and tissue homogenates, *J.Chromatogr.*, **1993**, *621*, 65–76.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL MeOH:MeCN 2:1 and 1 mL water. Acidify 1 mL plasma with 200 μL 1 M NaH_2PO_4. Add 20-200 ng IS to 1 mL acidified plasma, add to the SPE cartridge, wash with 1 mL water, wash with 1 mL 100 mM pH 3 NaH_2PO_4, elute with 400 μL mobile phase, discard first 100 μL eluate, inject a 200 μL aliquot of the remaining eluate (from J. Chromatogr. 1987, 421, 327; modifications may be necessary).

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Dioxane:water 20:80 containing 100 mM NaH_2PO_4 and 0.44 mM 9,10-dimethoxyanthracene-2-sulfonate, pH adjusted to 3 with phosphoric acid. (Caution! Dioxane is a carcinogen!) (After each series of analyses flush column with 200 mL MeOH then re-equilibrate with 120 mL mobile phase.)
Flow rate: 1
Injection volume: 200
Detector: F ex 380 em 452 following post-column extraction. The column effluent mixed with dichloroethane pumped at 1.6 mL/min and the mixture flowed through a 1 m × 0.25 mm i.d. stainless steel coil to a phase separator (Anal. Chim. Acta 1987, 192, 267) then the organic phase flowed through the detector.

CHROMATOGRAM
Internal standard: 3,17-didesacetyl vecuronium (Org 7402)
Limit of quantitation: 10 ng/mL

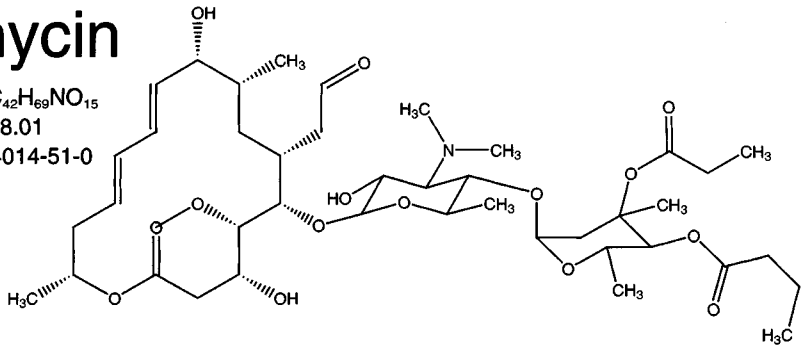
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; pharmacokinetics; post-column reaction; post-column extraction

REFERENCE
Cooper,R.A.; Maddineni,V.R.; Mirakhur,R.K.; Wierda,J.M.K.H.; Brady,M.; Fitzpatrick,K.T.J. Time course of neuromuscular effects and pharmacokinetics of rocuronium bromide (Org 9426) during isoflurane anaesthesia in patients with and without renal failure, *Br.J.Anaesth.*, **1993**, *71*, 222–226.

Rokitamycin

Molecular formula: $C_{42}H_{69}NO_{15}$
Molecular weight: 828.01
CAS Registry No.: 74014-51-0
Merck Index: 8408



SAMPLE

Matrix: blood

Sample preparation: Collect blood in tubes containing neostigmine methyl sulfate, final concentration 0.2 mM. 1 mL Plasma + 100 μL 2 μg/mL josamycin in MeOH, add 50 μL 100 mM pH 4.65 acetate buffer + 100 μL saturated NaCl + 100 μL 10 mM sodium lauryl sulfate + 5 mL hexane:isoamyl alcohol 90:10, shake for 15 min, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen with mild heating, reconstitute the residue in 200 μL 0.002% dansylhydrazine in toluene:MeOH:acetic acid 90:10:1.13 (freshly prepared), heat at 60° for 20 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute with 20 μL ethyl acetate, add 60 μL 100 mM HCl, inject a 20 μL aliquot of the lower aqueous phase. (Silanize glassware with 1% trimethylchlorosilane in toluene for 1 h at 70°, wash twice with MeOH.)

HPLC VARIABLES

Column: 150 × 4.6 3 μm Nucleosil C18
Mobile phase: MeCN:50 mM ammonium acetate 72:28
Column temperature: 32.5
Flow rate: 0.8
Injection volume: 20
Detector: F ex 352 em 537

CHROMATOGRAM

Retention time: 11.5
Internal standard: josamycin (9.5)
Limit of detection: 20 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

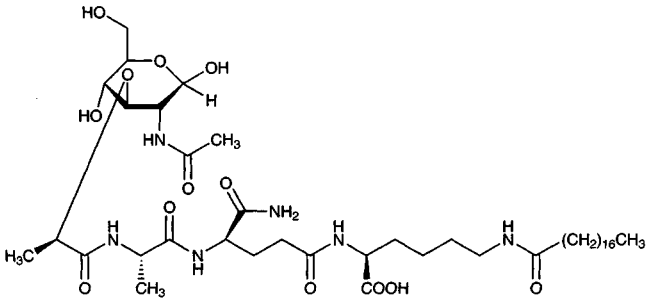
KEY WORDS

plasma; derivatization

REFERENCE

Tod,M.; Biarez,O.; Nicolas,P.; Petitjean,O. Sensitive determination of josamycin and rokitamycin in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1992**, *575*, 171–176.

Romurtide



Molecular formula: $C_{43}H_{78}N_6O_{13}$
Molecular weight: 887.12
CAS Registry No.: 78113-36-7
Merck Index: 8412

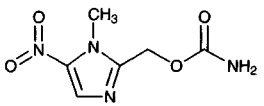
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm octadecyl silica
Mobile phase: MeCN:MeOH:20 mM ammonium acetate 66:4:40
Column temperature: 25
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 8.6

REFERENCE
Moroi,R.; Yamazaki,K.; Hirota,T.; Watanabe,S.; Kataoka,K.; Ichinose,M. Physico-chemical properties of muroctasin, *Arzneimittelforschung*, **1988**, *38*, 953–959.

Ronidazole



Molecular formula: $C_6H_8N_4O_4$
Molecular weight: 200.15
CAS Registry No.: 7681-76-7
Merck Index: 8413
Lednicer No.: 2 245

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

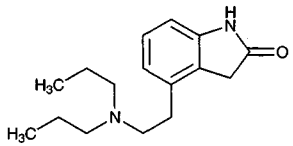
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 308

CHROMATOGRAM
Retention time: 8.265

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ropinirole



Molecular formula: $C_{16}H_{24}N_2O$
Molecular weight: 260.38
CAS Registry No.: 91374-21-9, 91374-20-8 (HCl)
Merck Index: 8416

SAMPLE
Matrix: microsomal incubations
Sample preparation: 250 μL Microsomal incubation + 50 μL 5% trichloroacetic acid, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ (Supelco)
Mobile phase: Gradient. A was MeCN. B was 100 mM pH 4 ammonium acetate. A:B from 0:100 to 15:85 over 10 min, to 100:0 over 12 min, maintain at 100:0 for 15 min
Column temperature: 40
Flow rate: 1
Detector: UV 250; Radioactivity, Ramona-5 (Lablogic, Inc., UK)

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
liver

REFERENCE
Bloomer,J.C.; Clarke,S.E.; Chenery,R.J. In vitro identification of the P450 enzymes responsible for the metabolism of ropinirole, *Drug Metab.Dispos.*, **1997**, *25*, 840–844.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)

Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

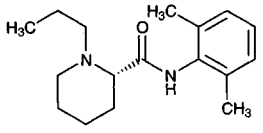
CHROMATOGRAM
Retention time: k' 0.95

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Ropivacaine



Molecular formula: $C_{17}H_{26}N_2O$
Molecular weight: 274.41
CAS Registry No.: 84057-95-4, 98717-15-8 (HCl), 132112-35-7 (HCl monohydrate)
Merck Index: 8417

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond Elut SCX SPE cartridge. Hydrolyze 1 mL urine with 6 M HCl in a water-bath at 95° for 1 hr, dilute with water 1:5. Acidify plasma with phosphoric acid. Add the sample to the SPE cartridge, wash with 200 mM pH 4.5 acetate buffer, wash with MeOH:buffer 50:50, elute with MeOH:2 M ammonia 80:20, evaporate to dryness, reconstitute with 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 2 Superspher RP-Select B (plasma) or 125 × 4 Superspher RP-Select B (urine)
Mobile phase: MeCN:pH 2 phosphate buffer 15-25:85-75 containing 5-15 mM octanesulfonic acid
Detector: UV 210

CHROMATOGRAM
Limit of quantitation: 10 ng/mL (plasma), 300 nM (urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Halldin,M.M.; Bredberg,E.; Angelin,B.; Arvidsson,T.; Askemark,Y.; Elofsson,S.; Widman,M. Metabolism and excretion of ropivacaine in humans, *Drug Metab.Dispos.*, **1996**, *24*, 962–968.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase to a ropivacaine hydrochloride concentration of 75 μg/mL, inject an aliquot.

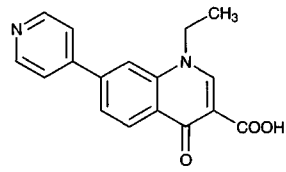
HPLC VARIABLES
Guard column: 10 × 3 chiral-AGP
Column: 100 × 4 5 μm chiral-AGP (Chromtech)
Mobile phase: Isopropanol:pH 7.2 phosphate buffer (μ = 0.05) 7:93
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 9 (R), 12 (S)

KEY WORDS
chiral; injections; comparison with capillary electrophoresis

REFERENCE
Sänger-van de Griend,C.E.; Wahlström,H.; Gröningsson,K.; Widahl-Näsman,M. A chiral capillary electrophoresis method for ropivacaine hydrochloride in pharmaceutical formulations: validation and comparison with chiral liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1051–1061.

Rosoxacin



Molecular formula: $C_{17}H_{14}N_2O_3$
Molecular weight: 294.31
CAS Registry No.: 40034-42-2
Merck Index: 8426
Lednicer No.: 3 185

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 20:80 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 5.13
Internal standard: sparfloxacin (8.3)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 271.2

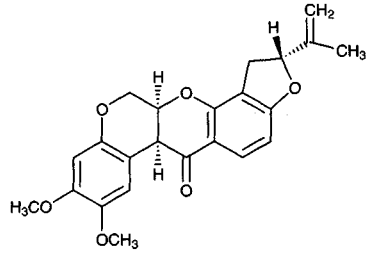
CHROMATOGRAM
Retention time: 13.467

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Rotenone

Molecular formula: $C_{23}H_{22}O_6$
Molecular weight: 394.42
CAS Registry No.: 83-79-4
Merck Index: 8427



SAMPLE
Matrix: feed
Sample preparation: 10 g Ground feed + 100 mL MeCN:glacial acetic acid 99:1, shake for 1 h, centrifuge, filter (0.45 μm) an aliquot of the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Simultaneous: rotenonone

REFERENCE
Kline,D.A.; Hanna,G.R.; Honaker,C.B.; Kuhn,G.O.; Jameson,C.W. Preparation and stability of animal feed mixtures dosed with rotenone, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 660–663.

SAMPLE
Matrix: sediment, tissue
Sample preparation: Fish, crayfish, mussels. Homogenize (Waring blender) with dry ice, place in freezer overnight. 10 g Sample + 60 (fish), 70 (fish offal, crayfish) or 100 (mussels) g anhydrous sodium sulfate, mix (Sorval Omni-mixer), place sample on top of 50 mm anhydrous sodium sulfate in a 22 mm diameter glass column, place a 50 mM layer of anhydrous sodium sulfate on top of the column, pass 100 mL diethyl ether through the column at 1.5 mL/min. Evaporate the eluate to dryness under reduced pressure at 30°, reconstitute the residue in 10 mL dichloromethane:cyclohexane 50:50, add a 5 mL aliquot to a 400 × 28 column packed with SX-3 BioBeads (Analytical BioChemistry Labs) in dichloromethane:cyclohexane 50:50, elute with dichloromethane:cyclohexane 50:50 at 4 mL/min, discard the first 112 mL of eluate, collect the second 112 mL and evaporate it to dryness under reduced pressure at 30°. Reconstitute with 5 mL benzene (Caution! Benzene is a carcinogen!) and add to the silica column, rinse flask with five 5 mL portions of benzene, add rinses to the column (do not allow column to go dry), elute with 70 mL benzene:acetone 97:3. Evaporate the eluate to dryness under reduced pressure at 30°, reconstitute in 5 mL MeOH, inject a 100 μL aliquot. Sediment. 10 g Sediment + 20 mL MeOH, mix (Sorval Omni-mixer), centrifuge at 1000 g for 5 min, repeat extraction 3 more times with 10 mL MeOH. Filter (Gelman type A/E glass fiber) the combined supernatants, evaporate the filtrate under reduced pressure at 30° to about 25 mL. Add the residue to 500 mL 100 mM HCl, extract three times with 20 mL hexane. Combine the hexane extracts and evaporate them to dryness under reduced pressure at 30°, reconstitute with 5 mL benzene (Caution! Benzene is a carcinogen!) and add to the silica column, rinse flask with five 5 mL portions of benzene, add rinses to the column (do not allow column to go dry), elute with 70 mL benzene:acetone 97:3. Evaporate the eluate to dryness under reduced pressure at 30°, reconstitute in 5 mL MeOH, inject a 100 μL aliquot. (Column was 5 g anhydrous sodium sulfate, 5 g 3% deactivated silica gel, and 5 g anhydrous sodium sulfate, all slurry packed in benzene. Silica gel was 40-140 mesh from J.T. Baker activated at 130° for 24 h.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:water 70:30 (60:40 for fish offal)
Flow rate: 1
Injection volume: 100
Detector: UV 295

CHROMATOGRAM
Retention time: 5 , 17 (for fish offal)
Limit of detection: 5 ng/g (tissue), 25 ng/g (sediment)

KEY WORDS
fish; crayfish; mussels; shellfish; sediment

REFERENCE
Dawson,V.K.; Allen,J.L. Liquid chromatographic determination of rotenone in fish, crayfish, mussels, and sediments, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1094–1096.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) water, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Zorbax ODS

Mobile phase: MeCN:water 70:30
Flow rate: 1.3
Injection volume: 200
Detector: UV 210

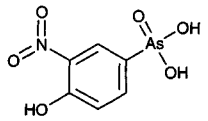
CHROMATOGRAM
Retention time: 6.5
Limit of quantitation: 7.5 ppb

OTHER SUBSTANCES
Simultaneous: rotenonone

KEY WORDS
drinking water; stream water; pond water; water

REFERENCE
Bushway,R.J. High-performance liquid chromatographic analysis of rotenone and rotenonone in water by direct injection, *J.Chromatogr.*, **1984**, *303*, 263–266.

Roxarsone



Molecular formula: $C_6H_6AsNO_6$
Molecular weight: 263.04
CAS Registry No.: 121-19-7
Merck Index: 8430

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass through No. 20 sieve. 15 g Ground feed + 50 mL 20 g/L K_2HPO_4, shake for 5 min, centrifuge at 2385 rpm for 10 min. REmove a 30 mL aliquot of the supernatant and add it to 1 mL 2.5 M HCl, let stand for 15 min, centrifuge for 10 min. Remove a 25 mL aliquot and add it to 1 mL 6 M NaOH, add 2 g activated charcoal (Darco G-60 or equivalent), swirl, let stand for 30 min with periodic swirling, filter (0.45 μm) an aliquot, add the filtrate to an activated carbon SPE cartridge (Analtech No. 01-97), discard the first 2 mL, inject a 15 μL aliquot of the next 500 μL of eluate.

HPLC VARIABLES
Guard column: Bondapak C18
Column: 8NVC18 Radial Pak radial compression (Waters)
Mobile phase: MeOH:water 25:75 containing 3% PIC-A (tetrabutylammonium sulfate)
Flow rate: 1.2
Injection volume: 15
Detector: UV 243

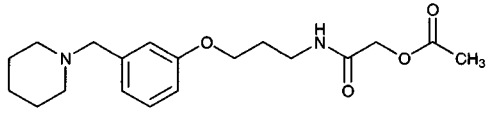
CHROMATOGRAM
Retention time: 8
Limit of detection: <0.3 ng

OTHER SUBSTANCES
Noninterfering: bacitracin, bambemycins, BHA, BHT, chlortetracycline, erythromycin, ethoxyquin, furazolidone, hygromycin B, monensin, niacin, nicarbazin, ormetoprim, oxytetracycline, riboflavin, sulfadimethoxine, tetracycline, thiamine, vitamin D3, vitamin E, pyridoxine, vitamin A

KEY WORDS
SPE

REFERENCE
Sapp,R.E.; Davidson,S. Determination of Roxarsone in feeds using solid phase extraction and liquid chromatography with ultraviolet detection, *J.AOAC Int.*, **1993**, *76*, 956–961.

Roxatidine acetate



Molecular formula: $C_{19}H_{28}N_2O_4$
Molecular weight: 348.44
CAS Registry No.: 78628-28-1, 93793-83-0 (HCl)
Merck Index: 8431
Lednicer No.: 5 26

SAMPLE
Matrix: solutions

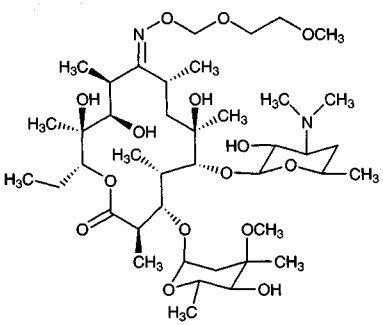
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.29

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Roxithromycin



Molecular formula: $C_{41}H_{76}N_2O_{15}$
Molecular weight: 837.06
CAS Registry No.: 80214-83-1
Merck Index: 8433

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 50 µL 20 µg/mL IS in MeOH + 2.5 mL hexane:isoamyl alcohol 95:5, vortex for 20 s, centrifuge at 1500 g for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 µL mobile phase, vortex for 20 s, sonicate for 1 min, centrifuge at 1500 g for 5 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 mm long Supelguard C18 (Supelco)
Column: 50 × 4.6 5 µm Supelcosil C18
Mobile phase: MeCN:MeOH:water 50:20:30 containing 4 g/L (?) ammonium acetate
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem 5100A, 5010 analytical cell, +0.89 V

CHROMATOGRAM

Retention time: 3.5
Internal standard: RU 29767 (7.5)
Limit of detection: 50 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

Nilsen,O.G.; Aamo,T.; Zahlsen,K.; Svarva,P. Macrolide pharmacokinetics and dose scheduling of roxithromycin, *Diagn.Microbiol.Infect.Dis.*, **1992**, *15*, 71S–76S.

SAMPLE

Matrix: blood
Sample preparation: Condition a 10 × 2 20 mg 30-40 µm Baker CN SPE cartridge with 2 mL MeOH, 2 mL MeOH:water 10:90, and 4 mL MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90 at 2 mL/min. Centrifuge plasma at 1300 g for 5 min, 100 µL plasma + 100 µL clarithromycin in MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90, mix, add a 20-100 µL aliquot to the SPE cartridge, wash SPE cartridge with MeCN:pH 10.5 phosphate buffer (I = 0.10) 10:90 at 0.5 mL/min, after 5 min backflush the contents of the SPE cartridge onto the column with the mobile phase, elute the column with the mobile phase and monitor the effluent.

HPLC VARIABLES

Column: 100 × 4.6 3 µm Hypersil BDS C18
Mobile phase: MeCN:water 54:46 containing 4.5 mM NaH_2PO_4 and 6.8 mM Na_2HPO_4, pH 7
Column temperature: 55
Flow rate: 1
Detector: E, ESA Coulochem II, Model 5011 dual analytical cell, upstream +0.65 V, downstream +0.85 V (monitored), analytical cell protected by an ESA carbon in-line filter

CHROMATOGRAM

Retention time: 7

Internal standard: clarithromycin (6)
Limit of quantitation: 500 nM

KEY WORDS
plasma; SPE

REFERENCE
Hedenmo,M.; Eriksson,B.-M. Liquid chromatographic determination of the macrolide antibiotics roxithromycin and clarithromycin in plasma by automated solid-phase extraction and electrochemical detection, *J.Chromatogr.A*, **1995**, *692*, 161–166.

SAMPLE
Matrix: blood, gastric juice, gastric mucosa, saliva, vitreous humor
Sample preparation: Homogenize 5-20 mg gastric mucosa in 300 μL 10 mM pH 7.4 sodium phosphate buffer with sonication. Add 500 ng clarithromycin in MeOH:water 50:50 to 500 μL plasma, serum, saliva, gastric juice, leucocytes lysate, vitreous humor or 300 μL gastric mucosa homogenate, vortex, add 200 μL 100 mM sodium carbonate and 3 mL MTBE, shake thoroughly (5 × 2 s in an SMI Multi-tube vortexer), centrifuge at 1000 g for 5 min, freeze the aqueous layer in liquid nitrogen or in a freezer at -70° for 15 min. Evaporate the upper organic layer to dryness in a centrifugal vacuum evaporator (Jouan RC 10.22), reconstitute the residue in 250 μL MeOH:water 50:50, inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax SB CN
Mobile phase: MeCN:MeOH:50 mM Na_2HPO_4 and NaH_2PO_4 buffer 53:41:6 (pH 7.0) (The mobile phase was a mixture of 350 mL MeCN, 50 mL MeOH and 450 mL 50 mM Na_2HPO_4 and NaH_2PO_4 buffer.)
Column temperature: 30
Flow rate: 1
Injection volume: 20-50
Detector: E, ESA Coulochem II, guard cell +1.0 V, screening cell E1 +0.50 V, analytical cell E2 +0.80 V

CHROMATOGRAM
Retention time: 11.5
Internal standard: clarithromycin (10)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: azithromycin

KEY WORDS
pharmacokinetics; plasma; saliva; serum; leucocytes

REFERENCE
Kees,F.; Spangler,S.; Wellenhofer,M. Determination of macrolides in biological matrices by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1998**, *812*, 287–293.

SAMPLE
Matrix: blood, saliva, urine
Sample preparation: Plasma. 2 mL Plasma + 20 μL 750 μg/mL roxithromycin in MeCN + 5 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 5 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot. Urine. 1.5 mL Urine + 100 μL 750 μg/mL roxithromycin in saturated K_2HPO_4 + 4 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 5 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot. Saliva. 1.5 mL Saliva + 100 μL 750 μg/mL roxithromycin in saturated K_2HPO_4 + 4 mL diethyl ether, shake vigorously for 3 min, centrifuge at 900 g at 4° for 15 min. Remove upper layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute residue with 100 μL MeCN, vortex 5 s, inject 40 μL aliquot.

HPLC VARIABLES
Column: Nova-Pak C18
Mobile phase: MeCN:MeOH:56 mM sodium acetate buffer 50:4:56, final pH adjusted to 7.0 with glacial acetic acid
Flow rate: 1.1
Injection volume: 40
Detector: E, Waters M460, +0.9 V versus Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 14.7
Internal standard: roxithromycin
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Simultaneous: 4″-acetylerythromycin, 6-O-methylerythromycin, erythromycin base, erythromycin B, erythromycin estolate, erythromycin ethylsuccinate

KEY WORDS
plasma; roxithromycin is IS

REFERENCE
Croteau,D.; Vallée,F.; Bergeron,M.G.; LeBel,M. High-performance liquid chromatographic assay of erythromycin and its esters using electrochemical detection, *J.Chromatogr.*, **1987**, *419*, 205–212.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 1:2 with isotonic NaCl. 200 μL Plasma or diluted urine + 100 μL 10 μg/mL erythromycin in water + 600 μL pH 9 phosphate buffer + 3 mL dichloromethane, shake for 10 min, centrifuge at 2000 g for 5 min. Remove 2.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, vortex for 10 s, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:83 mM ammonium acetate 55:22:23, pH adjusted to 7.5 with acetic acid
Flow rate: 1
Injection volume: 15
Detector: E, ESA Coulochem Model 5100A, Model 5020 guard cell 1.0 V (before injector), Model 5010 dual-electrode cell, screen electrode E1 + 0.7 V, sample electrode E2 +0.9 V, 0.5 μm ESA carbon filters placed before guard and analytical cells

CHROMATOGRAM
Retention time: 9.8
Internal standard: erythromycin (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, clomipramine, disopyramide, erythromycin estolate, erythromycin ethylsuccinate, erythromycin stearate, imipramine, josamycin, lidocaine, spiramycin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Demotes-Mainaird,F.M.; Vinçon,G.A.; Jarry,C.H.; Albin,H.C. Micro-method for the determination of roxithromycin in human plasma and urine by high-performance liquid chromatography using electrochemical detection, *J.Chromatogr.*, **1989**, *490*, 115–123.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.833

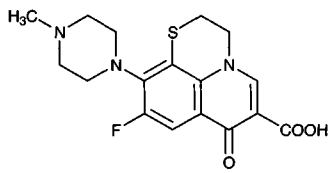
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Rufloxacin



Molecular formula: $C_{17}H_{18}FN_3O_3S$
Molecular weight: 363.41
CAS Registry No.: 101363-10-4
Merck Index: 8448

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Plasma. 500 µL Plasma + 25 µL 350 µg/mL pipemidic acid in water + 50 µL 70% perchloric acid, vortex for 20 s, centrifuge at 14926 g for 5 min, inject a 10 µL aliquot of the supernatant. Urine. 500 µL Urine + 500 µL water + 1 mL 35 µg/mL pipemidic acid in water + 1.5 g Na_2HPO_4:NaH_2PO_4 50:50, mix, add 8 mL dichloromethane, agitate horizontally at low sped for 40 min, centrifuge at 6590 g for 5 min. Remove 5 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject a 10 µL aliquot. Alternatively, dilute 1 mL urine to 50 mL with water, inject a 10 µL aliquot. Bile. Centrifuge 500 µL bile at 14926 g for 15 min, filter (0.2 µm) the supernatant, inject a 5 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 3.2 7 µm Polymer RP (Brownlee)
Column: 50 × 4.1 10 µm PRP1 poly(styrene-divinylbenzene) (Hamilton)
Mobile phase: MeCN:0.17% phosphoric acid 12:88, adjust to pH 5.6 with triethylamine then add 5 mL/L THF
Flow rate: 1
Injection volume: 5-10
Detector: F ex 350 em 510 or UV 300

CHROMATOGRAM
Retention time: 8.9
Internal standard: pipemidic acid (3.5)
Limit of detection: 10 ng/mL (F)
Limit of quantitation: 50 μg/mL (F)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; protect from light

REFERENCE
Lombardi,F.; Ardemagni,R.; Colzani,V.; Visconti,M. High-performance liquid chromatographic determination of rufloxacin and its main active metabolite in biological fluids, *J.Chromatogr.*, **1992**, *576*, 129–134.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 20 μL 100 μg/mL IS in 20 mM NaOH. Add 500 μL 50 mM pH 7.0 phosphate buffer, vortex for 1 min, add 2 mL dichloromethane, vortex for 1 min, shake for 5 min. Centrifuge at 100 g for 10 min, separate the organic layer, repeat the extraction procedure twice, evaporate the combined organic lowers to dryness under reduced pressure. Reconstitute with 200 μL 20 mM NaOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Vydac AXGU
Column: 250 × 4.6 5 μm Supelcosil LC-SAX
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.3
Internal standard: fenbufen (3.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: furprofen

KEY WORDS
SPE; plasma

REFERENCE
Carlucci,G.; Mazzeo,P. Simultaneous determination of furprofen and rufloxacin in human plasma by high-performance liquid chromatography, *J.Chromatogr.Sci.*, **1996**, *34*, 182–184.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate..

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 19:81 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 248

CHROMATOGRAM
Retention time: 4.68
Internal standard: enrofloxacin (7.53)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 2 mg/mL IS in MeOH, mix, add 2 mL dichloromethane:diethyl ether 80:20, vortex for 15 s, centrifuge at 1500 g for 5 min, remove a 1.7 mL aliquot of the organic phase, repeat the extraction twice more with 2 mL portions of dichloromethane:diethyl ether 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Pelliguard C18 (Supelco)
Column: 250 × 4.6 5 μm Viosfer C18 (Violet, Rome)
Mobile phase: MeCN:buffer 15:85 (Buffer was 100 mM phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 6.2
Internal standard: 2-[4-(2'-furoyl)phenyl]propionic acid (7.3)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: theophylline

KEY WORDS
plasma

REFERENCE
Carlucci,G.; Mazzeo,P.; Palumbo,G. Simultaneous determination of rufloxacin and theophylline by high-performance liquid chromatography in human plasma, *Analyst*, **1995**, *120*, 2493–2495.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum + 1 mL 100 mM pH 7 phosphate buffer + 100 (serum) or 150 (urine) μL 5 μg/mL ofloxacin in 500 mM NaOH, mix, add 2.5 mL dichloromethane, shake for 10 min, centrifuge at 1500 g for 10 min, repeat the extraction twice. Combine the organic layers and evaporate them to dryness with nitrogen under vacuum, reconstitute the residue in 200 μL mobile phase, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm AXGU anion-exchange (Rainin)
Column: 250 × 4.6 10 μm anion-exchange (Vydac)
Mobile phase: MeCN:50 mM pH 7 phosphate buffer 20:80
Flow rate: 1.8
Injection volume: 20
Detector: UV 296

CHROMATOGRAM
Retention time: 7.5
Internal standard: ofloxacin (5.6)
Limit of detection: 50 ng/mL (urine), 100 ng/mL (serum)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Carlucci,G.; Palumbo,G. Analytical procedure for the determination of rufloxacin, a new pyridobenzothiazine, in human serum and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *564*, 346–351.

SAMPLE
Matrix: urine
Sample preparation: Dilute ten times with water, inject a 20 μL aliquot. Deconjugate by heating 100 μL urine, 100 μL 50000 U/mL β-glucuronidase (Type II from limpets Patella vulgata, Sigma), and 800 μL pH 3.8 KH_2PO_4 buffer at 37° for 16 h.

HPLC VARIABLES
Guard column: 75 × 2.1 10 μm pellicular reversed phase (Chrompack)
Column: 250 × 4.6 5 μm CP Spher 5-ODS (Chrompack)
Mobile phase: Gradient. MeCN:buffer from 4:96 to 26:74 over 37 min, return to initial conditions over 5 min. (Buffer was 6.75 mL orthophosphoric acid and 2 mL diethylamine in 1 L water, pH 3.1.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 246

CHROMATOGRAM
Retention time: 22
Limit of detection: 500 ng/mL

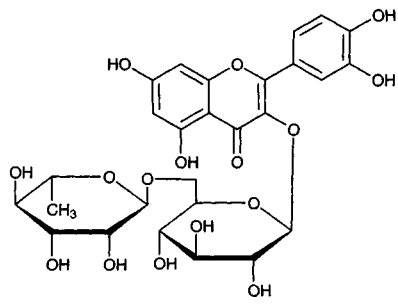
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
monkey; pharmacokinetics

REFERENCE
Vree,T.B.; Van den Biggelaar-Martea,M.; Peeters,A.; Imbimbo,B.P. High-performance liquid chromatography and preliminary pharmacokinetics of rufloxacin and its metabolites, N-desmethylrufloxacin and rufloxacin-sulfoxide, in urine of rhesus monkey *Macaca mulatta*, *J.Chromatogr.*, **1992**, *573*, 168–172.

Rutin

Molecular formula: $C_{27}H_{30}O_{16}$
Molecular weight: 610.53
CAS Registry No.: 153-18-4
Merck Index: 8456



SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

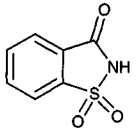
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 10.1

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Saccharin



Molecular formula: $C_7H_5NO_3S$
Molecular weight: 183.19
CAS Registry No.: 81-07-2, 6485-34-3 (Ca salt), 6381-91-5 (Ca salt hydrate), 128-44-9 (Na salt), 6155-57-3 (Na salt dihydrate)
Merck Index: 8463

SAMPLE
Matrix: beverage
Sample preparation: Sonicate 25 mL beverage for 15-20 min, filter (0.45 μm) if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 10:20:70:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: benzoic acid, hydroquinine, quinine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

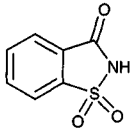
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 10.1

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Saccharin



Molecular formula: $C_7H_5NO_3S$
Molecular weight: 183.19
CAS Registry No.: 81-07-2, 6485-34-3 (Ca salt), 6381-91-5 (Ca salt hydrate), 128-44-9 (Na salt), 6155-57-3 (Na salt dihydrate)
Merck Index: 8463

SAMPLE
Matrix: beverage
Sample preparation: Sonicate 25 mL beverage for 15-20 min, filter (0.45 μm) if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 10:20:70:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: benzoic acid, hydroquinine, quinine

KEY WORDS
tonic water; soft drinks

REFERENCE
Valenti,L.P. Liquid chromatographic determination of quinine, hydroquinine, saccharin, and sodium benzoate in quinine beverages, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 782–784.

SAMPLE
Matrix: beverages, sweetener
Sample preparation: Sweetener. Dissolve 30 mg powdered tabletop sweetener in water and dilute to 25 mL, filter (0.2 μm PTFE). Beverages. Dilute fruit juice 1:10 with water. Degas carbonated beverages in a ultrasonic bath for 5 min, dilute 1:10 with water, filter. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 Dionex IonPak AG4A-SC
Column: 250 × 4 Dionex IonPak AS4A-SC
Mobile phase: Gradient. A was 1 mM sodium carbonate. B was 12.5 mM sodium carbonate. A:B 100:0 for 4.5 min, from 100:0 to 0:100 over 1 min, maintain at 0:100 for 22.5 min, from 0:100 to 100:0 over 0.1 min
Flow rate: 1
Injection volume: 50
Detector: UV 190 for 6 min, UV 206 22 min, then UV 190; Conductivity, Dionex ED40 in conductivity mode preceded by a Dionex ASRS-I suppressor (external water mode, 300 mA)

CHROMATOGRAM
Retention time: 18
Limit of detection: 19 ng/mL (UV), 260 ng/mL (conductivity)

OTHER SUBSTANCES
Simultaneous: acesulfame, aspartame

REFERENCE
Chen,Q.-C.; Mou,S.; Liu,K.; Yang,Z.; Ni,Z. Separation and determination of four artificial sweeteners and citric acid by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *771*, 135–143.

SAMPLE
Matrix: beverages, syrup
Sample preparation: Dilute syrup ten fold. Filter (0.45 μm) beverages and diluted syrup, inject a 10-20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:water 20:5:75
Flow rate: 2
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 30 ng

OTHER SUBSTANCES
Simultaneous: acesulfame, benzoic acid, caffeine, dulcin, p-hydroxybenzoic acid, vanillin

REFERENCE
Veerabhadrarao,M.; Narayan,M.S.; Kapur,O.; Sastry,C.S. Reverse phase liquid chromatographic determination of some food additives, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 578–582.

SAMPLE
Matrix: blood, feces, feed, milk, urine

Sample preparation: Condition a 3 mL quaternary amine SPE cartridge (J.T. Baker) with 3 mL MeOH, 3 mL concentrated ammonium hydroxide, and 3 mL 200 mM HCl. Feed, feces. Air dry feces at 50° for 17 h. 350 mg Feed or dried feces + 350 μL 100 mM NaOH, mix, add 2.5 mL 100 mM NaOH, mix, centrifuge at 500 g for 10 min, repeat extraction twice with 2.5 mL aliquots and once with a 2 mL aliquot of 100 mM NaOH. Combine the supernatants and make up to 10 mL with water. Remove a 500 μL aliquot and add it to the SPE cartridge, wash with two 1 mL aliquots of MeOH, wash with 2 mL water (add 3 μL 2-octanol to top of water as an anti-foaming agent), elute with two 2 mL aliquots of 200 mM pH 8.8 K_2HPO_4, add 80 μL phosphoric acid to the eluate, make up to 5 mL with water, inject an aliquot. Urine. 1 mL Urine + 8.5 mL 100 mM NaOH, make up to 10 mL with water. Remove a 500 μL aliquot and add it to the SPE cartridge, wash with two 1 mL aliquots of MeOH, wash with 2 mL water (add 3 μL 2-octanol to top of water as an anti-foaming agent), elute with two 2 mL aliquots of 200 mM pH 8.8 K_2HPO_4, add 80 μL phosphoric acid to the eluate, make up to 5 mL with water, inject an aliquot. Serum. 500 μL Serum + 4.25 mL 100 mM NaOH, make up to 5 mL with water. Remove a 500 μL aliquot and add it to the SPE cartridge, wash with two 1 mL aliquots of MeOH, wash with 2 mL water (add 3 μL 2-octanol to top of water as an anti-foaming agent), elute with two 2 mL aliquots of 200 mM pH 8.8 K_2HPO_4, add 80 μL phosphoric acid to the eluate, make up to 5 mL with water, inject an aliquot. Milk. Add 200 μL milk to the SPE cartridge, wash with two 1 mL aliquots of MeOH, wash with 2 mL water (add 3 μL 2-octanol to top of water as an anti-foaming agent), elute with two 2 mL aliquots of 200 mM pH 8.8 K_2HPO_4, add 80 μL phosphoric acid to the eluate, make up to 5 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:10 mM pH 2.2 KH_2PO_4 16:84
Column temperature: 30
Flow rate: 1
Detector: UV 254 or F ex 232 em 432

CHROMATOGRAM

Retention time: 1.9
Limit of quantitation: 2.45 pmole (F), 245 pmole (UV)

KEY WORDS

rat; SPE

REFERENCE

Tibbels,T.S.; Smith,R.A.; Cohen,S.M. Determination of saccharin in diet and biological materials, *J.Chromatogr.*, **1988**, *441*, 448–453.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 5.907

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: food
Sample preparation: 2 g Soy sauce or 1 g sugared fruit or roast beef + 10 g NaCl, make up to 50 mL with acetone, swirl vigorously, let stand for 30 min, filter, wash the solid with acetone, make up filtrate to 50 mL with acetone, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm monomeric C18 (Shoko, Kyoto)
Mobile phase: MeCN:50 mM pH 4.5 α-hydroxyisobutyric acid in water 22:34 containing 2.5 mM hexadecyltrimethylammonium bromide
Flow rate: 1
Injection volume: 20
Detector: UV 233

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Simultaneous: acesulfame-K, benzoic acid, 3-t-butyl-4-hydroxyanisole, butyl p-hydroxybenzoate, t-butylhydroxyquinone, dulcin, ethyl p-hydroxybenzoate, isobutyl p-hydroxybenzoate, isopropyl p-hydroxybenzoate, methyl p-hydroxybenzoate, sodium dehydroacetate, sorbic acid

KEY WORDS
soy sauce; roast beef; sugared fruit

REFERENCE
Chen,B.H.; Fu,S.C. Comparison of extraction methods and column types for the determination of additives by liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 625–643.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets and weigh out 1 g, add 1 mL formic acid, add 25 mL MeOH, shake mechanically for 10 min, make up to 50 mL with methanol. Remove 10 mL and centrifuge. 5 mL Supernatant + 5 mL 0.0025% p-hydroxybenzoic acid in MeOH:water 20:80, make up to 25 mL with water, inject an aliquot. (Analyze within 1 h.)

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb RP8
Mobile phase: MeOH:200 mM pH 3.5 phosphate buffer:water 20:10:70
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: p-hydroxybenzoic acid (18)

OTHER SUBSTANCES
Simultaneous: aspirin, p-aminophenol, 3-O-acetylascorbic acid, 2-O-acetylascorbic acid, Ascorbic acid, acetaminophen, O-acetyl-p-aminophenol, salicylic acid (UV 280), diacetyl-p-aminophenol (UV 280)

KEY WORDS
tablets

REFERENCE
Thomis,R.; Roets,E.; Hoogmartens,J. Analysis of tablets containing aspirin, acetaminophen, and ascorbic acid by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 1830–1833.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, add 80 mL water, shake at 240 oscillations/min for 30 min, make up to 100 mL with water, mix well, centrifuge at 1500 rpm, dilute supernatant with water, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 3:97 (Buffer was 250 mM NaH_2PO_4 adjusted to pH 2.5 with phosphoric acid.) (Flush column with MeCN:water 5:95 at the end of each day.)
Flow rate: 2
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: pantothenic acid, pantoyllactone, panthenol

KEY WORDS
tablets

REFERENCE
Timmons,J.A.; Meyer,J.C.; Steible,D.J.; Assenza,S.P. Reverse phase liquid chromatographic assay for calcium pantothenate in multivitamin preparations and raw materials, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 510–513.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1 mL syrup to 50 mL with mobile phase, filter (0.45 μm), inject 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeCN:water:formic acid:methanesulfonic acid 500:500:1:1, pH adjusted to 3.5 with 10% NaOH
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: guaifenesin, dextromethorphan, benzoic acid

KEY WORDS
syrup

REFERENCE

Chen,T.M.; Pacifico,J.R.; Daly,R.E. High-pressure liquid chromatographic assay of dextromethorphan hydrobromide, guaifenesin, and sodium benzoate in an expectorant syrup, *J.Chromatogr.Sci.*, **1988**, *26*, 636–639.

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM

Retention time: 7.5

OTHER SUBSTANCES

Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, riboflavin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS

tablets

REFERENCE

Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam,

mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: yogurt

Sample preparation: 500 mg Homogenized yogurt + 7 mL buffer, sonicate for 2 min, shake mechanically for 20 min, centrifuge at 2000 rpm for 10 min, repeat extraction twice more. Combine the supernatants and make up to 25 mL with buffer. Remove a 5 mL extract and add it to 5 mL 10 mM tri-n-octylamine in chloroform, shake for 20 min, centrifuge at 2000 rpm for 10 min. Remove 2.5 mL of the organic phase and add it to 2.5 mL 100 mM sodium perchlorate in water, extract, centrifuge, inject an aliquot of the aqueous phase. (Buffer was 24.650 g $NaH_2PO_4.H_2O$ and 1.260 g $Na_2HPO_4.2H_2O$ made up to 2 L with water, pH 5.5.)

HPLC VARIABLES

Column: 250 × 4 10 μm RP-18 (Merck)

Mobile phase: MeOH:buffer 40:60 (Buffer was 900 μL 1 M phosphoric acid and 27.598 g $NaH_2PO_4.H_2O$ made up to 2 L with water, pH 4.5.)

Injection volume: 100

Detector: UV 270 for 4 min then UV 240

CHROMATOGRAM

Retention time: 2

Limit of detection: 20 μg/g

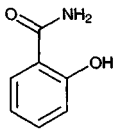
OTHER SUBSTANCES

Simultaneous: benzoic acid, sorbic acid

REFERENCE

Puttemans,M.L.; Branders,C.; Dryon,L.; Massart,D.L. Extraction of organic acids by ion-pair formation with tri-n-octylamine. Part 6. Determination of sorbic acid, benzoic acid, and saccharin in yogurt, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 80–82.

Salicylamide



Molecular formula: $C_7H_7NO_2$
Molecular weight: 137.14
CAS Registry No.: 65-45-2
Merck Index: 8480
Lednicer No.: 1 109

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Plasma. 200 μL Plasma + 100 μL 1 M perchloric acid, mix, centrifuge at 12000 g for 3 min, inject a 100 μL aliquot of the supernatant. Bile. Dilute bile 1:10 with water, inject a 20 μL aliquot. Urine. Filter (0.45 μm) urine, dilute with water 1:4, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: 40 μm μBondapak C18
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: 85 mM KH_2PO_4, pH adjusted to 3.35 with glacial acetic acid
Flow rate: 0.8 for 34 min, to 1.8 over 5 min, maintain at 1.8 for 11 min
Injection volume: 20-100
Detector: UV 313

CHROMATOGRAM

Retention time: 48
Limit of detection: 1.09 nmole

OTHER SUBSTANCES

Extracted: metabolites, conjugates

KEY WORDS

rat; plasma; whole blood

REFERENCE

Xu,X.; Pang,K.S. High-performance liquid chromatographic method for the quantitation of salicylamide and its metabolites in biological fluids, *J.Chromatogr.*, **1987**, *420*, 313–327.

SAMPLE

Matrix: blood
Sample preparation: 3 mL Whole blood + 6 mL ethyl acetate + 100 μL 10 μg/mL p-nitrophenol, shake, centrifuge at 3000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 40 μm μBondapak C18
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:0.5% acetic acid 40:60
Flow rate: 0.6
Injection volume: 20
Detector: UV 313

CHROMATOGRAM

Internal standard: p-nitrophenol
Limit of detection: 83 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
rat; whole blood

REFERENCE
Xu,X.; Pang,K.S. High-performance liquid chromatographic method for the quantitation of salicylamide and its metabolites in biological fluids, *J.Chromatogr.*, **1987**, *420*, 313–327.

SAMPLE
Matrix: blood, urine
Sample preparation: Whole blood. Mix whole blood with 2 volumes cold MeOH, centrifuge, inject an aliquot of the supernatant. Urine. Dilute 24 h urine with 5 mL water, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 25 × 3.9 37-50 μm Bondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: THF:1% acetic acid 5:95 containing 1.5 mM tetrabutylammonium hydroxide
Flow rate: 1.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Extracted: metabolites, conjugates

KEY WORDS
mouse; whole blood

REFERENCE
Howell,S.R.; Kotkoskie,L.A.; Dills,R.L.; Klaassen,C.D. 3-Hydroxylation of salicylamide in mice, *J.Pharm.Sci.*, **1988**, *77*, 309–313.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compounds in MeOH, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 3 μm Hitachi-Gel 3011 porous polymer (Hitachi)
Mobile phase: MeOH:ammonia 99:1
Flow rate: 0.03
Injection volume: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.16

OTHER SUBSTANCES
Also analyzed: acetaminophen, caffeine, bucetin (3-hydroxy-p-butyrophenetidine), phenacetin, dipyrone (sulpyrin), mefenamic acid, aspirin, salicylic acid, ethenzamide (o-ethoxybenzamide), theobromine, theophylline

KEY WORDS
semi-micro; porous polymer

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 30:1.5:0.5:68
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.19

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 230, 296

CHROMATOGRAM
Retention time: 2.4
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES
Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE
Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphet-

amine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in the mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm Lichrosorb RP 18
Mobile phase: MeOH:water 45:55 containing 1% acetic acid
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 4.20

OTHER SUBSTANCES

Simultaneous: acetaminophen, aspirin, phenacetin, salicylic acid

REFERENCE
Nivaud-Guernet,E.; Guernet,M.; Ivanovic,D.; Medenica,M. Effect of eluent pH on the ionic and molecular forms of the non-steroidal anti-inflammatory agents in reversed-phase high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2343–2357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 8 μm Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)
Mobile phase: MeCN:water 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: acetaminophen, aspirin, phenacetin

REFERENCE
Jedrejewski,P.T.; Taylor,L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1995**, *33*, 438–445.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Cole-Parmer Model 4420) one hemisphere with 4 mL 100 mM pH 5.0 sodium phosphate buffer and p-nitrophenol at 1000 rpm for 2 min, add 5 mL ethyl acetate, vortex for 2 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 5 μm Partisil-5 ODS-3 RAC
Mobile phase: MeOH:25 mM KH_2PO_4:acetic acid 30:69:1
Flow rate: 2
Injection volume: 10
Detector: UV 313

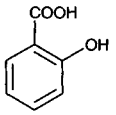
CHROMATOGRAM
Retention time: 7.5
Internal standard: p-nitrophenol (12.5)

OTHER SUBSTANCES
Extracted: gentisamide

KEY WORDS
rat; brain

REFERENCE
Morris,M.E.; Levy,G. Pharmacodynamics of the hypnotic effect of salicylamide in rats, *J.Pharm.Sci.*, **1985**, *74*, 599–602.

Salicylic acid



Molecular formula: $C_7H_6O_3$
Molecular weight: 138.12
CAS Registry No.: 69-72-7, 54-21-7 (Na salt)
Merck Index: 8484

SAMPLE

Matrix: blood
Sample preparation: Mix 100 μL plasma with 100 μL 2.5 μM IS, add 200 μL EtOH, vortex for 2 min, centrifuge at 1600 g for 15 min, dilute 50 μL of the supernatant with 950 μL mobile phase, filter (0.45 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 30 × 4.6 10 μm Spheri-10 RP18 (Alltech)
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:buffer 15:85 (Buffer was 30 mM sodium citrate and 30 mM sodium acetate, pH 5.45.)
Flow rate: 1
Injection volume: 50
Detector: UV 295

CHROMATOGRAM

Retention time: 7.2
Internal standard: 2.6-dihydroxybenzoic acid (11.4)
Limit of detection: 3.89 μM

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Coudray,C.; Mangournet,C.; Bouhadjeb,S.; Faure,H.; Favier,A. Rapid high-performance liquid chromatographic assay for salicylic acid in plasma without solvent extraction, *J.Chromatogr.Sci.*, **1996**, *34*, 166–173.

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 200 mM orthophosphoric acid to 250 μL chilled plasma within 10 min of centrifuging (if fresh plasma) or within 10 min of thawing (if frozen plasma), vortex for 20 s, centrifuge at 5800 g for 3 min. Inject a 200 μL aliquot onto column A and elute to waste with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 4.3 30 μm Hypersil C18 PEEK cartridge (Shandon, England); B 10 × 4 30 μm Hypersil C8 + 250 × 4.6 5 μm Nucleosil C8
Mobile phase: A Water:orthophosphoric acid 1000:1, pH 2.5; B MeCN:MeOH:water:orthophosphoric acid 150:200:650:1 (pH 2.6)
Flow rate: 1
Injection volume: 200
Detector: UV 225

CHROMATOGRAM

Retention time: 60
Limit of detection: 40 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: aspirin
Noninterfering: barbital, butobarbital, caffeine, 8-chlorotheophylline, clonazepam, cocaine, diazepam, flurazepam, furosemide, hydralazine, imipramine, nitrazepam, phenytoin, pindolol, propranolol, quinidine, theophylline
Interfering: xylazine, prazosin

KEY WORDS
column-switching; plasma

REFERENCE
McMahon,G.P.; Kelly,M.T. Determination of aspirin and salicylic acid in human plasma by column-switching liquid chromatography using on-line solid-phase extraction, *Anal.Chem.*, **1998**, *70*, 409–414.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, vortex for 30 s, centrifuge at 900 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:water 22:5:73
Flow rate: 2.6
Injection volume: 100
Detector: UV 280

CHROMATOGRAM
Retention time: 7.0
Limit of quantitation: 5000 ng/mL

OTHER SUBSTANCES
Extracted: aspirin
Noninterfering: acetaminophen, albuterol, aminophylline, amitriptyline, amoxicillin, ampicillin, amobarbital, beclomethasone, carbamazepine, carbenicillin, chlordiazepoxide, cimetidine, clonazepam, cyproheptadine, debrisoquine, dextropropoxyphene, diazepam, digoxin, dihydroxyanthraquinone, ergotamine, ethosuximide, fluphenazine, furosemide, gentamicin, gentisic acid, guaifenesin, haloperidol, heparin, hydrocortisone, indomethacin, methdilazine, methyclothiazide, methylphenobarbitone, methysergide, metoclopramide, naproxen, nitrazepam, nystatin, penicillin, pentobarbitone, phenytoin, phenytoin, pizotifen, prazosin, prednisone, prochlorperazine, propranolol, spironolactone, sulfamethoxazole, theophylline, trifluoperazine, trimethoprim, valproic acid

KEY WORDS
plasma

REFERENCE
Cham,B.E.; Ross-Lee,L.; Bochner,F.; Imhoff,D.M. Measurement and pharmacokinetics of acetylsalicylic acid by a novel high performance liquid chromatographic assay, *Ther.Drug Monit.*, **1980**, *2*, 365–372.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3

Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 8.0
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS
serum

REFERENCE
Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 15 μg/mL β-hydroxyethyltheophylline in MeCN, mix for 30 s, centrifuge at 13000 g for 5 min. Inject the supernatant (about 20 μL).

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:buffer 9.75:90.25 (Buffer was 100 mM KH_2PO_4 adjusted to pH 4.0 with phosphoric acid.) (At the end of each day clean with water for 20 min and MeOH for 30 min.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: β-hydroxyethyltheophylline (5.8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: aspirin
Simultaneous: dyphylline, theophylline, caffeine, acetaminophen, procainamide, N-acetylprocainamide
Noninterfering: benzoic acid

KEY WORDS
serum

REFERENCE
Ou,C.-N.; Frawley,V.L. Theophylline, dyphylline, caffeine, acetaminophen, salicylate, acetylsalicylate, procainamide, and N-acetylprocainamide determined in serum with a single liquid-chromatographic assay, *Clin.Chem.*, **1982**, *28*, 2157–2160.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1-2 mL Plasma + 600-1000 mg potassium carbonate plasma + 1 mL MeOH, shake for 1 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 25-50 μL aliquot.

HPLC VARIABLES
Column: 610 × 2 25-50 μm Bondapak AX/Corasil anion exchange
Mobile phase: MeOH:pH 5.6 phosphate buffer 15:85
Flow rate: 0.5
Injection volume: 10-50
Detector: UV 254, 280

CHROMATOGRAM
Retention time: 11.2 (at UV 280)
Internal standard: sodium salicylate

OTHER SUBSTANCES
Extracted: dipyrone (at UV 254)

KEY WORDS
plasma; sodium salicylate is IS

REFERENCE
Asmardi,G.; Jamali,F. High-performance liquid chromatography of dipyrone and its active metabolite in biological fluids, *J.Chromatogr.*, **1983**, *277*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Whole blood + 1 mL 1 M HCl + 200 μL 1 mg/mL β-hydroxyethyltheophylline in MeOH + 10 mL dichloromethane, rotate for 10 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS (Altex)
Mobile phase: MeOH:water:glacial acetic acid 40:66:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 6.4
Internal standard: β-hydroxyethyltheophylline (2)
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: methyl salicylate, theophylline
Noninterfering: acetaminophen, amobarbital, butalbital, caffeine, carbamazepine, glutethimide, ibuprofen, indomethacin, meprobamate, methaqualone, pentobarbital, phenobarbital, phenylbutazone, phenytoin, primidone, secobarbital

KEY WORDS
whole blood

REFERENCE
Levine,B.; Caplan,Y.H. Liquid chromatographic determination of salicylate and methyl salicylate in blood and application to a postmortem case, *J.Anal.Toxicol.*, **1984**, *8*, 239–241.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 0.2 mL 1 M HCl + 10 mL diethyl ether, gently mix for 10 min, centrifuge at 1500 rpm for 4 min. Remove the organic phase, evaporate it to dryness at 0° under a stream of nitrogen, add 200 μL mobile phase, vortex 90 s, inject a 5-100 μL aliquot.

HPLC VARIABLES
Guard column: 23 × 3.9 μBondapak C18/Porasil B

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:1-butanol:orthophosphoric acid 270:720:10:0.13
Column temperature: 47
Flow rate: 1.8
Injection volume: 5-100
Detector: UV 234

CHROMATOGRAM
Retention time: 8.0
Internal standard: m-anisic acid (9.6)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: aspirin
Noninterfering: acetaminophen, albuterol, aminophylline, amitriptyline, atenolol, beclomethasone, bromazepam, caffeine, carbamazepine, chloral hydrate, chlordiazepoxide, cimetidine, clonazepam, codeine, desipramine, dexamethasone, dextropropoxyphene, diazepam, dicyclomine, digoxin, disopyramide, doxycycline, ergotamine, ethosuximide, furosemide, gentisic acid, haloperidol, hydrocortisone, imipramine, indomethacin, levodopa, lignocaine, lithium carbonate, meperidine, methdilazine, methylphenobarbitone, methylprednisolone, methysergide, metoclopramide, metoprolol, mexiletine, midazolam, naphthoxyacetic acid, nitrazepam, nitroglycerin, nortripyline, oxazepam, oxpranolol, pentobarbitone, pethidine, phenytoin, prednisolone, prednisone, primidone, procainamide, prochlorperazine, propranolol, quinidine, salicyluric acid, spironolactone, sulfamethoxazole, theophylline, trimethoprim, valproic acid, verapamil, warfarin
Interfering: methyclothiazide

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Brandon,R.A.; Eadie,M.J.; Smith,M.T. A sensitive liquid chromatographic assay for plasma aspirin and salicylate concentrations after low doses of aspirin, *Ther.Drug Monit.*, **1985**, *7*, 216–221.

SAMPLE
Matrix: blood
Sample preparation: Activate a 1 mL Bond-Elut C8 SPE cartridge with 2 mL MeOH then 1 mL 10 mM HCl, do not allow it to dry completely. Sonicate 1 mL whole blood for 20-30 min then apply to cartridge. Wash with 100 μL water, elute with three 500 μL portions of MeOH:MeCN:1% aqueous ammonium hydroxide 50:20:30, combine eluents and evaporate to dryness under a stream of nitrogen at 40°. Redissolve in 1 mL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Spherisorb ODS
Mobile phase: MeCN:MeOH:buffer 35:13:52 (Buffer was water adjusted to pH 3.2 with orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: ketoprofen, acetaminophen, naproxen, fenoprofen, ibuprofen, indomethacin

KEY WORDS
whole blood; SPE

REFERENCE
Moore,C.M.; Tebbett,I.R. Rapid extraction of anti-inflammatory drugs in whole blood for HPLC analysis, *Forensic Sci.Int.*, **1987**, *34*, 155–158.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Blood + 1 mL water + 50 μL 76 mg/L allobarbital in EtOH:water 10:90 + 5 mL ethyl acetate, shake by hand, add 2 mL of 0.1 M HCl. Mix by inversion with a mechanical shaker for 5 min. Centrifuge at 2700 rpm for 5-10 min. Remove ethyl acetate and evaporate to dryness under a stream of nitrogen at room temperature. Take up in 200 μL MeOH, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pak precolumn insert
Column: 200 × 4.6 5 μm Hypersil octadecylsilane
Mobile phase: Gradient. MeCN:1 mM pH 3.2 phosphate buffer from 20:80 to 40:60 over 10 min, stay at 40:60 for 6 min, to 20:80 over 4 min
Column temperature: 60
Flow rate: 3
Injection volume: 20
Detector: UV 202

CHROMATOGRAM
Retention time: 3.6
Internal standard: allobarbital (2.9)
Limit of quantitation: 10000 ng/mL pharmacokinetics

OTHER SUBSTANCES
Extracted: butalbital
Simultaneous: caffeine, aspirin

REFERENCE
Drost,M.L.; Walter,L. Blood and plasma concentrations of butalbital following single oral doses in man, *J.Anal.Toxicol.*, **1988**, *12*, 322–324.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 400 μL 100 μg/mL 8-chlorotheophylline in MeCN, vortex for 10 s, centrifuge at 15000 rpm for 3 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water:glacial acetic acid 4:84:12 containing 4.84 g/L Trizma, pH 2.3
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.26
Internal standard: 8-chlorotheophylline (5.29)
Limit of quantitation: 8 μg/mL

OTHER SUBSTANCES
Extracted: acetaminophen, theophylline
Simultaneous: caffeine, cefazolin, cimetidine, ergotamine, glutethimide, heparin, methamphetamine, propranolol, sulfamethoxazole, theobromine, tobutamide, trimethoprim
Noninterfering: amitriptyline, amobarbital, ampicillin, butabarbital, butalbital, celbenine, chlordiazepoxide, chlorpromazine, clorazepate, desipramine, diazepam, doxepin, ethchlorvynol, fluphenazine, hydroxyzine, ibuprofen, imipramine, isoniazid, lidocaine, mephobarbital, mesoridazine, methaqualone, methyluric acid, naprotyline, nordiazepam, nortriptyline, oxazepam, pentobarbital, perphenazine, phenelzine, phenmetrazine, phenobarbital, phenylbutazone, phenytoin, prednisolone, prednisone, procainamide, prochlorperazine, promazine, promethazine, propoxyphene, protriptyline, pyrilamine, secobarbital, thioridazine, thiothixene, timolol, trazodone, triazolam, trifluoperazine

KEY WORDS
serum

REFERENCE
Osterloh,J.; Yu,S. Simultaneous ion-pair and partition liquid chromatography of acetaminophen, theophylline and salicylate with application to 500 toxicologic specimens, *Clin.Chim.Acta*, **1988**, *175*, 239–248.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 2.5 mL 10 μL/mL salicylamide in MeOH, stir, centrifuge. Remove a 1.8 mL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:50 mM acetic acid 40:60
Flow rate: 1
Detector: UV 237

CHROMATOGRAM
Retention time: 5.6
Internal standard: salicylamide (7.7)
Limit of detection: 500 ng/mL

KEY WORDS
plasma; rat; dog; pharmacokinetics; also for humans (see Int.J.Clin.Pharmacol.Ther.Toxicol. 1988, 26, 421)

REFERENCE
Ramis,J.; Mis,R.; Forn,J. Pharmacokinetics of fosfosal in rats and dogs, *Arzneimittelforschung*, **1989**, *39*, 74–77.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL anisic acid + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:0.5 mM phosphoric acid 30:70
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Internal standard: anisic acid

KEY WORDS
plasma; rat

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: blood

Sample preparation: Add o-anisic acid to 1 mL plasma, acidify with HCl, extract with diethyl ether. Remove the organic layer and evaporate it to dryness, reconstitute the residue, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-8
Mobile phase: MeCN:20 mM phosphoric acid 15:85
Flow rate: 1.6
Detector: UV 237

CHROMATOGRAM
Internal standard: o-anisic acid
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: aspirin

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Benedek,I.H.; Joshi,A.S.; Pieniaszek,H.J.; King,S.-Y.P.; Kornhauser,D.M. Variability in the pharmacokinetics and pharmacodynamics of low dose aspirin in healthy male volunteers, *J.Clin.Pharmacol.*, **1995**, *35*, 1181–1186.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.22 μm), inject a 2 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: MeCN:50 mM pH 3.0 phosphate buffer 1.5:98.5
Flow rate: 0.5
Injection volume: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Extracted: aspirin

KEY WORDS
serum; direct injection

REFERENCE
Nimura,N.; Itoh,H.; Kinoshita,T. Diol-bonded silica gel as a restricted access packing forming a binary-layered phase for direct injection of serum for the determination of drugs, *J.Chromatogr.A*, **1995**, *689*, 203–210.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 200 μL Plasma + 200 μL 5 μg/mL IS in 200 mM HCL:200 mM orthophosphoric acid 50:50, vortex for 1-2 s, add 400 μL MeCN, vortex, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 100-120 mg NaCl, vortex briefly, let stand at 4° for 10 min, vortex, centrifuge at 10500 g for 1 min, inject a 10 μL aliquot of the upper organic layer. Whole blood. 200 μL Lysed whole blood + 400 μL 5 μg/mL IS in 200 mM HCL:200 mM orthophosphoric acid 50:50, vortex for 1-2 s, add 600 μL MeCN, vortex, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 200 mg NaCl, vortex briefly, let stand at 4° for 10 min, vortex, centrifuge at 10500 g for 1 min, inject a 10 μL aliquot of the upper organic layer.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:water:85% orthophosphoric acid 18:74:0.09 (Before use prime column by recycling 200 mL mobile phase + 400 μL di-n-butylamine overnight at 0.3 mL/min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10
Detector: UV 237

CHROMATOGRAM

Retention time: 6.8
Internal standard: 2-methylbenzoic acid (8.9)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, aspirin, gentisic acid, salicyluric acid

KEY WORDS

plasma; whole blood; pharmacokinetics

REFERENCE

Kees,F.; Jehnich,D.; Grobecker,H. Simultaneous determination of acetylsalicylic acid and salicylic acid in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *677*, 172–177.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 5.2
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, luminal contents, tissue
Sample preparation: Homogenize intestinal tissue with water, make up to 25 mL with water, sonicate for 20 min. 100 μL Whole blood, luminal contents or tissue homogenate + 200 μL MeCN + 100 μL 5 mM HCl, vortex for 30 s, centrifuge at 3000 rpm for 5 min, inject a 30 μL aliquot of the clear supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:glacial acetic acid 35:65:4
Flow rate: 1.5
Injection volume: 30
Detector: UV 313

CHROMATOGRAM
Limit of detection: <1 μg/mL

KEY WORDS
rat; whole blood; intestine; pharmacokinetics

REFERENCE
Choi,Y.M.; Chung,S.M.; Chiou,W.L. First-pass accumulation of salicylic acid in gut tissue after absorption in anesthetized rat, *Pharm.Res.*, **1995**, *12*, 1323–1327.

SAMPLE
Matrix: blood, plants, urine, water
Sample preparation: Urine, plasma, water. Acidify 2 mL urine, plasma, or drinking water to pH 1 with 2 M HCl, shake with two 5 mL aliquots of diethyl ether, centrifuge. Remove the organic layer and add it to a few drops of saturated sodium bicarbonate solution, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL MeOH, inject a 25 μL aliquot. Hay, grain. Homogenize 30 g hay or grain with 10 mL 10% NaOH and 290 mL water, heat on a steam bath for 1 h, filter. Acidify a 10 mL aliquot of the filtrate to pH 1 with 2 M HCl, shake with two 5 mL aliquots of diethyl ether, centrifuge. Remove the organic layer and add it to a few drops of saturated sodium bicarbonate solution, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18 radial compression
Mobile phase: MeCN:0.05% phosphoric acid 30:70 (plasma) or MeCN:0.2% acetic acid 20:80 (urine)
Flow rate: 1.5
Injection volume: 25
Detector: F ex 313 em 425

CHROMATOGRAM
Limit of detection: 100 ng/mL (plasma), 5 μg/mL (urine)

KEY WORDS
horse; plasma; hay; grain; pharmacokinetics

REFERENCE
Beaumier,P.M.; Fenwick,J.D.; Stevenson,A.J.; Weber,M.P.; Young,L.M. Presence of salicylic acid in standardbred horse urine and plasma after various feed and drug administrations, *Equine.Vet.J.*, **1987**, *19*, 207–213.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 900 μL 270 mM HCl + 100 μL 100 μg/mL α-phenylcinnamic acid in MeOH + 10 mL dichloromethane, shake at 125 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot. Urine. 2 mL Urine + 900 μL 270 mM HCl + 100 μL 100 μg/mL α-phenylcinnamic acid in MeOH + 10 mL hexane, shake at 125 cycles/

min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:1% acetic acid 60:40
Flow rate: 2
Injection volume: 25
Detector: UV 300

CHROMATOGRAM
Retention time: 3.0
Internal standard: α-phenylcinnamic acid (8.0)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: aspirin (UV 280), salsalate

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Harrison,L.I.; Funk,M.L.; Ober,R.E. High-pressure liquid chromatographic determination of salicylsalicylic acid, aspirin, and salicylic acid in human plasma and urine, *J.Pharm.Sci.*, **1980**, *69*, 1268–1271.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 200 μL 330 mM perchloric acid, mix thoroughly, centrifuge at 2600 g for 10 min, inject an aliquot of the supernatant. Urine. 100 μL Urine + 900 μL 200 mM pH 5 phosphate buffer, mix, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 8 μm Cp Spher C8 (Chrompack) + 250 × 4.6 5 μm Spherisorb 5 ODS
Mobile phase: Gradient. MeCN:buffer from 1:99 to 35:65 over 35 min, return to initial conditions over 3 min, re-equilibrate for 2 min. (Buffer was 6 g orthophosphoric acid and 1 mL glacial acetic acid in 1 L distilled water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 236

CHROMATOGRAM
Retention time: 32.09
Limit of quantitation: 5 μg/mL (urine), 200 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites, gentisic acid, hippuric acid, salicylic acid acyl glucuronide, salicylic acid phenolic glucuronide, salicyluric acid, salicyluric acid phenolic glucuronide

KEY WORDS
pharmacokinetics

REFERENCE
Vree,T.B.; van Ewijk-Beneken Kolmer,E.W.J.; Verwey-van Wissen,C.P.W.G.M.; Hekster,Y.A. Direct gradient reversed-phase high-performance liquid chromatographic determination of salicylic acid, with the corresponding glycine and glucuronide conjugates in human plasma and urine, *J.Chromatogr.B*, **1994**, *652*, 161–170.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Adjust pH of plasma to 3 with 43% phosphoric acid. 500 μL Acidified plasma + 300 μL 10 μg/mL m-hydroxybenzoic acid in MeCN + 1.2 mL MeCN, vortex

for 1-2 min, centrifuge at 2000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 500 μL mobile phase, centrifuge at 1500 g for 2 min, inject a 50 μL aliquot of the supernatant. Urine. Dilute 25 μL urine to 500 μL with 40 μg/mL m-hydroxybenzoic acid in MeCN:water 10:90, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 (Brownlee)
Column: 150 × 4.6 5 μm Axxiom ODS (Springfield VA)
Mobile phase: MeCN:MeOH:25 mM acetic acid 8.5:8.5:83
Flow rate: 1
Injection volume: 25-50
Detector: UV 310

CHROMATOGRAM
Retention time: 16.5
Internal standard: m-hydroxybenzoic acid (8.2)
Limit of quantitation: 5 μg/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: etodolac, ibuprofen, ketorolac

KEY WORDS
plasma; paper also contains details of preparative HPLC; pharmacokinetics

REFERENCE
Liu,J.-H.; Smith,P.C. Direct analysis of salicylic acid, salicyl acyl glucuronide, salicyluric acid and gentisic acid in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 61–70.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 12.12

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in dilute HCl (pH 2), sonicate if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil-BDS C18
Mobile phase: MeOH:THF:buffer 11:4:85 (Buffer was 8.6 g $NaH_2PO_4.H_2O$, 8.2 g NaCl, 2.2 g sodium 1-heptanesulfonate, and 5.75 mL 85% phosphoric acid in 2 L water, pH 2.0.)
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 38

OTHER SUBSTANCES
Simultaneous: impurities, 5-aminosalicylic acid

KEY WORDS
impurity in 5-aminosalicylic acid

REFERENCE
Kersten,B.S.; Catalano,T.; Rozenman,Y. Ion-pairing high-performance liquid chromatographic method for the determination of 5-aminosalicylic acid and related impurities in bulk chemical, *J.Chromatogr.*, **1991**, *588*, 187–193.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 20 mg/mL solution of bulk aspirin in dichloromethane, inject a 10 μL aliquot as soon as dissolution is complete. Tablets. Prepare a 20 mg/mL solution of ground aspirin tablets in dichloromethane, filter (0.45 μm) immediately, immediately inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 6 μm Zorbax SIL
Mobile phase: Hexane:chloroform:acetic acid 80:19:3 (Before first use pump 10 column volumes of dichloromethane:acetic acid:2,3-dimethoxypropane 96:2:2 through column at 3 mL/min.)
Flow rate: 3
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 1.6
Limit of detection: 14 ppm

OTHER SUBSTANCES
Simultaneous: aspirin, salsalate

KEY WORDS
normal phase; tablets

REFERENCE
Pfeiffer,C.D.; Pankey,J.W. Determination of related compounds in aspirin by liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 511–514.

SAMPLE
Matrix: formulations
Sample preparation: Vaccine. Centrifuge vaccine at 3400 g for 15 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 5 × 4 5 μm Hypersil C18
Column: 210 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:water:orthophosphoric acid 35:35:0.9, pH 2.5
Flow rate: 0.6
Injection volume: 25
Detector: UV 222

CHROMATOGRAM
Retention time: 6.5
Internal standard: salicylic acid

OTHER SUBSTANCES
Simultaneous: dithiosalicylic acid, thimerosal, thiosalicylic acid,

KEY WORDS
vaccine; salicylic acid is IS

REFERENCE
Tleugabulova,D.; Gonzalez Perez,I. Reversed-phase high-performance liquid chromatographic study of thimerosal stability in Cuban recombinant hepatitis B vaccine, *J.Chromatogr.A*, **1996**, *729*, 219–227.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 75 mg powdered tablets with 25 mL mobile phase for 15 min, filter (paper), inject a 135 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Scharlau Science, Spain)
Mobile phase: MeOH:20 mM pH 4.0 KH_2PO_4 30:70 adjusted to pH 4.0 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 135
Detector: UV 224

CHROMATOGRAM
Retention time: 9.7
Limit of quantitation: 1.5 mg/L

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, thiamine

KEY WORDS
tablets

REFERENCE
Gámiz-Gracia,L.; Luque de Castro,M.D. An HPLC method for the determination of vitamin B1, caffeine, acetylsalicylic acid, and the impurities of salicylic acid in a pharmaceutical preparation, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2123–2133.

SAMPLE
Matrix: formulations
Sample preparation: Place 5 tablets in MeCN:MeOH:85% phosphoric acid 92:8:0.5, sonicate 15 min, shake 15 min, dilute to 250 mL. Centrifuge an aliquot in 50 mL tube at 2000 rpm for 15 min and filter supernatant (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve (Waters)
Mobile phase: MeCN:water:85% phosphoric acid 24:76:0.5
Column temperature: 35
Flow rate: 2
Injection volume: 10
Detector: UV 295

CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, acetaminophen

KEY WORDS
film coated tablets; tablets

REFERENCE
Fogel,J.; Epstein,P.; Chen,P. Simultaneous high-performance liquid chromatography assay of acetylsalicylic acid and salicylic acid in film-coated aspirin tablets, *J.Chromatogr.*, **1984**, *317*, 507–511.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder and add about 250 mg aspirin to 100 mL chloroform saturated with citric acid containing 500 μL formic acid, add 500 mg solid citric acid, sonicate for 2 min, centrifuge or filter, inject an aliquot. (If buffers or antacid are present add ground tablets equivalent to about 500 mg aspirin to 3 g acid-washed siliceous earth, mix, add 2 mL 6 M HCl, mix, add to a 200 × 25 column, dry wash container with siliceous earth, add to column, elute column with chloroform saturated with citric acid at 10 mL/min. Collect 150 mL eluent, add 1 mL formic acid, make up to 200 mL with chloroform saturated with citric acid, add 500 mg citric acid, shake, inject an aliquot.)

HPLC VARIABLES
Column: 250 × 4.6 5μm Zorbax-Sil
Mobile phase: Chloroform:methylene chloride:acetonitrile:formic acid 700:300:30:4 (At the end of the day wash the column with 200 mL MeOH.)
Flow rate: 2
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: aspirin, acetylsalicylsalicylic acid, acetylsalicylic acid anhydride, excipients

KEY WORDS
normal phase; tablets; SPE

REFERENCE
Galante,R.N.; Visalli,A.J.; Grim,W.M. Stabilized normal-phase high-performance liquid chromatographic analysis of aspirin and salicylic acid in solid pharmaceutical dosage forms, *J.Pharm.Sci.*, **1984**, *73*, 195–197.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets and weigh out 1 g, add 1 mL formic acid, add 25 mL MeOH, shake mechanically for 10 min, make up to 50 mL with methanol. Remove 10 mL and centrifuge. 5 mL Supernatant + 5 mL 0.0025% p-hydroxybenzoic acid in MeOH:water 20:80, make up to 25 mL with water, inject an aliquot. (Analyze within 1 h.)

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb RP8
Mobile phase: MeOH:200 mM pH 3.5 phosphate buffer:water 20:10:70
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 3
Internal standard: p-hydroxybenzoic acid (18)

OTHER SUBSTANCES
Simultaneous: aspirin (UV 254), p-aminophenol (UV 254), 3-O-acetylascorbic acid (UV 254), 2-O-acetylascorbic acid (UV 254), saccharin (UV 254), acetaminophen (UV 254), O-acetyl-p-aminophenol (UV 254), Ascorbic acid (UV 254), diacetyl-p-aminophenol

KEY WORDS
tablets

REFERENCE
Thomis,R.; Roets,E.; Hoogmartens,J. Analysis of tablets containing aspirin, acetaminophen, and ascorbic acid by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 1830–1833.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: phenyl
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM KH_2PO_4 containing 0.52% tetrabutylammonium phosphate, pH adjusted to 7.1 with KOH.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 6
Internal standard: salicylic acid

OTHER SUBSTANCES
Simultaneous: nitroprusside

KEY WORDS
injections; 5% dextrose; salicylic acid is IS

REFERENCE
Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

SAMPLE
Matrix: formulations
Sample preparation: Condition a 500 mg Extract Clean silica SPE cartridge (Alltech stock no. 209250) with 2 mL hexane. Allow a solution of aspirin in 10 mM sorbitan trioleate in CFC-11 to evaporate, dissolve the residue in 5 mL hexane. Add 1 mL to the SPE cartridge, elute with two 2 mL portions of mobile phase, make up eluate to 5 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 3040 μm Perisorb RP-8 Pellicular (Upchurch)
Column: 250 × 4.6 5 μm Econosphere C8
Mobile phase: MeOH:THF:1 M phosphoric acid:water 44:5:5:46
Flow rate: 1

Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 10.6

OTHER SUBSTANCES
Extracted: aspirin, degradation products

KEY WORDS
aerosols; SPE

REFERENCE
Blondino,F.E.; Byron,P.R. The quantitative determination of aspirin and its degradation products in a model solution aerosol, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 111–119.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out amount containing 100 mg salicylic acid, make up to 50 mL with mobile phase. Remove a 2 mL aliquot and dilute it to 10 mL with mobile phase, filter, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water:96% acetic acid 55:44:1, pH 3.0
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.44

OTHER SUBSTANCES
Simultaneous: triamcinolone acetonide

KEY WORDS
topical solution

REFERENCE
Kedor-Hackmann,E.R.M.; Gianotto,E.A.S.; Santoro,M.I.R.M. Determination of triamcinolone acetonide and salicylic acid in pharmaceutical formulations by high performance liquid chromatography, *Pharmazie*, **1996**, *51*, 63–63.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: Lichrospher 100 RP-18
Mobile phase: MeOH:water containing 10 mM citric acid 60:40
Column temperature: 45
Flow rate: 1
Detector: F ex 300 em 408

KEY WORDS
rat; intestine; Caco-2 monolayer

REFERENCE
Takagi,M.; Taki,Y.; Sakane,T.; Nadai,T.; Sezaki,H.; Oku,N.; Yamashita,S. A new interpretation of salicylic acid transport across the lipid bilayer: Implication of pH-dependent but not carrier-mediated absorption from the gastrointestinal tract, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 1175–1180.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5μm Brownlee C18 (Applied Biosystems)
Mobile phase: MeCN:0.03% phosphoric acid:triethylamine 64:35:1, pH 2.0
Flow rate: 1
Injection volume: 20
Detector: UV 237

OTHER SUBSTANCES
Simultaneous: diflunisal

REFERENCE
Hung,D.Y.; Mellick,G.D.; Anissimov,Y.G.; Weiss,M.; Roberts,M.S. Hepatic disposition and metabolite kinetics of a homologous series of diflunisal esters, *J.Pharm.Sci.*, **1998**, *87*, 943–951.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeOH:50 mM phosphoric acid 25:75
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: indoleacetic acid, phenylacetic acid

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:8 mM pH 7.0 phosphate buffer 5:95
Flow rate: 1.2
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: lodoxamide

KEY WORDS
aerosol

REFERENCE
Havel,H.A.; Beaubien,L.J.; Haaland,P.D. Analysis of the variance components in a pharmaceutical aerosol product: lodoxamide tromethamine, *J.Pharm.Sci.*, **1985**, *74*, 978–982.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compounds in MeOH, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 3 μm Hitachi-Gel 3011 porous polymer (Hitachi)
Mobile phase: MeOH:ammonia 99:1
Flow rate: 0.03
Injection volume: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.56

OTHER SUBSTANCES
Also analyzed: acetaminophen, caffeine, bucetin (3-hydroxy-p-butyrophenetidine), phenacetin, dipyrone (sulpyrin), mefenamic acid, aspirin, salicylamide, ethenzamide (o-ethoxybenzamide), theobromine, theophylline

KEY WORDS
semi-micro; porous polymer

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions
Sample preparation: Add 500 μL of a solution in MeCN to 100 mg finely powdered potassium carbonate, add 250 μL 3.8 mM 18-crown-6 in MeCN, add 250 μL 0.8 mM reagent in MeCN, heat at 80° in the dark for 20 min, cool, inject a 5 μL aliquot. (Synthesize the reagent, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone, as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°).)

HPLC VARIABLES
Column: 100 × 4 10 μm Radial-Pak C18 (Waters)
Mobile phase: Gradient. MeOH:water from 57:43 to 100:0 over 20 min, maintain at 100:0 for 12 min
Flow rate: 2
Injection volume: 5
Detector: F ex 370 em 450

CHROMATOGRAM
Retention time: 7.1
Limit of detection: 0.3-1 fmole

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, arachidic acid, arachidonic acid, benzoic acid, butyric acid, capric acid, caproic acid, caprylic acid, deoxyuridine, glucuronic acid, imidazole-4-acetic acid, lauric acid, linoleic acid, linolenic acid, margaric acid, 1-methyl-4-imidazoleacetic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, propionic acid, stearic acid, thymidine, uridine, valeric acid

KEY WORDS
derivatization

REFERENCE
Yamaguchi,M.; Hara,S.; Matsunaga,R.; Nakamura,M.; Ohkura,Y. 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as a new fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *346*, 227–236.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 35:1.5:0.5:63
Flow rate: 1.5
Injection volume: 10
Detector: UV 261

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: physostigmine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: React the carboxylic acid, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in MeCN at 45° for 2 h, inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH satu-

rated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.6 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6
Limit of detection: 1 pmole

OTHER SUBSTANCES
Simultaneous: benzoic acid, quinoxaline-2-carboxylic acid

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: 10 μL Solution + 500 μL micelle solution + 25 μL 28 mg/mL 9-bromomethylacridine in acetone, mix, heat at 60° for 6 min, inject a 20 μL aliquot. (Micelle solution was 25 mM Arkopal N-130 (a polyoxyethylene(13)nonylphenol, Hoechst Holland, Amsterdam) in 10 mM pH 7.0 phosphate buffer containing 6 mM tetrakis(decyl)ammonium bromide. Synthesize 9-bromomethylacridine as follows. Heat 10 g diphenylamine, 10 mL glacial acetic acid, and 40 g anhydrous zinc chloride to 220°, evaporate excess acetic acid with stirring, heat at 220-230° for 6 h, digest with hot 10% sulfuric acid, make strongly alkaline with 25% ammonia to dissolve the zinc chloride. Extract the insoluble residue with toluene. Extract the organic layer with 10% sulfuric acid, make the aqueous layer alkaline with aqueous ammonia. Collect the yellow precipitate that separates and recrystallize it twice from petroleum ether to give 9-methyl acridine as pale yellow needles. Reflux 560 mg 9-methylacridine, 445 mg N-bromosuccinimide, and 10 mg benzoyl peroxide in 30 mL carbon tetrachloride for more than 2 h, cool, chromatograph on silica gel with benzene:ethyl acetate 30:1 (Caution! Benzene is a carcinogen!) to obtain 9-bromomethylacridine as yellow crystals (mp 147-151°) (Anal. Lett. 1987, 20, 1581).)

HPLC VARIABLES
Guard column: 10 × 2.1 40 μm Chromsep C18 (Chrompack)
Column: 100 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: Gradient. MeOH:water from 20:80 to 100:0 over 13 min.
Injection volume: 20
Detector: UV 254, F ex 362 em 418

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: cholic acid, ibuprofen, valproic acid

KEY WORDS
derivatization

REFERENCE
van der Horst,F.A.L.; Post,M.H.; Holthuis,J.J.M.; Brinkman,U.A.T. Derivatization of carboxylic acids with 9-bromomethylacridine using micellar phase-transfer catalysis, *Chromatographia*, **1989**, *28*, 267–273.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 16.5

OTHER SUBSTANCES
Simultaneous: acetophenone, amphetamine, desipramine, ethylmorphine, imipramine, mefenamic acid, methamphetamine, morphine, phenylbutazone

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spheri-5 RP-8
Mobile phase: MeOH:buffer 30:70 (Prepare buffer by mixing 4 mM Na_2HPO_4 and 7 mM KH_2PO_4 to achieve pH 7.)
Flow rate: 1
Injection volume: 50
Detector: F ex 355 em 460 (408 nm cutoff filter) following post-column extraction. The column effluent mixed with 50 μg/mL reagent in mobile phase pumped at 0.5 mL/min and then with chloroform pumped at 1 mL/min and the mixture flowed through a 1.8 m × 0.3 mm ID knitted PTFE coil to a 50 μL membrane phase separator using a polyethylene-backed 0.5 μm Fluoropore membrane filter (design in paper). The organic phase flowed to the detector. (Synthesize the reagent, α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate, as follows. Stir 20 mmoles 3,4-dimethoxyphenylacetonitrile and 20 mmoles p-toluamide in 50 mL EtOH at 50°, add 5 mL 50% aqueous KOH slowly, stir at 50° for 5 min, cool to room temperature, filter, dry the precipitate of α-(3,4-dimethoxyphenyl)-4'- methylcinnamonitrile. Dissolve 20 mmoles α-(3,4-dimethoxyphenyl)-4'-methylcinnamonitrile, 20 mmoles N-bromosuccinimide, and 20 mg benzoyl peroxide in 100 mL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), reflux with stirring for 1.5 h , cool, filter, evaporate to dryness under reduced pressure, recrystallize from MeOH to give α-(3,4-dimethoxyphenyl)-4'-bromomethylcinnamonitrile. Vigorously stir 30 mmoles anhydrous dimethylamine in 100 mL dry benzene (Caution! Benzene is a carcinogen!) at 0°, very slowly add 10 mmoles α-(3,4-dime-

thoxyphenyl)-4'-bromomethylcinnamonitrile while stirring at 0°, stir at room temperature overnight, add 150 mL water, remove the organic phase, extract the aqueous phase twice with 100 mL portions of diethyl ether, wash the organic layers with saturated NaCl solution, dry over anhydrous magnesium sulfate, evaporate under reduced pressure to give α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile (J.Chem.Eng.Data 1987, 32, 387). Reflux 10 mmoles α-(3,4-dimethoxyphenyl)-4'-dimethylaminomethylcinnamonitrile, 20 mmoles dimethyl sulfate (Caution! Dimethyl sulfate is a carcinogen and acutely toxic!), and 5 g potassium carbonate in 50 mL acetone for 1 h, cool to room temperature, filter, dry the precipitate under vacuum at room temperature overnight, recrystallize from chloroform containing 2-3 drops of 95% EtOH to give α-(3,4-dimethoxyphenyl)-4'-trimethylammoniummethylcinnamonitrile methosulfate (mp 212-215°). Protect solutions from light.)

CHROMATOGRAM
Retention time: k' 0.2791
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: ibuprofen, ketoprofen, mefenamic acid, naproxen, probenecid, valproic acid

KEY WORDS
post-column extraction; post-column reaction

REFERENCE
Kim,M.; Stewart,J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α-phenylcinnamonitrile quaternary ammonium salt as a new fluorescent ion-pair reagent, *J.Liq.Chromatogr.*, **1990**, *13*, 213–237.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin,

mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in the mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm Lichrosorb RP 18
Mobile phase: MeOH:water 45:55 containing 1% acetic acid
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 7.63

OTHER SUBSTANCES

Simultaneous: acetaminophen, aspirin, phenacetin, salicylamide

REFERENCE

Nivaud-Guernet,E.; Guernet,M.; Ivanovic,D.; Medenica,M. Effect of eluent pH on the ionic and molecular forms of the non-steroidal anti-inflammatory agents in reversed-phase high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2343–2357.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL Solution + 50 μL pH 7.4 PBS + 100 μL MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 20:80
Flow rate: 1
Injection volume: 50
Detector: UV 220

CHROMATOGRAM

Internal standard: salicylic acid

OTHER SUBSTANCES
Simultaneous: atenolol

KEY WORDS
buffer; Earle's balanced salt solution; salicylic acid is IS

REFERENCE
Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.20 (A), 4.35 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 mm long 5 μm Microsorb-MV C18
Mobile phase: MeOH:water 30:70
Flow rate: 1
Detector: UV 300

REFERENCE
Phillips,C.A.; Michniak,B.B. Transdermal delivery of drugs with differing lipophilicities using azone analogs as dermal penetration enhancers, *J.Pharm.Sci.*, **1995**, *84*, 1427–1433.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.50

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: wine
Sample preparation: Adjust pH of wine to 7-8 with potassium bicarbonate. Remove a 1 mL aliquot and add it to 1 mL 170 mM phenacyl bromide in acetone, add 1 mL 17 mM 18-crown-6 in acetone, add 1 mL acetone, heat in a boiling water bath for 75 min, cool, inject a 10 μL aliquot. (Recrystallize phenacyl bromide from n-heptane.)

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/Corasil
Column: 250 × 4 7 μm RP-18 (Merck)
Mobile phase: Gradient. MeOH:water from 35:65 to 85:15 over 20 min.
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11.7

OTHER SUBSTANCES

Extracted: acetic acid, anisic acid, benzilic acid, benzoic acid, butyric acid, caprylic acid, cinnamic acid, citramalic acid, citric acid, enanthic acid, fumaric acid, galacturonic acid, gallic acid, glutaric acid, glycolic acid, glyoxylic acid, p-hydroxybenzoic acid, isocitric acid, α-ketoglutaric acid, lactic acid, malic acid, mandelic acid, phenylacetic acid, propionic acid, protocatechuic acid, pyruvic acid, sorbic acid, succinic acid, tartaric acid, valeric acid, vanillic acid, ascorbic acid

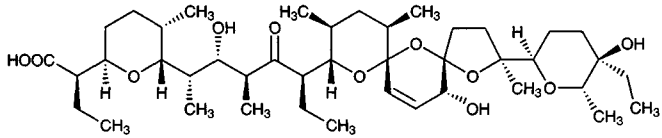
KEY WORDS

derivatization

REFERENCE

Mentasti,E.; Gennaro,M.C.; Sarzanini,C.; Baiocchi,C.; Savigliano,M. Derivatization, identification and separation of carboxylic acids in wines and beverages by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *322*, 177–189.

Salinomycin



Molecular formula: $C_{42}H_{70}O_{11}$
Molecular weight: 751.01
CAS Registry No.: 53003-10-4, 55721-31-8 (sodium salt)
Merck Index: 8488

SAMPLE

Matrix: albumen, eggs, feed, premix, tissue
Sample preparation: Eggs, feed, premix, tissue. 1 g Sample + 15 mL acetone, vortex for 5 min, centrifuge, decant the acetone layer. Re-extract residue (2x), evaporate the combined acetone layers to dryness. Partition the residue between 25 mL aqueous EtOH (water:EtOH 80:20) and 25 mL petroleum ether (50-110°). Evaporate EtOH fraction to dryness, dissolve residue in MeOH, adjust volume to 500 μL. Inject a 50 or 100 μL aliquot. Albumen. 1 g Sample + 15 mL MeOH, extract for 15 min, centrifuge at 1290 × g at -5°. Evaporate to dryness, dissolve residue in MeOH, adjust to a final volume of 500 μL. Inject a 50 or 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Inertsil ODS-2 (Mandel Scientific)
Column: 250 × 3.2 5 μm CSC-Inertsil 150A/ODS-2 (Mandel Scientific)
Mobile phase: MeOH:water:acetic acid 95:5:0.1
Flow rate: 0.5
Injection volume: 50-100
Detector: UV 520 nm following post-column reaction. The column effluent mixed with vanillin reagent pumped at 1 mL/min. The mixture flowed through a Teflon knitted reactor coil (10 m × 0.5 mm i.d., total volume 2 mL) at 95° to the detctor. (Prepare the vanillin reagent as follows. Slowly and carefully add 20 mL concentrated sulphuric acid (95-98%) to 950 mL chilled MeOH, mix well, allow to cool to room temperature. Add 30 g vanillin with constant stirring. Filter solution, degas using vacuum, and store in amber-colored bottle.)

CHROMATOGRAM

Retention time: <10
Limit of detection: 5 ng/g
Limit of quantitation: 10 ng/g

KEY WORDS

post-column reaction

OTHER SUBSTANCES

Extracted: acetic acid, anisic acid, benzilic acid, benzoic acid, butyric acid, caprylic acid, cinnamic acid, citramalic acid, citric acid, enanthic acid, fumaric acid, galacturonic acid, gallic acid, glutaric acid, glycolic acid, glyoxylic acid, p-hydroxybenzoic acid, isocitric acid, α-ketoglutaric acid, lactic acid, malic acid, mandelic acid, phenylacetic acid, propionic acid, protocatechuic acid, pyruvic acid, sorbic acid, succinic acid, tartaric acid, valeric acid, vanillic acid, ascorbic acid

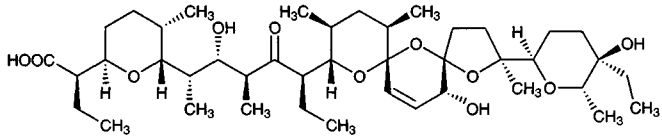
KEY WORDS

derivatization

REFERENCE

Mentasti,E.; Gennaro,M.C.; Sarzanini,C.; Baiocchi,C.; Savigliano,M. Derivatization, identification and separation of carboxylic acids in wines and beverages by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *322*, 177–189.

Salinomycin



Molecular formula: $C_{42}H_{70}O_{11}$
Molecular weight: 751.01
CAS Registry No.: 53003-10-4, 55721-31-8 (sodium salt)
Merck Index: 8488

SAMPLE

Matrix: albumen, eggs, feed, premix, tissue
Sample preparation: Eggs, feed, premix, tissue. 1 g Sample + 15 mL acetone, vortex for 5 min, centrifuge, decant the acetone layer. Re-extract residue (2x), evaporate the combined acetone layers to dryness. Partition the residue between 25 mL aqueous EtOH (water:EtOH 80:20) and 25 mL petroleum ether (50-110°). Evaporate EtOH fraction to dryness, dissolve residue in MeOH, adjust volume to 500 μL. Inject a 50 or 100 μL aliquot. Albumen. 1 g Sample + 15 mL MeOH, extract for 15 min, centrifuge at 1290 × g at -5°. Evaporate to dryness, dissolve residue in MeOH, adjust to a final volume of 500 μL. Inject a 50 or 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Inertsil ODS-2 (Mandel Scientific)
Column: 250 × 3.2 5 μm CSC-Inertsil 150A/ODS-2 (Mandel Scientific)
Mobile phase: MeOH:water:acetic acid 95:5:0.1
Flow rate: 0.5
Injection volume: 50-100
Detector: UV 520 nm following post-column reaction. The column effluent mixed with vanillin reagent pumped at 1 mL/min. The mixture flowed through a Teflon knitted reactor coil (10 m × 0.5 mm i.d., total volume 2 mL) at 95° to the detctor. (Prepare the vanillin reagent as follows. Slowly and carefully add 20 mL concentrated sulphuric acid (95-98%) to 950 mL chilled MeOH, mix well, allow to cool to room temperature. Add 30 g vanillin with constant stirring. Filter solution, degas using vacuum, and store in amber-colored bottle.)

CHROMATOGRAM

Retention time: <10
Limit of detection: 5 ng/g
Limit of quantitation: 10 ng/g

KEY WORDS

post-column reaction

REFERENCE

Akhtar,M.H.; abou el-Sooud,K.; Shehata,M.A.A. Concentrations of salinomycin in eggs and tissues of laying chickens fed medicated feed for 14 days followed by withdrawal for 3 days, *Food Addit.Contam.*, **1996**, *13*, 897–907.

SAMPLE

Matrix: blood

Sample preparation: Extract plasma with isooctane, add the organic layer to a silica SPE cartridge, wash with dichloromethane, wash with dichloromethane:MeOH (?) 98.5:1.5, elute with dichloromethane:MeOH 90:10, evaporate to dryness, reconstitute with dichloromethane, oxidize with pyridinium dichromate, concentrate, inject an aliquot on to column A and elute to waste with mobile phase A, after 1.85 min divert the effluent from column A on to column B (?), after another 1.8 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 75 mm long C18 (Waters); B 250 mm long C18 (Beckman)
Mobile phase: A MeCN:0.01% HCl 90:10; B MeCN:0.01% HCl 96:4
Detector: UV 225

CHROMATOGRAM

Limit of detection: 5 ng/mL

KEY WORDS

derivatization; plasma; column-switching; heart cut; SPE

REFERENCE

Wei,A.T.; Dimenna,G.P.; Karnes,H.T. HPLC analysis of sodium salinomycin in human plasma using derivatization and heart cut column switching, *Pharm.Res.*, **1992**, *9*, S21.–S21..

SAMPLE

Matrix: eggs, tissue

Sample preparation: 5 g Pulverized frozen tissue or 5 g homogenized whole eggs + 2 mL water + 13 mL MeOH, homogenize for 30 s. Sonicate for 10 min and centrifuge at 2000 g for 10 min. Add 4 mL 100 mM NaOH to a 2 mL aliquot of the supernatant, extract with 2 mL and 1 mL hexane:toluene 50:50 (v/v) for 30 s by inversion, centrifuge at 1500 g for 10 min. Evaporate the combined extracts to dryness under a stream of nitrogen at 60°. Dissolve the residue in 200 μL MeCN:water 75:25. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (GL Sciences, Japan)
Mobile phase: MeCN:MeOH:THF:water:trifluoroacetic acid 67:10:10:13:0.1
Flow rate: 1
Injection volume: 20
Detector: MS, VG Platform, Megaflow electrospray probe, positive ion mode, source at 125°, cone voltage 25 V, m/z 773

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 0.5-1 ng/g
Limit of quantitation: 2 ng/g

OTHER SUBSTANCES

Extracted: monensin, narasin

KEY WORDS

domestic fowl; muscle; liver

REFERENCE

Blanchflower,W.J.; Kennedy,D.G. Determination of monensin, salinomycin, and narasin in muscle, liver and eggs from domestic fowl using liquid chromatography-electrospray mass spectrometry, *J.Chromatogr.B*, **1996**, *675*, 225–233.

SAMPLE
Matrix: feed
Sample preparation: 20 g Ground Feed + 200 mL hexane:ethyl acetate 90:10, stir at high speed for 2 h, let stand. Remove an aliquot equivalent to 1 g feed and evaporate it to dryness under reduced pressure at 40°, reconstitute with 2 mL MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 60 × 4.6 3 μm C18 (Hewlett-Packard)
Mobile phase: MeOH:5% acetic acid 90:10
Flow rate: 0.5
Injection volume: 20-25
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 1.5 mL reaction coil (Kratos Model 510) at 95° to the detector. (Reagent was 40 g/L vanillin in MeOH:sulfuric acid 100:2. Keep in an ice bath and prepare fresh daily.)

CHROMATOGRAM
Retention time: 6.7
Limit of detection: 1 ppm

OTHER SUBSTANCES
Extracted: monensin, narasin

KEY WORDS
post-column reaction

REFERENCE
Lapointe,M.R.; Cohen,H. High-speed liquid chromatographic determination of monensin, narasin, and salinomycin in feeds, using post-column derivatization, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 480–484.

SAMPLE
Matrix: feed
Sample preparation: Pulverize feed in a grinder, mix with EtOH, sonicate for 5 min, filter (0.45 μm), dilute filtrate with EtOH if necessary. 5 mL Filtrate + 1 mL 600 μg/mL 2,4-dinitrophenylhydrazine in MeOH + 1 drop concentrated HCl, heat at 50° for about 3 min, cool to room temperature, make up to 10 mL with EtOH, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeOH:1.5% aqueous acetic acid 94:6
Flow rate: 1
Injection volume: 20
Detector: UV 380, UV 419

CHROMATOGRAM
Retention time: 7.8
Limit of detection: 2-5 ng

KEY WORDS
derivatization; maximum sensitivity at 419 nm

REFERENCE
Mathur,A.K. Determination of salinomycin by high-performance liquid chromatography using a precolumn derivatization technique, *J.Chromatogr.A*, **1994**, *664*, 284–288.

SAMPLE
Matrix: feed, premix
Sample preparation: Feed. Shake 5 g feed with 15 mL MeOH for 2 h, filter, evaporate the filtrate to 3 mL and make up to 10 mL with MeOH, inject a 3 μL aliquot. Premix. Shake 0.5

g premix with 15 mL MeOH for 2 h, filter, make up the filtrate to 50 mL with MeOH, inject a 3 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Separon SGX C18 glass column (Tessek Prague)
Mobile phase: MeOH:water:glacial acetic acid 94:5.9:0.1
Flow rate: 0.02
Injection volume: 3
Detector: UV 592 following post-column derivatization. The column effluent mixed with the reagent pumped at 0.015 mL/min and the mixture flowed through a 150 × 1 reactor containing 40-70 μm acid-washed glass beads at 75° to the detector. (The reagent was 500 mM 4-dimethylaminobenzaldehyde in 1.2 M sulfuric acid in MeOH.)

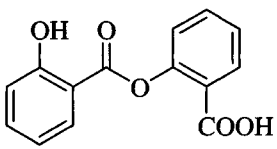
CHROMATOGRAM
Retention time: 16
Limit of detection: 2.2 μg/mL

OTHER SUBSTANCES
Extracted: monensin, narasin

KEY WORDS
derivatization; microbore; post-column reaction

REFERENCE
Fejglova,Z.; Dolezal,J.; Hrdlicka,A.; Frgalova,K. Microbore HPLC determination of polyether antibiotics using postcolumn derivatization with benzaldehyde reagents, *J.Liq.Chromatogr.*, **1994**, *17*, 359–372.

Salsalate



Molecular formula: $C_{14}H_{10}O_5$
Molecular weight: 258.23
CAS Registry No.: 552-94-3
Merck Index: 8491
Lednicer No.: 2 90

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 900 μL 270 mM HCl + 100 μL 100 μg/mL α-phenylcinnamic acid in MeOH + 10 mL dichloromethane, shake at 125 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot. Urine. 2 mL Urine + 900 μL 270 mM HCl + 100 μL 100 μg/mL α-phenylcinnamic acid in MeOH + 10 mL hexane, shake at 125 cycles/min for 15 min, centrifuge at 750 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:1% acetic acid 60:40
Flow rate: 2
Injection volume: 25
Detector: UV 300

CHROMATOGRAM
Retention time: 5.8
Internal standard: α-phenylcinnamic acid (8.0)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: aspirin (UV 280), salicylic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Harrison,L.I.; Funk,M.L.; Ober,R.E. High-pressure liquid chromatographic determination of salicylsalicylic acid, aspirin, and salicylic acid in human plasma and urine, *J.Pharm.Sci.*, **1980**, *69*, 1268–1271.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 20 mg/mL solution of bulk aspirin in dichloromethane, inject a 10 μL aliquot as soon as dissolution is complete. Tablets. Prepare a 20 mg/mL solution of ground aspirin tablets in dichloromethane, filter (0.45 μm) immediately, immediately inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 6 μm Zorbax SIL
Mobile phase: Hexane:chloroform:acetic acid 80:19:3 (Before first use pump 10 column volumes of dichloromethane:acetic acid:2,3-dimethoxypropane 96:2:2 through column at 3 mL/min.)
Flow rate: 3
Injection volume: 10
Detector: UV 254

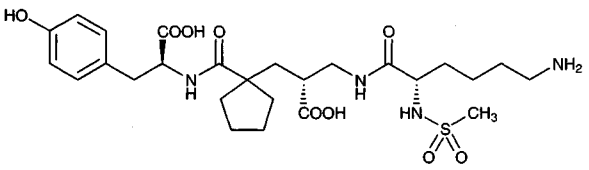
CHROMATOGRAM
Retention time: 2.2
Limit of detection: 5 ppm

OTHER SUBSTANCES
Simultaneous: aspirin, salicylic acid

KEY WORDS
normal phase; tablets

REFERENCE
Pfeiffer,C.D.; Pankey,J.W. Determination of related compounds in aspirin by liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 511–514.

Sampatrilat



Molecular formula: $C_{26}H_{40}N_4O_9S$
Molecular weight: 584.69
CAS Registry No.: 129981-36-8

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut Certify II SPE cartridge with 1 mL MeOH and 1 mL pH 7 phosphate buffer and a Bond-Elut C18 SPE cartridge with 1 mL MeOH and 1 mL water. Add 20 μL 1 μg/mL IS in water and 1 mL pH 7 phosphate buffer to 1 mL plasma, vortex. Add the mixture to the Bond-Elut Certify II cartridge. Wash with 1 mL pH 7 phosphate buffer, 1 mL MeOH:water:trifluoroacetic acid 15:85:0.1, dry under vacuum, elute with 1 mL MeOH: water:trifluoroacetic acid 80:20:0.1. Centrifuge briefly and evaporate to dryness under a stream of nitrogen at 37°. Reconstitute the residue with 1 mL 12% boron trifluoride in MeOH, incubate at 65° for 30 min. Add 2 mL water, evaporate to approximately 1 mL under a stream of nitrogen at 65°, add to the Bond-Elut C18 cartridge. Rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with three 1 mL portions of water, elute into MTBE-rinsed polypro-

pylene tapered tubes with two 1 mL portions of MeOH. Centrifuge briefly, evaporate to dryness under a stream of nitrogen at 37°. Reconstitute in HPLC injection solution, vortex, centrifuge at 10000 rpm for 5 min. Inject an 80 μL aliquot. (HPLC injection solution was MeOH:water 50:50 containing 20 mM ammonium acetate and 5 mM triethylamine.)

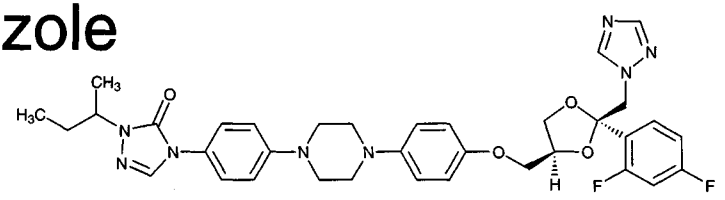
HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S5C6 (A), 30 × 4.6 3 μm (3 × 3CR C18) Perkin-Elmer (B)
Mobile phase: MeCN:20 mM pH 2.25 sodium phosphate buffer containing 2.5 mM octanesulfonic acid 35:65 (A); MeOH:water 80:20 containing 20 mM ammonium acetate and 5 mM triethylamine (B)
Flow rate: 1
Injection volume: 80
Detector: E, ESA Coulochem, analytical electrode 700 mV (A); MS, Perkin-Elmer Sciex API III-plus triple quadrupole, heated nebulizer, positive ion APCI mode, nebulizer 500°, collision gas argon, dwell time 100 ms, Q1 at m/z 613.1 and m/z 597.1, Q3 at m/z 211.4 (B)

CHROMATOGRAM
Internal standard: UK-79.942 (N-(1-(2-carboxy-3-(N2-acetyllysylamino)propyl)-1-cyclopentylcarbonyl)tyrosine, Pfizer Central Research, UK)
Limit of detection: 100 pg
Limit of quantitation: 500 pg/mL

KEY WORDS
plasma; derivatization; SPE

REFERENCE
Venn,R.F.; Kaye,B.; Macrae,P.V.; Saunders,K.C. Clinical analysis of sampatrilat, a combined renal endopeptidase and angiotensin-converting enzyme inhibitor. I: Assay in plasma of human volunteers by atmospheric-pressure ionisation mass-spectrometry following derivatisation with BF3-methanol, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 875–881.

Saperconazole



Molecular formula: $C_{35}H_{38}F_2N_8O_4$
Molecular weight: 672.74
CAS Registry No.: 110588-57-3
Merck Index: 8510

SAMPLE
Matrix: blood
Sample preparation: 250 μL serum + 50 μL 0.3 N barium hydroxide + 50 μL 0.4 N zinc sulfate + 1 mL MeCN, vortex, centrifuge at 3521 g for 15 min, evaporate the supernatant to dryness under a stream of nitrogen, reconstitute with 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 7.5 × 4.6 5 μm Alltech Alltima C18
Column: 250 × 4.6 5 μm Alltech Alltima C18
Mobile phase: MeCN:MeOH:50 mM pH 6.7 phosphate buffer 47:8:45
Column temperature: 37
Flow rate: 1
Detector: UV 263

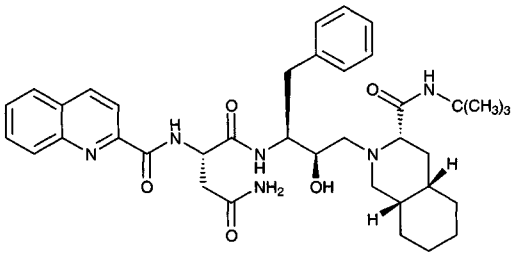
CHROMATOGRAM
Internal standard: saperconazole

OTHER SUBSTANCES
Extracted: itraconazole

KEY WORDS
serum; saperconazole is IS

REFERENCE
Christensen,K.J.; Gubbins,P.O.; Gurley,B.J.; Bowman,J.L.; Buice,R.G. Relative bioavailability of itraconazole from an extemporaneously prepared suspension and from the marketed capsules, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 261–265.

Saquinavir



Molecular formula: $C_{38}H_{50}N_6O_5$, $C_{38}H_{50}N_6O_5.CH_4O_3S$ (mesylate)
Molecular weight: 670.85
CAS Registry No.: 127779-20-8, 149845-06-7 (mesylate)
Merck Index: 8516

SAMPLE
Matrix: blood, CSF, saliva
Sample preparation: CSF, plasma. Condition a 1 mL 200 mg C2 SPE cartridge with 1 mL MeCN and 1 mL 100 mM ammonium acetate at 1 mL/min. 600 μL CSF or plasma + 600 μL 100 mm ammonium acetate solution, vortex for 10 s, centrifuge at 10500 g for 3 min. Add 1 mL of the diluted sample to the SPE cartridge using vacuum. Wash with 1 mL MeCN:100 mM ammonium acetate 30:70, suck dry using vacuum for 1 min. Elute with 400 μL MeCN:2.5 mM ammonium acetate 80:20, evaporate the eluate to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue in 150 μL mobile phase, vortex for 60 s, centrifuge at 10500 g for 3 min, inject an aliquot. Saliva. Condition a 1 mL 200 mg C2 SPE cartridge with 1 mL MeCN and 1 mL 100 mM ammonium acetate at 1 mL/min. 600 μL Saliva + 600 μL blank plasma + 1.2 mL 100 mm ammonium acetate, mix, centrifuge at 10500 g for 3 min. Add 2 mL diluted sample to the SPE cartridge using a vacuum. Wash with 1 mL MeCN:100 mM ammonium acetate 30:70, suck dry using a vacuum for 1 min. Elute with 400 μL MeCN:2.5 mM ammonium acetate 80:20, evaporate the eluate to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue in 150 μL mobile phase, vortex for 60 s, centrifuge at 10500 g for 3 min, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Chromguard C18 (Chrompack Netherlands)
Column: 75 × 4.6 3.5 μm Zorbax SB-C18
Mobile phase: MeCN:buffer 40.5:59.5 (Buffer was water containing 25 mM sodium acetate and 25 mM hexane-1-sulfonic acid, adjusted to pH 4.0 with 37% HCl)
Flow rate: 1
Injection volume: 100
Detector: UV 239

CHROMATOGRAM
Retention time: 7.5

Limit of detection: 1.0 ng/mL
Limit of quantitation: 2.5 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Hoetelmans,R.M.W.; van Essenberg,M.; Meenhorst,P.L.; Mulder,J.W.; Beijnen,J.H. Determination of saquinavir in human plasma, saliva, and cerebrospinal fluid by ion-pair high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1997**, *698*, 235–241.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Delta-pak C4 (Waters)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM ammonium dihydrogen phosphate and 1 mM 1-heptanesulfonic acid sodium salt, pH adjusted to 4.8 with ammonium hydroxide.)
Flow rate: 0.6
Injection volume: 35
Detector: UV 210

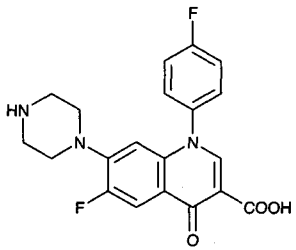
CHROMATOGRAM
Retention time: 22-27

OTHER SUBSTANCES
Simultaneous: indinavir, nelfinavir, ritonavir
Noninterfering: didanosine, lamivudine, stavudine, zalcitabine, zidovudine

REFERENCE
Iayewardene,A.L.; Zhu,F.; Aweeka,F.T.; Gambertoglio,J.G. Simple high-performance liquid chromatographic determination of the protease inhibitor indinavir in human plasma, *J.Chromatogr.B*, **1998**, *707*, 203–211.

Sarafloxacin

Molecular formula: $C_{20}H_{17}F_2N_3O_3$
Molecular weight: 385.37
CAS Registry No.: 98105-99-8, 91296-87-6 (HCl)
Merck Index: 8517



SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg Bond Elut LRC PRS SPE cartridge with 5 mL MeOH and 5 mL extracting solution 65:35. Add 25 mL extracting solution to 5 mL milk, shake for 15 s, add 4 g anhydrous sodium sulfate, shake for 15 s, centrifuge at 3000 rpm at 5° for 5 min. Remove the supernatant and repeat the extraction with 25 mL extracting solution as before except do not add any more sodium sulfate, mix mechanically, centrifuge, combine the supernatants, add 25 mL 1% acetic acid, shake for 10-15 s. Freeze for 30 min to facilitate precipitation, centrifuge at 2500 rpm at 5° for 10 min. Add 75 mL to the SPE cartridge, pass the entire sample through the cartridge, then add 2 mL MeOH, wash with 5 mL water, wash with 2 mL MeOH. Elute with 2.5 mL 25% ammonium hydroxide-MeOH. Evaporate to dryness under nitrogen at 55°, dissolve the residue in 2 mL 1% acetic acid, sonicate for 1 min, vortex for 20 s, filter (0.45 μm), inject an aliquot. (Extracting solution was 1% aqueous acetic acid:EtOH 1:99.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil
Mobile phase: MeCN:2% acetic acid 15:85
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 278 em 450, with a 418 nm cut-off filter

CHROMATOGRAM
Retention time: 5.6
Limit of detection: 1.2 ppb
Limit of quantitation: 5 ppb

OTHER SUBSTANCES
Extracted: ciprofloxacin, difloxacin, enrofloxacin

KEY WORDS
SPE

REFERENCE
Roybal,J.E.; Pfenning,A.P.; Turnipseed,S.B.; Walker,C.C.; Hurlbut,J.A. Determination of four fluoroquinolones in milk by liquid chromatography, *J.AOAC Int.*, **1997**, *80*, 982–987.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 10 mL 500 mg Bond Elut LRC PRS SPE cartridge with 2 mL MeOH and 2 mL equilibrating solution. 2 g Catfish muscle + 18 mL extracting solution, homogenize for 20 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add another 18 mL extracting solution to the pellet and homogenize again, centrifuge at 3000 rpm for 5 min, combine the supernatants. Add 20 mL 1% glacial acetic acid, freeze for 30 min, centrifuge at 2500 rpm at 4° for 10 min. Add the extracts to the SPE cartridge, wash with 2 mL MeOH, 5 mL water, and 2 mL MeOH. Let the SPE cartridge dry for 30 s. Elute with 2 mL MeOH:30% ammonium hydroxide 80:20, dry the eluate under nitrogen at 50°. Reconstitute the residue in 500 μL mobile phase, filter (0.45 μm), inject an aliquot. (The extracting solution was EtOH:water:glacial acetic acid 98:1:1. The equilibrating solution was extracting solution:1% glacial acetic acid 35:20.)

HPLC VARIABLES
Column: 150 × 2 5 μm Inertsil Phenyl
Mobile phase: MeCN:2% formic acid 14:86
Column temperature: 40
Flow rate: 0.35
Injection volume: 50
Detector: MS, Hewlett-Packard 5989, Model 59987A electrospray, nitrogen drying gas 40 mL/min, 260°, nebulizing gas nitrogen, 80 psi, m/z 342

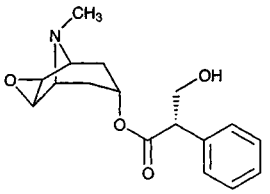
CHROMATOGRAM
Retention time: 7.33-7.75
Limit of detection: 10 ppb
Limit of quantitation: 20 ppb

OTHER SUBSTANCES
Extracted: difloxacin

KEY WORDS
catfish; muscle; SPE

REFERENCE
Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Pfenning,A.P.; Hurlbut,J.A. Confirmation of fluoroquinolones in catfish muscle by electrospray liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1998**, *81*, 554–562.

Scopolamine



Molecular formula: $C_{17}H_{21}NO_4$
Molecular weight: 303.36
CAS Registry No.: 51-34-3, 6533-68-2 (HBr trihydrate), 114-49-8 (HBr anhydrous)
Merck Index: 8550

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL MeOH, vortex briefly, add 50 μL 1 M ammonium hydroxide, mix, add 5 mL dichloromethane, shake horizontally for 5 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 5 μm BDS C18 (Keystone)
Column: 50 × 3 3 μm BDS C18 (Keystone)
Mobile phase: MeCN:MeOH:10 mM ammonium acetate 62.5:37.5:15
Flow rate: 0.5
Injection volume: 20
Detector: MS, Perkin Elmer Sciex API III-Plus triple quadrupole, APCI, nebulizer 400° and 80 psi, auxiliary nitrogen 1.2 L/min, curtain gas 1.2 L/min, interface 55°, collision gas argon, electron multiplier 3000 V, declustering potential 35 V, collision energy 35 eV

CHROMATOGRAM

Retention time: 0.8
Internal standard: scopolamine

OTHER SUBSTANCES

Extracted: hyoscyamine

KEY WORDS

plasma; protect from light; scopolamine is IS

REFERENCE

Xu,A.; Havel,J.; Linderholm,K.; Hulse,J. Development and validation of an LC/MS/MS method for the determination of L-hyoscyamine in human plasma, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 33–42.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 7.39

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.9

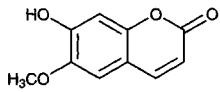
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine,

thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Scopoletin



Molecular formula: $C_{10}H_8O_4$
Molecular weight: 192.17
CAS Registry No.: 92-61-5
Merck Index: 8552

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

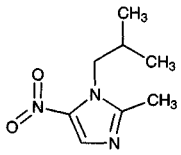
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine,

pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Secnidazole



Molecular formula: $C_7H_{11}N_3O_3$
Molecular weight: 185.18
CAS Registry No.: 3366-95-8
Merck Index: 8562

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 318.8

CHROMATOGRAM

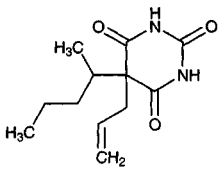
Retention time: 9.668

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Secobarbital



Molecular formula: $C_{12}H_{18}N_2O_3$
Molecular weight: 238.29
CAS Registry No.: 76-73-3, 309-43-3 (Na salt)
Merck Index: 8563
Lednicer No.: 1 269

SAMPLE

Matrix: blood
Sample preparation: 200 µL Serum + 200 µL 50 µg/mL hexobarbital in MeCN + 25 µL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 µL aliquot of the supernatant.

HPLC VARIABLES

Column: µBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM

Retention time: 25.8
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 50 µL 7 µg/mL IS in water + 1 mL buffer, vortex for 10 s, add 5 mL n-hexane:ether:n-propanol 49:49:2, shake gently for 20 min, centrifuge at 1000 g for 5 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 µL mobile phase, inject a 50-100 µL aliquot. (Buffer was 10 mM sodium acetate:10 mM acetic acid 88.5:11.5, pH 5.5.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm Partisil 5 ODS-3
Mobile phase: MeCN:buffer 28:72 (Buffer was 300 µL 1 M KH_2PO_4 and 50 µL 900 mM phosphoric acid in 1.8 L water, pH 4.4.)
Column temperature: 50
Flow rate: 2.8
Injection volume: 50-100
Detector: UV 195

CHROMATOGRAM
Retention time: 8.5
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (11.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, ethosuximide, phenytoin
Simultaneous: mephobarbital, paramethadione, phenobarbital, primidone
Noninterfering: chlorazepate, clonazepam, diazepam, thioridazine, valproic acid

KEY WORDS
serum; pharmacokinetics

REFERENCE
Levine,H.L.; Cohen,M.E.; Duffner,P.K.; Kustas,K.A.; Shen,D.D. An improved high-pressure liquid chromatographic assay for secobarbital in serum, *J.Pharm.Sci.*, **1982**, *71*, 1281–1283.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C8 SPE cartridge with 2 volumes of MeOH, 2 volumes of water, and 1 volume of 100 mM pH 5.59 Sörensen's phosphate buffer. Add 500 μL plasma to the SPE cartridge, wash with 2 volumes of 100 mM pH 5.59 Sörensen's phosphate buffer, wash with 1 volume of water, elute with 500 μL MeOH. Evaporate the eluate to dryness under vacuum, reconstitute in 50 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak C18 (Waters)
Column: 100 × 8 10 μm Radial-Pak C8 (Waters)
Mobile phase: MeOH:THF:100 mM pH 7.72 Sörensen's phosphate buffer 28:16:52
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.39
Internal standard: secobarbital

OTHER SUBSTANCES
Extracted: methohexital, pentobarbital, thiopental
Noninterfering: ketamine

KEY WORDS
plasma; dog; secobarbital is IS; SPE

REFERENCE
Avram,M.J.; Krejcie,T.C. Determination of sodium pentobarbital and either sodium methohexital or sodium thiopental in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1987**, *414*, 484–491.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)

Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 7.31
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, theophylline, thiopental
Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: methyl salicylate

KEY WORDS
serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 13.80
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, centrifuge at 10000 g, dilute supernatant with an equal volume of water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 110 × 4.7 5 μm PartiSphere C18 (Whatman)
Mobile phase: MeCN:15 mM pH 7.0 phosphate buffer 30:70
Flow rate: 0.8
Injection volume: 50
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted coil of PTFE tubing irradiated by an 8 W low-pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 8.7

OTHER SUBSTANCES
Extracted: aprobarbital, butethal, pentobarbital

KEY WORDS
plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Wolf,C.; Schmid,R.W. Enhanced UV-detection of barbiturates in HPLC analysis by on-line photochemical reaction, *J.Liq.Chromatogr.*, **1990**, *13*, 2207–2216.

SAMPLE
Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 12.82
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 17.42

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Dissolve injection in mobile phase to give a secobarbital sodium concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 75 × 4.6 5 μm Chromegabond C18 or 150 × 4.6 7 μm Zorbax ODS

Mobile phase: MeOH:buffer:polyethylene glycol 300 60:40:0.4 (Buffer was 4.1 g anhydrous sodium acetate and 15 mL acetic acid in 1 L water.)

Flow rate: 1

Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rugged; injections

REFERENCE
Reif,V.D.; Kaufmann,K.L.; DeAngelis,N.J.; Frankhouser,M.C. Liquid chromatographic assays for barbiturate injections, *J.Pharm.Sci.*, **1986**, *75*, 714–716.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 20-200 μg/mL solution in acetone with 50 μL of a 0.4-1.6 mg/mL solution of 2-bromo-2'-acetonaphthone in acetone, add 5-10 mg cesium carbonate, heat at 30° for 30 min, add 50 μL glacial acetic acid, mix, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 2
Detector: UV 249

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: amobarbital, butobarbital, heptobarbital, hexobarbital, mephobarbital, pentobarbital, phenobarbital
Interfering: barbital

KEY WORDS
derivatization

REFERENCE
Hulshoff,A.; Roseboom,H.; Renema,J. Improved detectability of barbiturates in high-performance liquid chromatography by pre-column labelling and ultraviolet detection, *J.Chromatogr.*, **1979**, *186*, 535–541.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate a solution in water, MeOH, or diethyl ether to dryness, add a 3-fold molar excess of triethylamine, add 0.5-3 mL MeCN, add a 3-fold molar excess of N-chloromethyl-4-nitrophthalimide, heat at 60° for 1 h, inject an aliquot. (Preparation of N-chloromethyl-4-nitrophthalimide is as follows. Suspend 130 g 4-nitrophthalimide in 80 mL 40% formaldehyde solution, add 200 mL water, reflux for 4 h, filter while hot, N-(hydroxymethyl-4-nitrophthalimide crystallizes on cooling (cf. J. Am. Chem. Soc. 1922, 44, 817). Mix a suspension of 2.26 g N-hydroxymethyl-4-nitrophthalimide in 10-15 mL ether with a suspension of 2.1 g phosphorus pentachloride in 10-15 mL ether, after 10 min heat on a water bath, cool in an ice-salt mixture, add ice-water dropwise with shaking, filter to obtain N-chloromethyl-4-nitrophthalimide, dry under vacuum (cf. Chem. Ber. 1959, 9, 1258).)

HPLC VARIABLES
Column: 7 μm LiChrosorb RP8

Mobile phase: MeCN:water 60:40
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.6
Limit of detection: 4 ng

OTHER SUBSTANCES
Extracted: amobarbital
Simultaneous: cyclobarbital, methylphenobarbital, phenobarbital

KEY WORDS
derivatization

REFERENCE
Lindner,W.; Santi,W. N-chloromethylphthalimides as derivatization reagents for high-performance liquid chromatography, *J.Chromatogr.*, **1979**, *176*, 55–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: allobarbital, amobarbital, barbital, barbituric acid, butabarbital, mephobarbital, methabarbital, methohexital, phenobarbital, phenytoin, thiamylal

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mm KH_2PO_4 + 5 mM 1-decanesulfonic acid 30:70, adjusted to pH 3.2 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 13.6
Internal standard: methyl paraben (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, butalbital, aprobarbital, mephobarbital, pentobarbital, phenobarbital, talbutal, vinbarbital

KEY WORDS
stability-indicating

REFERENCE
Ibrahim,F.B. Simultaneous determination and separation of several barbiturates and analgesic products by ion-pair high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2835–2851.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 2.04

OTHER SUBSTANCES
Simultaneous: aprobarbital, pentobarbital, amobarbital, butabarbital, butalbital, thiopental, phenobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, imino-

stilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.19 (A), 5.91 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, met-

ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.17

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 1.0
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone
Interfering: caffeine, cotinine, benzoylecgonine, oxazepam, phenobarbital, nordiazepam

KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine
Sample preparation: 2 mL Urine +1 mL 500 mM pH 5.5 phosphate buffer, add to an Extrelut 3 SPE cartridge, let stand for 10 min, elute with 15 mL dichloromethane:isopropanol 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm Lichrospher 100 RP8
Column: 250 × 4 5 μm Lichrospher 100 RP8
Mobile phase: Gradient. MeCN:10 mM pH 4.4 phosphate buffer from 30:70 to 40:60 over 8 min, maintain at 40:60 for 6 min, to 30:70 over 1 min
Flow rate: 1
Injection volume: 20
Detector: UV 212

CHROMATOGRAM

Retention time: 13.0
Limit of detection: 150 ng/mL

OTHER SUBSTANCES

Extracted: barbital, allobarbital, butabarbital, phenobarbital, pentobarbital
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nic-

otine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, triflupromazine, trimethoprim, trimipramine

KEY WORDS
SPE

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

Secretin

Molecular formula: $C_{130}H_{220}N_{44}O_{41}$
Molecular weight: 3055.45
CAS Registry No.: 1393-25-5
Merck Index: 8564

1 9
His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-
10 18
Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Asp-
19 27
Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in 1% trifluoroacetic acid adjusted to pH 2.5 with triethylamine, filter (0.2 μm), inject a 80 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Whatman)
Column: 300 mm long MCH-10 C18 (Varian)
Mobile phase: Gradient. A was 1% trifluoroacetic acid adjusted to pH 2.5 with triethylamine. B was isopropanol:trifluoroacetic acid 99:1 containing the same amount of triethylamine as A. A:B 80:20 for 5 min, to 65:35 over 50 min, to 40:60 over 10 min, maintain at 40:60 for 10 min. At the end of each run cycle repeatedly from 60:40 to 40:60 for 15 min.
Flow rate: 0.5
Injection volume: 80
Detector: RIA of fractions

CHROMATOGRAM
Retention time: 60

OTHER SUBSTANCES
Simultaneous: motilin, sincalide

REFERENCE
Chang,T.-M.; Erway,B.; Chey,W.Y. Rapid, small-scale preparation of gastrointestinal hormones by high-performance liquid chromatography on a C18 column, *J.Chromatogr.*, **1985**, *326*, 121–127.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in water, inject a 2 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:0.01% HCl 25:65
Flow rate: 1
Injection volume: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Tsuda,T.; Uchiyama,M.; Sato,T.; Yoshino,H.; Tsuchiya,Y.; Ishikawa,S.; Ohmae,M.; Watanabe,S.; Miyake,Y. Identification of secretin diastereoisomers produced during synthesis, *J.Pharm.Sci.*, **1989**, *78*, 91–94.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL Secretin in pH 4 or 7 Buffer (μ = 0.5) + 300 μL MeCN:1% perchloric acid 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 5 mM pH 3 phosphate buffer containing 200 mM sodium perchlorate.)
Flow rate: 1
Injection volume: 10
Detector: UV 210

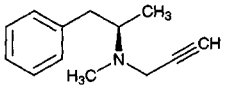
CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
buffer

REFERENCE
Tsuda,T.; Uchiyama,M.; Sato,T.; Yoshino,H.; Tsuchiya,Y.; Ishikawa,S.; Ohmae,M.; Watanabe,S.; Miyake,Y. Degradation peptides of secretin after storage in acid and neutral aqueous solutions, *J.Pharm.Sci.*, **1990**, *79*, 53–56.

Selegiline



Molecular formula: $C_{13}H_{17}N$
Molecular weight: 187.28
CAS Registry No.: 2323-36-6, 14611-51-9
Merck Index: 8569

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM
Retention time: 10.712

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM $(NH_4)_3PO_4$ 20:80 adjusted to pH 3.1 with concentrated phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 1 (relative retention time)
Internal standard: methamphetamine (relative retention time = 0.71)

REFERENCE
Vargay,Z.; Horváth,G.; Korponay,K.; Kovács,G.; Kálmánne Máthé,I.; Bánki,A. Attekintes a selegilin hatóanyag analitikájáról [Survey of the analysis of selegiline], *Acta Pharm.Hung.*, **1992**, *62*, 212–217.

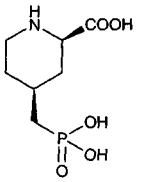
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsorb RP-18
Mobile phase: MeCN:buffer 20:80 (Buffer was 100 mM $(NH_4)H_2PO_4$ and 0.08% triethylamine adjusted to pH 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 205

OTHER SUBSTANCES
Simultaneous: degradation products, methamphetamine

REFERENCE
Chafetz,L.; Desai,M.P.; Sukonik,L. Trace decomposition of selegiline. Use of worst-case kinetics for a stable drug, *J.Pharm.Sci.*, **1994**, *83*, 1250–1252.

Selfotel



Molecular formula: $C_7H_{14}NO_5P$
Molecular weight: 223.17
CAS Registry No.: 110347-85-8

SAMPLE

Matrix: urine

Sample preparation: 50 μL Urine + 25 μL 100 μg/mL IS in water + 1 mL buffer, vortex for 5 s, add 100 μL reagent, vortex for 10 s, let stand for 1.5 min, add 200 μL 100 mg/mL iodoacetamide in MeCN, vortex for 15 s, let stand for 1.5 min, add 500 μL 2 mg/mL 9-fluorenylmethyl chloroformate in acetone, vortex for 20 s, let stand for 2 min, add 5 mL ether, vortex for 1 min, repeat the ether wash. Remove traces of organic solvent from the aqueous layer with a stream of nitrogen, vortex the aqueous layer for 2 s. Remove a 50 μL aliquot and add it to 500 μL mobile phase, inject a 50 μL aliquot onto column A and elute to waste with mobile phase A, divert the fraction containing selfotel and the IS onto column B, after 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Prepare buffer by dissolving 15.5 g boric acid in 500 mL water, adjust pH to 9.5 with NaOH. Prepare reagent by adding 32 μL 3-mercaptopropionic acid to 1 mL 50 mg/mL o-phthalaldehyde in MeCN. Primary amino acids are removed by derivatization with o-phthalaldehyde/3-mercaptopropionic acid.)

HPLC VARIABLES

Column: A 75 × 4.6 5 μm Inertsil ODS-2; B 250 × 4.6 10 μm Chiralcel OD-R

Mobile phase: A MeCN:100 mM pH 2.50 phosphate buffer 35:65; B MeCN:100 mM pH 2.00 phosphate buffer 35:65

Column temperature: 30

Flow rate: A 0.5 for 16 min, to 2.0 over 0.3 min, maintain at 2.0 for 18.4 min, return to 0.5 over 0.3 min; B 0.5

Injection volume: 50

Detector: F ex 262 em 314

CHROMATOGRAM

Retention time: 19.2, 22.0 (enantiomers)

Internal standard: cis-4-(phosphonoethyl)-2-piperidinecarboxylic acid (CGS 20005) (36 (first eluting peak))

Limit of quantitation: 250 ng/mL

KEY WORDS

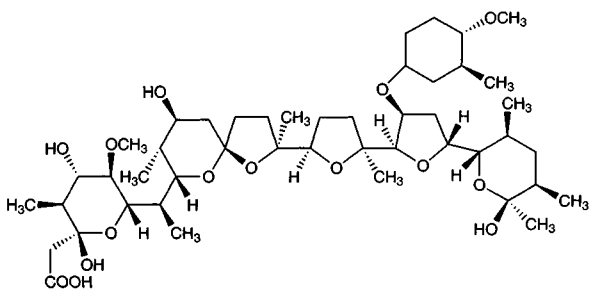
derivatization; chiral; column-switching

REFERENCE

Knoche,B.; Milosavljev,S.; Gropper,S.; Brunner,L.A.; Powell,M.L. Enantioselective determination of selfotel in human urine by high-performance liquid chromatography on a chiral stationary phase after derivatization with 9-fluorenylmethyl chloroformate, *J.Chromatogr.B*, **1997**, *695*, 355–363.

Semduramicin

Molecular formula: $C_{45}H_{76}O_{16}$
Molecular weight: 873.09
CAS Registry No.: 113378-31-7, 119068-77-8 (sodium salt)
Merck Index: 8587



SAMPLE

Matrix: tissue

Sample preparation: Condition a 200 mg BondElut LRC C8 SPE cartridge with 5 mL MeCN, 5 mL MeOH, add 5 mL water, do not allow to dry. Condition a 500 mg BondElut LRC silica SPE cartridge with 5 mL chloroform and 5 mL isooctane:dichloromethane 50:50, do not allow to dry. Vortex 1.25 g homogenized liver with 7.5 mL MeOH:water:ammonium hydroxide 80:20:1 for 3 min, heat at 55° for 1 h, centrifuge at 4000 rpm for 5 min, decant the supernatant, rinse the tube with 1-2 mL MeOH. Combine the rinse and the supernatant and evaporate them to 2-3 mL under a stream of nitrogen at 55°, add 5 mL water, vortex, sonicate for 5 min, add to the C8 SPE cartridge, rinse the tube with 1-2 mL water, add the rinse to the C8 SPE cartridge, wash with 3 mL water, wash with 1 mL MeOH:water 25:75, elute with 5 mL ethyl acetate, evaporate to dryness under a stream of nitrogen at 55°, reconstitute with 6 mL isooctane:dichloromethane 50:50, vortex, sonicate for 5 min, add to the silica SPE cartridge, rinse the tube with 1.5 mL isooctane:dichloromethane 50:50, add the rinse to the SPE cartridge, wash with 2.5 mL isooctane:dichloromethane 50:50, wash with 1 mL ethyl acetate, elute with 5 mL dichloromethane:MeOH 90:10, evaporate to dryness under a stream of nitrogen at 55°, reconstitute with 150 μL isooctane:ethyl acetate 60:40, vortex, sonicate for 2 min, inject a 75 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long 40 μm LC-Si (Supelco)
Column: 250 × 4.6 Zorbax silica
Mobile phase: Isooctane:ethyl acetate:glacial acetic acid:triethylamine:MeOH 35:65:0.4:0.2:0.1 (At the end of each day flush column with isooctane:ethyl acetate 40:60 for at least 3 h.)
Flow rate: 0.6
Injection volume: 75
Detector: UV 522 following post-column reaction. The column effluent mixed with the reagent pumped at 0.3 mL/min and the mixture flowed through a 15 m × 0.25 mm ID stainless steel coil at 95 ± 1° to the detector. At the end of each day flush the pump with MeOH for at least 3 h. (Prepare reagent by cautiously adding 20 mL concentrated sulfuric acid to 500 mL EtOH, cool to room temperature, add 30 g vanillin dissolved in 500 mL EtOH, mix, protect from light, prepare every other day.)

CHROMATOGRAM

Retention time: 11.3
Limit of detection: 25 ng/g

OTHER SUBSTANCES

Simultaneous: maduramicin, monensin, narasin, salinomycin

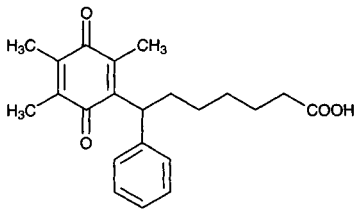
KEY WORDS

post-column reaction; normal phase; SPE; chicken; liver

REFERENCE

Ericson,J.F.; Calcagni,A.; Lynch,M.J. Determination of senduramicin sodium in poultry liver by liquid chromatography with vanillin postcolumn derivatization, *J.AOAC Int.*, **1994**, *77*, 577–582.

Seratrodast



Molecular formula: $C_{22}H_{26}O_4$
Molecular weight: 354.45
CAS Registry No.: 112665-43-7
Merck Index: 8603

SAMPLE

Matrix: blood
Sample preparation: Add IS to plasma, acidify with 300 mM HCl. Extract the samples with 5 mL hexane:ethyl acetate 20:80, evaporate the organic layer to dryness, reconstitute with 150 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm Adsorbosphere CN
Column: 250 × 4.6 5 μm Phenomenex PhenoSphere C8
Mobile phase: MeCN:MeOH:isopropanol:1M pH 4.0 sodium perchlorate 35:20:5:40
Flow rate: 1.0
Injection volume: 100
Detector: UV 266

CHROMATOGRAM

Internal standard: A-68500
Limit of quantitation: 3.3 ng/mL

KEY WORDS

plasma

REFERENCE

el-Shourbagy,T.; Hsu-Beischer,R.; Sapochak,L.; Chu,S. An HPLC method for the simultaneous determination of CEP-2563 (KT-8391) and its active metabolites CEP-2547, CEP-751, and CEP-701, in human plasma using fluorometric detection (Abstract 3354), *Pharm.Res.*, **1997**, *14*, S582.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 150 μL 100 mM HCl to 500 μL serum, add 6 mL 167 ng/mL idebenone in ethyl acetate, extract, centrifuge, add 200 μL 5% propylene glycol in ethyl acetate to the organic layer, evaporate to dryness under a stream of nitrogen at 40°, add 2 mL iron(III) chloride solution to the residue, vortex for 10 s. Add 4 mL ethyl acetate, extract, add 200 μL 5% propylene glycol solution to the separated organic layer, evaporate to dryness under a stream of nitrogen at 40°, dissolve the residue in 300 μL MeCN:50 mM KH_2PO_4 55:45, inject a 100 μL aliquot. Urine. Add 750 μL enzyme solution (667 U/mL β-glucuronidase (type B-3, H-1, and IX) and 33.3 U/mL sulfatase (type VIII) in 200 mM pH 5.0 phosphate buffer) to 250 μL urine, incubate the mixture at 37° for 1 h. Add 6 mL 167 ng/mL idebenone in ethyl acetate solution to the hydrolyzed urine or to 500 μL urine, extract, centrifuge, add 200 μL 5% propylene glycol in ethyl acetate to the organic layer, evaporate to dryness under a stream of nitrogen at 40°, add 2 mL iron(III) chloride solution to the residue, vortex for 10 s. Add 4 mL ethyl acetate, extract, add 200 μL 5% propylene glycol in ethyl acetate to the separated organic layer, evaporate to dryness under a stream of nitrogen at 40°, dissolve the residue in 300 μL MeCN:50 mM pH 3.0 phosphate buffer 55:45, inject a 100 μL aliquot. (The compounds are oxidized by the iron(III) chloride.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm YMC Pack ODS, A-302
Mobile phase: Gradient. A was 50 mM KH_2PO_4 (serum) or 50 mM pH 3.0 phosphate buffer (urine). B was MeCN:50 mM KH_2PO_4 60:40 (serum) or MeCN:50 mM pH 3.0 phosphate buffer 60:40 (urine). A:B from 50:50 to 0:100 in 50 min, maintain at 0:100 for 5 min, from 0:100 to 50:50 for 5 min, maintain at 50:50 for 15 min
Column temperature: 25

Flow rate: 1
Injection volume: 100
Detector: UV 266

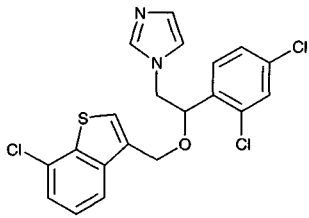
CHROMATOGRAM
Retention time: 44.5
Internal standard: idebenone (40)
Limit of detection: 5 ng/mL (serum, urine), 10 ng/mL (hydrolyzed urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics; serum; derivatization

REFERENCE
Ohta,R.; Amano,T.; Yamashita,K.; Motohashi,M. High-performance liquid chromatographic determination of seratrodast and its metabolites in human serum and urine, *J.Chromatogr.B*, **1997**, *704*, 325–331.

Sertaconazole



Molecular formula: $C_{20}H_{15}Cl_3N_2OS$
Molecular weight: 437.78
CAS Registry No.: 99592-32-2
Merck Index: 8610

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 96 μg/mL solution in MeCN, inject a 25 μL aliquot. Cream. Disperse 2 g cream in 15 mL MeCN:MeOH 80:20 using a spatula, sonicate for 10 min, make up to 50 mL with MeCN, centrifuge at 4500 rpm for 10 min. Dilute 1 mL of the supernatant to 10 mL with MeCN, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Spherisorb CN
Mobile phase: MeCN:10 mM NaH_2PO_4 37:63
Column temperature: 35
Flow rate: 1.6
Injection volume: 25
Detector: UV 260

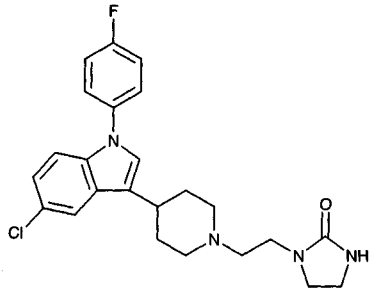
CHROMATOGRAM
Retention time: 19.3

OTHER SUBSTANCES
Simultaneous: impurities, degradation products

KEY WORDS
cream

REFERENCE
Albet,C.; Fernandez,J.M.; Rozman,E.; Perez,J.A.; Sacristan,A.; Ortiz,J.A. Determination of sertaconazole nitrate, a new imidazole antifungal, by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 205–211.

Sertindole



Molecular formula: $C_{24}H_{26}ClFN_4O$
Molecular weight: 440.95
CAS Registry No.: 106516-24-9
Merck Index: 8611

SAMPLE

Matrix: blood

Sample preparation: Condition a 200 mg Bond Elut C8 SPE cartridge with two 2 mL portions of MeCN, three 3 mL portions of MeOH, and two 2 mL portions of water. Briefly vortex 1 mL (human), 500 μL (dog), 200 μL (rat), or 100 μL (mouse) plasma with 50 μL 100 ng/mL IS in EtOH and 10 mM pH 8.5 dibasic potassium phosphate as required for sample transfer. Add to the SPE cartridge, wash with three 2 mL portions of water, and with three 1 mL portions of MeCN, dry with vacuum for 1-2 min. Elute with two 1 mL portions of MeOH:glacial acetic acid 98:2, evaporate the eluate to dryness under a gentle stream of air or nitrogen at 25-35°. Reconstitute the residue with 50 μL mobile phase, centrifuge at 3000 rpm for 5 min, maintain extract at 5° or less until injection, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: guard cartridge
Column: 150 × 2.1 5 μm YMC basic (YMC Inc., Wilmington, NC)
Mobile phase: MeCN:100 mM ammonium acetate 42:58 adjusted to pH 6.8 with glacial acetic acid
Flow rate: 0.4
Injection volume: 20
Detector: MS, Sciex API III, positive ion mode at 450°, nebulizing gas nitrogen 80 psi, auxiliary gas nitrogen at 2-3 mL/min, curtain gas nitrogen at 1.8 mL/min, m/z 441-113

CHROMATOGRAM

Retention time: 3.9
Internal standard: Lu-26-009
Limit of quantitation: 100 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

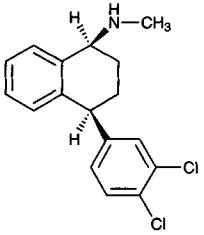
KEY WORDS

SPE; plasma; human; rat; dog; mouse; pharmacokinetics

REFERENCE

Menacherry,S.D.; Stamm,G.E.; Chu,S.-Y. A sensitive and specific method for assay of sertindole and its metabolites in human, rat, dog, and mouse plasma using HPLC with tandem mass spectrometric detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2241–2257.

Sertraline



Molecular formula: $C_{17}H_{17}Cl_2N$
Molecular weight: 306.23
CAS Registry No.: 79617-96-2, 79559-97-0 (HCl)
Merck Index: 8612
Lednicer No.: 4 57

SAMPLE

Matrix: blood, tissue, vitreous humor
Sample preparation: Blood, vitreous humor. Mix 1 mL sample with 500 ng IS, add 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 200 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer. Tissue. Mix 500 μL liver homogenate with 5.0 μg IS, add 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 400 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 3.9 5 μm NovaPak-Phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 55:45, adjusted to pH 3.0
Flow rate: 1.5
Injection volume: 30
Detector: UV 214

CHROMATOGRAM

Internal standard: pentazocine
Limit of quantitation: 100 ng/mL (blood), 2.5 μm/g (liver)

OTHER SUBSTANCES

Extracted: pimozide

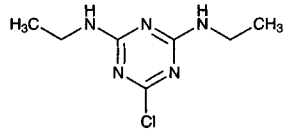
KEY WORDS

liver

REFERENCE

McIntyre,I.M.; King,C.V.; Staikos,V.; Gall,J.; Drummer,O.H. A fatality involving moclobemide, sertraline, and pimozide, *J.Forensic Sci.*, **1997**, *42*, 951–953.

Simazine



Molecular formula: $C_7H_{12}ClN_5$
Molecular weight: 201.66
CAS Registry No.: 122-34-9
Merck Index: 8681

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

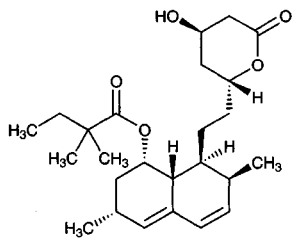
CHROMATOGRAM
Retention time: 15.752

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Simvastatin

Molecular formula: $C_{25}H_{38}O_5$
Molecular weight: 418.57
CAS Registry No.: 79902-63-9
Merck Index: 8686



SAMPLE
Matrix: blood
Sample preparation: Condition a 2.8 mL 500 mg Bond Elut C8 SPE cartridge with 2 mL MeOH and 2.5 mL water. Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 1.5 mL MeCN and 2 mL water. 1 mL Plasma + 2.5 ng IS, mix, add to the C8 SPE cartridge, wash with 2 mL MeCN:water 10:90, wash with 1 mL MeOH:water 30:70, wash with 2 mL MeOH:water 60:40 (the ring-opened active metabolite elutes in this fraction), elute with 2 mL MeCN, add 100 μL 20 mM potassium carbonate to the eluate, evaporate to dryness under reduced pressure at 40° (this hydrolyses the lactone), dry under vacuum for more than 30 min, reconstitute with 100 μL 10 mM 1-(bromoacetyl)pyrene in DMF, add 100 μL 10 mM 18-crown-6 in DMF, mix, let stand at room temperature for 30 min, add 2 mL MeCN:triethylamine 90:10, add to a 10 mL 100 mg Bond Elut LRC PBA SPE cartridge, wash with 4 mL MeOH, wash with 2 mL MeCN, elute with 2 mL MeCN:propylene glycol 60:40, dilute the eluate with 1 mL water, add to the C18 SPE cartridge, wash with 2 mL MeCN:water 70:30, elute with 3 mL MeCN. Evaporate the eluate to dryness, reconstitute the residue in 300 μL MeCN:water 70:30, inject a 150 μL aliquot onto column A and elute to waste with mobile phase A, after 12.5 min elute column A onto column B with mobile phase A, after another 5 min remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B. Flush column A with MeOH then re-equilibrate with mobile phase A for 6 min. (The procedure can also be modified to determine the active metabolite.)

HPLC VARIABLES
Column: A 150 × 4.6 5 μm Bondesil CH (Varian); B 150 × 4.6 5 μm Capcell Pak C18 UG 120 (Shiseido)

Mobile phase: A MeOH:water 80:20; B MeCN:water 80:20
Column temperature: 40
Flow rate: 1
Injection volume: 150
Detector: F ex 360 em 430

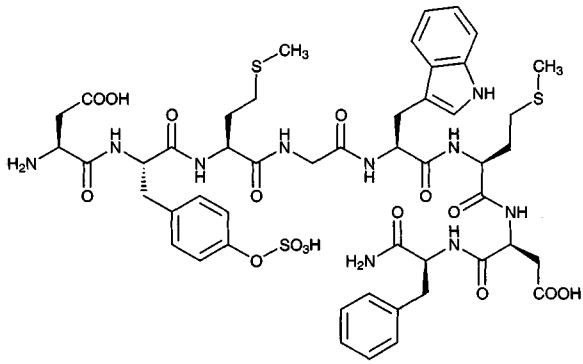
CHROMATOGRAM
Retention time: 27.5
Internal standard: 2-ethyl-2-methylbutanoate ester analog of simvastatin (31)
Limit of detection: 20 pg/mL

KEY WORDS
derivatization; plasma; column-switching; SPE; heart-cut; pharmacokinetics

REFERENCE
Ochiai,H.; Uchiyama,N.; Imagaki,K.; Hata,S.; Kamei,T. Determination of simvastatin and its active metabolite in human plasma by column-switching high-performance liquid chromatography with fluorescence detection after derivatization with 1-bromoacetylpyrene, *J.Chromatogr.B*, **1997**, *694*, 211–217.

Sincalide

Molecular formula: $C_{49}H_{62}N_{10}O_{16}S_3$
Molecular weight: 1143.29
CAS Registry No.: 25126-32-3
Merck Index: 8689



SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeCN and 10 mL 0.1% acetic acid. Add 3 mL plasma to the SPE cartridge, wash with 5 mL 0.1% acetic acid, elute with 6 mL MeCN:0.1% acetic acid 50:50, lyophilize the eluate. Reconstitute with 400 μL water:0.1% trifluoroacetic acid 50:50, filter (Millex-HV, Millipore), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK ODS-120 T
Mobile phase: MeCN:0.1% trifluoroacetic acid 34:66
Flow rate: 1
Detector: UV 214 or RIA

CHROMATOGRAM
Retention time: 6.5

KEY WORDS
plasma; SPE; dog

REFERENCE
Lindén,A.; Uvnäs-Moberg,K. Plasma levels of cholecystokinin (CCK-8 and CCK-33-39) in response to feeding and during pregnancy in dogs, *Scand.J.Gastroenterol.*, **1987**, *22*, 859–864.

SAMPLE
Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeCN and 10 mL 0.1% acetic acid. Add 2 mL plasma to the SPE cartridge, wash with 5 mL 0.1% acetic acid, elute with 6 mL MeCN:0.1% acetic acid 50:50, lyophilize the eluate. Reconstitute with 200 μL 1% pH 8.0 ammonium bicarbonate, incubate with 5 μg protease (from Staphylococcus aureus V.8, Miles Scientific) at 37° for 6 h, lyophilize. Reconstitute with 500 μL water:0.1% trifluoroacetic acid 50:50, filter (Millex-HV, Millipore), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK ODS-120 T (LKB)
Mobile phase: MeCN:0.1% trifluoroacetic acid containing 155 mM NaCl 32:68
Flow rate: 1
Detector: UV 215 or RIA

CHROMATOGRAM
Retention time: 9.5

KEY WORDS
rat; plasma; SPE

REFERENCE
Lindén,A.; Carlquist,M.; Hansen,S.; Uvnäs-Moberg,K. Plasma concentrations of cholecystokinin, CCK-8, and CCK-33, 39 in rats, determined by a method based on enzyme digestion of gastrin before HPLC and RIA detection of CCK, *Gut*, **1989**, *30*, 213–222.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in 1% trifluoroacetic acid adjusted to pH 2.5 with triethylamine, filter (0.2 μm), inject a 80 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Whatman)
Column: 300 mm long MCH-10 C18 (Varian)
Mobile phase: Gradient. A was 1% trifluoroacetic acid adjusted to pH 2.5 with triethylamine. B was isopropanol:trifluoroacetic acid 99:1 containing the same amount of triethylamine as A. A:B 80:20 for 5 min, to 65:35 over 50 min, to 40:60 over 10 min, maintain at 40:60 for 10 min. At the end of each run cycle repeatedly from 60:40 to 40:60 for 15 min.
Flow rate: 0.5
Injection volume: 80
Detector: RIA of fractions

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Simultaneous: motilin, secretin

REFERENCE
Chang,T.-M.; Erway,B.; Chey,W.Y. Rapid, small-scale preparation of gastrointestinal hormones by high-performance liquid chromatography on a C18 column, *J.Chromatogr.*, **1985**, *326*, 121–127.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) frozen brain tissue with 5 volumes of ice-cold MeOH:water 90:10, centrifuge at 10000 g for 5 min, lyophilize the supernatant, reconstitute with 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 7 μm LiChrosorb RP-18
Mobile phase: Isopropanol:150 mM pH 5.5 phosphate buffer 11.5:88.5
Column temperature: 45
Flow rate: 0.6

Injection volume: 20
Detector: E, BAS LC-4, TL-5 glassy carbon electrode +1.0 V, Ag/AgCl reference electrode

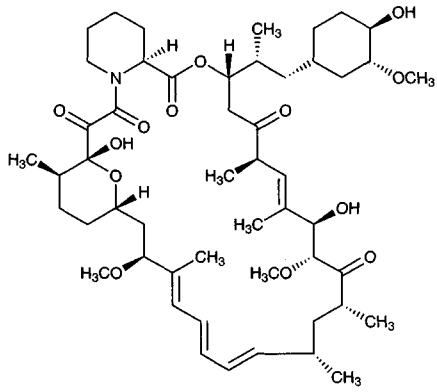
CHROMATOGRAM
Retention time: 5
Limit of quantitation: 0.2 ng

KEY WORDS
rat; brain

REFERENCE
Sauter,A.; Frick,W. Determination of cholecystokinin tetrapeptide and cholecystokinin octapeptide sulfate in different rat brain regions by high-pressure liquid chromatography with electrochemical detection, *Anal.Biochem.*, **1983**, *133*, 307–313.

Sirolimus

Molecular formula: $C_{51}H_{79}NO_{13}$
Molecular weight: 914.19
CAS Registry No.: 53123-88-9
Merck Index: 8288



SAMPLE
Matrix: blood, tissue
Sample preparation: Vortex 0.05-1 mL whole blood or hepatic microsomes with 1 μg IS and 1 mL 100 mM sodium carbonate for 10 s. Add 10 mL MTBE, shake horizontally for 30 min. Centrifuge at 1500 g for 10 min at 4°, remove the organic layer, evaporate to dryness under a stream of nitrogen. Reconstitute the residue in 200 μL mobile phase. Centrifuge at 2500 g at 4°. Inject a 150 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 mm 37-53 μm C18 pellicular (Upchurch)
Column: 250 × 4.6 5 μm Supelco LC-318 C18
Mobile phase: MeOH:water 70:30
Column temperature: 45
Flow rate: 1.0
Injection volume: 150
Detector: UV 278

CHROMATOGRAM
Retention time: 22, 32 (isomers)
Internal standard: N-undecyl-o-toluamide (Prepare by dispersing N-undecylamine in cold NaOH and adding an equimolar amount of o-toluoyl chloride, shake vigorously. Remove the product by filtration, wash, air dry, recrystallize from EtOH/water.) (27.5)
Limit of detection: 1 ng
Limit of quantitation: 2.5 ng

OTHER SUBSTANCES
Simultaneous: beclomethasone, corticosterone, cyclosporine A and G, erythromycin, ethinyl estradiol, hydrocortisone, ketoconazole, lorazepam, methylprednisolone, norethindrone, prednisolone, prednisone, propranolol, rifampicin, tacrolimus

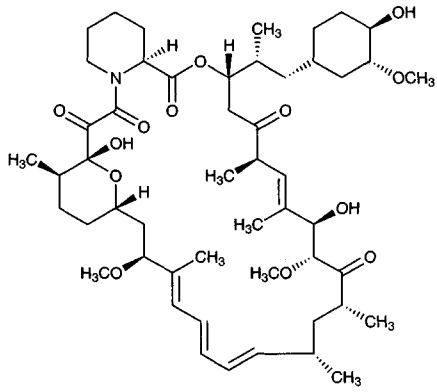
Injection volume: 20
Detector: E, BAS LC-4, TL-5 glassy carbon electrode +1.0 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 0.2 ng

KEY WORDS
rat; brain

REFERENCE
Sauter,A.; Frick,W. Determination of cholecystokinin tetrapeptide and cholecystokinin octapeptide sulfate in different rat brain regions by high-pressure liquid chromatography with electrochemical detection, *Anal.Biochem.*, **1983**, *133*, 307–313.

Sirolimus



Molecular formula: $C_{51}H_{79}NO_{13}$
Molecular weight: 914.19
CAS Registry No.: 53123-88-9
Merck Index: 8288

SAMPLE
Matrix: blood, tissue
Sample preparation: Vortex 0.05-1 mL whole blood or hepatic microsomes with 1 μg IS and 1 mL 100 mM sodium carbonate for 10 s. Add 10 mL MTBE, shake horizontally for 30 min. Centrifuge at 1500 g for 10 min at 4°, remove the organic layer, evaporate to dryness under a stream of nitrogen. Reconstitute the residue in 200 μL mobile phase. Centrifuge at 2500 g at 4°. Inject a 150 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 mm 37-53 μm C18 pellicular (Upchurch)
Column: 250 × 4.6 5 μm Supelco LC-318 C18
Mobile phase: MeOH:water 70:30
Column temperature: 45
Flow rate: 1.0
Injection volume: 150
Detector: UV 278

CHROMATOGRAM
Retention time: 22, 32 (isomers)
Internal standard: N-undecyl-o-toluamide (Prepare by dispersing N-undecylamine in cold NaOH and adding an equimolar amount of o-toluoyl chloride, shake vigorously. Remove the product by filtration, wash, air dry, recrystallize from EtOH/water.) (27.5)
Limit of detection: 1 ng
Limit of quantitation: 2.5 ng

OTHER SUBSTANCES
Simultaneous: beclomethasone, corticosterone, cyclosporine A and G, erythromycin, ethinyl estradiol, hydrocortisone, ketoconazole, lorazepam, methylprednisolone, norethindrone, prednisolone, prednisone, propranolol, rifampicin, tacrolimus

KEY WORDS
rat; rabbit; human; whole blood; microsomes

REFERENCE
Ferron,G.M.; Conway,W.D.; Jusko,W.J. Lipophilic benzamide and anilide derivatives as high-performance liquid chromatography internal standards: application to sirolimus (rapamycin) determination, *J.Chromatogr.B*, **1997**, *703*, 243–251.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 3 mL C18 SPE cartridge (LiChroprep, Merck) with 3 mL MeCN and 3 mL pH 3.0 sulfuric acid. Add 500 μL MeCN to 1.5 mL microsomal incubation. Centrifuge at 2500 g for 2 min. Add the supernatant to the SPE cartridge, wash with 3 mL MeOH:pH 3.0 sulfuric acid 50:50 and 500 μL hexane. Dry cartridge by drawing air through it and elute with 1.5 mL dichloromethane. Evaporate the eluate under a stream of nitrogen at 40°. Reconstitute the residue in 300 μL MeCN:pH 3.0 sulfuric acid 75:25, wash with 500 :gmL hexane. Inject a 125 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 3 μm Hypersil C8
Mobile phase: Gradient. A was MeCN. B was pH 3.0 sulfuric acid. A:B maintain at 47:53 for 7 min, to 50:50 over 13 min, to 55:45 over 20 min, to 61:39 over 5 min, wash with 95:5 for 5 min, re-equilibrate at initial conditions for 7 min.
Column temperature: 40
Flow rate: 0.7
Injection volume: 125
Detector: UV 276

CHROMATOGRAM
Retention time: 33
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, aspirin, amphotericin B, bromocryptine, captopril, corticosterone, cyclosporine, dexamethasone, diclofenac, erythromycin, ergotamine tartrate, diethyldithiocarbamate, disulfiram, ethinyl estradiol, josamycin, ketoconazole, lidocaine, methylprednisolone, miconazole, α-naphthoflavone, naringin, nifedipine, omeprazole, phenytoin, prednisolone, progesterone, propranolol, quinidine, ranitidine, sulfaphenazole, trimethoprim, troleandomycin, verapamil

KEY WORDS
rat; pharmacokinetics; human; pig; small intestine; liver; SPE

REFERENCE
Lampen,A.; Zhang,Y.; Hackbarth,I.; Benet,L.Z.; Sewing,K.-F.; Christians,U. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 1104–1112.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 3 mL C8 SPE cartridge (Recipe, Germany) with 3 mL MeCN and 1.5 mL water. Mix 1.5 mL microsomal incubation with 2 mL MeOH:saturated zinc sulfate in water 50:50, add 10 μL 1 mM IS in MeCN:pH 3.0 sulfuric acid 75:25. Centrifuge at 2500 g. Add the supernatant to the SPE cartridge, wash with 3 mL water. Dry cartridge by drawing air through it for 3 min, elute with 400 μL MeCN:0.1% formic acid 90:10 by centrifuging at 800 g for 2 min. Inject a 25 μL aliquot of the extract.

HPLC VARIABLES
Column: 250 × 4 3 μm Hypersil C8
Mobile phase: MeOH:0.1% formic acid 90:10

Column temperature: 35
Flow rate: 0.5
Injection volume: 25
Detector: MS, HP 5989A, ESI 59887A, drying gas 350°, quadrupole 120°, capillary exit voltage 200 V, positive ion mode, multiplier voltage 2740 V, X-ray 10000 V, m/z 936

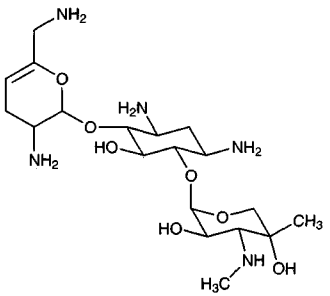
CHROMATOGRAM
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; pharmacokinetics; human; pig; small intestine; liver; SPE

REFERENCE
Lampen,A.; Zhang,Y.; Hackbarth,I.; Benet,L.Z.; Sewing,K.-F.; Christians,U. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 1104–1112.

Sisomicin



Molecular formula: $C_{19}H_{37}N_5O_7$
Molecular weight: 447.53
CAS Registry No.: 32385-11-8, 53179-09-2 (sulfate)
Merck Index: 8695

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

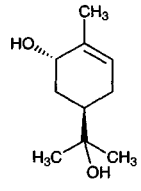
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.032

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Sobrerol



Molecular formula: $C_{10}H_{18}O_2$
Molecular weight: 170.25
CAS Registry No.: 498-71-5
Merck Index: 8707

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP18
Mobile phase: EtOH:water 20:80 with 10 mM β-cyclodextrin and 0.5 mM tri-O-methyl-β-cyclodextrin
Column temperature: 25
Flow rate: 0.04
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: k' 3.4

OTHER SUBSTANCES
Extracted: morsuximide, mephenytoin

KEY WORDS
no chiral separation

REFERENCE
Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

Sodium nitroprusside

$Na_2[Fe(CN)_5NO]$

Molecular formula: $C_5FeN_6Na_2O$
Molecular weight: 261.92
CAS Registry No.: 14402-89-2, 13755-38-9 (dihydrate)
Merck Index: 8794

SAMPLE
Matrix: blood
Sample preparation: 100 μL Whole blood or plasma + 100 μL 3 M perchloric acid, mix, let stand at 0° for 5 min, centrifuge at 8000 g for 5 min. Remove a 150 μL aliquot of the supernatant and add it to 250 μL 1 M K_2HPO_4, mix vigorously, centrifuge for 8000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4 Asahipak BEST 502Q
Mobile phase: 100 mM pH 6.0 Acetate buffer containing 300 mM sodium perchlorate
Flow rate: 0.5
Injection volume: 20
Detector: F ex 583 em 607 following post-column reaction. The column effluent mixed with 5 mM dithiothreitol in 50 mM pH 9.0 Tris-HCl buffer containing 5 mM EDTA pumped at 0.1 mL/min and this mixture flowed through a 4.5 m × 0.5 mm ID coil at 90°. The effluent from this coil mixed with 0.5% Chloramine T in water pumped at 0.1 mL/min and flowed through a mixing coil. The effluent from this coil mixed with reagent pumped at 0.1 mL/min and this mixture flowed through a mixing coil to the detector. (Reagent was a mixture of 1.5 g barbituric acid, 15 mL pyridine, 3 mL concentrated HCl, and 82 mL water. Nitroprusside is reduced to cyanide with dithiothreitol. The cyanide is converted to cyanogen chloride with chloramine T, the cyanogen chloride reacts with the pyridine to form pent-2-en-1,5-dial, and this compound reacts with barbituric acid to form the fluorescent 5,5'-(1,3-pentadiene-1-yl-5-ylidene) dibarbituric acid.)

CHROMATOGRAM
Retention time: 17
Limit of detection: 200 fmole

OTHER SUBSTANCES
Noninterfering: cyanide, thiocyanate

KEY WORDS
post-column reaction; whole blood; plasma; rat; pharmacokinetics

REFERENCE
Watanabe,T.; Nagamine,Y.; Toida,T.; Koshiishi,I.; Imanari,T. Sensitive determination of nitroprusside in blood by high performance liquid chromatography, *Anal.Sci.*, **1988**, *4*, 203–206.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot of the solution.

HPLC VARIABLES
Column: 300 × 3.9 10 μm phenyl (Waters)
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM KH_2PO_4 and 5 mM tetrabutylammonium hydroxide adjusted to pH 7.1 with phosphoric acid.)
Flow rate: 2
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 6
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: degradation products, ferricyanide, ferrocyanide

KEY WORDS
stability-indicating; injections; 5% dextrose

REFERENCE
Baaske,D.M.; Smith,M.D.; Karnatz,N.; Carter,J.E. High-performance liquid chromatographic determination of sodium nitroprusside, *J.Chromatogr.*, **1981**, *212*, 339–346.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: Partisil 10-SAX
Mobile phase: 500 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.4
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 5

KEY WORDS
injections; 5% dextrose; saline; lactated Ringer's solution

REFERENCE
Mahony,C.; Brown,J.E.; Stargel,W.W.; Verghese,C.P.; Bjornsson,T.D. In vitro stability of sodium nitroprusside solutions for intravenous administration, *J.Pharm.Sci.*, **1984**, *73*, 838–839.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: phenyl
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM KH_2PO_4 containing 0.52% tetrabutylammonium phosphate, pH adjusted to 7.1 with KOH.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 7.2
Internal standard: salicylic acid (6)

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

Somatomedin

Molecular weight: 7649
CAS Registry No.: 67763-96-6
Merck Index: 8862

SAMPLE
Matrix: blood
Sample preparation: Prepare a 240:10 serum:concentrated formic acid mixture, let stand for 1 h at room temperature or 37°, filter (0.22 μm) or centrifuge at 12000 g for 5 min, inject a 200 μL aliquot of the filtrate or supernatant.

HPLC VARIABLES
Guard column: 100 × 7.5 10 μm TSK 2000 SW (Toyo Soda)
Column: 600 × 7.5 10 μm TSK 2000 SW (Toyo Soda)
Mobile phase: 100 mM Ammonium formate adjusted to pH 3.0 with concentrated formic acid
Flow rate: 0.7
Injection volume: 200
Detector: UV 280 or bioassay

CHROMATOGRAM
Retention time: 14

KEY WORDS
serum; SEC; human; rat

REFERENCE
Goldstein,S.; Stivaletta,L.A.; Phillips,L.S. Separation of somatomedins and somatomedin inhibitors by size exclusion high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *339*, 388–393.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge cells in modified Eagle's medium containing 10% fetal bovine serum, 20 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin at 2500 g, to each 1 liter of supernatant add 390 g ammonium sulfate, dissolve, let stand overnight, centrifuge at 4° at 10000 g for 30 min. Dissolve the precipitate in 25-30 mL 50 mM pH 7.8 ammonium bicarbonate, purify 40-50 mL aliquots on a 1000 × 50 column of Sephacryl S-300, elute with 50 mM pH 7.8 ammonium bicarbonate buffer at 0.7 mL/min at 4°, adjust pH of eluate to 6.5 with acetic acid, inject a 10-15 mL aliquot at 0.5 mL/min.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Aquapore RP-300 C8 (Brownlee)
Mobile phase: Gradient. MeCN:buffer from 0:100 to 20:80 over 10 min, to 60:40 over 75 min. (Buffer was 100 mM pH 6.5 ammonium acetate, pass through a C18 Sep-Pak to remove impurities.)
Flow rate: 0.5 (?)
Injection volume: 10000-15000
Detector: UV 280

CHROMATOGRAM
Retention time: 40

REFERENCE
Powell,D.R.; Lee,P.D.K.; Shively,J.E.; Eckenhausen,M.; Hintz,R.L. Method for purification of an insulin-like growth factor -binding protein produced by human HEP G2 hepatoma cells, *J.Chromatogr.*, **1987**, *420*, 163–170.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 4.6 Beckman RPSC
Mobile phase: Gradient. MeCN:10 mM trifluoroacetic acid from 10:90 to 60:40 over 40 min
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 33

REFERENCE
Saito,Y.; Yamada,H.; Niwa,M.; Ueda,I. Production and isolation of recombinant somatomedin C, *J.Biochem.(Tokyo)*, **1987**, *101*, 123–134.

SAMPLE
Matrix: solutions
Sample preparation: Adjust pH to 3.0 with trifluoroacetic acid (if necessary), filter (0.22 μm) (if necessary), inject a 200 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.9 Aquapore RP-300 (Brownlee)

Mobile phase: Gradient. A was water containing 0.1% trifluoroacetic acid. B was MeCN:water 80:20 containing 0.1% trifluoroacetic acid. A:B from 30:70 to 50:50 over 20 min, maintain at 50:50 for 3 min, to 0:100 over 3 min, return to initial conditions over 2 min.
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 12 (isoform 1), 14 (isoform 2), 2 (reduced form)

REFERENCE
Meng,H.; Burleigh,B.D.; Kelly,G.M. Reduction studies on bacterial recombinant somatomedin C/insulin-like growth factor-1, *J.Chromatogr.*, **1988**, *443*, 183–192.

Somatrem

Molecular formula: $C_{995}H_{1537}N_{263}O_{301}S_8$
Molecular weight: 22256.39
CAS Registry No.: 82030-87-3
Merck Index: 8864

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm C4 (Vydac)
Mobile phase: Isopropanol:500 mM pH 6.5 KH_2PO_4 29:71
Column temperature: 45
Flow rate: 1
Detector: F ex 295 em 348

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: somatropin

REFERENCE
Oroszlan,P.; Wicar,S.; Teshima,G.; Wu,S.L.; Hancock,W.S.; Karger,B.L. Conformational effects in the reversed-phase chromatographic behavior of recombinant human growth hormone (rhGH) and N-methionyl recombinant human growth hormone (Met-hGH), *Anal.Chem.*, **1992**, *64*, 1623–1631.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac C4 Protein Pak
Mobile phase: Gradient. MeCN:0.05% trifluoroacetic acid from 43:57 to 55:45 over 30 min
Column temperature: 45
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Simultaneous: somatropin

REFERENCE
Arcelloni,C.; Fermo,I.; Banfi,G.; Pontiroli,A.E.; Paroni,R. Capillary electrophoresis for protein analysis: separation of human growth hormone and human insulin molecular forms, *Anal.Biochem.*, **1993**, *212*, 160–167.

Somatropin

Molecular formula: $C_{990}H_{1529}N_{263}O_{299}S_7$
Molecular weight: 22124.21
CAS Registry No.: 9002-72-6, 12629-01-5 (human)
Merck Index: 8864

SAMPLE
Matrix: bulk
Sample preparation: Prepare a solution in 20 mM sodium borate containing 1.44 mM EDTA adjusted to pH 9.5 with NaOH, inject an aliquot.

HPLC VARIABLES
Column: Vydac 218TP104
Mobile phase: Gradient. A was MeCN:0.1% trifluoroacetic acid:water 10.5:70:19.5. B was MeCN:0.1% trifluoroacetic acid:water 21:70:9. A:B from 100:0 to 0:100 in 30 min
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 22.5 (monomer), 25 (dimer)

REFERENCE
Chang,J.P.; Ferguson,T.H.; Record,P.A.; Dickson,D.A.; Kiehl,E.; Kennington,A.S. Improved potency assay for recombinant bovine somatotropin by high-performance size-exclusion chromatography, *J.Chromatogr.A*, **1996**, *736*, 97–104.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a solution in 20 mM sodium borate containing 1.44 mM EDTA adjusted to pH 9.5 with NaOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 21.5 TSK G3000SW
Mobile phase: 20 mM Sodium borate containing 1.44 mM EDTA, adjusted to pH 7.3 with HCl
Flow rate: 0.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 13.3 (dimer), 14.5 (monomer)

REFERENCE
Chang,J.P.; Ferguson,T.H.; Record,P.A.; Dickson,D.A.; Kiehl,E.; Kennington,A.S. Improved potency assay for recombinant bovine somatotropin by high-performance size-exclusion chromatography, *J.Chromatogr.A*, **1996**, *736*, 97–104.

SAMPLE
Matrix: fermentation broth

Sample preparation: Centrifuge 1.5 mL fermentation broth at 16000 g for 2 min, put the pellet on ice. Re-suspend the pellet in 150 μL ice-cold 10 mM pH 7.5 Tris-HCl containing 20% (w/v) sucrose. Add 5 μL 500 mM pH 8.0 EDTA, incubate on ice for 10 min. Microcentrifuge the cells and re-suspend the pellets in 100 μL cold 1 mM pH 7.5 Tris-HCl solution. Incubate the mixture for 10 min on ice and centrifuge again for 5 min. Remove the supernatant and inject an aliquot.

HPLC VARIABLES
Guard column: Vydac 214 FSK 54
Column: 250 × 4.6 5 μm Vydac 214 TP 54 C4
Mobile phase: n-Propanol:50 mM pH 7.5 Tris-hydrochloric acid buffer 29:71 (Place a column of 7.9-12.4 μm LiChrosorb Si 60 between the pump and injector.)
Column temperature: 45
Flow rate: 0.5
Injection volume: 40-70
Detector: UV 220

CHROMATOGRAM
Retention time: 29

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
comparison with SEC

REFERENCE
Dalmora,S.; Ezequiel de Oliveira,J.; Affonso,R.; Gimbo,E.; Ribela,M.T.C.P.; Bartolini,P. Analysis of recombinant human growth hormone directly in osmotic shock fluids, *J.Chromatogr.A*, **1997**, *782*, 199–210.

SAMPLE
Matrix: fermentation broth
Sample preparation: Centrifuge 1.5 mL fermentation broth at 16 000 g for 2 min, put the pellet on ice. Re-suspend the pellet in 150 μL ice-cold 10 mM pH 7.5 Tris-HCl containing 20% (w/v) sucrose. Add 5 μL 500 mM pH 8.0 EDTA, incubate on ice for 10 min. Microcentrifuge the cells and re-suspend the pellets in 100 μL cold 1 mM pH 7.5 Tris-HCl solution. Incubate the mixture for 10 min on ice and centrifuge again for 5 min. Remove the supernatant and inject an aliquot.

HPLC VARIABLES
Guard column: 75 × 7.5 10 μm SW (TosoHaas, USA)
Column: 600 × 7.5 G2000SW or 600 × 7.5 G3000SW (TosoHaas, USA)
Mobile phase: 25 mM pH 7.0 ammonium bicarbonate
Flow rate: 1
Injection volume: 10-100
Detector: UV 220 or Radioimmunoassay

CHROMATOGRAM
Retention time: 13.93

OTHER SUBSTANCES
Extracted: size isomers

KEY WORDS
comparison with RP-HPLC

REFERENCE
Dalmora,S.; Ezequiel de Oliveira,J.; Affonso,R.; Gimbo,E.; Ribela,M.T.C.P.; Bartolini,P. Analysis of recombinant human growth hormone directly in osmotic shock fluids, *J.Chromatogr.A*, **1997**, *782*, 199–210.

SAMPLE
Matrix: formulations

Sample preparation: Reconstitute somatropin with diluent (Diluent for Humatrope, 5-mL vial, Lilly) to a concentration of 3.33 mg/mL. Dilute 200 μL 3.33 mg/mL solution to 800 μL with highly purified water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 7.5 10 μm G3000SW (TosoHaas, Montgomeryville, PA)
Mobile phase: 10 mM pH 7.3 Sodium phosphate buffer
Flow rate: 0.6
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 12.5-12.8

KEY WORDS
stability-indicating; injections

REFERENCE
Ray,L.R.; Chen,D.A. Stability of somatropin stored in plastic syringes for 28 days, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1508–1511.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute somatropin with diluent (Diluent for Humatrope, 5-mL vial, Lilly) to a concentration of 3.33 mg/mL. Dilute 200 μL 3.33 mg/mL solution to 800 μL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Vydac C4
Mobile phase: 1-Propanol:50 mM pH 7.5 Tris buffer 29:71
Column temperature: 45
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

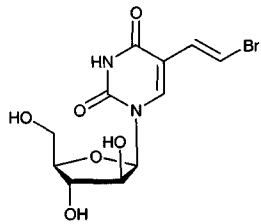
CHROMATOGRAM
Retention time: 31.3-36.3

KEY WORDS
stability-indicating; injections

REFERENCE
Ray,L.R.; Chen,D.A. Stability of somatropin stored in plastic syringes for 28 days, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1508–1511.

Sorivudine

Molecular formula: $C_{11}H_{13}BrN_2O_6$
Molecular weight: 349.14
CAS Registry No.: 77181-69-2
Merck Index: 8875



SAMPLE
Matrix: blood

Sample preparation: 250-500 μL Serum + 100 μL 10 μg/mL IS in water, add 5 mL MeCN while mixing, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES

Guard column: 37-53 μm silica gel (Whatman)
Column: 250 × 4.6 5 μm alkyl phenyl (ES Industries)
Mobile phase: MeCN:MeOH:buffer 15:5:80 (Buffer was 50 mM ammonium acetate adjusted to pH 5.0 with glacial acetic acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 295

CHROMATOGRAM

Retention time: 12.5
Internal standard: 1-β-D-arabinofuranosyl-E-5-(2-chlorovinyl)uracil (10)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

protect from light; serum; pharmacokinetics

REFERENCE

Whigan,D.B.; Cohen,A.I. High-performance liquid chromatographic determination of 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil and its metabolite (E)-5-(2-bromovinyl)uracil in serum, *J.Chromatogr.*, **1991**, *568*, 385–392.

SAMPLE

Matrix: cell suspensions
Sample preparation: Cell suspension + 200 μL ice-cold 4 M perchloric acid, vortex for 1 min, let stand in an ice bath for 5 min, centrifuge at 4° at 2000 g for 10 min. Remove the supernatant and add it to 200 μL 150 μg/mL IS, neutralize with 2 M K_2HPO_4, centrifuge at 4° at 2000 g for 10 min. Free-dry the supernatant, reconstitute in water, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.6 Spherisorb ODS2
Mobile phase: Gradient. MeOH:buffer from 25:75 to 50:50 over 24 min. (Buffer was 50 mM NaH_2PO_4 containing 12.5 mM tetrabutylammonium bromide, adjusted to pH 5 with NaOH. Use a 120 × 4.6 20 μm Partisil column before the injector.)
Column temperature: 48
Flow rate: 1
Injection volume: 10
Detector: UV 292

CHROMATOGRAM

Retention time: 5
Internal standard: E-5-(2-iodovinyl)-2'-deoxyuridine (8)

OTHER SUBSTANCES

Simultaneous: metabolites, phosphorylated species

REFERENCE

Ayisi,N.K.; Wall,R.A.; Wanklin,R.J.; Machida,H.; De Clercq,E.; Sacks,S.L. Comparative metabolism of E-5-(2-bromovinyl)-2'-deoxyuridine and 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil in herpes simplex virus-infected cells, *Mol.Pharmacol.*, **1987**, *31*, 422–429.

SAMPLE

Matrix: cell suspensions

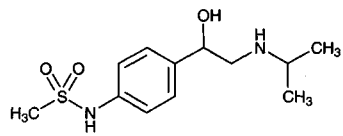
HPLC VARIABLES
Column: Chemopack 300-10C18
Mobile phase: MeOH:10 mM KH_2PO_4 10:50
Flow rate: 1
Detector: UV 290

CHROMATOGRAM
Retention time: 9.64
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Suzuki,S.; Machida,H.; Saneyoshi,M. Antiviral activity of various 1-β-D-arabinofuranosyl-*E*-5-halogenovinyluracils and *E*-5-bromovinyl-2'-deoxyuridine against salmon herpes virus, *Oncorhynchus masou* virus (OMV), *Antiviral Res.*, **1987**, *7*, 79–86.

Sotalol



Molecular formula: $C_{12}H_{20}N_2O_3S$
Molecular weight: 272.37
CAS Registry No.: 3930-20-9, 959-24-0 (HCl)
Merck Index: 8876
Lednicer No.: 1 66; 5 23

SAMPLE
Matrix: blood

HPLC VARIABLES
Column: 100 × 2.1 10 μm Chiralpak AD (Chiral Technologies, Exton, PA)
Mobile phase: EtOH:n-hexane:isopropanol:diethylamine 28.5:63:8.5:0.17
Column temperature: 20
Flow rate: 0.1
Injection volume: 10
Detector: MS, SCIEX API 300 tandem mass, positive ion mode, nebulizer 440°, scan 273.0/213.0

CHROMATOGRAM
Retention time: 5.27, 6.58 (enantiomers)

KEY WORDS
plasma; chiral; small-bore

REFERENCE
Alebic-Kolbah,T.; Zavitsanos,A.P. Chiral bioanalysis by normal phase high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry, *J.Chromatogr.A*, **1997**, *759*, 65–77.

SAMPLE
Matrix: blood
Sample preparation: Condition a Baker 10 C18 SPE cartridge with two 1.5 mL aliquots of MeOH and two 1.5 mL aliquots of 170 mM pH 9.4 N,N-bis(2-hydroxyethyl)glycine buffer. 1 mL Serum + 500 μL 500 mM pH 9.4 N,N-bis(2-hydroxyethyl)glycine buffer + 2 μg IS, vortex, add to the SPE cartridge, wash with two 1.5 mL aliquots of 170 mM pH 9.4 N,N-bis(2-hydroxyethyl)glycine buffer, elute with three 1 mL aliquots of MeCN:ethyl acetate 2:1, freeze the eluate in dry ice/MeOH for 5 s. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm ODS C18 (Altex)
Mobile phase: 10 mM K_2HPO_4 containing 2 mM nonylamine adjusted to pH 2.4 with phosphoric acid (Apply a conditioning injection of 100 μg sotalol and 100 μg IS at the start of the day.)
Column temperature: 40
Flow rate: 2
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 4.5
Internal standard: MJ-6564-1 (Bristol Meyers) (3.4)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amiodarone, amitriptyline, carbamazepine, desipramine, digoxin, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, lithium, methotrexate, mexiletine, nortriptyline, phenobarbital, phenytoin, primidone, salicylic acid, theophylline, tobramycin, valproic acid

KEY WORDS
serum; SPE

REFERENCE
Hoyer,G.L. Improved high-performance liquid chromatographic method for the analysis of serum sotalol, *J.Chromatogr.*, **1988**, *427*, 181–187.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 μg/mL bisoprolol in water + 200 μL pH 9.2 bicine (N,N-bis(2-hydroxyethyl)glycine) + 4 mL ethyl acetate, shake vigorously for 5 min, centrifuge at 2000 *g* for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeOH, inject an aliquot of 100 μL or less.

HPLC VARIABLES
Guard column: 10 mm long LiChrosorb CN
Column: 250 × 4 10 μm LiChrosorb CN
Mobile phase: MeOH:isopropanol:1.16 M perchloric acid 75:25:0.5
Flow rate: 2.5
Injection volume: ≤100
Detector: F ex 235 em 310

CHROMATOGRAM
Retention time: 4.4
Internal standard: bisoprolol (3.6)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: verapamil
Noninterfering: acebutolol, amiodarone, disopyramide, propafenone, hydroquinidine, quinidine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Poirier,J.-M.; Lebot,M.; Cheymol,G. Rapid and sensitive column liquid chromatographic determination of sotalol in plasma, *J.Chromatogr.*, **1989**, *493*, 409–413.

SAMPLE
Matrix: blood

Sample preparation: 500 μL Serum or plasma + 50 μL 25 μg/mL atenolol in water + 100 μL buffer + 5 mL dichloromethane:isopropanol, rotate at 40 rpm for 5 min, centrifuge at 800 g for 3 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 μL 50 mM sulfuric acid, vortex for 15 s, inject a 30-50 μL aliquot. (Buffer was prepared by adjusting the pH of a saturated solution of disodium tetraborate to 9 with 6 M HCl.)

HPLC VARIABLES
Column: 150 × 4.5 5 μm ODS Hypersil
Mobile phase: MeCN:10 mM pH 3.2 phosphate buffer 20:80 containing 3 mM sodium 1-octanesulfonate
Flow rate: 1
Injection volume: 30-50
Detector: UV 226

CHROMATOGRAM
Retention time: 5.5
Internal standard: atenolol (4.0)
Limit of detection: 10 ng/mL

KEY WORDS
serum; plasma

REFERENCE
Urech,R.; Chan,L.; Duffy,P. High-performance liquid chromatographic assay of sotalol: improved procedure and investigation of peak broadening, *J.Chromatogr.*, **1990**, *534*, 271–278.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 50 μL water:70% perchloric acid 75:25, vortex for 30 s, let stand on ice for 5-10 min, vortex, centrifuge at 3500 g for 5 min, add 30 μL 4 M K_2HPO_4, shake gently by hand, centrifuge at 2500 g for 2 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm C18 glass-lined column (Scientific Glass Engineering)
Mobile phase: MeCN:80 mM pH 4.6 KH_2PO_4 6:94
Flow rate: 0.8
Injection volume: 20
Detector: F ex 235 em 310

CHROMATOGRAM
Retention time: 7.1
Limit of quantitation: 80 ng/mL

OTHER SUBSTANCES
Simultaneous: atenolol
Noninterfering: N-acetylprocainamide, disopyramide, flecainide, metoprolol, procainamide, propranolol, quinidine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Boutagy,J.; Shenfield,G.M. Simplified procedure for the determination of sotalol in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *565*, 523–528.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 5000 rpm for 5 min, dilute the supernatant with an equal volume of water, shake vigorously, inject an aliquot onto column A with mobile phase A and elute to waste with mobile phase A, after 10 min backflush the contents of column A onto

column B with mobile phase B, elute with mobile phase B and monitor the effluent. (Two concentration columns (A) are used, one column is being flushed with mobile phase B then mobile phase A while the other is involved in the analysis.)

HPLC VARIABLES
Column: A 40 × 4.6 37-50 μm Bondapak Corasil C18; B 125 × 4.6 5 μm ODS (Shandon)
Mobile phase: A water; B MeCN:10 mM KH_2PO_4 30:70 containing 0.135% dodecylsulfonic acid, adjusted to pH 6.0 with dilute phosphoric acid or KOH
Column temperature: 35
Flow rate: 1
Injection volume: 500
Detector: UV 227

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; column-switching; pharmacokinetics

REFERENCE
Herrmann,R. Automated HPLC assay for sotalol in human plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 329–333.

SAMPLE
Matrix: blood
Sample preparation: Evaporate 100 (?) μL 10 μg/mL atenolol in MeOH in to the bottom of a tube, add 500 μL plasma, add 200 μL 1 M pH 9.3 carbonate buffer, add 6 mL ethyl acetate, shake for 4 min, centrifuge at 1000 g for 4 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue with 2 μL 1 M pH 9.3 carbonate buffer and 200 μL 0.005% R-(-)-1-(1-naphthyl)ethyl isocyanate in chloroform (prepared fresh daily), vortex for 15 s, let stand at room temperature for 1 h, evaporate to dryness with a stream of air, add 200 μL MeCN:water 39:61, vortex for 10 s, let stand at room temperature for 1 h, inject a 10 μL aliquot on to column A and column B in series and elute with mobile phase. Column A is subsequently removed from the circuit and backflushed with MeCN:water 50:50 for 3 min, with MeCN for 12 min, with MeCN:water 50:50 for 7.5 min, and with mobile phase for 7.5 min (this is very unclear in the paper).

HPLC VARIABLES
Column: A 75 × 3.9 4 μm Nova-Pak C18; B 100 × 8 4 μm Nova-Pak C18 Radial Compression
Mobile phase: MeCN:water 39:61
Flow rate: 1.5
Injection volume: 10
Detector: F ex 280 em 320

CHROMATOGRAM
Retention time: 23 (+), 26 (-)
Internal standard: atenolol (13)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites, N-desisopropylpropranolol, 4-hydroxypropranolol, labetalol, 4-methylpropranolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, propranolol glycol, timolol

KEY WORDS
plasma; chiral; pharmacokinetics; derivatization; column-switching

REFERENCE
Hooper,W.D.; Baker,P.V. Enantioselective analysis of sotalol in plasma by reversed-phase high-performance liquid chromatography using diastereomeric derivatives, *J.Chromatogr.B*, **1995**, *672*, 89–96.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 229

CHROMATOGRAM

Retention time: 3.58

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma, whole blood. Condition a Bond-Elut C8 SPE cartridge with MeOH and water. Mix plasma or whole blood, IS, and 50 mM pH 9 borate buffer, add to the SPE cartridge. Wash with water and MeCN. Elute with MeOH. Evaporate the eluate to dryness and reconstitute in 400 μL mobile phase. Inject a 50μL aliquot. Tissue. Homogenize (Braun micro-dismembrator) 100 mg tissue with 100 μL IS solution and 400 μL water while frozen in liquid nitrogen, thaw, rinse twice with 250 μL 1 M pH 3 potassium phosphate buffer. Centrifuge at 2740 g at 20° for 20 min, separate supernatant (S1). Extract pellet with 1 mL MeOH for 15 min with sonication. Centrifuge at 20° for 10 min, evaporate the supernatant to dryness under a stream of nitrogen at 40°. Reconstitute residue in 500 μL 15 mM pH 3 potassium phosphate buffer add to S1, centrifuge. Suck sample slowly through a Bond-Elut C8 SPE cartridge. Wash twice with water, elute twice with 200 μL MeOH, evaporate the eluate to dryness under a stream of nitrogen at 40°. Reconstitute in 500 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 17 × 4 5 μm Spherisorb C6
Column: 150 × 4.6 5 μm Spherisorb C6
Mobile phase: MeCN:15 mM pH 3 potassium phosphate buffer 17:83 (plasma) or 10:90 (tissue, blood)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 5.8 (plasma), 7.8 (whole blood, tissue)
Internal standard: atenolol (4.4 (plasma), 6.8 (whole blood, tissue))
Limit of quantitation: 26.5 ng/mL (whole blood, plasma); 270 ng/g tissue

KEY WORDS
whole blood; plasma; heart; SPE

REFERENCE
Laer,S.; Neumann,J.; Scholz,H.; Uebeler,P.; Zimmermann,N. Determination of sotalol in human cardiac tissue by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 291–298.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 2 mL Plasma + 100 μL 100 μg/mL procainamide in water + 660 μL 2 M perchloric acid, shake briefly, centrifuge at 3000 rpm for 5 min. Remove 1.5 mL of the supernatant and adjust the pH to 9 with 150 μL 4 M NaOH and 4 mL 500 mM boric acid/KCl buffer, add 12 mL water-saturated n-pentanol:chloroform 20:60, shake for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic phase and dry it over anhydrous sodium sulfate, add 8 mL to 300 μL 100 mM HCl, shake for 10 min, centrifuge at 3000 rpm for 10 min, inject a 25-100 μL aliquot of the aqueous phase. Urine. Dilute urine with pH 9 borate buffer, add 12 mL water-saturated n-pentanol:chloroform 20:60, shake for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic phase and dry it over anhydrous sodium sulfate, add 8 mL to 300 μL 100 mM HCl, shake for 10 min, centrifuge at 3000 rpm for 10 min, inject a 25-100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:water:acetic acid 38:61:1 containing 1-heptanesulfonic acid (PIC B7)
Flow rate: 1.5
Injection volume: 25-100
Detector: F ex 235 em no filter

CHROMATOGRAM
Retention time: 5.2
Internal standard: procainamide (7)
Limit of detection: 20 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lefebvre,M.A.; Girault,J.; Saux,M.C.; Fourtillan,J.B. Fluorometric high-performance liquid chromatographic determination of sotalol in biological fluids, *J.Pharm.Sci.*, **1980**, *69*, 1216–1217.

SAMPLE
Matrix: blood, urine

Sample preparation: 1 mL Plasma + 200 μL 2.5 μg/mL S-(-)atenolol + 660 μL 2 M perchloric acid, shake briefly, centrifuge at 2000 g for 5 min. Remove 1 mL of the supernatant and adjust the pH to 9 with 1 mL 2 M Tris-HCl buffer and 250 μL 2 M NaOH, add 5 mL chloroform: isopropanol 75:25, vortex for 1 min, centrifuge at 2000 g for 10 min, repeat the extraction. Combine the organic layers and dry them over about 3 g anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL saturated sodium carbonate solution, add 200 μL 40 μL/mL (-)-menthylchloroformate in MeCN (prepare fresh daily), vortex for 30 s, add 1 mL water, add 2 mL chloroform, vortex for 1 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, centrifuge for 5 min, inject a 40 μL aliquot. Urine. Dilute 1 mL (?) urine 20 times with blank urine, add 200 μL 7.5 μg/mL S-(-)atenolol, adjust the pH to 9 with 1 mL 2 M Tris-HCl buffer, add 5 mL chloroform:isopropanol 75:25, vortex for 1 min, centrifuge at 2000 g for 10 min, repeat the extraction. Combine the organic layers and dry them over about 3 g anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL saturated sodium carbonate solution, add 200 μL 40 μL/mL (-)-menthylchloroformate in MeCN (prepare fresh daily), vortex for 30 s, add 1 mL water, add 2 mL chloroform, vortex for 1 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, centrifuge for 5 min, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C8 (Jones)
Mobile phase: MeCN:MeOH:water 15:50:35
Flow rate: 2
Injection volume: 40
Detector: F ex 235 em 300

CHROMATOGRAM
Retention time: 14 (l), 15 (d)
Internal standard: S-(-)-atenolol (12.3)
Limit of detection: 12.5 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: alprenolol, captopril, metoprolol, pindolol, propafenone

Noninterfering: acetaminophen, alginic acid, amiloride, amoxicillin, aspirin, diazoxide, digoxin, domperidone, flurazepam, furosemide, glyburide, heparin, hydralazine, hydrochlorothiazide, indapamide, lidocaine,lorazepam, lovastatin, mexiletine, nifedipine, nitroglycerin, omeprazole, oxazepam, procainamide, propranolol, propoxyphene, quinidine, ranitidine, timolol, triamterene, verapamil, warfarin

KEY WORDS
plasma; chiral; derivatization

REFERENCE
Fiset,C.; Philippon,F.; Gilbert,M.; Turgeon,J. Stereoselective high-performance liquid chromatographic assay for the determination of sotalol enantiomers in biological fluids, *J.Chromatogr.*, **1993**, *612*, 231–237.

SAMPLE
Matrix: blood, urine

Sample preparation: Condition a 500 mg Sep-Pak C18 SPE cartridge with 3 mL MeOH and 3 mL 200 mM sodium tetraborate. Dilute urine 10 times with 200 mM sodium tetraborate. Briefly vortex 1 mL plasma with 500 μL saturated pH 9.3 sodium tetraborate. Add 1 mL diluted urine or 1.5 mL diluted plasma to the SPE cartridge, wash with 2 mL 20 mM sodium tetra-

borate, wash with 2 mL water, wash with 1 mL dichloromethane, elute with 5 mL isopropanol. Add 100 μL 100 μg/mL isoamyl p-hydroxybenzoate in MeCN:water 10:90, 100 μL 4 mg/mL 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate in MeCN, and 100 μL 10 mM pH 8.0 $(NH_4)_2HPO_4$ to the eluate, heat at 50° for 3 h, evaporate to dryness under reduced pressure, reconstitute with 200 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pak Resolve C18 (Waters)
Column: 150 × 4.6 5 μm STR ODS II (Shimadzu)
Mobile phase: MeCN:20 mM pH 4.6 $(NH_4)H_2PO_4$ 40:60
Column temperature: 25
Flow rate: 1
Injection volume: 40
Detector: UV 225

CHROMATOGRAM
Retention time: 11.4 ((-)-R), 14.3 ((+)-S)
Internal standard: isoamyl p-hydroxybenzoate (25.5)
Limit of quantitation: 220 ng/mL (urine), 22 ng/mL (plasma)

KEY WORDS
chiral; rat; human; mouse; rabbit; plasma; SPE; derivatization; pharmacokinetics

REFERENCE
Shimizu,T.; Hiraoka,M.; Nakanomyo,H. Enantioselective determination of sotalol enantiomers in biological fluids using high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *674*, 77–83.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.842

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: k' 5.01, k' 6.53 (enantiomers)

OTHER SUBSTANCES
Interfering: atenolol

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: formulations
Sample preparation: Take up in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb C2
Mobile phase: MeCN:buffer 35:65 (1 mL 100 mM HCl + 1200 mL water + 5.84 g NaCl, mix to dissolve, add 700 mL MeOH, make up to 2 L, apparent pH 4.5.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Simultaneous: atenolol, nadolol, alprenolol, acebutolol, propranolol, metoprolol, practolol, oxprenolol
Interfering: pindolol, timolol

KEY WORDS
tablets

REFERENCE
Patel,B.R.; Kirschbaum,J.J.; Poet,R.B. High-pressure liquid chromatography of nadolol and other β-adrenergic blocking drugs, *J.Pharm.Sci.*, **1981**, *70*, 336–338.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 500 μL to 10 mL with water. 100 μL Diluted sample + 100 μL 2.5 μg/mL sotalol + 100 μL saturated sodium tetraborate adjusted to pH 9 with HCl + 500 μL

water + 5 mL dichloromethane:isopropanol 3:1, agitate on mechanical shaker for 5 min, centrifuge at 800 g for 3 min. Evaporate organic layer to dryness at 45° under a stream of nitrogen. Dissolve residue in 200 μL 50 mM sulfuric acid, mix for 30 s, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: Novapak C18 guard insert
Column: 100 × 5 Novapak C18
Mobile phase: MeCN:10 mM potassium phosphate buffer adjusted to pH 3.2 with 0.2 M phosphoric acid 20:80 containing 3 mM 1-octanesulfonic acid
Flow rate: 1
Injection volume: 30
Detector: UV 226

CHROMATOGRAM
Retention time: 5
Internal standard: sotalol

OTHER SUBSTANCES
Simultaneous: atenolol

KEY WORDS
stability indicating; oral liquid; sotalol is IS

REFERENCE
Garner,S.S.; Wiest,D.B.; Reynolds,E.R.,Jr. Stability of atenolol in an extemporaneously compounded oral liquid, *Am.J.Hosp.Pharm.*, **1994**, *51*, 508–511.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine,

methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 amylose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Detector: UV

CHROMATOGRAM

Retention time: 16 (+), 24 (-)

KEY WORDS

chiral

REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE

Matrix: solutions
Sample preparation: Evaporate an aliquot of a solution in MeCN containing 625 ng drug to dryness under a stream of nitrogen at room temperature, add 200 μL saturated sodium carbonate, add 200 μL 4% (-)-menthyl chloroformate in MeCN, vortex for 30 s, add an excess amount of 4-hydroxy-L-proline, vortex for 30 s, centrifuge for 3 min, inject a 10-25 μL aliquot of the upper layer.

HPLC VARIABLES

Guard column: 50 × 4.6 Pellicular ODS (Whatman)
Column: 100 × 4.6 5 μm Partisil 5 ODS3
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10-25
Detector: F ex 232 em no emission filter

CHROMATOGRAM

Retention time: 28 (-), 31 (+)

OTHER SUBSTANCES
Simultaneous: metaproterenol

KEY WORDS
derivatization; chiral

REFERENCE
Mehvar,R. Stereospecific liquid chromatographic analysis of racemic adrenergic drugs utilizing precolumn derivatization with (-)-menthyl chloroformate, *J.Chromatogr.*, **1989**, *493*, 402–408.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES
Column: 100 × 4.7 7 μm Hypercarb (Shandon)
Mobile phase: MeOH containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH
Column temperature: 17
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: k' 9.8 (first enantiomer)

KEY WORDS
chiral; α = 1.13

REFERENCE
Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonyl-glyclyl-L-proline as counter ion in methanol, *J.Chromatogr.A*, **1995**, *705*, 275–287.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.71

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.96 (A), 3.41 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Pecosphere CR C18
Mobile phase: MeCN:MeOH:water acidified to pH 2.7 18:2:80 containing 2 g/L sodium octanesulfonate
Flow rate: 4
Injection volume: 10
Detector: UV 238

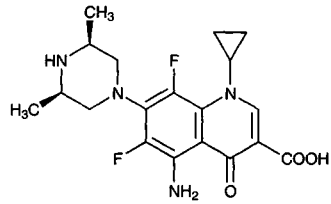
CHROMATOGRAM
Retention time: 0.8

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
high-speed

REFERENCE
Muller,M.C.; Caude,M.; Dauphin,J.F.; Lecointre,L.; Saint-Germain,J. Use of high speed liquid chromatography (HSLC) in the pharmaceutical industry. Practical aspects and limitations, *Chromatographia*, **1995**, *40*, 394–398.

Sparfloxacin



Molecular formula: $C_{19}H_{22}F_2N_4O_3$
Molecular weight: 392.41
CAS Registry No.: 110871-86-8
Merck Index: 8884

SAMPLE
Matrix: aqueous humor, blood, vitreous humor
Sample preparation: Plasma. Acidify plasma with 200 μL 1 M HCl. Filter (Amicon MPS1) while centrifuging at 1000 g for 1 h, inject a 500 μL aliquot of the ultrafiltrate. Vitreous humor. Filter (Amicon MPS1) while centrifuging at 1000 g for 1 h. Acidify ultrafiltrate with one tenth the volume of 330 mM HCl, inject a 200 μL aliquot. Aqueous humor. Filter (Amicon MPS1) while centrifuging at 1000 g for 1 h. Acidify ultrafiltrate with one tenth the volume of 100 mM HCl, inject a 100 μL aliquot

HPLC VARIABLES
Guard column: Corasil C18
Column: μBondapak C18
Mobile phase: MeCN:buffer 13.6:86.4 (Buffer was 25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide.)
Flow rate: 1.5
Injection volume: 100-500
Detector: UV 313 (aqueous humor), UV 365 (vitreous humor, plasma)

CHROMATOGRAM
Limit of detection: 10 ng/mL

KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

REFERENCE
Bron,A.M.; Pechinot,A.P.; Garcher,C.P.; Guyonnet,G.A.; Kazmierczak,A.M.; Schott,D.A.; Lecoeur,H. The ocular penetration of oral sparfloxacin in humans, *Am.J.Ophthalmol.*, **1994**, *117*, 322–327.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 50 μg/mL IS solution and 2 mL pH 7.5 phosphate buffer to 250 μL plasma. Extract twice with 5 mL portions of ethyl acetate. Evaporate the organic layer. Reconstitute the residue with mobile phase and inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil C8

Mobile phase: MeCN:2 mM phosphoric acid:triethylamine 15:85:0.15, pH 3.0
Flow rate: 1
Detector: UV 308

CHROMATOGRAM
Retention time: 20
Internal standard: ciprofloxacin (10)
Limit of quantitation: 200 ng/mL

KEY WORDS
plasma

REFERENCE
Bhatti,M.M.; Hanson,G.D. Determination of cisapride in human plasma by high-performance liquid chromatography with ultraviolet detection (Abstract 2504), *Pharm.Res.*, **1997**, *14*, S378.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate..

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 20:80 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 299

CHROMATOGRAM
Retention time: 8.46
Internal standard: rosoxacin (5.73)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Homogenize rat brain in 4 volumes water. 0.05-1 mL Whole blood, serum, CSF or brain homogenate + 1 mL 400 ng/mL IS in 100 mM pH 7.4 phosphate buffer + 5 mL dichloromethane:diethyl ether 80:20, shake for 10 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm YMC Pack A-312 (YMC)
Mobile phase: MeCN:MeOH:5% acetic acid 15:15:70
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 364

CHROMATOGRAM
Internal standard: 5-amino-7-(3-amino-3-ethyl-1-pyrrolidinyl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid
Limit of detection: 5 ng/mL

KEY WORDS
rat; serum; brain; pharmacokinetics

REFERENCE
Naora,K.; Katagiri,Y.; Iwamoto,K.; Tanaka,K.; Yamaguchi,T.; Sekine,Y. Effect of fenbufen on the pharmacokinetics of sparfloxacin in rats, *J.Antimicrob.Chemother.*, **1992**, *30*, 673–683.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Homogenize brain tissue in 20 mM sodium phosphate buffer. Add an equal volume of 4% perchloric acid to homogenate, serum, or CSF, vortex, centrifuge, filter (0.45 μm), add IS, inject an aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:MeOH:5% acetic acid 11:15:74
Flow rate: 3
Injection volume: 100
Detector: UV 364

CHROMATOGRAM
Internal standard: RP41983 (Rhone Poulenc)
Limit of detection: 150 ng/mL

KEY WORDS
brain; serum; pharmacokinetics

REFERENCE
Davey,P.G.; Charter,M.; Kelly,S.; Varma,T.R.K.; Jacobson,I.; Freeman,A.; Precious,E.; Lambert,J. Ciprofloxacin and sparfloxacin penetration into human brain tissue and their activity as antagonists of GABAA receptor of rat vagus nerve, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1356–1362.

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Serum. 500 μL Serum + 100 μL 50 ng/mL ofloxacin in water + 1 mL MeCN, shake mechanically for 30 s, centrifuge at 10000 g for 5 min. Remove 1.5 mL of the supernatant and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 300 μL mobile phase, inject a 25 μL aliquot. Urine. Dilute urine with 4 volumes buffer, inject a 50 μL aliquot. Feces. Suspend 100 mg of feces in 9 mL mobile phase, shake for 15 min, centrifuge at 1500 g for 10 min, repeat the extraction twice more, inject a 25 μL aliquot of each extract.

HPLC VARIABLES
Guard column: 30 × 4 30-40 μm Perisorb RP-18 (Merck)
Column: 125 × 4 5 μm Nucleosil 100 SA
Mobile phase: MeCN:buffer 75:25 adjusted to pH 3.82 with concentrated phosphoric acid, final sodium concentration 23 mM (Buffer was 6.74 mL concentrated phosphoric acid and 40 mL 2 M NaOH made up to 990 mL with water, adjust pH to 2.92 with concentrated phosphoric acid, make up to 1 L with water.)
Flow rate: 1.5
Injection volume: 25-50
Detector: F ex 295 em 525

CHROMATOGRAM
Retention time: 4.7
Internal standard: ofloxacin (8.0)
Limit of detection: 140 ng/mL (feces), 460 ng/mL (urine), 50 ng/mL (serum)

KEY WORDS
serum; protect from light

REFERENCE

Borner,K.; Borner,E.; Lode,H. Determination of sparfloxacin in serum and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *579*, 285–289.

SAMPLE

Matrix: blood, intestinal efflux

Sample preparation: Intestinal efflux. Freeze intestinal efflux at -80°, lyophilize, reconstitute with 1 mL ofloxacin in MeOH:100 mM phosphoric acid 50:50, centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot. Serum. Deproteinize serum with MeOH containing ofloxacin, extract with dichloromethane at pH 7.5.

HPLC VARIABLES

Column: 150 × 3.9 Novapack C18

Mobile phase: MeOH:buffer 35:65 (Buffer was 10 mM pH 3.0 potassium phosphate buffer containing 25 mM sodium heptanesulfonate (PIC B7) and 20 mM triethylamine.)

Flow rate: 1.5

Injection volume: 20

Detector: UV 364

CHROMATOGRAM

Internal standard: ofloxacin

Limit of detection: 100 ng/mL

KEY WORDS

serum; rat

REFERENCE

Rubinstein,E.; Dautrey,S.; Farinoti,R.; St.Julien,L.; Ramon,J.; Carbon,C. Intestinal elimination of sparfloxacin, fleroxacin, and ciprofloxacin in rats, *Antimicrob.Agents Chemother.*, **1995**, *39*, 99–102.

SAMPLE

Matrix: blood, tissue

Sample preparation: Homogenize (Ultra-Turrax T25) mouse lung in 1-3 mL pH 6.8 Soerensen phosphate buffer, centrifuge. Extract serum or lung homogenate supernatant, extract using a Bond Elut C2 SPE cartridge, inject a 100 μL aliquot of the extract.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrabase C8 (SFCC, Neuilly Plaisance, France)

Mobile phase: MeCN:MeOH:5% acetic acid 15:10:75

Flow rate: 1

Injection volume: 100

Detector: UV 280

CHROMATOGRAM

Retention time: 6

Internal standard: sparfloxacin

Limit of detection: 15 ng

OTHER SUBSTANCES

Extracted: temafloxacin

KEY WORDS

serum; lung; mouse; pharmacokinetics; SPE; sparfloxacin is IS

REFERENCE

Vallée,E.; Azoulay-Dupuis,E.; Bauchet,J.; Pocidalo,J.-J. Kinetic disposition of temafloxacin and ciprofloxacin in a murine model of pneumococcal pneumonia. Relevance for drug efficacy, *J.Pharmacol.Exp.Ther.*, **1992**, *262*, 1203–1208.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition an AASP SPE cartridge (Varian) with MeOH. Centrifuge plasma at 1000 g for 10 min. 500 μL Plasma + 50 μL 20 μg/mL IS, add to the SPE cartridge, wash with water, elute the contents of the SPE cartridge onto the analytical column with mobile phase. Urine. Add internal standard, inject an aliquot directly. Hydrolyze conjugates with 1 M NaOH for 10 min.

HPLC VARIABLES

Column: 150 × 6 Asahi PAK OD1,50 reverse phase
Mobile phase: 5% methanol-acetonitrile-acetic acid (11.6/11.6/76.8) (sic) [Perhaps MeCN:MeOH: 5% acetic acid 11.6:11.6:76.8]
Detector: UV 364

CHROMATOGRAM

Internal standard: 1-ethyl-6-chloro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid
Limit of quantitation: 250 ng/mL (urine), 15 ng/mL (plasma)

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Fillastre,J.P.; Montay,G.; Bruno,R.; Etienne,I.; Dhib,M.; Vivier,N.; Le Roux,Y.; Guimart,C.; Gay,G.; Schott,D. Pharmacokinetics of sparfloxacin in patients with renal impairment, *Antimicrob.Agents Chemother.*, **1994**, *38*, 733–737.

SAMPLE

Matrix: growth medium
Sample preparation: 500 μL Sample + 500 μL 100 μg/mL IS in cold (4°) MeCN, vortex, centrifuge at 3000 g for 5 min. Remove a 500 μL aliquot of the supernatant, filter (0.45 μm Acrodisc syringe filter), inject a 30 μL aliquot. (Protect all specimens from light.)

HPLC VARIABLES

Guard column: C18 5U (Alltech)
Column: 150 × 4.6 7 μm Adsorbosphere HS C18 7U
Mobile phase: MeCN:20 mM pH 3.0 phosphate buffer 35:65 containing 0.2% triethylamine and 0.2% sodium dodecyl sulfate, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1.75
Injection volume: 30
Detector: UV 280

CHROMATOGRAM

Retention time: 7.09
Internal standard: ciprofloxacin (4.67)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Also analyzed: ciprofloxacin, clinafloxacin, levofloxacin, ofloxacin, temafloxacin, trovafloxacin

KEY WORDS

Mueller-Hinton broth

REFERENCE

Wright,D.H.; Herman,V.K.; Konstantinides,F.N.; Rotschafer,J.C. Determination of quinolone antibiotics in growth media by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *709*, 97–104.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Centrifuge 250 μL microsomal incubation at 13000 g for 5 min, neutralize perchloric acid lysates with 1 M potassium carbonate, add IS, inject an aliquot directly.

HPLC VARIABLES
Column: Asahipack ODP 50
Mobile phase: Gradient. MeCN:water from 10:90 to 40:60 over 10 min
Detector: UV

CHROMATOGRAM
Internal standard: ciprofloxacin

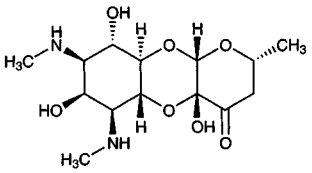
REFERENCE
Rispal,P.; Grellet,J.; Celerier,C.; Breilh,D.; Dorian,M.; Pellegrin,J.L.; Saux,M.C.; Leng,B. Comparative uptake of sparfloxacin and ciprofloxacin into human THP 1 monocytic cells, *Arzneimittelforschung*, **1996**, *46*, 316–319.

SAMPLE
Matrix: solution

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 35:65 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 291

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

Spectinomycin



Molecular formula: $C_{14}H_{24}N_2O_7$
Molecular weight: 332.35
CAS Registry No.: 1695-77-8, 22189-32-8 (di HCl pentahydreate), 21736-83-4 (di HCl)
Merck Index: 8890

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 400 μL MeCN:trifluoroacetic acid 97:3, vortex, centrifuge at 2000 g for 3 min. Remove a 250 μL aliquot of the supernatant and add it to 200 μL 5 mg/mL 2,4-dinitrophenylhydrazine in MeCN, mix, heat at 70° for 1 h, cool on ice for 2 min, add 30 μL acetone, mix, heat at 70° for 10 min, cool on ice, filter (Ultrafree MC 30000 molecular weight limit) while centrifuging at 2000 g, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 33 × 4.6 3 μm Pecosphere C18 CR
Mobile phase: Gradient. A was MeCN:water 60:40. B was MeCN:MeOH 19:1. A:B 100:0 for 1 min, to 0:100 over 10 min, maintain at 0:100 for 2 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1.2
Injection volume: 20
Detector: UV 405

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 2 μg/mL

KEY WORDS
plasma; turkey; derivatization

REFERENCE
Burton,S.D.; Hutchins,J.E.; Fredericksen,T.L.; Ricks,C.; Tyczkowski,J.K. High-performance liquid chromatographic method for the determination of spectinomycin in turkey plasma, *J.Chromatogr.*, **1991**, *571*, 209–216.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL C18 SPE cartridge (J.T. Baker) with 3 mL MeOH, 2 mL 20 mM sodium dioctylsulfosuccinate (slowly!), and 3 mL 20 mM pH 5.6 citric acid, do not allow to go dry. 2 mL Plasma + 8 mL water + 625 μL MeOH, adjust pH to 5.2-5.7 with about 60 μL 1 M HCl, vortex for 30 s, centrifuge at 2500 g for 10 min, remove the supernatant, resuspend the solid in 3 mL 20 mM pH 5.6 citric acid, centrifuge. Combine the supernatants and add them to the SPE cartridge at 1-2 mL/min, wash with two 3 mL portions of 20 mM pH 5.6 citric acid, allow to go dry. Centrifuge the SPE cartridge at 3000 g for 15 min, dry with a stream of nitrogen for 20 min, elute with 3 mL MeOH at 2 drops/s. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 2 mL 10 g/L sodium bicarbonate containing 100 μL/L 1-methylpyrrole, vortex for 30 s, add 3 mL 12 mg/mL 2-naphthalenesulfonyl chloride in MeCN (prepare just before derivatization), vortex for 30 s, heat at 100° for 15 min, cool to room temperature, add 1 mL n-butyl chloride, vortex twice for 20 s, centrifuge at 1500 g for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 1 mL n-butyl chloride, vortex for 30 s, centrifuge at 2500 g for 10 min, inject a 50 μL aliquot onto column A and elute to waste with mobile phase A, after 8 min divert the effluent from column A containing spectinomycin onto column B, after 2 min remove column A form the circuit, elute column B with mobile phase B, monitor the effluent from column B. Backflush column A with mobile phase A for 15 min.

HPLC VARIABLES
Column: A 10 × 2.1 40 μm pellicular silica + 100 × 3 5 μm Chromspher silica glass cartridge (Chrompack); B two 100 × 3 5 μm Chromspher silica glass cartridges in series (Chrompack)
Mobile phase: A Dichloromethane:MeCN:ethyl acetate:acetic acid 75:7.5:1.8:0.425; B Dichloromethane:MeCN:ethyl acetate:acetic acid 100:20:3.6:0.85
Flow rate: A 0.4; B 0.6
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 14
Limit of quantitation: 40 ng/mL

KEY WORDS
derivatization; cow; calf; chicken; pig; plasma; SPE; use glass not plastic tubes; column-switching; heart-cut; normal phase

REFERENCE
Haagsma,N.; Keegstra,J.R.; Scherpenisse,P. High-performance liquid chromatographic determination of spectinomycin in swine, calf and chicken plasma, *J.Chromatogr.*, **1993**, *615*, 289–295.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL High-hydrophobic C18 SPE cartridge (J.T. Baker) with 3 mL MeOH, 2 mL 20 mM sodium dioctyl sulfosuccinate (homogenize before use, pass slowly through cartridge), and 4 mL 20 mM pH 5.6 citric acid buffer, do not allow to go dry. 2 mL Plasma + 8 mL water + 625 μL MeOH, adjust pH to 5.2-5.7 with ca. 60 μL 1 M HCl, vortex for 30 s, centrifuge at 2500 g for 10 min, remove the supernatant, rinse the residue with 3 mL citric acid buffer, centrifuge. Combine the rinse and the supernatant and add them to the SPE cartridge at 1-2 mL/min, wash with two 3 mL portions of citric acid buffer, allow to run dry. Centrifuge the SPE cartridge at 3000 g for 20 min then dry it in a stream of nitrogen for 20 min, elute with 4 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen at

room temperature, reconstitute with 1 mL MeOH:water 20:80, vortex, centrifuge at 2500 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 × 4.6 cation-exchange (Phase-Sep)
Column: 250 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeCN:100 mM pH 2.6 sodium sulfate 20:80
Flow rate: 1.5
Injection volume: 50
Detector: F ex 340 em 460 following post-column reaction. The column effluent mixed with reagent A pumped at 0.25 mL/min and reagent B pumped at 0.75 mL/min and the mixture flowed through a 2.2 m × 0.5 mm i.d. coil at 70°. The effluent from the coil was mixed with reagent C pumped at 1 mL/min and this mixture flowed through a 1.7 m × 0.3 mm i.d. PTFE coil at 25° to the detector. Reagent A was prepared by adding 10 mL sodium hypochlorite solution (13% active chlorine) and 10 mL 100 mM pH 7.0 potassium phosphate buffer to 980 mL water. Reagent B was 400 mM pH 10.2 boric acid buffer prepared by dissolving 24.4 g boric acid and 20.0 g KOH in 1 L water. Reagent C was prepared by adding 10 mL 80 mg/mL o-phthaldialdehyde in EtOH and 1 mL 2-mercaptoethanol to 990 mL 300 mM pH 10.2 boric acid buffer. (The 300 mM pH 10.2 boric acid buffer was prepared by dissolving 18.55 g boric acid and 15.0 g KOH in 1 L water.)

CHROMATOGRAM

Retention time: 9
Limit of quantitation: 60 ng/mL

KEY WORDS

pig; cow; chicken; plasma; SPE; post-column reaction

REFERENCE

Haagsma,N.; Scherpenisse,P.; Simmonds,R.J.; Wood,S.A.; Rees,S.A. High-performance liquid chromatographic determination of spectinomycin in swine, calf and chicken plasma using post-column derivatization, *J.Chromatogr.B*, **1995**, *672*, 165–171.

SAMPLE

Matrix: bulk
Sample preparation: 10 mL 250 μg/mL Spectinomycin solution in buffer + 10 mL 10 mg/mL 2-naphthalenesulfonyl chloride in MeCN containing 200 μg/mL methylprednisolone acetate (prepare fresh daily), shake, heat at 100° for 10 min, cool to room temperature, make up to 50 mL with mobile phase, shake vigorously for 10 min, centrifuge at <300 g for 3-5 min, inject a 40 μL aliquot of the organic layer. (Buffer was 4.2 g/L sodium bicarbonate containing 100 μL/L 1-methylpyrrole.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb SI-60
Mobile phase: Butyl chloride:THF:ethyl acetate:isopropanol:acetic acid 86:3.7:3:2.5:5 (Butyl chloride was 50% water-saturated.)
Flow rate: 1
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Retention time: 10
Internal standard: methylprednisolone acetate (18)
Limit of detection: 4.2 ng

OTHER SUBSTANCES

Simultaneous: actinosapectinoic acid

KEY WORDS

derivatization; normal phase

REFERENCE
Tsuji,K.; Jenkins,K.M. Derivatization of secondary amines with 2-naphthalene-sulfonyl chloride for high-performance liquid chromatographic analysis of spectinomycin, *J.Chromatogr.*, **1985**, *333*, 365–380.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject an aliquot.

HPLC VARIABLES
Guard column: 43 × 4.2 Perisorb RP-8 (Merck)
Column: 250 × 4.6 LiChrosorb RP-8
Mobile phase: 0.1% Acetic acid containing 20 mM sodium heptanesulfonate and 200 mM sodium sulfate
Flow rate: 2
Injection volume: 15
Detector: F ex 350 em 450 following post-column reaction. The column effluent mixed with 10 mM sodium hypochlorite in 400 mM pH 10.4 potassium borate buffer pumped at 0.5 mL/min and the mixture flowed through a 2 m × 0.5 mm i.d. PTFE coil at 100° and was mixed with the reagent pumped at 0.5 mL/min. This mixture passed through a 2 m × 0.5 mm i.d. PTFE coil at ambient temperature to the detector. (The reagent was prepared by adding 10 mL 80 mg/mL o-phthalaldehyde in 95% EtOH to 1 L 400 mM boric acid solution containing 2 mL 2-mercaptoethanol adjusted to pH 9.7 with KOH, then adding 1 g/L Brij. (Proc. Nat. Acad. Sci. USA 1975, 72, 619).)

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: actinamine, actinospectinoic acid, dihydrospectinomycin

KEY WORDS
post-column reaction

REFERENCE
Myers,H.N.; Rindler,J.V. Determination of spectinomycin by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1979**, *176*, 103–108.

SAMPLE
Matrix: solutions
Sample preparation: Inject 50 μL of a solution in 150 mM sodium acetate.

HPLC VARIABLES
Guard column: 25 × 4 IonPac CG3 (Dionex)
Column: 250 × 4 IonPac CS3 (Dionex)
Mobile phase: 150 mM pH 6 sodium acetate buffer
Column temperature: 21
Flow rate: 1
Injection volume: 50
Detector: E following post-column reaction, Dionex pulsed-amperometric detector, gold working electrode, stainless steel auxiliary electrode, Ag/AgCl reference electrode, rise-time filter 3.0 s, output range 1 μA. Pulse sequence was +0.05 V sampling potential with 100 ms delay-time and 380 ms measuring time followed by +0.60 V oxidation potential to clean the electrode for 120 ms and -0.70 V reduction potential to activate the electrode for 60 ms. The column effluent mixed with 100 mM NaOH pumped at 0.5 mL/min and the mixture flowed to the detector.

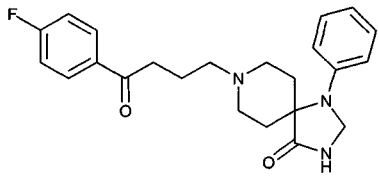
CHROMATOGRAM
Retention time: 10.7
Limit of detection: 20 ng
Limit of quantitation: 60 ng

OTHER SUBSTANCES
Simultaneous: actinamine, actinospecinoic acid

KEY WORDS
post-column reaction

REFERENCE
Phillips,J.G.; Simmonds,C. Determination of spectinomycin using cation-exchange chromatography with pulsed amperometric detection, *J.Chromatogr.A*, **1994**, *675*, 123–128.

Spiperone



Molecular formula: $C_{23}H_{26}FN_3O_2$
Molecular weight: 395.48
CAS Registry No.: 749-02-0
Merck Index: 8903

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 500 mM pH 8.5 phosphate buffer, extract with 2.5 mL heptane:isoamyl alcohol 98:2. Shake for 5 min, centrifuge at 1700 g for 10 min. Mix 2.0 mL organic layer with 100 μL 3 M acetic acid, shake for 20 min, centrifuge at 1700 g for 10 min. Aspirate organic layer, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm EicomPak MA-5ODS (Eicom, Japan)
Mobile phase: MeCN:MeOH:buffer 20:15:65 containing 500 μg/L disodium EDTA (Buffer was 100 mM KH_2PO_4 adjusted to pH 3.5 with 100 mM phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: E, Eicom ECD-100, glassy carbon electrode +1 V, Ag/AgCl reference electrode

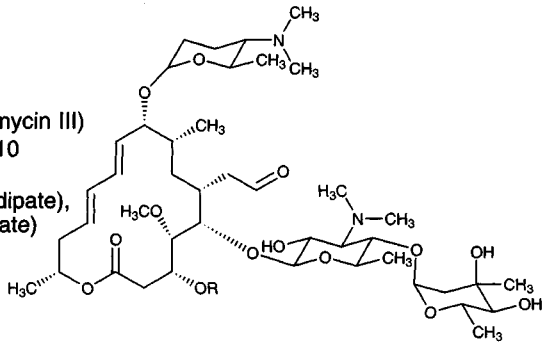
CHROMATOGRAM
Retention time: 14.9
Internal standard: spiperone

KEY WORDS
plasma; rat; spiperone is IS

REFERENCE
Takayasu,T.; Kakubari,I.; Fukamachi,A.; Mafune,E.; Takasugi,N.; Takayama,K.; Nagai,T. Determination of timiperone in rat plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1996**, *679*, 161–165.

Spiramycin

Molecular formula: $C_{43}H_{74}N_2O_{14}$ (Spiramycin I), $C_{45}H_{76}N_2O_{15}$ (Spiramycin II), $C_{46}H_{78}N_2O_{15}$ (Spiramycin III)
Molecular weight: 843.07 (Spiramycin I), 885.10 (Spiramycin II), 899.13 (Spiramycin III)
CAS Registry No.: 8025-81-8, 24916-50-5 (I adipate), 24916-51-6 (II diacetate), 24916-52-7 (III diacetate)
Merck Index: 8904



Spiramycin I R = H
Spiramycin II R = $COCH_3$
Spiramycin III R = $COCH_2CH_3$

SAMPLE

Matrix: tissue
Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm C18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
Flow rate: 0.5
Injection volume: 200
Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 475.3-684.4 in NCI and 843.5-684.4 in PCI

CHROMATOGRAM

Retention time: 5.2
Limit of detection: 50 μg/kg

OTHER SUBSTANCES

Extracted: erythromycin, josamycin, tilmicosin, tylosin

KEY WORDS

muscle; cow; SPE

REFERENCE

Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut SCX SPE cartridge (Varian) with 5 mL MeOH and 10 mL 100 mM pH 4.4 KH_2PO_4 buffer. Homogenize 5 g tissue with 100 mL MeOH: 0.3% metaphosphoric acid 30:70 at high speed for 2 min, filter through 2 mm Hyflo Super-Cel coated on a suction funnel (when filtering liver or kidney add several grams of Hyflo Super-Cel to the homogenized solution before filtration). Evaporate the filtrate to ca. 20 mL under

reduced pressure at 45°, add to the SPE cartridge, wash with 10 mL distilled water and 5 mL 100 mM pH 8.9 K_2HPO_4 buffer, elute with 10 mL MeOH, evaporate the eluate to dryness under reduced pressure at 45°, dissolve the residue in 1 mL MeCN:50 mM pH 4.5 NaH_2PO_4 buffer 30:70, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Puresil 5C18 (Waters)
Mobile phase: Gradient. A:B from 60:40 to 0:100 over 16 min. A was buffer. B was MeCN:buffer 40:60 (Buffer was 2.5 g KH_2PO_4 dihydrate and 0.65 mL 85% phosphoric acid dissolved in 1 L distilled water, pH 2.5.)
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 232 for 9 min, UV 287 for 2 min, UV 232 for 4 min

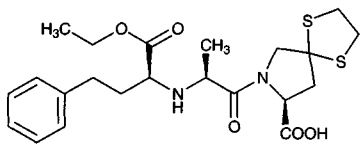
CHROMATOGRAM
Retention time: 3.23
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Extracted: josamycin, leucomycin (kitasamycin), mirosamicin, tylosin

KEY WORDS
meat; SPE

REFERENCE
Horie,M.; Saito,K.; Ishii,R.; Yoshida,T.; Haramaki,Y.; Nakazawa,H. Simultaneous determination of five macrolide antibiotics in meat by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 295–302.

Spirapril



Molecular formula: $C_{22}H_{30}N_2O_5S_2$
Molecular weight: 466.62
CAS Registry No.: 83647-97-6, 94841-17-5
Merck Index: 8905
Lednicer No.: 4 83

SAMPLE
Matrix: solutions

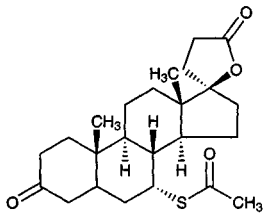
HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee RP-18
Mobile phase: MeCN:water 50:50 containing 0.5 mM tetramethylammonium hydroxide, adjusted to pH 2
Column temperature: 70
Detector: UV 217

CHROMATOGRAM
Retention time: 3.6

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Steuer,W.; Grant,I.; Erni,F. Comparison of high-performance liquid chromatography, supercritical fluid chromatography and capillary zone electrophoresis in drug analysis, *J.Chromatogr.*, **1990**, *507*, 125–140.

Spironolactone



Molecular formula: $C_{24}H_{32}O_4S$
Molecular weight: 416.58
CAS Registry No.: 52-01-7
Merck Index: 8917
Lednicer No.: 1 206

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 500 ng/mL methyltestosterone in MeCN to 100 μL serum, vortex for 15 s, centrifuge at 5000 rpm for 3 min, inject 100 μL of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Spherisorb ODS-2
Column: 250 × 4 5 μm Spherisorb ODS-2
Mobile phase: MeOH:water 67:33
Flow rate: 1
Injection volume: 100
Detector: UV 238

CHROMATOGRAM
Retention time: 6.61
Internal standard: methyltestosterone (12.65)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; serum

REFERENCE
Kaukonen,A.M.; Vuorela,P.; Vuorela,H.; Mannermaa,J.-P. High-performance liquid chromatography methods for the separation and quantitation of spironolactone and its degradation products in aqueous formulations and of its metabolites in rat serum, *J.Chromatogr.A*, **1998**, *797*, 271–281.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with two 2.5 mL portions of MeOH and two 2.5 mL portions of water. Condition a Bond-Elut CN SPE cartridge with two 2.5 mL portions of MeOH and two 2.5 mL portions of hexane. Plasma. 1 mL Plasma + 100 μL 1 μg/mL IS in water + 1 mL water, add to the C18 SPE cartridge, wash with two 1.5 mL portions of water, wash with 1 mL MeOH:water 40:60, elute with two 1 mL portions of MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 100 μL ethyl acetate, add 1.5 mL hexane, mix, add to the CN SPE cartridge, wash with 2 mL hexane, elute with two 750 μL portions of MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot. Urine. 1 mL Urine + 1 mL IS in water, add to the C18 SPE cartridge, wash with two 1.5 mL portions of water, wash with 1 mL MeCN:water 35:65, elute with two 1 mL portions of MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 100 μL ethyl acetate, add 1.5 mL hexane, mix, add to the CN SPE cartridge, wash with 2 mL hexane, elute with two 750 μL portions of MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 5 μm Spherisorb S5 ODS1
Mobile phase: MeOH:water 65:35, pH adjusted to 3.4 with phosphoric acid (plasma) or Gradient. MeOH:water acidified to pH 3.4 with phosphoric acid from 55:45 to 65:35 over 10 min (concave curve). (urine)

Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 3.0 (plasma), 8.3 (urine)
Internal standard: 16α,17α-epoxyprogesterone (7 (plasma), 14 (urine))
Limit of quantitation: 31.25 ng/mL (urine), 6.25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites, canrenone

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Varin,F.; Tu,T.M.; Benoît,F.; Villeneuve,J.P.; Théorêt,Y. High-performance liquid chromatographic determination of spironolactone and its metabolites in human biological fluids after solid-phase extraction, *J.Chromatogr.*, **1992**, *574*, 57–64.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 239.3

CHROMATOGRAM
Retention time: 20.68

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare spironolactone solutions in MeCN:water 50:50 containing 2 μg/mL testosterone, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 4 μm Sentry guard column (Waters)

Column: 150 × 3.9 4 μm mean pore diameter 60 Å Nova-Pak Phenyl
Mobile phase: THF:water 21.5:78.5
Flow rate: 1
Injection volume: 30
Detector: UV 250

CHROMATOGRAM
Retention time: 39.5
Internal standard: testosterone (22.5)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Kaukonen,A.M.; Vuorela,P.; Vuorela,H.; Mannermaa,J.-P. High-performance liquid chromatography methods for the separation and quantitation of spironolactone and its degradation products in aqueous formulations and of its metabolites in rat serum, *J.Chromatogr.A*, **1998**, *797*, 271–281.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 3.42

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.50 (A), 9.12 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphen-

oxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES
Column: 250 × 4.6 HP LiChrosorb RP-18

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230

CHROMATOGRAM
Retention time: 17.5

Internal standard: β-hydroxyethyltheophylline (4.4)

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, probenecid, canrenone, flumethiazide, bumetanide, ethacrynic acid

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 15:15:70:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM

Retention time: 9.4

Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES

Extracted: probenecid, bumetanide, ethacrynic acid

REFERENCE

Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE

Matrix: urine

Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 21.8

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, triamterene, trichlormethiazide

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN: water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 7.8

Internal standard: 7-propyltheophylline (4.5)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, clopamide, chlorthalidone, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, morazone, piretanide, polythiazide, probenecid, torsemide, triamterene

Interfering: mesocarb

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 Hypersil ODS-C18

Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 10.8

Limit of detection: 20 ng/mL.

OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

SAMPLE

Matrix: urine

Sample preparation: Inject 50 μL urine on to column A and elute to waste with mobile phase A, after 1.5 min elute the contents of column A on to column B with mobile phase B and start the gradient.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm HP-LiChrospher 100 RP 18
Mobile phase: A water; B Gradient. MeCN:water from 0:100 to 60:40 over 3 min, maintain at 60:40 for 3.5 min
Flow rate: 1
Injection volume: 50
Detector: UV 230

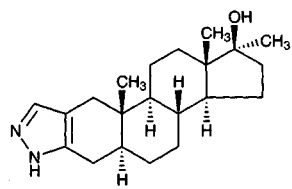
CHROMATOGRAM
Retention time: 7.2
Limit of detection: 20 ng/mL
Limit of quantitation: 70 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, canrenone (UV 300)
Simultaneous: bendroflumethiazide, chlorthalidone, hydrochlorothiazide

KEY WORDS
column-switching

REFERENCE
Herráez-Hernández,R.; Soriano-Vega,E.; Campíns-Falcó,P. High-performance liquid chromatographic determination of spironolactone and its major metabolite canrenone in urine using ultraviolet detection and column-switching, *J.Chromatogr.B*, **1994**, *658*, 303–310.

Stanozolol



Molecular formula: $C_{21}H_{32}N_2O$
Molecular weight: 328.50
CAS Registry No.: 10418-03-8
Merck Index: 8951
Lednicer No.: 1 174

SAMPLE
Matrix: formulations
Sample preparation: Weigh out amount of powdered tablets equivalent to 3.36 mg stanozolol, add 20 mL EtOH:100 mM HCl 70:30, stir magnetically filter, repeat extraction. Combine the filtrates and make up to 50 mL with EtOH:100 mM HCl 70:30. Remove a 5 mL aliquot and add it to 1 mL 115 μg/mL 4-acetylbiphenyl in MeOH, make up to 10 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Hypersil RP-18
Mobile phase: MeOH:50 mM $(NH_4)H_2PO_4$ 85:15
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 5.3
Internal standard: 4-acetylbiphenyl (4.2)

KEY WORDS
tablets

REFERENCE
Cavrini,V.; Di Pietra,A.M.; Augusta Raggi,M.; Grazia Maioli,M. Determination of stanozolol in tablets by derivative ultraviolet spectrophotometry and high-performance liquid chromatography, *Analyst*, **1987**, *112*, 1671–1674.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 16.4
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, benzyl benzoate, trenbolone acetate, nandrolone acetate
Interfering: testosterone acetate, oxymetholone

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

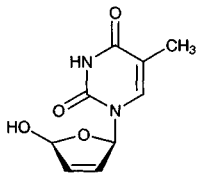
CHROMATOGRAM
Retention time: 15.5

OTHER SUBSTANCES
Simultaneous: boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, danazol (UV 280), fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE
Lurie,I.S.rling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

Stavudine



Molecular formula: $C_{10}H_{12}N_2O_4$
Molecular weight: 224.22
CAS Registry No.: 3056-17-5
Merck Index: 8958

SAMPLE
Matrix: amniotic fluid, blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 2 mL MeOH and two 2 mL portions of water. 100 μL Plasma or amniotic fluid + 20 μL 10 μg/mL 3-hydroxyacetamidophenol in water, mix, add to the SPE cartridge, wash with two 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute with 100 μL MeCN:50 mM pH 3.3 ammonium phosphate buffer 6:94, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:50 mM pH 3.3 ammonium phosphate buffer 6:94. B was MeCN:50 mM pH 3.3 ammonium phosphate buffer 25:75. A:B from 100:0 to 0:100 over 17 min, maintain at 0:100 for 5 min, return to initial conditions over 3 min, re-equilibrate for 17 min.
Flow rate: 1
Injection volume: 50
Detector: UV 266

CHROMATOGRAM
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: antipyrine

KEY WORDS
monkey; plasma; pharmacokinetics; SPE

REFERENCE
Odinecs,A.; Nosbisch,C.; Keller,R.D.; Baughman,W.L.; Unadkant,J.D. In vivo maternal-fetal pharmacokinetics of stavudine (2',3'-didehydro-3'-deoxythymidine) in pigtailed macaques (*Macaca nemestrina*), *Antimicrob.Agents Chemother.*, **1996**, *40*, 196–202.

SAMPLE
Matrix: blood

Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of water. 250 μL Plasma + 100 μL 125 μg/mL IS in water, vortex, add to the SPE cartridge, wash with 2 column volumes of water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute with 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 37-53 μm pellicular ODS (Whatman)
Column: 250 × 4.6 5 μm Apex octadecyl (Jones Chromatography)
Mobile phase: MeOH:50 mM KH_2PO_4 20:80
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.1
Internal standard: thymidine oxetane (7.7)
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS
rat; monkey; plasma; SPE; pharmacokinetics

REFERENCE
Kaul,S.; Dandekar,K.A.; Pittman,K.A. Analytical method for the quantification of 2',3'-didehydro-3'-deoxythymidine, a new anti-human immunodeficiency virus (HIV) agent, by high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection in rat and monkey plasma, *Pharm.Res.*, **1989**, *6*, 895–899.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bakerbond C18 SPE cartridge with 2 mL MeOH and two 2 mL portions of water. Evaporate 50 μL 5 ng/mL didanosine in MeOH into the bottom of a tube at 60° under a stream of nitrogen, add 500 μL plasma, vortex for 10 s, add to the SPE cartridge, wash with 1 mL water, allow to dry under vacuum for 10 min, elute with two 500 μL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex for 30 s, centrifuge at 800 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 LiChroCART 4-4 RP-8 (Merck)
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeOH:5 mM pH 6.8 phosphate buffer 10:90
Flow rate: 1
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 9
Internal standard: didanosine (10.5)
Limit of detection: 10 ng/mL

KEY WORDS
plasma; rat; human; pharmacokinetics; SPE

REFERENCE
Burger,D.M.; Rosing,H.; van Gijn,R.; Meenhorst,P.L.; van Tellingen,O.; Beijnen,J.H. Determination of stavudine, a new antiretroviral agent, in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1992**, *584*, 239–247.

SAMPLE
Matrix: blood

Sample preparation: 200 μL Plasma + 1 mL ice-cold MeCN, mix vigorously for 30 s, centrifuge at 9000 g for 7 min. Remove the supernatant and add it to excess crystalline magnesium sulfate, mix for 2 min, centrifuge at 9000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 15-150 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 2.3 PRP-1 (Hamilton)
Column: 250 × 4.1 10 μm PRP-1 (Hamilton)
Mobile phase: MeCN:5 mM pH 11.1 tetrabutylammonium hydroxide 16:84
Flow rate: 1.5
Injection volume: 15-150
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: stavudine

OTHER SUBSTANCES
Extracted: 4-deoxy-5-fluorouracil (UV 313), 5-fluorouracil, tegafur

KEY WORDS
plasma; rat; stavudine is IS

REFERENCE
Jarugula,V.R.; Boudinot,F.D. High-performance liquid chromatographic determination of 5-fluorouracil and its prodrugs, tegafur and 4-deoxy-5-fluorouracil, in rat plasma, *J.Chromatogr.B*, **1996**, *677*, 199–203.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Plasma. Mix 100 μL plasma with 50 μL 10% acetic acid and 500 μL 1 μg/mL IS in ethyl acetate, centrifuge at 9000 g for 3 min, remove a 400 μL aliquot of the organic layer, repeat the extraction with 400 μL fresh ethyl acetate. Combine the ethyl acetate extracts and evaporate them to dryness under a stream of nitrogen at 50° in less than 5 min, reconstitute with 100 μL mobile phase, inject an aliquot (J.Pharm.Sci. 1993, 82, 1232). Tissue, CSF. Homogenize nasal tissue with pH 7.4 phosphate buffer to form a 4% (w/v) mixture. Mix 300 μL CSF or tissue homogenate with 4 μL isotonic phosphate buffer at 37°. Remove 50 μL aliquot of the reaction mixture, add 50 μL MeOH, mix, centrifuge, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 Lichrospher 100 RP-18e
Mobile phase: MeOH:water:acetic acid 16:84:0.2
Column temperature: 40
Flow rate: 1.0
Detector: UV 265

CHROMATOGRAM
Retention time: 4.2
Internal standard: 1-(2-deoxy-3,5-epoxy-β-D-threo-pentofuranosyl)thymine (5.4)

KEY WORDS
plasma; rat; pharmacokinetics; nose

REFERENCE
Yajima,T.; Juni,K.; Saneyoshi,M.; Hasegawa,T.; Kawaguchi,T. Direct transport of 2',3'-didehydro-3'-deoxythymidine (D4T) and its ester derivatives to the cerebrospinal fluid via the nasal mucous membrane in rats, *Biol.Pharm.Bull.*, **1998**, *21*, 272–277.

SAMPLE
Matrix: blood, urine

Sample preparation: Dilute 100 μL urine to 1 mL with water. Evaporate 20 μL 5 μg/mL IS in MeOH in the bottom of a tube at 45° under reduced pressure, add 1 mL plasma or diluted urine, add 8 mL dichloromethane:isopropanol 95:5, shake horizontally at 180 cycles/min for 10 min, centrifuge at 750 g. Remove the organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 100 μL mobile phase, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18 (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:10 mM pH 4.60 $(NH_4)H_2PO_4$ 6.5:93.5 (7:93 for rabbit plasma)
Flow rate: 0.75
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Retention time: 9.7
Internal standard: thymidine oxetane (BMY-33644) (14.3)
Limit of detection: 12.2 (urine), 2.58 (plasma) ng/mL
Limit of quantitation: 35.2 (urine), 6.7 (plasma) ng/mL

KEY WORDS

human; rabbit; plasma; pharmacokinetics

REFERENCE

Wong,S.L.; Sawchuk,R.J. High-performance liquid chromatographic determination of 2',3'-didehydro-3'-deoxythymidine (d4T) in human and rabbit plasma and urine and its application to pharmacokinetic studies in the rabbit, *Pharm.Res.*, **1991**, *8*, 619–623.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Condition a 1 mL Bond Elut C18 SPE cartridge with MeOH and water. 500 μL Plasma + 50 μL IS solution, add to the SPE cartridge, wash with 2 column volumes of water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 125 μL mobile phase, inject a 100 μL aliquot. Urine. Condition a 3 mL Bond Elut phenyl SPE cartridge with 3 mL MeOH and two column volumes of 20 mM pH 8.0 potassium phosphate buffer. 500 μL Urine + 50 μL IS solution, add to the SPE cartridge, wash with 1 column volume of 20 mM pH 4.1 potassium phosphate buffer, wash with 1 column volume of 20 mM pH 8.0 potassium phosphate buffer, wash with 1 column volume of water, elute with two 500 μL portions of MeOH:water 70:30 containing 1.4 mM triethylamine, dilute the eluate with 500 μL 20 mM pH 7.2 potassium phosphate buffer, vortex briefly, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Apex octadecyl (Jones)
Mobile phase: MeCN:10 mM ammonium phosphate 10:90 containing 7.2 mM triethylamine, pH adjusted to 2.5 with 85% phosphoric acid
Flow rate: 0.8
Injection volume: 75-100
Detector: UV 266

CHROMATOGRAM

Retention time: 7 (plasma), 7.5 (urine)
Internal standard: thymidine oxetane (9 (plasma), 10.5 (urine))
Limit of detection: 150 ng/mL (urine), 6 ng/mL (plasma)
Limit of quantitation: 500 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES

Simultaneous: dapsone, fluconazole, gangiclovir, ketoconazole, sulfamethoxazole
Interfering: ethambutol

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE
Janiszewski,J.S.; Mulvana,D.E.; Kaul,S.; Dandekar,K.A.; Barbhaiya,R.H. High-performance liquid chromatographic determination of 2',3'-didehydro-3'-deoxythymidine, a new anti-human immunodeficiency virus agent, in human plasma and urine, *J.Chromatogr.*, **1992**, *577*, 151–156.

SAMPLE
Matrix: solutions

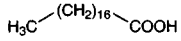
HPLC VARIABLES
Column: 220 × 4.6 5μm Brownlee C18
Mobile phase: MeOH:40 mM KH_2PO_4 containing 0.2% triethylamine 15:85, pH adjusted to 4.0 with 85% phosphoric acid (A) or MeCN:MeOH:40 mM KH_2PO_4 containing 0.2% triethylamine 3:15:85, pH adjusted to 4.0 with 85% phosphoric acid (B)
Column temperature: -10
Flow rate: 0.7
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 26.3 (A), 16,5 (B)

OTHER SUBSTANCES
Simultaneous: didanosine

REFERENCE
Stancato,F.A.; Srinivas,N.R.; Knupp,C.A. Effect of temperature on the high-performance liquid chromatographic separation of the anti-HIV agents, didanosine and stavudine, *Biomed.Chromatogr.*, **1996**, *10*, 29–31.

Stearic acid



Molecular formula: $C_{18}H_{36}O_2$
Molecular weight: 284.48
CAS Registry No.: 57-11-4
Merck Index: 8959

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 2 mmoles stearic acid in 10 mL diethyl ether, add 10 mmoles 1-benzyl-3-p-tolyltriazene in 5 mL diethyl ether, stir at 36° for 3 h, cool, wash with two 5 mL portions of 10% HCl, wash with two 5 mL portions of 10% sodium carbonate, dry over anhydrous magnesium sulfate, evaporate to dryness, prepare a solution in mobile phase, inject an aliquot. (Synthesis of 1-benzyl-3-p-tolyltriazene is as follows. Stir 50.2 g p-toluidine in an ice/salt bath, add a mixture of 250 g crushed ice and 140 mL concentrated HCl, slowly add a solution of 46.8 g potassium nitrite in 150 mL water over 1-2 h until a positive starch/KI is obtained (stop addition 1-2 min before each test), stir for 1 h, allow to warm to 0°, adjust pH to 6.8-7.2 with cold concentrated sodium carbonate solution to give a solution of p-toluenediazonium chloride (Org.Syn., Coll.Vol. V, 797). Stir 107 g benzylamine in water in an ice/salt bath and slowly add the diazonium solution, extract with ether, recrystallize 1-benzyl-3-p-tolyltriazene from diethyl ether:n-hexane 50:50 to give yellow crystals (mp 77°) (Berichte 1888, 21, 1016). (Caution! 1-Benzyl-3-p-tolyltriazene explodes when heated to 90-100°!))

HPLC VARIABLES
Column: 1830 mm long Corasil II
Mobile phase: Heptane:chloroform 50:50
Detector: UV 254

OTHER SUBSTANCES
Also analyzed: heptadecanoic acid, palmitic acid

KEY WORDS
derivatization; normal phase

REFERENCE
Politzer,I.R.; Griffin,G.W.; Dowty,B.J.; Laseter,J.L. Enhancement of ultraviolet detectability of fatty acids for purposes of liquid chromatographic-mass spectrometric analyses, *Anal.Lett.*, **1973**, *6*, 539–546.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 3 μmole compound in 125 μL dichloromethane, add 125 μL 72 mM O-(p-nitrobenzyl)-N,N'-diisopropylisourea in dichloromethane, heat at 80° for 2 h, cool, inject an aliquot. (Synthesis of O-(p-nitrobenzyl)-N,N'-diisopropylisourea is as follows. Mix 15.3 g 4-nitrobenzyl alcohol, 12.6 g diisopropylcarbodiimide, 10 mg copper(II) chloride, and 10 mL DMF, let stand at room temperature for 96 h, remove the solvent by distillation (45°/10 mm Hg), dissolve the residue in petroleum ether (bp 40-80°), chromatograph on a 200 × 30 column of aluminum oxide, elute with petroleum ether until the eluate does not turn moistened litmus paper blue. Remove the petroleum ether under reduced pressure to give O-(p-nitrobenzyl)-N,N'-diisopropylisourea as yellow crystals (mp 42° (softening after 38°)) (Liebigs Ann. Chem. 1965, 685, 161).

HPLC VARIABLES
Column: 250 mm long 5 μm MicroPak silica
Mobile phase: Hexane:chloroform 80:20
Detector: UV 254

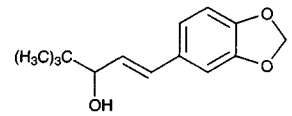
CHROMATOGRAM
Limit of detection: 4 pmole

OTHER SUBSTANCES
Also analyzed: lauric acid, myristic acid, palmitic acid

KEY WORDS
derivatization; normal phase

REFERENCE
Knapp,D.R.; Krueger,S. Use of O-p-nitrobenzyl-N,N'-diisopropylisourea as a chromogenic reagent for liquid chromatographic analysis of carboxylic acids, *Anal.Lett.*, **1975**, *8*, 603–610.

Stiripentol



Molecular formula: $C_{14}H_{18}O_3$
Molecular weight: 234.29
CAS Registry No.: 49763-96-4

SAMPLE
Matrix: blood
Sample preparation: 300 μL Blood + 15 μg piperonyl alcohol + 3 mL ethyl acetate, extract, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue with three 50 μL portions of anhydrous toluene, add 2 μL triethylamine, add 0.5 μL di-n-butyltin dilaurate, add 5 μL phenyl isocyanate, let stand overnight at room temperature, add EtOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Bakerbond DNBPG chiral

Mobile phase: Hexane:isopropanol 95:5
Flow rate: 2
Detector: F ex 290 em 355

CHROMATOGRAM
Retention time: 6.4 (S), 7.3 (R)
Internal standard: piperonyl alcohol (9.0)
Limit of detection: 8 ng/mL

KEY WORDS
derivatization; rat; whole blood; pharmacokinetics; chiral

REFERENCE
Zhang,K.; Tang,C.; Rashed,M.; Cui,D.; Tombret,F.; Botte,H.; Lepage,F.; Levy,R.H.; Baillie,T.A. Metabolic chiral inversion of stiripentol in the rat. I. Mechanistic studies, *Drug Metab.Dispos.*, **1994**, *22*, 544–553.

Streptokinase

CAS Registry No.: 9002-01-1
Merck Index: 8981

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 7.5 Bio-Gel TSK-phenyl-5-PW
Mobile phase: Gradient from 100 mM pH 7.0 to water over 11 min (?)
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 7

REFERENCE
Jackson,K.W.; Malke,H.; Gerlach,D.; Ferretti,J.J.; Tang,J. Active streptokinase from the cloned gene in Streptococcus sanguis is without the carboxyl-terminal 32 residues, *Biochem.*, **1986**, *25*, 108–114.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Bakerbond C4
Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 0:100 to 60:40 over 90 min
Flow rate: 1
Injection volume: 200
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: alteplase, urokinase, anistreplase

REFERENCE
Werner,R.G.; Bassarab,S.; Hoffmann,H.; Schlüter,M. Quality aspects of fibrinolytic agents based on biochemical characterization, *Arzneimittelforschung*, **1991**, *41*, 1196–1200.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 600 × 7.5 Spherogel 3000 SW
Mobile phase: 230 mM pH 6.8 phosphate buffer containing 0.1% sodium dodecyl sulfate
Flow rate: 0.5
Detector: UV 280

CHROMATOGRAM
Retention time: 27

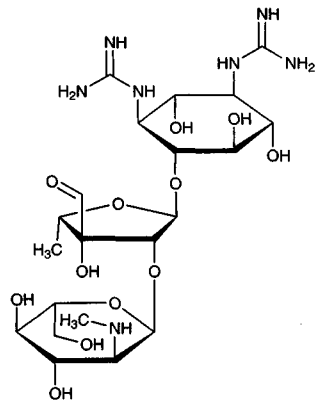
OTHER SUBSTANCES
Simultaneous: aggregates
Also analyzed: anistreplase, urokinase, alteplase

KEY WORDS
SEC; GPC

REFERENCE
Werner,R.G.; Bassarab,S.; Hoffmann,H.; Schlüter,M. Quality aspects of fibrinolytic agents based on biochemical characterization, *Arzneimittelforschung*, **1991**, *41*, 1196–1200.

Streptomycin

Molecular formula: $C_{21}H_{39}N_7O_{12}$
Molecular weight: 581.58
CAS Registry No.: 57-92-1, 3810-74-0 (sulfate)
Merck Index: 8983



SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 20 mL MeOH and 20 mL water. 400 µL Serum + 2 mL buffer + 100 µL 100 µg/mL dihydrostreptomycin sulfate in water, add to the SPE cartridge, wash with 2 mL water, centrifuge at 2300 g for 5 min, elute with 5 mL MeOH. Concentrate the eluate to 200 µL under vacuum at 30°, add 200 µL mobile phase, inject a 100 µL aliquot. (Buffer was 50 mM sodium 1-hexanesulfonate and 25 mM Na_3PO_4, pH adjusted to 2.0 with phosphoric acid.)

HPLC VARIABLES
Guard column: 30 × 4.6 10 µm Spheri-10 RP-8 (Brownlee)
Column: 250 × 4 5 µm LiChrosorb RP-18
Mobile phase: MeCN:buffer 8:92 (Buffer was 3.76 g sodium 1-hexanesulfonate and 9.50 g $Na_3PO_4.12H_2O$ in 900 mL water, adjust pH to 3.0 with phosphoric acid, make up to 1 L with water.)
Column temperature: 55
Flow rate: 1
Injection volume: 100
Detector: UV 195

CHROMATOGRAM
Retention time: 18
Internal standard: dihydrostreptomycin (20)

Limit of detection: 2000 ng/mL

KEY WORDS
serum; SPE

REFERENCE
Kurosawa,N.; Kuribayashi,S.; Owada,E.; Ito,K.; Nioka,M.; Arakawa,M.; Fukuda,R. Determination of streptomycin in serum by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 379–385.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 50 μL 3.5% perchloric acid, vortex for a few s, centrifuge at 10000 g for 1 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 10 μm Radial-PAK C18 radial compression (Waters)
Mobile phase: MeCN:water 20:80 containing 20 mM disodium 1,2-ethanedisulfonate, 5 mM sodium octanesulfonate, and 5 mM ninhydrin, adjusted to pH 3.3 with acetic acid
Flow rate: 1.5
Injection volume: 50
Detector: F ex 302 em 420 (cut-off filter) following post-column reaction. The column effluent mixed with 300 mM NaOH pumped at 0.5 mL/min and flowed through a 10 m × 0.5 mm i.d. stainless steel coil at 80° to the detector.

CHROMATOGRAM
Retention time: 12
Limit of detection: 1 μg/mL

KEY WORDS
serum; post-column reaction

REFERENCE
Kubo,H.; Li,H.; Kobayashi,Y.; Kinoshita,T. Fluorometric determination of streptomycin in serum by high-performance liquid chromatography using mobile phase containing fluorogenic reagent, *Anal.Biochem.*, **1987**, *162*, 219–223.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 4 mg/mL a solution in water, dilute a 3 mL aliquot to 50 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 8:92, pH 6.0 (Prepare by dissolving 3.8 g sodium 1-hexanesulfonate and 9.5 g Na_3PO_4 in 850 mL water and 80 mL MeCN, adjust pH to 6.0 with phosphoric acid, make up to 1 L with water. (Connect a 250 × 4.6 column of Bondapak C18/Corasil or Co:Pell ODS between pump and injector. Flush column with MeOH:water 50:50 at the end of the day.)
Column temperature: 25
Flow rate: 1.3
Injection volume: 25
Detector: UV 195

CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Simultaneous: dihydrostreptomycin, impurities

REFERENCE
Whall,T.J. Determination of streptomycin sulfate and dihydrostreptomycin sulfate by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *219*, 89–100.

SAMPLE

Matrix: milk, tissue

Sample preparation: Tissue. Condition a 5 mL 500 mg 40 μm Bakerbond aromatic sulfonic acid SPE cartridge with 5 mL water. Homogenize (Polytron Model PT 10/35) 5 g tissue with 10 mL 3.6% perchloric acid for 10-15 s, shake horizontally for 5 min, centrifuge at 2000 g for 5 min, add the supernatant to the SPE cartridge at 2 mL/min, wash with 3 mL water, allow to dry, elute with 9 mL buffer. Add 500 μL 200 mM 1-hexanesulfonic acid in water and 150 μL perchloric acid to the eluate, make up to 10 mL with water, filter (0.45 μm), inject a 2 mL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Milk. 10 g Milk + 3 mL 3.6% perchloric acid, shake horizontally for 30 s, centrifuge at 2000 g for 5 min. Add the supernatant to 70 μL 5 M NaOH and 500 μL 200 mM 1-hexanesulfonic acid, make up to 10 mL with water, filter (0.45 μm), inject a 2 mL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B (J. AOAC Int. 1994, 77, 765). (Prepare the buffer by dissolving 33.46 g K_2HPO_4 and 1.05 g KH_2PO_4 in 1 L water, pH 8.0. At the end of each day wash with MeCN:water 50:50 at 1.5 mL/min for 10 min.)

HPLC VARIABLES

Column: A 40 × 4.6 5 μm Inertsil C8; B 250 × 4.6 5 μm LC8-DB (Supelco)

Mobile phase: A 10 mM 1-Hexanesulfonic acid adjusted to pH 3.3 with acetic acid; B MeCN: water 17:83 containing 10 mM 1-hexanesulfonic acid and 400 μM 1,2-naphthoquinone-4-sulfonic acid, adjust pH to 3.3 with acetic acid

Flow rate: A 1; B 1.5

Injection volume: 2000

Detector: F ex 347 em 418 (tissue) or ex 365 em 418 (milk) following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.5 mL/min and the mixture flowed through a 2 mL reaction coil (Pickering, Mountain View CA) at 50° to the detector. (At the end of each day wash the post-column reaction system with 1% acetic acid for 10 min and with water for 10 min.)

CHROMATOGRAM

Retention time: 22

Limit of detection: 10 ppb

OTHER SUBSTANCES

Extracted: dihydrostreptomycin

KEY WORDS

post-column reaction; column-switching; SPE; pig; cow; muscle; kidney

REFERENCE

Gerhardt,G.C.; Salisbury,C.D.C.; MacNeil,J.D. Determination of streptomycin and dihydrostreptomycin in animal tissue by on-line sample enrichment liquid chromatography, *J.AOAC Int.*, **1994**, *77*, 334–337.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 10 μm RP-18

Column: 150 × 4.6 5 μm Supelcosil LC-8-DB

Mobile phase: MeOH:buffer 1.5:98.5 (Buffer was 10 mM sodium 1-pentanesulfonate, 56 mM sodium sulfate, and 7 mM acetic acid.)

Flow rate: 1.5

Detector: F ex 340 em 455 following post-column reaction with derivatization reagent pumped at 0.9 mL/min. (Derivatization reagent was commercially available (Pierce) or prepared by adding 2.5 mL 2-mercaptoethanol and 2.5 mL Brij-35 to 850 mg o-phthalaldehyde in 10 mL MeOH, mix until decolorization is complete, add 1 L buffer, filter (0.45 μm), and refrigerate until used. Buffer was prepared by adjusting pH of 250 mM boric acid to 9.5 with 5 M KOH.)

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: dihydrostreptomycin, neomycin, paromomycin

KEY WORDS
post-column reaction

REFERENCE
Shaikh,B.; Allen,E.H.; Gridley,J.C. Determination of neomycin in animal tissues, using ion-pair liquid chromatography with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 29–36.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond Elut Certify II SPE cartridge with 3 mL MeCN, 1 mL water, and three 1 mL aliquots of buffer B, do not allow to dry. Homogenize (Ultra Turrax TP 18/10) 8 g Kidney or meat, 5 mL buffer A, and 1 mL 85% trichloroacetic acid in water for 6 s, centrifuge at 5000 rpm for 3 min, add 2 mL dichloromethane, mix for 6 s, centrifuge at 5000 rpm for 5 min. Remove a 7 mL aliquot of the supernatant and add it to 900 μL 4 M NaOH, blend, centrifuge at 4000 rpm for 5 min. Remove the upper layer and add it to 900 μL 500 mM phosphoric acid, adjust the pH to 5.5-5.8 with 1 M NaOH or 500 mM phosphoric acid, add 2.5 mL buffer B, add to the SPE cartridge at 1 mL/min, rinse out the tube with 1 mL buffer A, add the rinse to the SPE cartridge, wash with two 5 mL portions of buffer A, suck dry for 2 s after each wash, wash with three 5 mL portions of 25% ammonia, suck dry for 2 s after each wash, wash with two 1 mL portions of water, suck dry for 2 s after each wash, wash with 1 mL water, suck dry for 10 s, elute with two 1 mL portions of 20% formic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeOH, mix for 3-4 s, evaporate to dryness, reconstitute with 400 μL buffer A, add 200 μL chloroform, mix vigorously for 10 s, centrifuge for 3 min. Filter (Costar Spin X with 0.22 μm nylon membrane) the aqueous layer while centrifuging at 5600 g for 2 min, inject a 25 μL aliquot of the filtrate. (Prepare buffer A by dissolving 4.45 g sodium 1-heptanesulfonate and 1.8 g Na_2HPO_4 in 750 mL water, adjust pH to 5.9 with 5 M phosphoric acid, adjust pH to 5.5 with 500 mM phosphoric acid, make up to 1 L with water, adjust pH to 5.5 with 500 mM phosphoric acid. Prepare buffer B by dissolving 13.35 g sodium 1-heptanesulfonate and 1.8 g Na_2HPO_4 in 750 mL water, adjust pH to 5.9 with 5 M phosphoric acid, adjust pH to 5.5 with 500 mM phosphoric acid, make up to 1 L with water, adjust pH to 5.5 with 500 mM phosphoric acid.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ + Plus
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ + Plus
Mobile phase: MeCN:buffer 30:70 (Prepare buffer by dissolving 8.65 g sodium octanesulfonate and 110 mg potassium 1,2-naphthoquinone-4-sulfonate in 750 mL water, make up to 1 L with water, filter (0.45 μm), adjust pH to 3.24 with 1 mL acetic acid.)
Column temperature: 31
Flow rate: 0.6 for 0.5 min, 0.9 for 4 min, 0.6 for 9 min
Injection volume: 25
Detector: F ex 375 em 412 following post-column reaction. The column effluent mixed with 300 mM NaOH pumped at 0.3 mL/min in a 1.2 μL vortex mixer (Kratos PCRS 520) and the mixture flowed through a 15 m × 0.5 mm ID knitted coil at 40° and a room temperature heat exchanger to the detector.

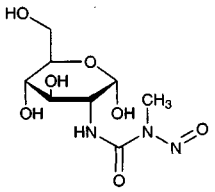
CHROMATOGRAM
Retention time: 17.3

OTHER SUBSTANCES
Extracted: dihydrostreptomycin

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Hormazal,V.; Yndestad,M. High performance liquid chromatographic determination of dihydrostreptomycin sulfate in kidney and meat using post column derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2259–2268.

Streptozocin



Molecular formula: $C_8H_{15}N_3O_7$
Molecular weight: 265.22
CAS Registry No.: 18883-66-4
Merck Index: 8991

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Precipitate proteins with EtOH, centrifuge at 2000 rpm for 10 min. Remove the supernatant and adjust the pH to 4.0 with 6 M HCl, inject a 10 μL aliquot. Urine. Reconstitute lyophilized urine in MeOH:acetone 75:25, centrifuge, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 2 Durapak 3,3'-oxypropionylnitrile
Mobile phase: Hexane:isopropanol 75:25
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 17
Limit of detection: 6 μg/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Adolphe,A.B.; Glasofer,E.D.; Troetel,W.M.; Weiss,A.J.; Manthei,R.W. Preliminary pharmacokinetics of streptozotocin, an antineoplastic antibiotic, *J.Clin.Pharmacol.*, **1977**, *17*, 379–388.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 1 mg/mL solution in 100 mM pH 4.0 acetate buffer. Remove a 5 mL aliquot and add it to 5 mL 2 mg/mL potassium acid phthalate, mix, inject a 4 μL aliquot. Powders. Reconstitute the contents of a vial with 10-15 mL water, make up solution to 1 mL with water. Remove a 5 mL aliquot and add it to 5 mL 2 mg/mL potassium acid phthalate, mix, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 3:97 containing 100 mM acetic acid, pH adjusted to 4.0 with 50% NaOH
Flow rate: 0.5
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 5 (β-anomer), 7 (α-anomer)
Internal standard: potassium hydrogen phthalate (21)

OTHER SUBSTANCES
Noninterfering: degradation products

KEY WORDS
powders; mutarotation occurs in solution

REFERENCE
Oles,P.J. High-pressure liquid chromatographic separation and determination of anomeric forms of streptozocin in a powder formulation, *J.Pharm.Sci.*, **1978**, *67*, 1300–1302.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:20 mM sodium acetate 26:74, pH adjusted to 4.0 with acetic acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 2.01

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

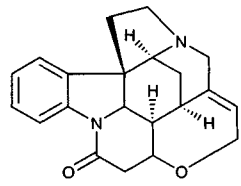
SAMPLE
Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: 200 mM pH 4.4 KH_2PO_4
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 9.8
Limit of detection: 500 ng/mL

REFERENCE
Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

Strychnine



Molecular formula: $C_{21}H_{22}N_2O_2$
Molecular weight: 334.42
CAS Registry No.: 57-24-9
Merck Index: 9020

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 9.202

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

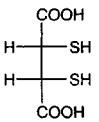
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone,

butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Succimer



Molecular formula: $C_4H_6O_4S_2$
Molecular weight: 182.22
CAS Registry No.: 304-55-2
Merck Index: 9034

SAMPLE

Matrix: solutions

Sample preparation: 100-200 μL Solution + 1.75-1.85 mL buffer + 50 μL reagent (final volume 2 mL), purge head space with nitrogen, shake at room temperature in the dark for 5 min, add 2 mL dichloromethane, shake for 10 s, centrifuge for 2 min. Remove the aqueous phase and adjust the pH to 7 with 15 μL 6 M HCl, inject a 20 μL aliquot. (Buffer was 100 mM pH 8.0 ammonium bicarbonate purged with nitrogen for at least 1 h before use. Reagent was 40 mM bromobimane in MeCN.)

HPLC VARIABLES
Guard column: 45 × 4.6 10 μm Ultrasphere IP C18
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: Gradient. A was 20 mM tetrabutylammonium bromide in MeOH. B was 20 mM tetrabutylammonium bromide in water. A:B 55:45 for 11 min, to 75:25 in 1 min, maintain at 75:25 for 7 min, return to initial conditions over 1 min, re-equilibrate for 15 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 356 em 450

CHROMATOGRAM
Retention time: k' 2.55

OTHER SUBSTANCES
Simultaneous: 4-sec-butyl-5-ethyl-2-thiobarbituric acid, 2,3-dimercaptopropane-1-sulfonic acid, N-(2,3-dimercaptopropyl)phthalamidic acid, 2-mercaptoethanesulfonic acid, mercaptosuccinic acid, sodium sulfite

KEY WORDS
protect from light; derivatization

REFERENCE
Maiorino,R.M.; Weber,G.L.; Aposhian,H.V. Fluorometric determination of 2,3-dimercaptopropane-1-sulfonic acid and other dithiols by precolumn derivatization with bromobimane and column liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 297–310.

SAMPLE
Matrix: urine
Sample preparation: 50 μL Urine + 900 μL buffer + 50 μL reagent, stir under nitrogen for 45 min, add 50 μL 80 mM monobromobimane in MeCN, let stand under nitrogen in the dark for 10 min, add 2 mL dichloromethane, extract for 15 s, centrifuge for 2 min, repeat extraction. Remove the aqueous phase and add 5 μL 6 M HCl, inject a 20 μL aliquot. (Buffer was 100 mM ammonium bicarbonate purged with nitrogen for 1 h. Reagent was 50 mM dithiothreitol in nitrogen-purged water.) [Procedures for electrolytic reduction and reduction with sodium borohydride are also described.]

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS RP-18
Mobile phase: Gradient. A was 20 mM tetrabutylammonium bromide in MeOH. B was 20 mM tetrabutylammonium bromide in 10 mM pH 4.1 acetate buffer. A:B 47.5:52.5 for 12 min, to 90:10 over 5 min, maintain at 90:10 for 7 min, re-equilibrate at initial conditions for 11 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 356 em 450

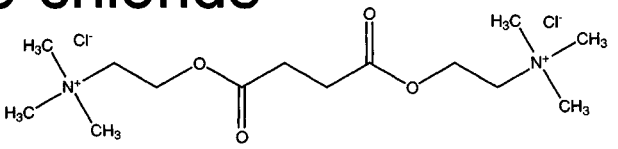
CHROMATOGRAM
Retention time: 10

KEY WORDS
pharmacokinetics; derivatization

REFERENCE
Maiorino,R.M.; Barry,T.J.; Aposhian,H.V. Determination and metabolism of dithiol-chelating agents: electrolytic and chemical reduction of oxidized dithiols in urine, *Anal.Biochem.*, **1987**, *160*, 217–226.

Succinylcholine chloride

Molecular formula: $C_{14}H_{30}Cl_2N_2O_4$
Molecular weight: 361.31
CAS Registry No.: 71-27-2, 6101-15-1 (dihydrate), 55-94-7 (bromnide), 541-19-5 (iodide)
Merck Index: 9044



SAMPLE
Matrix: formulations
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 0.26 silica A (Perkin-Elmer)
Mobile phase: MeOH:water:500 mM tetramethylammonium sulfate 65:25:10
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 5.1
Limit of detection: 100 μg/mL

OTHER SUBSTANCES
Simultaneous: degradation products, choline, methyl p-hydroxybenzoate, succinic acid, succinylmonocholine

KEY WORDS
injections; saline

REFERENCE
Schmutz,C.W.; Mühlebach,S.F. Stability of succinylcholine chloride injection, *Am.J.Hosp.Pharm.*, **1991**, *48*, 501–506.

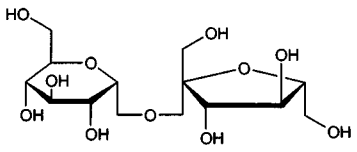
SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in saline, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 40-60 (Buffer was 20 mL Low-UV PIC B-7 (Waters) diluted with 480 mL water (10 mM 1-heptanesulfonic acid).)
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 7.8

REFERENCE
Woodman,T.F.; Johnson,B.; Marwaha,R.K. Determination of methacholine chloride by ion-pair high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1982**, *5*, 1341–1348.

Sucrose



Molecular formula: $C_{12}H_{22}O_{11}$
Molecular weight: 342.30
CAS Registry No.: 57-50-1
Merck Index: 9051

SAMPLE
Matrix: beverages, plants
Sample preparation: Beverages. Dilute 50-fold, filter (0.22 μm), inject an aliquot of the filtrate. Plants. Heat 1 g barley leaves and 10 mL EtOH:water 80:20 at 100° in a sealed tube for 15-30 min. Evaporate the liquid phase to dryness, reconstitute with water, pass through Analytichem trimethylaminopropyl and cyclohexyl SPE cartridges, inject an aliquot.

HPLC VARIABLES
Column: 300 × 6.5 Sugar-Pak I (Waters)
Mobile phase: water
Column temperature: 70
Flow rate: 0.4
Injection volume: 10
Detector: F ex 360 em 470 following post-column reaction. The effluent from the column passed through a 75 × 3.8 reactor containing Dowex 50 W × 2 sulfonic-acid type styrene divinylbenzene copolymer at 100° and mixed with 30 mM benzamidine in 1 M KOH pumped at 1 mL/min. This mixture flowed through a 530 μL reaction coil (Varian PCR-1) at 100° to the detector.

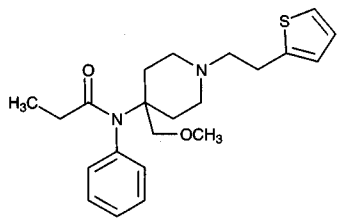
CHROMATOGRAM
Retention time: 15.30
Limit of detection: 22 pmole

OTHER SUBSTANCES
Extracted: dextrose, fructose

KEY WORDS
barley; SPE

REFERENCE
Coquet,A.; Haerdi,W.; Degli Agosti,R.; Veuthey,J.-L. Determination of sugars by liquid chromatography with post-column catalytic derivatization and fluorescence detection, *Chromatographia*, **1994**, *38*, 12–16.

Sufentanil



Molecular formula: $C_{22}H_{30}N_2O_2S$
Molecular weight: 386.56
CAS Registry No.: 56030-54-7, 60561-17-3 (citrate)
Merck Index: 9056
Lednicer No.: 3 118

SAMPLE
Matrix: blood, urine
Sample preparation: 50 μL Plasma or urine + 50 μL 4 M NaOH + 100 μL MeCN + 500 μL n-hexane, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 4 μm Nova pak cyano
Mobile phase: MeCN:5 mM pH 3.2 phosphate buffer 70:30
Flow rate: 2.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 7.08
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: fentanyl, alfentanil

KEY WORDS
plasma

REFERENCE
Bansal,R.; Aranda,J.V. Simultaneous microassay of alfentanil, fentanil, and sufentanil by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 339–348.

SAMPLE
Matrix: formulations
Sample preparation: Direct injection.

HPLC VARIABLES
Column: 100 × 3 Chromspher C18 (Chrompack)
Mobile phase: MeCN:MeOH:0.5% ammonium acetate in water 36.4:36.4:27.2
Flow rate: 0.6
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 3

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Roos,P.J.; Glerum,J.H.; Meilink,J.W. Stability of sufentanil citrate in a portable pump reservoir, a glass container and a polyethylene container, *Pharm.Weekbl.[Sci].*, **1992**, *14*, 196–200.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Injection solution + 400 μL 2.5 μg/mL haloperidol in water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:10 mM NaH_2PO_4 24:31:45, pH adjusted to 5.0 with 2 M KOH
Flow rate: 1.7
Injection volume: 100
Detector: UV 210

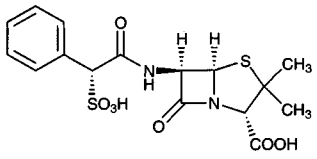
CHROMATOGRAM
Retention time: 15.46
Internal standard: haloperidol (9.74)

OTHER SUBSTANCES
Extracted: fentanyl

KEY WORDS
injections

REFERENCE
Dewell,W.M.,Jr.; Khandaghabadi,M.; D'Souza,M.J.; Solomon,H.M. High-performance liquid chromatographic determination of fentanyl and sufentanil returned from the operating room, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2374–2375.

Sulbenicillin



Molecular formula: $C_{16}H_{18}N_2O_7S_2$
Molecular weight: 414.46
CAS Registry No.: 41744-40-5
Merck Index: 9059

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elute-Certify II SPE cartridge with 5 mL MeOH:10% LiCl 40:60, 2 mL MeOH, and 2 mL water. Mix 500 μL plasma with 2 mL 50 mM ammonium acetate, add 100 μL 100 μg/mL carbenicillin in water. Add the mixture to the SPE cartridge, draw it through the cartridge under vacuum, wash with 3 mL MeCN:500 mM acetic acid 50:50 and 2 mL MeOH:100 mM ammonium acetate 50:50, elute with 1mL MeOH:10% LiCl 40:60. Inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil C18-AR (Nacalai Tesque Co., Kyoto)
Mobile phase: MeOH:50 mM ammonium acetate 10:70
Flow rate: 0.9
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Internal standard: carbenicillin
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: probenecid

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Itoh,T.; Watanabe,N.; Ishida,M.; Tsuda,Y.; Koyano,S.; Tsunoi,T.; Shimada,H.; Yamada,H. Stereoselective disposition of sulbenicillin in humans, *Antimicrob.Agents Chemother.*, **1998**, *42*, 325–331.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond Elut-SAX SPE cartridge with 2 mL MeOH and 2 mL water. Mix 500 μL plasma or diluted urine with 5 mL 50 mM ammonium acetate and 100 μL 100 μg/mL carbenicillin in water. Add the mixture to the SPE cartridge, draw it through the cartridge under vacuum, wash with 3 mL MeCN:500 mM acetic acid 50:50 and 2 mL MeOH:100 mM ammonium acetate 50:50, elute with 500 μL MeOH:10% LiCl 40:60. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil C18-AR (Nacalai Tesque Co., Kyoto)
Mobile phase: MeOH:50 mM pH 7.0 phosphate buffer 10:80

Flow rate: 0.9
Injection volume: 20
Detector: UV 254

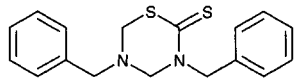
CHROMATOGRAM
Internal standard: carbenicillin
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Interfering: probenecid

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Itoh,T.; Watanabe,N.; Ishida,M.; Tsuda,Y.; Koyano,S.; Tsunoi,T.; Shimada,H.; Yamada,H. Stereoselective disposition of sulbenicillin in humans, *Antimicrob.Agents Chemother.*, **1998**, *42*, 325–331.

Sulbentine



Molecular formula: $C_{17}H_{18}N_2S_2$
Molecular weight: 314.48
CAS Registry No.: 350-12-9
Merck Index: 9061

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject an aliquot.

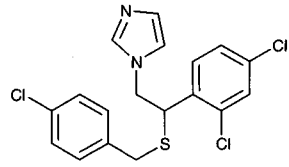
HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select-B (A) or 125 × 3 5 μm LiChrospher 60 RP-Select-B (B)
Mobile phase: MeCN: pH 2 trifluoroacetic acid 55:45
Flow rate: 1 (A) or 0.5 (B)
Detector: UV 290 (A), UV 240 (B)

CHROMATOGRAM
Retention time: 11.3 (A), 12.1 (B)

OTHER SUBSTANCES
Simultaneous: cloxyquin, chlorphenesin, naftifine, tolnaftate, degradation products

REFERENCE
Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

Sulconazole



Molecular formula: $C_{18}H_{15}Cl_3N_2S$
Molecular weight: 397.75
CAS Registry No.: 61318-90-9, 61318-91-0 (nitrate)
Merck Index: 9062
Lednicer No.: 3 133

SAMPLE

Matrix: blood
Sample preparation: 400 μL Plasma + 400 μL water + 50 μL 100 μg/mL miconazole in MeOH + 100 μL 1 M KOH + 6 mL hexane:dichloromethane 50:50, shake for 3 min, centrifuge at 4000 rpm for 6 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 80 × 4 CoPell ODS
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:10 mM pH 8.0 NaH_2PO_4 buffer 66:34
Flow rate: 2
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 7
Internal standard: miconazole (10)
Limit of quantitation: 500 ng/mL

KEY WORDS

plasma; dog; pharmacokinetics

REFERENCE

Fass,M.; Zaro,B.; Chaplin,M.; Matin,S. Reversed-phase high-pressure liquid chromatographic analysis of sulconazole in plasma, *J.Pharm.Sci.*, **1981**, *70*, 1338–1340.

SAMPLE

Matrix: formulations
Sample preparation: Dissolve 0.5 g cream containing 1% sulconazole nitrate in 5 mL THF, add fluocinonide, dilute to 100 mL with MeOH, inject a 25 μL aliquot onto column A with mobile phase A and allow components to elute from column A to column B for 7 min. After 7 min remove column A from circuit, monitor effluent from column B. Back-flush column A with mobile phase B for 3 min, equilibrate column A with mobile phase A for 5 min before next injection.

HPLC VARIABLES

Column: A 30 × 4.6 10 μm Partisil-10-ODS-3; B 70 × 2.1 Whatman Co:Pell ODS + 250 × 4.6 10 μm Partisil-10-ODS-3
Mobile phase: A MeCN:water:acetic acid 48:51:1 containing 10 mM $KClO_4$; B MeOH:THF 75:25
Flow rate: A 1.5; B 1
Injection volume: 25
Detector: UV 236

CHROMATOGRAM

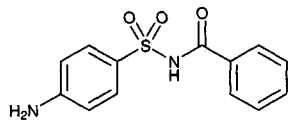
Retention time: 16
Internal standard: fluocinonide (12.5)

KEY WORDS

creams; column-switching

REFERENCE
Conley,D.L.; Benjamin,E.J. Automated high-performance liquid chromatographic column switching technique for the on-line clean-up and analysis of drugs in topical cream formulations, *J.Chromatogr.*, **1983**, *257*, 337–344.

Sulfabenzamide



Molecular formula: $C_{13}H_{12}N_2O_3S$
Molecular weight: 276.32
CAS Registry No.: 127-71-9
Merck Index: 9065
Lednicer No.: 2 112

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 8.56

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE

Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 1.34

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM

Retention time: 16.79

OTHER SUBSTANCES

Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE

Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1

Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM
Retention time: 8.8
Limit of detection: 2 ppb

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; liver; kidney

REFERENCE
Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 52.5

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

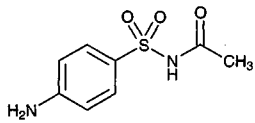
CHROMATOGRAM
Retention time: 47

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

Sulfacetamide



Molecular formula: $C_8H_{10}N_2O_3S$
Molecular weight: 214.25
CAS Registry No.: 144-80-9, 127-56-0 (Na salt), 6209-17-2 (Na salt monohydrate)
Merck Index: 9067
Lednicer No.: 1 123

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 6% trichloroacetic acid, vortex for 1 min, add 1 mL MeCN, centrifuge at 2500 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex for 1 min, shake for 10 min, centrifuge, inject a 100 μL aliquot of the aqueous phase. Analyze for free ceforanide by centrifuging serum at 3000 rpm for 20 min through an Amicon micropartition system with YMT membranes, 200 μL ultrafiltrate + 20 μL 1 mg/mL sulfacetamide, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 mm long C18 (Alltech)
Mobile phase: MeOH:100 mM pH 4.0 sodium acetate buffer 10:90
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Internal standard: sulfacetamide

OTHER SUBSTANCES
Extracted: ceforanide

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

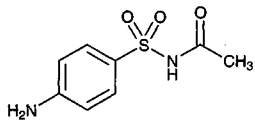
CHROMATOGRAM
Retention time: 47

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

Sulfacetamide



Molecular formula: $C_8H_{10}N_2O_3S$
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CAS Registry No.: 144-80-9, 127-56-0 (Na salt), 6209-17-2 (Na salt monohydrate)
Merck Index: 9067
Lednicer No.: 1 123

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 6% trichloroacetic acid, vortex for 1 min, add 1 mL MeCN, centrifuge at 2500 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex for 1 min, shake for 10 min, centrifuge, inject a 100 μL aliquot of the aqueous phase. Analyze for free ceforanide by centrifuging serum at 3000 rpm for 20 min through an Amicon micropartition system with YMT membranes, 200 μL ultrafiltrate + 20 μL 1 mg/mL sulfacetamide, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 mm long C18 (Alltech)
Mobile phase: MeOH:100 mM pH 4.0 sodium acetate buffer 10:90
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Internal standard: sulfacetamide

OTHER SUBSTANCES
Extracted: ceforanide

KEY WORDS
plasma; pharmacokinetics; sulfacetamide is IS

REFERENCE
DiPiro,J.T.; Bayoumi,S.M.; Vallner,J.J.; Nesbit,R.R.; Gokhale,R.; Rissing,J.P. Intraoperative ceforanide pharmacokinetics and protein binding, *Antimicrob.Agents Chemother.*, **1985**, *27*, 487–490.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10 mg/mL solution in MeOH:water 20:80, dilute a 3 mL aliquot to 100 mL with MeOH:water 20:80, inject a 90 μL aliquot. Ophthalmic solutions. Dilute a 1 mL aliquot to 100 mL with MeOH:water 20:80, inject a 90 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:glacial acetic acid 10:89:1
Flow rate: 1.5
Injection volume: 90
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: sulfanilamide

KEY WORDS
ophthalmic solutions

REFERENCE
Hall,L.; Chadwick,V. Quantitative determination of sulfanilamide in sodium sulfacetamide raw material and ophthalmic solutions by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *478*, 438–445.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24"long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM
Retention time: k' 1.0
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES
Extracted: dapsone, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfaquinoxaline, sulfathiazole, sulfatroxazole
Noninterfering: chloramphenicol, trimethoprim
Interfering: sulfanilamide, sulfaguanidine

KEY WORDS
post-column reaction; meat; column-switching; dialysis

REFERENCE
Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 4
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfadiazine, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfathiazole
Noninterfering: benzocaine

KEY WORDS
tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE
Garcia-Alvarez-Coque,M.C.; Simo-Alfonso,E.F.; Ramis-Ramos,G.; Esteve-Romero,J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245.

SAMPLE
Matrix: milk, urine

Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 3.43

OTHER SUBSTANCES
Extracted: sulfabenzamide, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: sulfabenzamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854,

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 6.49

OTHER SUBSTANCES

Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfachloropyridazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

Interfering: sulfadiazine

REFERENCE

Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole,

sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.5 5 μm Ultrasphere octyl
Mobile phase: MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, pH 4.35
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 1.69
Internal standard: naproxen (3.89)

OTHER SUBSTANCES

Simultaneous: bacitracin, cortisone acetate, diazepam, diclofenac, fluorometholone, flurbiprofen, hydrocortisone acetate, imipramine, indomethacin, ketoprofen, ketorolac tromethamine, meclofenamic acid, metipranolol, neomycin, prednisolone acetate, proparacaine, propranolol, suprofen
Noninterfering: acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide
Interfering: levobunolol, salicylic acid

REFERENCE

Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 14

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 14.5

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfaben-

zamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM

Retention time: 4
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole
Interfering: sulfadiazine

KEY WORDS

post-column reaction; muscle; liver; kidney; SPE

REFERENCE

Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE

Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 2.5
Internal standard: niacin (3.3)

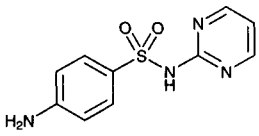
OTHER SUBSTANCES

Extracted: sulfathiazole, sulfamethazine, sulfamethoxazole, sulfadiazine, sulfamerazine, sulfamonomethoxine

KEY WORDS
wastewater

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfadiazine



Molecular formula: $C_{10}H_{10}N_4O_2S$
Molecular weight: 250.28
CAS Registry No.: 68-35-9, 22199-08-2 (Ag salt), 547-32-0 (Na salt)
Merck Index: 9071
Lednicer No.: 1 124

SAMPLE
Matrix: amniotic fluid, blood, tissue
Sample preparation: Homogenize (Ultraturrax) tissue with four volumes of physiological saline, centrifuge at 1000 g for 20 min. 200 μL Serum, tissue supernatant, or amniotic fluid + 300 μL 300 mM perchloric acid, vortex, centrifuge at 4000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 8 μm 6.0 nm Dynamax C8 (Rainin)
Mobile phase: Gradient. MeCN:1% acetic acid from 10:90 to 28:72 over 18 min
Flow rate: 1.5
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Limit of quantitation: 200 ng/mL

KEY WORDS
serum; monkey; pharmacokinetics; placenta; brain; heart; liver; spleen; lung

REFERENCE
Schoondermark-Van de Ven,E.; Galama,J.; Vree,T.; Camps,W.; Baars,I.; Eskes,T.; Meuwissen,J.; Melchers,W. Study of treatment of congenital *Toxoplasma gondii* infection in rhesus monkeys with pyrimethamine and sulfadiazine, *Antimicrob.Agents Chemother.*, **1995**, *39*, 137–144.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 1 mL sulfamethazine in MeCN, vortex for 30 s, centrifuge at 10000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LichroCart 100-RP18 (Merck)
Mobile phase: MeCN:1% acetic acid 13:87
Flow rate: 1
Injection volume: 20
Detector: UV 269

CHROMATOGRAM
Internal standard: sulfamethazine

KEY WORDS
rabbit; plasma; pharmacokinetics

REFERENCE

Hsu,K.-Y.; Song,D.-J.; Ho,Y. The influence of pyruvic acid on the pharmacokinetics of sulphadiazine in rabbits, *Biopharm.Drug Dispos.*, **1995**, *16*, 233–244.

SAMPLE

Matrix: blood, milk

Sample preparation: 1 mL Serum or milk + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL p-aminobenzoic acid in 0.01% trichloroacetic acid, centrifuge at 1000 g for 10 min. Remove a 500 μL aliquot of the clear layer and add it to 100 μL 1 mg/mL fluorescamine in acetone (prepared fresh each day), mix for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM

Retention time: 6.5
Internal standard: p-aminobenzoic acid (5.5)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES

Extracted: sulfadimethoxine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, sulfathiazole

KEY WORDS

cow; serum; derivatization

REFERENCE

Tsai,C.-E.; Kondo,F. Liquid chromatographic determination of fluorescent derivatives of six sulfonamides in bovine serum and milk, *J.AOAC Int.*, **1995**, *78*, 674–678.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. 500 μL Plasma + 100 μL 30 μg/mL sulfamethazine (sulfadimidine) in EtOH + 150 μL 3% trichloroacetic acid in EtOH + 100 μL EtOH, vortex, freeze at -20° for 5 min, centrifuge, freeze at -20° for 10 min, centrifuge through a Spin-X filter tube, inject a 10 μL aliquot of the supernatant. Tissue. 1-3 g Tissue + 3 (muscle) or 6 (liver) μL 1 mg/mL sulfamethoxazole in EtOH + 2 (liver) or 3 (muscle) mL 0.7% trichloroacetic acid in acetone, mix in Whirlmixer, sonicate for 10 min at 40°, add 2 mL 10 mM pH 6 Na_2HPO_4, sonicate for 5 min, add 100 μL 500 mM NaOH, add 9 (muscle) or 10 (liver) dichloromethane, mix thoroughly for 1 min, centrifuge at 2240 g for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness at 40° under a stream of nitrogen. Dissolve the residue in 400 (muscle) or 800 (liver) μL MeCN:10 mM pH 2.8 phosphate buffer 20:80, sonicate, extract with 1 mL hexane. Sonicate the aqueous phase for 1 min, centrifuge through a Spin-X filter tube, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18 DB
Column: 250 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeCN:buffer 23:77 (plasma) or 20:80 (tissue) with 0.1% triethylamine added (Buffer was 25 mM sodium phosphate and 20 mM sodium 1-hexanesulfonate, pH adjusted to 2.8 with 5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 6 (plasma), 7 (tissue)
Internal standard: sulfamethazine (sulfadimidine) (8), sulfamethoxazole (18)
Limit of quantitation: 30 ng/g (liver), 25 ng/mL (plasma), 15 ng/g (muscle)

OTHER SUBSTANCES
Simultaneous: trimethoprim

KEY WORDS
plasma; fish; salmon; trout; muscle; liver

REFERENCE
Hormazabal,V.; Rogstad,A. Simultaneous determination of sulfadiazine and trimethoprim in plasma and tissues of cultured fish for residual and pharmacokinetic studies, *J.Chromatogr.*, **1992**, *583*, 201–207.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute 1 mL urine with 5 or 10 mL water. 1 mL Serum, plasma, or diluted urine + 200 μL 1 M pH 6.8 KH_2PO_4 buffer + 6 mL ethyl acetate, vortex for 3 min, centrifuge at 2400 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL 30 μg/mL sulfadimethoxine in mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 25-40 μm LiChroprep Si 60 (Merck)
Column: 250 × 4 10 μm LiChrosorb Si 60
Mobile phase: Dichloromethane:MeOH:ammonia 80:19:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 289

CHROMATOGRAM
Retention time: 6.7
Internal standard: sulfadimethoxine (3.7)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, N-acetylsulfadiazine, trimethoprim

KEY WORDS
normal phase; serum; plasma; pharmacokinetics

REFERENCE
Ascalone,V. Assay of trimethoprim, sulfadiazine and its N4-acetyl metabolite in biological fluids by normal-phase high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *224*, 59–66.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 200 μL MeCN, centrifuge at 3000 g for 5 min, inject a 50 μL aliquot of the supernatant. Urine. Centrifuge urine at 3000 g, dilute the supernatant with 5 volumes of 0.6% acetic acid, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4.6 8 μm C8 (Meyvis, Bergen op Zoom, Netherlands)
Column: 150 × 4.6 8 μm Dynamax 60 Å C8 (?) (Rainin)
Mobile phase: Gradient. A was MeCN. B was 0.5% acetic acid containing 0.5 g/L ammonium acetate, pH 3.3. A:B from 0:100 to 10:90 over 5 min, to 18:82 over 13 min, return to initial conditions over 1 min, re-equilibrate for 4 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Retention time: 12.70
Limit of quantitation: 310 ng/mL (plasma), 800 ng/mL (urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; monkey; plasma; pharmacokinetics

REFERENCE
Vree,T.B.; Schoondermark-Van de Ven,E.; Verwey-van Wissen,C.P.W.G.M.; Baars,A.M.; Swolfs,A.; van Galen,P.M.; Amatdjais-Groenen,H. Isolation, identification and determination of sulfadiazine and its hydroxy metabolites and conjugates from man and Rhesus monkey by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *670*, 111–123.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 8.375

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cell cultures
Sample preparation: Condition a cyclohexyl-bonded silica Bond-elut SPE cartridge with 2 mL MeOH and 2 mL water. Centrifuge cell cultures at 6000 g at 4° for 15 min, add 100 μL supernatant and 100 μL 2 μg/mL sulfamerazine to the SPE cartridge, wash with 1 mL water, elute with 1.5 mL MeOH. Evaporate the eluate to dryness under a stream of air at 60°, reconstitute the residue in 100 μL water, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm ODS Hypersil

Mobile phase: MeOH:10 mM pH 2.5 phosphate buffer 5:95 containing 40 mM tetrabutylammonium bromide
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: sulfamerazine (7.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: p-aminobenzoic acid, trimethoprim, dibromopropamidine isethionate

KEY WORDS
SPE

REFERENCE
Taylor,R.B.; Richards,R.M.E.; Xing,D.K.-I. Determination of antibacterial agents in microbiological cultures by high-performance liquid chromatography, *Analyst*, **1990**, *115*, 797–799.

SAMPLE
Matrix: cell suspensions
Sample preparation: Cool cell suspension in an ice bath, centrifuge at 800 g at 4° for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water 20:80
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Also analyzed: sulfanilamide, sulfamethoxazole, sulfamerazine

REFERENCE
Climax,J.; Lenehan,T.J.; Lambe,R.; Kenny,M.; Caffrey,E.; Darragh,A. Interaction of antimicrobial agents with human peripheral blood leucocytes: uptake and intracellular localization of certain sulphonamides and trimethoprims, *J.Antimicrob.Chemother.*, **1986**, *17*, 489–498.

SAMPLE
Matrix: eggs, honey, milk
Sample preparation: Honey. Dissolve 1 g honey in 10 mL water, homogenize, filter (0.45 μm), inject a 50 μL aliquot. Milk, eggs. 5 mL Milk or 0.4 g lyophilized eggs + 10 mL trichloroacetic acid solution (so as to give a final trichloroacetic acid concentration of 3%), homogenize, centrifuge at 5000 rpm for 5 min. Re-extract the residue with 10 mL 3% trichloroacetic acid. Combine the aqueous phases and make up to 25 mL with trichloroacetic acid solution, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. MeCN:water 3:97 for 5 min, to 40:60 over 15 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (Wash column with MeCN:ethyl acetate 5:95 at the end of each day.)
Flow rate: 1
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 12
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: sulfaguanidine, sulfamethoxazole, sulfapyridine, sulfathiazole

REFERENCE
Viñas,P.; López Erroz,C.; Hernández Canals,A.; Hernández Córdoba,M. Liquid chromatographic analysis of sulfonamides in foods, *Chromatographia*, **1995**, *40*, 382–386.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM
Retention time: k' 2.0
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES
Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole
Noninterfering: chloramphenicol, trimethoprim

KEY WORDS
post-column reaction; meat; column-switching; dialysis

REFERENCE
Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Homogenize 3 g milk and 500 μL 30% trichloroacetic acid, centrifuge at 5000 rpm for 5 min. Remove the aqueous phase and extract the residue with 4 mL 3% trichloroacetic acid. Combine the aqueous layers and make up to 10 mL with trichloroacetic acid, filter (0.45 μm), inject a 50 μL aliquot. Fish, eggs. Homogenize (Ultra-Turrax) 3 g fish or 4 g eggs with 4 mL 3% trichloroacetic acid, centrifuge at 5000 rpm for 5 min. Remove the aqueous phase and extract the residue with 4 mL 3% trichloroacetic acid. Combine the aqueous layers and make up to 10 mL with trichloroacetic acid, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Spherisorb ODS-2
Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. MeCN:water 3:97 for 5 min, to 40:60 over 15 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (At the end of each day wash with MeCN: ethyl acetate 5:95.)
Flow rate: 0.5
Injection volume: 50
Detector: F ex 302 em 412 following post-column reaction. The column effluent mixed with reagent 1 pumped at 0.25 mL/min and with reagent 2 pumped at 0.25 mL/min and this mixture flowed through a 2.5 m × 0.8 mm i.d. PTFE coil at 40° to the detector. (Reagent 1 was 10 mM o-phthalaldehyde in EtOH:700 mM phosphoric acid 2:98. Reagent 2 was 20 mM β-mercaptoethanol in EtOH:700 mM phosphoric acid 2:98.)

CHROMATOGRAM
Retention time: 20
Limit of detection: 16 ng/mL

OTHER SUBSTANCES
Extracted: sulfaguanidine, sulfamethoxazole, sulfanilamide, sulfapyridine
Noninterfering: sulfathiazole

KEY WORDS
post-column reaction

REFERENCE
Viñas,P.; Erroz,C.L.; Campillo,N.; Hernández-Córdoba,M. Determination of sulphonamides in foods by liquid chromatography with postcolumn fluorescence derivatization, *J.Chromatogr.A*, **1996**, *726*, 125–131.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Suspension + 100 mL MeOH:water 60:40, shake mechanically for 15 min, make up to 200 mL with MeOH:water 60:40, filter (0.45 μm silver membrane, Selas Corp.). Evaporate a 1 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL 200 μg/mL acetanilide in MeCN, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water 20:80
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: acetanilide (11)

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfanilamide, sulfanilic acid
Noninterfering: erythromycin ethylsuccinate

KEY WORDS
oral suspensions; suspensions

REFERENCE
Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 9
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfathiazole
Noninterfering: benzocaine

KEY WORDS
tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE
Garcia-Alvarez-Coque,M.C.; Simo-Alfonso,E.F.; Ramis-Ramos,G.; Esteve-Romero,J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45

Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2.5
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfathiazole, sulfamethazine, sulfisoxazole, sulfadimethoxine

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 7.7
Limit of detection: 0.9 ppb
Limit of quantitation: 2.4 ppb

OTHER SUBSTANCES
Extracted: sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfathiazole
Interfering: theobromine

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-

500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 6.4
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 3.77

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: saliva

Sample preparation: 1 mL Saliva + 1 mL MeCN + 400 mg potassium carbonate, vortex for 1 min, centrifuge at ≥1000 g for 10 min. Remove the upper MeCN layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm RP-18 (Brownlee)
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 mM $NaHPO_4$ (sic) containing 10 mM sodium 1-hexanesulfonate and 7.2 mM triethylamine, adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: F ex 395 em 470 following post-column reaction. The column effluent mixed with reagent pumped at 0.3 mL/min and the mixture flowed through a 4.8 m × 0.7 mm ID PTFE coil at 60° to the detector. (Prepare reagent by dissolving 400 mg fluorescamine in 250 mL MeOH, add 1 mL 2-mercaptoethanol, add 250 mL mobile phase.)

CHROMATOGRAM
Retention time: 5.66
Internal standard: sulfadiazine

OTHER SUBSTANCES
Extracted: sulfapyridine
Noninterfering: N-acetylsulfapyridine, 2-amino-3-phenyl-1-propanol, 5-aminosalicylic acid, amphetamine, furosemide, levallorphan, metoprolol, riboflavin, salicylic acid, sulfasalazine, viloxazine

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KEY WORDS
post-column reaction; sulfadiazine is IS

REFERENCE
Sista,H.S.; Dye,D.M.; Leonard,J. High-performance liquid chromatographic method for determination of sulfapyridine in human saliva using post-column, in-line derivatization with fluorescamine, *J.Chromatogr.*, **1983**, *273*, 464–468.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 mm long MicroPak CN-10
Mobile phase: Isooctane:chloroform:MeOH:acetic acid 30.5:65:4:0.5
Flow rate: 0.33
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.55

OTHER SUBSTANCES
Simultaneous: sulfabromomethazine, sulfadimethoxine, sulfaethoxypridazine, sulfamethazine
Noninterfering: sulfamerazine, sulfathiazole, sulfanilamide, sulfapyridine, sulfaquinoline
Interfering: sulfachlorpyridazine

REFERENCE
Seymour,D.; Rupe,B.D. High-pressure liquid chromatographic determination of sulfamethazine residues in beef tissues, *J.Pharm.Sci.*, **1980**, *69*, 701–703.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: sulfanilic acid, sulfanilamide, sulfapyridine, sulfamerazine, sulfamethizole, sulfamethazine, sulfamethoxazole, sulfisoxazole, sulfachlorpyridine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 6.50

OTHER SUBSTANCES

Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfachloropyridazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole
Interfering: sulfacetamide

REFERENCE

Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole,

sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Sentry (Waters)
Column: 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: MeOH:water:glacial acetic acid 20:79:1
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 3.7

OTHER SUBSTANCES

Simultaneous: sulfanilamide, sulfathiazole, sulfamerazine, sulfamethazine, succinylsulfathiazole

REFERENCE

Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 19.5

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS

capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 μL initial mobile phase, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 0.8
Injection volume: 50
Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5

mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 μL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 10
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Blend 3 g meat with 30 mL chloroform for 2 min in a Polytron homogenizer, shake for 10 min, centrifuge at 1600 g for 5 min, filter (5A filter paper). Add 5 mL filtrate to 1 mL 3 M HCl, shake for 10 min, centrifuge at 1600 g for 5 min. 250 μL Aqueous layer + 250 μL 3.5 M sodium acetate solution, vortex, add 100 μL 0.2% fluorescamine in acetone, vortex, let stand for 20 min at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:2% acetic acid 5:3
Column temperature: 55
Flow rate: 1
Injection volume: 10
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 6
Limit of detection: 0.005 ng/g

OTHER SUBSTANCES
Simultaneous: sulfisomidine, sulfamethoxazole, sulfamerazine, sulfamethazine (sulfadimidine), sulfamonomethoxine, sulfadimethoxine, sulfaquinoxaline

KEY WORDS
cow; pig; chicken; ham; sausage; roast beef; derivatization

REFERENCE
Takeda,N.; Akiyama,Y. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products, *J.Chromatogr.*, **1991**, *558*, 175–180.

SAMPLE
Matrix: tissue
Sample preparation: Cut tissue into small pieces and homogenize in blender. 20 g Homogenized tissue + 200 μL 10 μg/mL methyl p-aminobenzoate in water + 60 mL acetone:chloroform 50:50, shake vigorously on a mechanical shaker for 10 min, centrifuge at 3000 g for 10 min, filter (Whatman No. 41 paper) supernatant, repeat extraction. Combine the extracts, if the extract is not clear centrifuge at 3000 g for 10 min and discard the aqueous layer, evaporate to an oily residue at 45° under reduced pressure, add 5 mL MeCN to flask, let stand for 10 min, remove MeCN layer, add 5 mL hexane and 5 mL MeCN, shake, centrifuge at 3000 g for 10 min, remove

the MeCN layer, add 5 mL MeCN to the hexane layer, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer. If hexane layer is not clear centrifuge at 3000 g for 10 min and remove the clear portion. Add 400 μL 15% trichloroacetic acid to the hexane layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Evaporate the MeCN layers, transfer the oily residue to a small flask with 3 mL hexane, add the aqueous trichloroacetic acid layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Discard the hexane layer, add 100 μL saturated aqueous sodium citrate solution to the aqueous layer, mix, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP 18 (Brownlee)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 1% aqueous acetic acid. B was MeCN:water 80:20. A:B from 90:10 to 60:40 over 20 min, return to initial conditions over 5 min, re-equilibrate for 5 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 7 m × 0.25 mm i.d. coil of stainless steel tubing to the detector. (Prepare reagent by dissolving 1 g p-dimethylaminobenzaldehyde in 30 mL MeCN, make up to 100 mL with 5% trichloroacetic acid in water.)

CHROMATOGRAM

Retention time: 9.1
Internal standard: methyl p-aminobenzoate (18.6)
Limit of detection: 20 ng/g

OTHER SUBSTANCES

Extracted: sulfamerazine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfapyridine, sulfaquinoxaline

KEY WORDS

chicken; liver; pig; kidney; sheep; cow; post-column reaction

REFERENCE

Bui,L.V. Liquid chromatographic determination of six sulfonamide residues in animal tissues using postcolumn derivatization, *J.AOAC Int.*, **1993**, *76*, 966–976.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The

effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM

Retention time: 4
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole
Interfering: sulfacetamide

KEY WORDS

post-column reaction; muscle; liver; kidney; SPE

REFERENCE

Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 μL 20 μg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min. Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72
Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM

Retention time: 4.7
Internal standard: sulfaethoxypyridazine (12.8)

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfathiazole

KEY WORDS

cow; pig; muscle; liver; SPE

REFERENCE

Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 3 mL Bond Elut propylsulfonic acid strong cation exchange SPE cartridge with 4 mL MeCN, 16 mL 200 mM phosphoric acid, and 4 mL MeCN. Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase at 16000 rpm for 1 min, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to 2-3 mL, add 3 mL dichloromethane, add to the SPE cartridge, rinse flask with MeCN:dichloromethane 60:40, add rinse to the SPE cartridge, rinse flask with 5 mL MeCN, add rinse to the SPE cartridge, wash with 2 mL MeCN:200 mM phosphoric acid 10:90, elute with 5 mL MeCN:200 mM phosphoric acid 10:90, inject a 10 μL aliquot of the eluate. (Do not allow SPE cartridge to go dry at any time.)

HPLC VARIABLES

Guard column: 20 × 2 pellicular C18
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:2% acetic acid 10:90
Flow rate: 1
Injection volume: 10
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL (?) fluorescamine in MeCN:2% acetic acid 55:45 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 0.2 ng/g
Limit of quantitation: 1 ng/g

KEY WORDS

fish; salmon; post-column reaction; SPE

REFERENCE

Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Liquid chromatographic determination of sulfadiazine in salmon by postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1995**, *78*, 1161–1164.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (Ultra-Turrax) 3 g ground tissue with 30 mL chloroform for 2 min, centrifuge at 3000 g for 5 min, filter (paper). Remove a 10 mL aliquot of the filtrate and add it to 1 mL 3 M HCl, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove a 250 μL aliquot of the aqueous layer and add it to 250 μL 3.8 M sodium acetate, add 100 μL 1 mg/mL fluorescamine in MeCN, vortex, let stand at room temperature for 20 min, inject a 20 μL aliquot. (Sodium acetate should be a highly pure grade.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:20 mM pH 4 NaH_2PO_4 34:66 containing 20 mM sodium octanesulfonate
Column temperature: 30
Flow rate: 1.2
Injection volume: 20
Detector: F ex 405 em 495

CHROMATOGRAM

Retention time: 8
Limit of detection: 3 ng/g

OTHER SUBSTANCES

Extracted: sulfadimethoxine, sulfamethazine, sulfaquinoxaline

KEY WORDS
derivatization; chicken; muscle

REFERENCE
Simeonidou,E.J.; Botsoglou,N.A.; Psomas,I.E.; Fletouris,D.J. Liquid chromatographic analysis of multiple sulfonamide residues in chicken muscle using pre-column derivatization and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2349–2364.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM
Retention time: 6
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 10 mL 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18

Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 8.8
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: sulfamethizole, sulfathiazole
Interfering: sulfaguanidine, sulfamethoxazole

KEY WORDS
derivatization

REFERENCE
Simó-Alfonso,E.F.; Ramis-Ramos,G.; García-Alvarez-Coque,M.C.; Esteve-Romero,J.S. Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography, *J.Chromatogr.B*, **1995**, *670*, 183–187.

SAMPLE
Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

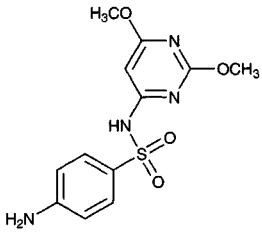
CHROMATOGRAM
Retention time: 4
Internal standard: niacin (3.3)

OTHER SUBSTANCES
Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfamethoxazole, sulfamerazine, sulfamonomethoxine

KEY WORDS
wastewater

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfadimethoxine



Molecular formula: $C_{12}H_{14}N_4O_4S$
Molecular weight: 310.33
CAS Registry No.: 122-11-2
Merck Index: 9073
Lednicer No.: 1 125

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 3 mL MeOH and 3 mL 50 mM pH 3.4 oxalate buffer. 1 mL Plasma + 1 mL 50 mM pH 3.4 oxalate buffer + 50 μL MeOH:water 50:50, mix, add to the SPE cartridge. Wash with 3 mL 50 mM pH 3.4 oxalate buffer, 1 mL MeOH:water 20:80, and 2 mL hexane:ether 80:20. Elute with two 1 mL portions of MeOH:25% ammonia solution 99:1. Evaporate the eluate to dryness under a gentle stream of nitrogen at 30°. Dissolve the residue in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 3.9 5 μm Symmetry C18 (Waters)
Column: 250 × 4.6 5 μm Symmetry C18 (Waters)
Mobile phase: MeCN:MeOH:water 25:10:65 containing 1% triethylamine, adjust to p 5.6 with phosphoric acid
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Internal standard: sulfadimethoxine

OTHER SUBSTANCES

Extracted: pyrimethamine, sulfadoxine
Simultaneous: acetaminophen, 4-chlorophenylbiguanide, cycloguanil, proguanil, quinine, sulfadiazine

KEY WORDS

plasma; SPE; sulfadimethoxine is IS

REFERENCE

Astier,H.; Renard,C.; Cheminel,V.; Soares,O.; Mounier,C.; Peyron,F.; Chaulet,J.F. Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction, *J.Chromatogr.B*, **1997**, *698*, 217–223.

SAMPLE

Matrix: blood, milk
Sample preparation: 1 mL Serum or milk + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL p-aminobenzoic acid in 0.01% trichloroacetic acid, centrifuge at 1000 g for 10 min. Remove a 500 μL aliquot of the clear layer and add it to 100 μL 1 mg/mL fluorescamine in acetone (prepared fresh each day), mix for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 18.5
Internal standard: p-aminobenzoic acid (5.5)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, sulfathiazole

KEY WORDS
cow; serum; derivatization

REFERENCE
Tsai,C.-E.; Kondo,F. Liquid chromatographic determination of fluorescent derivatives of six sulfonamides in bovine serum and milk, *J.AOAC Int.*, **1995**, *78*, 674–678.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 200 μL Plasma + 90 μL 24% trichloroacetic acid in MeOH + 10 μL 10 μg/mL sulfamethoxazole in MeOH, vortex for 30 s, centrifuge at 14000 g for 5 min, inject a 50 μL aliquot of the supernatant. Muscle. Homogenize 1 g muscle in 1.5 mL MeOH:buffer 20:80, add 50 μL 10 μg/mL sulfamethoxazole in MeOH, mix thoroughly for 1 min, centrifuge at 14000 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was 25 mM NaH_2PO_4 containing 15 mM sodium 1-heptanesulfonate adjusted to pH 2.8 with 5 M phosphoric acid.)

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm ODS-Hypersil
Column: 150 × 4.6 3 μm ODS-Hypersil C18
Mobile phase: MeCN:buffer:triethylamine 20:80:0.02 (Buffer was 25 mM NaH_2PO_4 containing 15 mM sodium 1-heptanesulfonate adjusted to pH 2.8 with 5 M phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 14
Internal standard: sulfamethoxazole (8)
Limit of detection: 30 ng/g (muscle), 15 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: ormetoprim

KEY WORDS
plasma; muscle; fish; salmon

REFERENCE
Samuelsen,O.B. Simultaneous determination of ormethoprim and sulphadimethoxine in plasma and muscle of Atlantic salmon *(Salmo salar)*, *J.Chromatogr.B*, **1994**, *660*, 412–417.

SAMPLE
Matrix: blood, tissue
Sample preparation: 1 mL Serum or homogenized tissue + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 15 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL sulfadiazine in 0.01% trichloroacetic acid, shake, add 100 μL hexane, shake, centrifuge at 1000 g for 15 min. Remove a 500 μL aliquot of the clear aqueous layer and add it to 100 μL 1 mg/mL fluorescamine in MeCN (freshly prepared), shake by hand for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pack C18

Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 18.2
Internal standard: sulfadiazine (7.1)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfamethoxazole, sulfamonomethoxine, sulfamethazine

KEY WORDS
serum; pig; derivatization; kidney; muscle; liver

REFERENCE
Tsai,C.-E.; Kondo,F. A sensitive high-performance liquid chromatographic method for detecting sulfonamide residues in swine serum and tissues after fluorescamine derivatization, *J.Liq.Chromatogr.*, **1995**, *18*, 965–976.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.735

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water

pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 5.0
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole
Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: eggs, tissue
Sample preparation: Homogenize (Nippon Seiki AM-1) 5 g tissue or eggs with 25 mL MeCN: water 90:10 and 20 mL hexane, centrifuge at 2100 g for 10 min, filter (Toyo Roshi No. 2 paper) the supernatant, repeat the extraction twice more. Leave the combined filtrate until phase separation is complete, dry the MeCN layer over anhydrous sodium sulfate, filter, add to the alumina column, wash with 30 mL MeCN, elute with 20 mL MeCN:water 90:10. Evaporate the eluate to dryness and reconstitute the residue with 1 mL mobile phase, inject a 20 μL aliquot. (Prepare the alumina column by adding 6 g 60-200 μm 90 active basic (activity I) aluminum oxide (Merck) to a 300 × 15 column, wash with 30 mL MeCN:water 90:10, wash with 30 mL MeCN.)

HPLC VARIABLES

Guard column: 4 × 4 7 μm LiChrosorb RP-18
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 25:75
Flow rate: 1

Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 12.4
Limit of detection: 10 ppb

OTHER SUBSTANCES
Extracted: metabolites, N-acetylsulfadimethoxine, N-acetylsulfamonomethoxine, sulfamonomethoxine

KEY WORDS
pig; cow; chicken; muscle; SPE

REFERENCE
Furusawa,N.; Mukai,T. Simultaneous high-performance liquid chromatographic determination of residual sulphamonomethoxine, sulphadimethoxine and their N4-acetyl metabolites in foods of animal origin, *J.Chromatogr.A*, **1994**, *677*, 81–85.

SAMPLE
Matrix: formulations
Sample preparation: Powder 20 tablets, dissolve a portion of the powder in water such that the concentration of penicillin V potassium is 0.6 mg/mL, mix well, filter. Mix 20 mL filtrate with 15 mL 0.4 mg/mL sulfadimethoxine in MeCN:water 50:50, dilute to 100 mL with water, mix well, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 mm long 30-50 μm Whatman Co:Pell ODS
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 21:4:75
Flow rate: 1-1.5
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Internal standard: sulfadimethoxine

OTHER SUBSTANCES
Simultaneous: penicillin V

KEY WORDS
tablets; collaborative study; sulfamdimethoxine is IS

REFERENCE
Mopper,B. Liquid chromatographic determination of penicillin V potassium in tablets: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 883–884.

SAMPLE
Matrix: milk
Sample preparation: Mix 5 mL milk with 20 μL 12 M HCl, sonicate, add 25 mL ethyl acetate, extract using a rotary shaker (REAX 2, Heidolph) for 10 min. Centrifuge at 1500 g for 5 min, evaporate 20 mL of the ethyl acetate extract to dryness, dissolve the residue in 10 mL 1 HCl. Wash the aqueous phase with 10 mL hexane, adjust to pH 5.5 with 900 μL 10 M NaOH and 5 mL 1 M pH 6.0 KH_2PO_4, extract with two 10 mL portions of dichloromethane. Evaporate the organic layer to dryness, dissolve the residue in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Novapak C18 (Waters)
Mobile phase: MeCN:10 mM pH 6.6 ammonium acetate 10:90
Flow rate: 1

Injection volume: 50
Detector: UV 271

CHROMATOGRAM
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Also analyzed: sulfadimidine (UV 265), sulfadoxine, sulfamethoxypyridazine (UV 265)

KEY WORDS
cow; milk

REFERENCE
Roudaut,B.; Moretain,J.P. Sulphonamide residues in milk of dairy cows following intravenous injection, *Food Addit.Contam.*, **1990**, *7*, 527–533.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 15.5
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfathiazole, sulfadiazine, sulfamethazine, sulfisoxazole

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 30:70
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 15.0
Limit of detection: 0.7 ppb
Limit of quantitation: 1.6 ppb

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfamethazine, sulfaquinoxaline

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 12.1
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfathiazole
Interfering: sulfaquinoxaline (distinguish by MS)

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk
Sample preparation: Sonicate (Branson Model 200 ultrasonic cell disruptor with a microtip probe) 50 mL milk for 2 min, cool to room temperature. Remove a 5 mL aliquot and add it to 25 mL dichloromethane:chloroform 75:25, invert 20 times, vortex horizontally at high speed for 15 s, centrifuge at 3300 g at 10° for 10 min. Remove 10 mL of the organic layer and evaporate it to just dryness under a stream of nitrogen at 32°, reconstitute the residue in 5 mL hexane, vortex for 30 s, add 1 mL 100 mM KH_2PO_4, shake briefly by hand, shake mechanically horizontally for 15 min, inject a 200 μL aliquot of the lower aqueous layer.

HPLC VARIABLES
Guard column: 50 mm long LC-18-DB (Supelco)
Column: 300 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:100 mM KH_2PO_4 40:60
Column temperature: 35
Flow rate: 1
Injection volume: 200
Detector: UV 269

CHROMATOGRAM
Retention time: 9.5
Limit of quantitation: 5 ppb

KEY WORDS
cow

REFERENCE
Weiss,G.; Laurencot,H.J.; MacDonald,A.; Duke,P.D.; Misra,K.; Horton,G.M.; Katz,S.E.; Brady,M.S. Determination of sulfadimethoxine withdrawal time from milk. Part I: Dosing, sampling and assay, *J.AOAC Int.*, **1995**, *78*, 358–371.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 10.78

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:10 mM phosphate buffer 52:48
Flow rate: 1.5
Detector: F ex 400 em 500

OTHER SUBSTANCES
Simultaneous: sulfachlorpyridazine, sulfadoxine, sulfamethazine, sulfaquinoxaline, sulfathiazole

REFERENCE
Thomas,G.K.; Millar,R.G.; Anstis,P.W. Stability of sulfonamide antibiotics in spiked pig liver tissue during frozen storage, *J.AOAC Int.*, **1997**, *80*, 988–995.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 mm long MicroPak CN-10
Mobile phase: Isooctane:chloroform:MeOH:acetic acid 30.5:65:4:0.5
Flow rate: 0.33
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.99

OTHER SUBSTANCES
Simultaneous: sulfabromomethazine, sulfachlorpyridazine, sulfadiazine, sulfaethoxypridazine, sulfamethazine
Noninterfering: sulfamerazine, sulfathiazole, sulfanilamide, sulfapyridine, sulfaquinoline

REFERENCE
Seymour,D.; Rupe,B.D. High-pressure liquid chromatographic determination of sulfamethazine residues in beef tissues, *J.Pharm.Sci.*, **1980**, *69*, 701–703.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 25-40 μm LiChroprep Si 60 (Merck)
Column: 250 × 4 10 μm LiChrosorb Si 60
Mobile phase: Dichloromethane:MeOH:ammonia 80:19:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 289

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Simultaneous: N-acetylsulfadiazine, sulfadiazine, trimethoprim

KEY WORDS
normal phase

REFERENCE

Ascalone,V. Assay of trimethoprim, sulfadiazine and its N4-acetyl metabolite in biological fluids by normal-phase high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *224*, 59–66.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 39

OTHER SUBSTANCES

Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE

Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 30:69:1
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5

OTHER SUBSTANCES

Simultaneous: sulfisoxazole

REFERENCE

Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 1182–1185.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.56

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm Hypersil ODS RP-C18
Column: 100 × 4 5 μm Hypersil ODS RP-C18
Mobile phase: MeOH:50 mM pH 6.67 phosphate buffer 10:90
Flow rate: 0.8
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Simultaneous: sulfamethazine (sulfadimidine)

REFERENCE
van 't Klooster,G.A.E.; van Seeventer,P.B.; Kolker,H.J.; Smit,L.A.; Witkamp,R.F. High-performance liquid chromatographic method for the routine determination of sulphadimidine, its hydroxy metabolites and N4-acetylsulphadimidine in body fluids and cell culture media, *J.Chromatogr.*, **1991**, *571*, 157–168.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 189.5

OTHER SUBSTANCES
Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadoxine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine, sulfisoxazole

REFERENCE
Nishikawa,M.; Takahashi,Y.; Ishihara,Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues, *J.Liq.Chromatogr.*, **1993**, *16*, 4031–4047.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, gbuprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac *IDI*-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)

Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 53

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 61

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: tissue
Sample preparation: 500 mg Tissue + 2 g C18 material + 5 μL MeOH + 5 μL 80 μg/mL sulfamethoxazole in MeOH, let stand for 2 min, grind gently with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane,

elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen at 40°, dissolve the residue in 500 μL mobile phase, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter supernatant (0.45 μm), inject a 25 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 300 × 4 MicroPak ODS
Mobile phase: MeCN:17 mM orthophosphoric acid 35:65
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 270

CHROMATOGRAM
Retention time: 8
Internal standard: sulfamethoxazole (6)
Limit of detection: 1.25 ng

KEY WORDS
muscle; fish; catfish; matrix solid phase dispersion

REFERENCE
Long,A.R.; Hsieh,L.C.; Malbrough,M.S.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of sulfadimethoxine in catfish (Ictalurus punctatus) muscle tissue, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 868–871.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. 5 g Fish + 1 mL 100 μg/mL carbamazepine diol in MeOH + 15 mL MeCN + 500 μL 50% trichloroacetic acid, homogenize (Brinkmann Polytron PT 10/35) at medium speed for 30 s, centrifuge at 4° at 7800 g for 25 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 5 mL water, vortex for 30 s, filter (13 mm dia. 8 μm Membra-Fil (Nucleopore)), add the filtrate to the SPE cartridge, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, vortex for 30 s, inject a 20 μL aliquot. (Flush injection valve with 1 mL mobile phase between analyses.)

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP-18
Column: 250 × 4.6 5 μm Ultrasphere
Mobile phase: MeCN:MeOH:100 mM pH 4.0 phosphate buffer 17:10:73
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 27.5
Internal standard: carbamazepine diol (10.5)
Limit of quantitation: 0.2 ppm

OTHER SUBSTANCES
Extracted: ormetoprim
Simultaneous: sulfacetamide, sulfadiazine, sulfamerazine, sulfamethazine, sulfisoxazole

KEY WORDS
fish; salmon; SPE; pharmacokinetics

REFERENCE
Walisser,J.A.; Burt,H.M.; Valg,T.A.; Kitts,D.D.; McErlane,K.M. High-performance liquid chromatographic analysis of Romet-30 in salmon following administration of medicated feed, *J.Chromatogr.*, **1990**, *518*, 179–188.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL water. Homogenize 5 g tissue with 100 mL MeOH:0.2% metaphosphoric acid 40:60 for 2 min, filter through a 1 mm layer of Hyflo Super-Cel. Evaporate the filtrate under reduced pressure at 40° to 10 mL, add the residue to the SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, take up the residue in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Newguard RP-8
Column: 150 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:5 mM oxalic acid 45:55
Flow rate: 0.5
Injection volume: 10
Detector: UV 265

CHROMATOGRAM

Retention time: 8
Limit of detection: 50 ng/g

OTHER SUBSTANCES

Extracted: sulfamonomethoxine, sulfisozole, nalidixic acid, oxolinic acid, piromidic acid, sodium nifurstyrenate, furazolidone

KEY WORDS

fish; SPE

REFERENCE

Horie,M.; Saito,K.; Hoshino,Y.; Nose,N.; Nakazawa,H.; Yamane,Y. Simultaneous determination of residual synthetic antibacterials in fish by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *538*, 484–491.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allow to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 μL initial mobile phase, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher 100 RP-18

Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 0.8

Injection volume: 50

Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 μL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 28
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadiazine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Brinkmann Polytron) 40 g salmon tissue and 200 mL acetone for 3 min, add 10 g Celite, add 20 g sodium sulfate, blend for 2 min, filter, wash the solid with three 15 mL portions of acetone. Evaporate the filtrate to dryness under educed pressure at 40°, reconstitute with 100 mL dichloromethane. Remove a 50 mL aliquot and add it to 100 mL 100 mM NaOH, shake vigorously, centrifuge at 10° at 12 g for 20 min. Remove the aqueous layer and neutralize it with HCl, freeze dry overnight, remove the remaining water under reduced pressure at 40°, reconstitute the remaining residue with 5 mL MeOH:water 25:75, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC 18DB
Mobile phase: MeCN:water 35:65 containing 0.1% formic acid
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 7.3
Limit of detection: 13 ng/g

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

KEY WORDS
salmon; fish

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: tissue
Sample preparation: Blend 3 g meat with 30 mL chloroform for 2 min in a Polytron homogenizer, shake for 10 min, centrifuge at 1600 g for 5 min, filter (5A filter paper). Add 5 mL filtrate to 1 mL 3 M HCl, shake for 10 min, centrifuge at 1600 g for 5 min. 250 μL Aqueous layer + 250 μL 3.5 M sodium acetate solution, vortex, add 100 μL 0.2% fluorescamine in acetone, vortex, let stand for 20 min at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:2% acetic acid 5:3
Column temperature: 55
Flow rate: 1
Injection volume: 10
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 14.5
Limit of detection: 0.005 ng/g

OTHER SUBSTANCES
Simultaneous: sulfisomidine, sulfamethoxazole, sulfamerazine, sulfadiazine, sulfamonomethoxine, sulfamethazine (sulfadimidine), sulfaquinoxaline

KEY WORDS
cow; pig; chicken; ham; sausage; roast beef; derivatization

REFERENCE
Takeda,N.; Akiyama,Y. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products, *J.Chromatogr.*, **1991**, *558*, 175–180.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM
Retention time: 15.5
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
post-column reaction; muscle; liver; kidney; SPE

REFERENCE
Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 μL 20 μg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min. Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72
Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM
Retention time: 23.0
Internal standard: sulfaethoxypyridazine (12.8)
Limit of detection: 10 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfathiazole

KEY WORDS
cow; pig; muscle; liver; SPE

REFERENCE
Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 3 g ground tissue with 30 mL chloroform for 2 min, centrifuge at 3000 g for 5 min, filter (paper). Remove a 10 mL aliquot of the filtrate and add it to 1 mL 3 M HCl, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove a 250 μL aliquot of the aqueous layer and add it to 250 μL 3.8 M sodium acetate, add 100 μL 1 mg/mL fluorescamine in MeCN, vortex, let stand at room temperature for 20 min, inject a 20 μL aliquot.)Sodium acetate should be a highly pure grade.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 120 C18

Mobile phase: MeCN:20 mM pH 4 NaH_2PO_4 34:66 containing 20 mM sodium octanesulfonate
Column temperature: 30
Flow rate: 1.2
Injection volume: 20
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 21
Limit of detection: 9 ng/g

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamethazine, sulfaquinoxaline

KEY WORDS
derivatization; chicken; muscle

REFERENCE
Simeonidou,E.J.; Botsoglou,N.A.; Psomas,I.E.; Fletouris,D.J. Liquid chromatographic analysis of multiple sulfonamide residues in chicken muscle using pre-column derivatization and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2349–2364.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

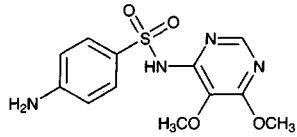
CHROMATOGRAM
Retention time: 23.5
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

Sulfadoxine



Molecular formula: $C_{12}H_{14}N_4O_4S$
Molecular weight: 310.33
CAS Registry No.: 2447-57-6
Merck Index: 9074
Lednicer No.: 1 125

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 3 mL MeOH and 3 mL 50 mM pH 3.4 oxalate buffer. 1 mL Plasma + 1 mL 50 mM pH 3.4 oxalate buffer + 50 µL 200 µg/mL IS in MeOH:water 50:50, mix, add to the SPE cartridge. Wash with 3 mL 50 mM pH 3.4 oxalate buffer, 1 mL MeOH:water 20:80, and 2 mL hexane:ether 80:20. Elute with two 1 mL portions of MeOH:25% ammonia solution 99:1. Evaporate the eluate to dryness under a gentle stream of nitrogen at 30°. Dissolve the residue in 250 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 3.9 5 µm Symmetry C18 (Waters)
Column: 250 × 4.6 5 µm Symmetry C18 (Waters)
Mobile phase: MeCN:MeOH:water 25:10:65 containing 1% triethylamine, adjusted to pH 5.6 with phosphoric acid
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Internal standard: sulfadimethoxine
Limit of detection: 14.61 ng/mL
Limit of quantitation: 22.08 ng/mL

OTHER SUBSTANCES

Extracted: pyrimethamine
Simultaneous: acetaminophen, 4-chlorophenylbiguanide, cycloguanil, proguanil, quinine, sulfadiazine,

KEY WORDS

plasma; SPE

REFERENCE

Astier,H.; Renard,C.; Cheminel,V.; Soares,O.; Mounier,C.; Peyron,F.; Chaulet,J.F. Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction, *J.Chromatogr.B*, **1997**, *698*, 217–223.

SAMPLE

Matrix: blood
Sample preparation: Add 150 µL 100 mM zinc sulfate to 600 µL plasma while vortexing over 15 s, add 700 µL MeCN containing 4 µm WR 184806 and 75 µM sulfadimethoxine while vortexing over 15 s, , let stand for 15 min, centrifuge at 10000 g for 10 min. Remove the supernatant and add it to 2 mL pH 9.0 phosphate buffer, add 2 mL 60 mM tetrabutylammonium hydroxide, add 5 mL MTBE, shake for 10 min, centrifuge at 1200 g for 5 min. Remove the upper organic layer and evaporate it to dryness at 50°, reconstitute the residue in 200 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 150 × 4 3 µm Spherisorb S3-ODS-1
Mobile phase: MeCN:100 mM phosphate buffer 48:52, adjusted to pH 3.5

Flow rate: 0.5
Injection volume: 100
Detector: UV 229

CHROMATOGRAM
Retention time: 5.31
Internal standard: sulfadimethoxine (6.22), 2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-tert-butylamino)propyl]quinoline phosphate (WR 184806) (Walter Reed) (21.00)
Limit of detection: 75 μM

OTHER SUBSTANCES
Extracted: mefloquine, pyrimethamine

KEY WORDS
plasma

REFERENCE
Bergqvist,Y.; Eckerbom,S.; Larsson,H.; Malekzadeh,M. Reversed-phase liquid chromatographic method for the simultaneous determination of the antimalarial drugs sulfadoxine, pyrimethamine, mefloquine and its major carboxylic metabolite in plasma, *J.Chromatogr.*, **1991**, *571*, 169–177.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma, whole blood, or red blood cells + 100 μL 50 μg/mL sulfamethoxazole in MeOH + 500 μL water + 100 μL buffer + 6 mL ethylene dichloride, shake on an orbital mixer for 20 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness at 60° on a vortex evaporator, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. (Buffer was prepared by adding 100 μL acetic acid to 9.9 mL phosphate buffer, pH 3.40.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:1 M perchloric acid:water 30:9:0.8:95
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: sulfamethoxazole (8)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, dapsone, primaquine, pyrimethamine, quinidine, quinine, sulfalene

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KEY WORDS
plasma; whole blood; red blood cells; pharmacokinetics

REFERENCE
Dua,V.K.; Sarin,R.; Sharma,V.P. Sulphadoxine concentrations in plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases after treatment with Fansidar using high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1317–1323.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Whole blood + 100 μL 200 mM zinc sulfate + 200 μL MeCN + sulfadimethoxine, vortex, centrifuge at 6000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL C18

Mobile phase: MeCN:MeOH:17 mM orthophosphoric acid 5:10:85
Column temperature: 45
Flow rate: 1.5
Detector: UV 270

CHROMATOGRAM
Internal standard: sulfadimethoxine

OTHER SUBSTANCES
Noninterfering: sulfalene, sulfamethoxazole

KEY WORDS
whole blood; comparison with colorimetric procedure

REFERENCE
Green,M.D.; Mount,D.L.; Todd,G.D. Determination of sulfadoxine concentrations in whole blood using C18 solid-phase extraction, sodium dodecyl sulfate and dimethylaminocinnamaldehyde, *Analyst*, **1995**, *120*, 2623–2626.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.345

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column

A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5

Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 2.6

Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole

Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long

cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 2.6
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole
Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: milk
Sample preparation: Mix 5 mL milk with 20 μL 12 M HCl, sonicate, add 25 mL ethyl acetate, extract using a rotary shaker (REAX 2, Heidolph) for 10 min. Centrifuge at 1500 g for 5 min, evaporate 20 mL of the ethyl acetate extract to dryness, dissolve the residue in 10 mL 1 HCl. Wash the aqueous phase with 10 mL hexane, adjust to pH 5.5 with 900 μL 10 M NaOH and 5 mL 1 M pH 6.0 KH_2PO_4, extract with two 10 mL portions of dichloromethane. Evaporate the organic layer to dryness, dissolve the residue in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Novapak C18 (Waters)
Mobile phase: MeCN:10 mM pH 6.6 ammonium acetate 10:90
Flow rate: 1
Injection volume: 50
Detector: UV 271

CHROMATOGRAM

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Also analyzed: sulfadimethoxine, sulfadimidine (UV 265), sulfamethoxypyridazine (UV 265)

KEY WORDS

cow; milk

REFERENCE

Roudaut,B.; Moretain,J.P. Sulphonamide residues in milk of dairy cows following intravenous injection, *Food Addit.Contam.*, **1990**, *7*, 527–533.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:10 mM phosphate buffer 52:48
Flow rate: 1.5
Detector: F ex 400 em 500

OTHER SUBSTANCES
Simultaneous: sulfachlorpyridazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline, sulfathiazole

REFERENCE
Thomas,G.K.; Millar,R.G.; Anstis,P.W. Stability of sulfonamide antibiotics in spiked pig liver tissue during frozen storage, *J.AOAC Int.*, **1997**, *80*, 988–995.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax-Sil
Mobile phase: Dichloromethane:MeOH:1 M perchloric acid 100:9:0.4
Flow rate: 0.8
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 7.4

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, dapsone, desethylchloroquine, dihydroquinidine, dihydroquinine, primaquine, proguanil, pyrimethamine, quinidine, quinine, sulfalene, sulfamethoxazole
Interfering: mefloquine

KEY WORDS
normal phase

REFERENCE
Dua,V.K.; Sarin,R.; Prakash,A. Determination of quinine in serum, plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 87–93.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 56.0

OTHER SUBSTANCES
Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadimethoxine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine, sulfisoxazole

REFERENCE
Nishikawa,M.; Takahashi,Y.; Ishihara,Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues, *J.Liq.Chromatogr.*, **1993**, *16*, 4031–4047.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 µL initial mobile phase, centrifuge, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 µm LiChrospher 100 RP-18
Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 0.8
Injection volume: 50
Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 µL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 24
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 µL 20 µg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min.

Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72
Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM

Retention time: 10.4
Internal standard: sulfaethoxypyridazine (12.8)

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfathiazole

KEY WORDS

cow; pig; muscle; liver; SPE

REFERENCE

Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 15
Limit of quantitation: 1 ng/g

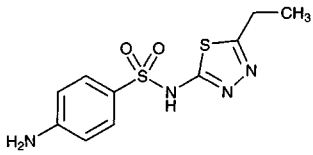
OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

Sulfaethidole



Molecular formula: $C_{10}H_{12}N_4O_2S_2$
Molecular weight: 284.36
CAS Registry No.: 94-19-9
Merck Index: 9075
Lednicer No.: 1 125

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

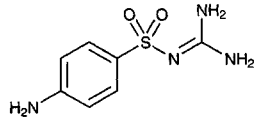
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine,

pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Sulfaguanidine



Molecular formula: $C_7H_{10}N_4O_2S$
Molecular weight: 214.25
CAS Registry No.: 57-67-0
Merck Index: 9076
Lednicer No.: 1 123

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

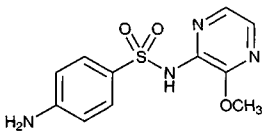
Retention time: 3.795

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Sulfalene



Molecular formula: $C_{11}H_{12}N_4O_3S$
Molecular weight: 280.31
CAS Registry No.: 152-47-6, 50933-06-7 (mixture with trimethoprim)
Merck Index: 9078
Lednicer No.: 1 125

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

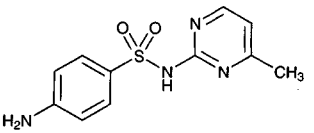
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.217

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Sulfamerazine



Molecular formula: $C_{11}H_{12}N_4O_2S$
Molecular weight: 264.31
CAS Registry No.: 127-79-7, 127-58-2 (monosodium salt)
Merck Index: 9081
Lednicer No.: 1 124

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: water

Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 6
Internal standard: niacin (3.3)

OTHER SUBSTANCES

Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamethoxazole, sulfamonomethoxine

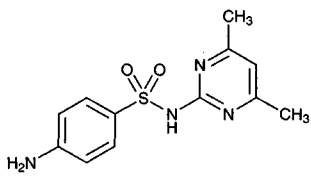
KEY WORDS

wastewater

REFERENCE

Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfamethazine



Molecular formula: $C_{12}H_{14}N_4O_2S$
Molecular weight: 278.33
CAS Registry No.: 57-68-1
Merck Index: 9083
Lednicer No.: 1 125

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 150 μL 3% trichloroacetic acid in EtOH + 100 μL EtOH, vortex, freeze at -20° for 5 min, centrifuge, freeze at -20° for 10 min, centrifuge through a Spin-X filter tube, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18 DB
Column: 250 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeCN:buffer 23:77 with 0.1% triethylamine added (Buffer was 25 mM sodium phosphate and 20 mM sodium 1-hexanesulfonate, pH adjusted to 2.8 with 5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 10
Detector: UV 270

CHROMATOGRAM

Retention time: 8
Internal standard: sulfamethazine (sulfadimidine)

Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 6
Internal standard: niacin (3.3)

OTHER SUBSTANCES

Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamethoxazole, sulfamonomethoxine

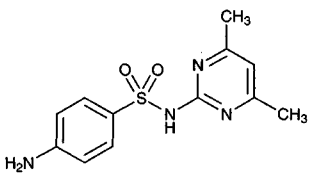
KEY WORDS

wastewater

REFERENCE

Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfamethazine



Molecular formula: $C_{12}H_{14}N_4O_2S$
Molecular weight: 278.33
CAS Registry No.: 57-68-1
Merck Index: 9083
Lednicer No.: 1 125

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 150 μL 3% trichloroacetic acid in EtOH + 100 μL EtOH, vortex, freeze at -20° for 5 min, centrifuge, freeze at -20° for 10 min, centrifuge through a Spin-X filter tube, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18 DB
Column: 250 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeCN:buffer 23:77 with 0.1% triethylamine added (Buffer was 25 mM sodium phosphate and 20 mM sodium 1-hexanesulfonate, pH adjusted to 2.8 with 5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 10
Detector: UV 270

CHROMATOGRAM

Retention time: 8
Internal standard: sulfamethazine (sulfadimidine)

OTHER SUBSTANCES
Simultaneous: sulfadiazine, trimethoprim

KEY WORDS
plasma; fish; salmon; trout; sulfamethazine is IS

REFERENCE
Hormazabal,V.; Rogstad,A. Simultaneous determination of sulfadiazine and trimethoprim in plasma and tissues of cultured fish for residual and pharmacokinetic studies, *J.Chromatogr.*, **1992**, *583*, 201–207.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut 40 μm C18 SPE cartridge with 2 mL MeOH and 2 mL pH 7.4 phosphate buffer. Add 1 mL plasma to the SPE cartridge, wash with 2 mL pH 7.4 phosphate buffer, elute with 250 μL MeOH, add 750 μL pH 6.4 phosphate buffer to the eluate, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher RP18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: MeOH:50 mM pH 6.4 phosphate buffer 25:75
Column temperature: 35
Flow rate: 0.9
Injection volume: 20
Detector: UV 262, UV 292

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 1.1 μg/mL
Limit of quantitation: 3.7 μg/mL

OTHER SUBSTANCES
Extracted: metabolites, acetylsulfamethazine

KEY WORDS
plasma; sheep; SPE; pharmacokinetics

REFERENCE
Hubert,P.; Chiap,P.; Evrard,B.; Delattre,L.; Crommen,J. Fully automated determination of sulfamethazine in ovine plasma using solid-phase extraction on disposable cartridges and liquid chromatography, *J.Chromatogr.*, **1993**, *622*, 53–60.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bakerbond C18 SPE cartridge with MeOH and 50 mM pH 5.5 citrate buffer. 500 μL Serum + 500 μL 50 mM pH 5.5 citrate buffer, vortex, add to the SPE cartridge, wash twice with 50 mM pH 5.5 citrate buffer, air dry, elute with MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:1% acetic acid 18:82
Flow rate: 2.5
Injection volume: 100
Detector: UV 240

CHROMATOGRAM
Retention time: 4.19
Internal standard: sulfamethazine

OTHER SUBSTANCES
Extracted: sulfamethoxazole, trimethoprim

KEY WORDS
serum; SPE; sulfamethazine is IS

REFERENCE
Moore,K.H.P.; Brouwer,K.L.R. High-performance liquid chromatographic evaluation of the effect of heat treatment on trimethoprim and sulfamethoxazole stability in serum, *Ther.Drug Monit.*, **1995**, *17*, 356–360.

SAMPLE
Matrix: blood, culture media, microsomal incubations, urine
Sample preparation: Plasma, urine, culture medium. 0.3 (Plasma, urine) or 2.5 (culture medium) mL sample + 1 mL 500 mM pH 4.5 acetate buffer + 20 mg limpet acetone powder (from Patella vulgata, Sigma), heat at 37° for 3 h, add 60 μL 4 M NaOH, add 1 mL 500 mM pH 6.0 phosphate buffer, add 200 μL 90 μg/mL sulfadimethoxine in MeOH:water 15:85, saturate with solid anhydrous ammonium sulfate, add 4 mL diethyl ether:dichloromethane:isopropanol 60:40:0.5, extract, centrifuge at 500 g for 10 min, repeat the extraction with 3 mL diethyl ether:dichloromethane:isopropanol 60:40:0.5. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 (urine) or 0.2 (plasma) mL mobile phase, inject a 20 μL aliquot. Microsomal incubations. Microsomal incubation + 1 mL 500 mM pH 6.0 phosphate buffer + 200 μL 90 μg/mL sulfadimethoxine in MeOH:water 15:85, saturate with solid anhydrous ammonium sulfate, add 4 mL diethyl ether:dichloromethane:isopropanol 60:40:0.5, extract, centrifuge at 500 g for 10 min, repeat the extraction with 3 mL diethyl ether:dichloromethane:isopropanol 60:40:0.5. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 (urine) or 0.2 (plasma) mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm Hypersil ODS RP-C18
Column: 100 × 4 5 μm Hypersil ODS RP-C18
Mobile phase: MeOH:50 mM pH 6.67 phosphate buffer 10:90
Flow rate: 0.8
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 16.5
Internal standard: sulfadimethoxine (28)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; goat; rat

REFERENCE
van 't Klooster,G.A.E.; van Seeventer,P.B.; Kolker,H.J.; Smit,L.A.; Witkamp,R.F. High-performance liquid chromatographic method for the routine determination of sulphadimidine, its hydroxy metabolites and N4-acetylsulphadimidine in body fluids and cell culture media, *J.Chromatogr.*, **1991**, *571*, 157–168.

SAMPLE
Matrix: blood, eggs
Sample preparation: 1 mL Serum or 1 g homogenized egg + 4 mL MeCN, vortex, centrifuge at 3000 rpm for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 3000 rpm for 15 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 0.01% trichloroacetic acid, centrifuge at 3000 rpm for 15 min. Remove a 500 μL aliquot and add it to 100 μL 1 mg/mL fluorescamine in MeCN (freshly prepared), shake, let stand for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: chemiluminescence following post-column reaction. The column effluent was mixed with reagent pumped at 0.5 mL/min and the mixture flowed to the detector. (Reagent was 1 mM bis[2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitrophenyl] oxalate (Wako) and 300 mM hydrogen peroxide in MeCN.)

CHROMATOGRAM
Retention time: 8.1
Limit of detection: 1 ng/mL

KEY WORDS
chicken; serum; derivatization

REFERENCE
Tsai,C.-E.; Kondo,F.; Ueyama,Y.; Azama,J. Determination of sulfamethazine residue in chicken serum and egg by high-performance liquid chromatography with chemiluminescence detection, *J.Chromatogr.Sci.*, **1995**, *33*, 365–369.

SAMPLE
Matrix: blood, milk
Sample preparation: 1 mL Serum or milk + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL p-aminobenzoic acid in 0.01% trichloroacetic acid, centrifuge at 1000 g for 10 min. Remove a 500 μL aliquot of the clear layer and add it to 100 μL 1 mg/mL fluorescamine in acetone (prepared fresh each day), mix for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 7.5
Internal standard: p-aminobenzoic acid (5.5)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfadimethoxine, sulfamethoxazole, sulfamonomethoxine, sulfathiazole

KEY WORDS
cow; serum; derivatization

REFERENCE
Tsai,C.-E.; Kondo,F. Liquid chromatographic determination of fluorescent derivatives of six sulfonamides in bovine serum and milk, *J.AOAC Int.*, **1995**, *78*, 674–678.

SAMPLE
Matrix: blood, tissue
Sample preparation: 1 mL Serum or homogenized tissue + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 15 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL sulfadiazine in 0.01% trichloroacetic acid, shake, add 100

μL hexane, shake, centrifuge at 1000 g for 15 min. Remove a 500 μL aliquot of the clear aqueous layer and add it to 100 μL 1 mg/mL fluorescamine in MeCN (freshly prepared), shake by hand for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pack C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 7.9
Internal standard: sulfadiazine (7.1)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfamethoxazole, sulfamonomethoxine, sulfadimethoxine

KEY WORDS
serum; pig; derivatization; kidney; muscle; liver

REFERENCE
Tsai,C.-E.; Kondo,F. A sensitive high-performance liquid chromatographic method for detecting sulfonamide residues in swine serum and tissues after fluorescamine derivatization, *J.Liq.Chromatogr.*, **1995**, *18*, 965–976.

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. 320 μL Whole blood + 450 μL urea (1:1), shake mechanically for 15 min, filter (Amicon Micropartition System MPS-1) while centrifuging at 4° at 4000 rpm for at least 3 h, inject a 300 μL aliquot of the ultrafiltrate. Urine. Dilute urine if necessary. Filter (Amicon Micropartition System MPS-1) urine while centrifuging at 4° at 4000 rpm for at least 3 h, inject a 300 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 mm long 5 μm Spherisorb ODS 2
Mobile phase: MeOH:MeCN:10 mM pH 5.0 sodium acetate buffer containing 4 mM triethylamine 14:14:72
Flow rate: 0.8
Injection volume: 300
Detector: UV 275

CHROMATOGRAM
Internal standard: sulfamethazine

OTHER SUBSTANCES
Extracted: trimethoprim

KEY WORDS
fish; whole blood; trout; rainbow trout; sulfamethazine is IS

REFERENCE
Tan,W.P.; Wall,R.A. Disposition kinetics of trimethoprim in rainbow trout (*Oncorhynchus mykiss*), *Xenobiotica*, **1995**, *25*, 315–329.

SAMPLE
Matrix: blood, urine, tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL dichloromethane and dry for 15 min under a gentle stream of nitrogen. Mix 5 g minced tissue or 0.5-2 mL plasma

or 0.2-1 mL urine (adjusted to pH 6.5-7.0 with 250 mM acetic acid) with 20 mL dichloromethane, vortex for 30 s, centrifuge at 2000 g for 10 min. Filter the supernatant, dry over glass wool and anhydrous sodium sulfate, weigh the filtered extract. Add the sample to the SPE cartridge, elute with 6 mL 50 mM pH 10 phosphate buffer, collect the first 4 mL eluate, weigh, mix, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 30 μm Perisorb C8 (Chrompack)
Column: 200 × 3 5 μm Chromspher C18
Mobile phase: MeCN:pH 5.3 ammonium acetate buffer 22:78
Flow rate: 0.5
Injection volume: 100
Detector: UV 261

CHROMATOGRAM

Limit of detection: 10 ng/g (tissue), 50 ng/mL (urine, plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pig; muscle; liver; kidney; SPE

REFERENCE

Haasnoot,W.; Korsrud,G.O.; Cazemier,G.; Manevals,F.; Keukens,H.; Nouws,J. Application of an enzyme immunoassay for the determination of sulphamethazine (sulphadimidine) residues in swine urine and plasma and their use as predictors of the level in edible tissue, *Food Addit.Contam.*, **1996**, *13*, 811–822.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 200 mg amoxicillin trihydrate in 8 mL 200 mM pH 11.0 phosphate buffer, add 10 mL 70 mg/L sulfamethazine in solvent, make up to 20 mL with solvent, inject an aliquot within 1 min. Dissolve 200 mg amoxicillin sodium salt in 8 mL solvent, add 10 mL 70 mg/L sulfamethazine in solvent, make up to 20 mL with solvent, inject an aliquot within 1 min. (Solvent was MeOH:200 mM pH 7.0 potassium phosphate buffer:water 5:5:90.)

HPLC VARIABLES

Guard column: 40 × 4.6 10 μm LiChrosorb RP-2
Column: 250 × 4.6 7μm Zorbax C8
Mobile phase: Gradient. A is MeOH:200 mM pH 7.0 potassium phosphate buffer:water 5:5:90. B is MeOH:200 mM pH 7.0 potassium phosphate buffer:water 50:5:45. A:B 95:5 for 5 min, to 35:65 over 30 min, to 95:5 over 7.5 min.
Column temperature: 30
Flow rate: 1
Injection volume: 25
Detector: UV 274

CHROMATOGRAM

Retention time: 35
Internal standard: sulfamethazine

OTHER SUBSTANCES

Simultaneous: amoxicillin, impurities, amoxicilloates, amoxicillin piperazine-2,5-dione, amoxicillin dimer, amoxicillin trimer

KEY WORDS

sulfamethazine is IS

REFERENCE

De Pourcq,P.; Hoebus,J.; Roets,E.; Hoogmartens,J.; Vanderhaeghe,H. Quantitative determination of amoxicillin and its decomposition products by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *321*, 441–449.

SAMPLE

Matrix: bulk, solutions, tissue

Sample preparation: Oil. Mix 200 μL oil with 100 μL 10 ng/mL sulphamerazine and dilute with 2 mL hexane. Extract with 1 mL 10 mM hydrochloric acid. Mix 200 μL acid layer with 100 μL MeCN, inject a 25 μL aliquot. Solutions. Directly inject an aliquot, use fluorescence or UV detection. Tissue. A. 5 g Tissue + IS + 25 mL 5% acetic acid in ethyl acetate, homogenize for 1 min, centrifuge at 3000 rpm for 5 min. Repeat this procedure. Decant supernatant onto NH2 and SCX SPE cartridges in series. Discard NH2 cartridge. Wash SCX cartridge with 5 mL water, wash with 10 mL acetone, wash with 10 mL MeCN. Elute with 10 mL MeOH:ammonia 50:50. Blow-down extract to dryness, add 2 mL 10 mM hydrochloric acid. Add 100 μL 2 mg/mL fluorescamine to a 200 μL aliquot, mix well and wait for 20 min. Inject a 10 μL aliquot, use fluorescence detection. B. Homogenize tissue in dichloromethane, dry with sodium sulfate, centrifuge at 1600 rcf for 5 min. Mix supernatant with hexane, add to an activated silica SPE cartridge. Dry SPE cartridge with nitrogen, elute with MeOH. Evaporate MeOH and reconstitute with 1 mL pH 6.5 mobile phase. Inject an aliquot, use UV detection.

HPLC VARIABLES

Column: 100 × 5 4 μm NovaPak C18

Mobile phase: MeCN:buffer 20:80 (Buffer was 77 mg ammonium acetate in 800 mL water)

Flow rate: 1

Injection volume: 25

Detector: F ex 405 em 495, UV 270

CHROMATOGRAM

Retention time: 6.8

Internal standard: sulphamerazine

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

kidney; liver; muscle; pig; derivatization; SPE

REFERENCE

Rose,M.D.; Farrington,W.H.; Shearer,G. The effect of cooking on veterinary drug residues in food: 3. Sulphamethazine (sulphadimidine), *Food Addit.Contam.*, **1995**, *12*, 739–750.

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 6.3
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole
Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: feed
Sample preparation: Blend 10-100 g feed with 200 mL chloroform:MeOH 75:25 for 3 min, filter through 25 mm Celite 545, re-extract residue with 200 mL chloroform:MeOH 75:25, add 50 mL chloroform:MeOH 75:25 to the residue, filter this mixture, wash the filter cake with several small portions of chloroform:MeOH 75:25. Evaporate the filtrate on a steam bath with a current of air to about 100 mL. Shake the organic layer with 50 mL 10% NaCl in 100 mM NaOH for 1 min, wash the aqueous phase with two or three 50 mL portions of chloroform (until wash is colorless), add 10 mL 1 M KH_2PO_4 solution to the aqueous phase, extract with three 50 mL portions of chloroform. Combine the extracts and filter them through a 25 mm layer of sodium sulfate, evaporate most of the filtrate on a steam bath with a current of air, evaporate the remainder with a current of air. Take up the residue in 100 mL MeOH, remove a 20 mL aliquot, evaporate most on a steam bath with a current of air, evaporate the remainder with a current of air, dissolve the residue in 100 μL MeOH, make up to 10 mL with MeCN:water 25:75, filter (0.45μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.5% acetic acid and 0.05% sodium 1-octanesulfonate.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5

OTHER SUBSTANCES

Simultaneous: carbadox

REFERENCE

McGary,E.D. Quantitative determination of sulphamethazine and carbadox in animal feeds by paired ion high-performance liquid, *Analyst*, **1986**, *111*, 1341–1342.

SAMPLE
Matrix: feed
Sample preparation: Condition a 3 mL 500 mg cation-exchange SPE cartridge (aromatic sulfonic acid, J.T. Baker) with 10 mL MeCN:water:glacial acetic acid 70:30:20, do not allow to go dry. Grind feed to pass through a 1 mm sieve. 10 g Ground feed + 10 mL MeCN:water 35:15, shake mechanically for 1 h, filter (Whatman glass fiber GF/A). Remove a 10 mL aliquot of the filtrate and add it to 5 mL acetic acid, shake gently, add to the SPE cartridge, rinse flask with 5 mL MeCN:water:glacial acetic acid 70:30:20, add rinse to the SPE cartridge, wash with 10 mL water, wash with 10 mL MeOH, pass nitrogen through the SPE cartridge for 15 min, elute with 30 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Rosil C18 (Alltech)
Mobile phase: MeCN:buffer:water 22.5:15:62.5 (Buffer was 19.27 g ammonium acetate and 30 mL acetic acid made up to 1 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 272

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Noninterfering: acinitrazole, amprolium, arprinocid, buquinolate, carbadox, clopidol, decoquinate, diaveridine, dimetridazole, dinitolmide, ethopabate, furazolidone, halofuginone, ipronidazole, methyl benzoquate, nifursol, nitrofurazone, nitrovin, olaquindox, pyrimethamine, robenidine, ronidazole, sulfanitran, sulfaquinoxaline

KEY WORDS
SPE

REFERENCE
Conway,B. Determination of sulphadimidine in animal feeds by high-performance liquid chromatography, *Analyst*, **1988**, *113*, 1397–1400.

SAMPLE
Matrix: feed
Sample preparation: 20 g Ground feed + 100 mL solvent + 5 mL 8 μg/mL sulfamerazine in diluent, shake for 1 h, chill an aliquot in an ice bath for 2 h, centrifuge at 1650 g for 5 min, filter (0.2 μm), inject a 200 μL aliquot of the filtrate. (Prepare solvent by mixing 250 mL MeOH, 300 mL water, and 25 mL HCl, mix, add 15 mL diethylamine, mix, make up to 1 L with water. Prepare diluent by mixing 250 mL MeOH, 300 mL water, and 12.5 mL HCl, mix, add 15 mL diethylamine, mix, make up to 1 L with water.)

HPLC VARIABLES
Guard column: C8 or C18
Column: 250 × 4.67 μm Lichrosorb RP-18
Mobile phase: MeCN:2% acetic acid 17:83
Flow rate: 1-1.3
Injection volume: 200
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.1 -0.5 mL/min and the mixture flowed through a 3 m × 0.5 mm i.d. PTFE coil to the detector. (Prepare reagent by dissolving 1.5 g dimethylaminobenzaldehyde in 100 mL glacial acetic acid, add 60 mL MeOH, mix well, add 40 mL water, mix well, prepare fresh daily.)

CHROMATOGRAM
Retention time: 8.5
Internal standard: sulfamerazine (7)
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: sulfathiazole

Simultaneous: sulfadimethoxine, sulfaquinoxaline
Noninterfering: amino acids, amprolium, apramycin, arsanilic acid, bacitracin, hygromycin B, neomycin, nystatin, ormetoprim, procaine

KEY WORDS
post-column reaction

REFERENCE
Smallidge,R.L.; Kentzer,E.J.; Stringham,K.R.; Kim,E.H.; Lehe,C.; Stringham,R.W.; Mundell,E.C. Sulfamethazine and sulfathiazole determination at residue levels in swine feeds by reverse-phase liquid chromatography with post-column derivatization, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 710–717.

SAMPLE
Matrix: feed
Sample preparation: Weigh out 1 g ground feed, add 3 mL trichloroacetic acid solution, mix well, sonicate at 40° for 10 min, make up to 500 mL with MeCN:10 mM Na_2HPO_4 adjusted to pH 3 with phosphoric acid 20:80, mix well, filter a 500 μL aliquot (Costar spin-X (low type) 0.22 μm cellulose acetate) with centrifuging for 1 min, inject a 10 μL aliquot of the filtrate. (Prepare trichloroacetic acid solution by mixing 87 g trichloroacetic acid with 13 g water, add 0.7 mL of this solution to 99.3 mL acetone.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil-LC-18-DB
Column: 250 × 4.6 5 μm Supelcosil-LC-18-DB
Mobile phase: MeCN containing 0.1% triethylamine:10 mM pH 2.8 Na_2HPO_4 21:79
Flow rate: 0.9
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 8
Internal standard: sulfamethazine (sulfadimidine)
Limit of quantitation: 250 μg/g

OTHER SUBSTANCES
Simultaneous: sulfadiazine, trimethoprim

KEY WORDS
sulfamethazine is IS

REFERENCE
Hormazabal,V.; Steffanak,I.; Yndestad,M. Simultaneous extraction and determination of sulfadiazine and trimethoprim in medicated fish feed by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *648*, 183–186.

SAMPLE
Matrix: feed, premix
Sample preparation: Shake premix or ground feed with 150 mM HCl in MeOH:water 25:75 for 1 h, dilute with 150 mM HCl in MeOH:water 25:75 to achieve a sulfonamide concentration of 5.5 μg/mL, filter (glass fiber), inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2 30-40 μm Perisorb RP-18
Column: 250 × 4.6 10 μm Partisil ODS-3
Mobile phase: MeOH:2% acetic acid 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 450 following post-column reaction. The column effluent mixed with reagent pumped at 0.5 mL/min and the mixture flowed through a 3 m × 0.5 mm ID coil of PTFE tubing to the detector. (Prepare reagent by dissolving 3 g dimethylaminobenzaldehyde in 100 mL glacial acetic acid, add 60 mL MeOH, add 40 mL water, mix well.)

CHROMATOGRAM
Retention time: 6
Limit of quantitation: 1.65 μg/mL

KEY WORDS
post-column reaction

REFERENCE
Stringham,R.W.; Mundell,E.C.; Smallidge,R.L. Use of post-column derivatization in liquid chromatographic determination of sulfamethazine and sulfathiazole in feeds and feed premixes, *J.Assoc.Off.Anal.Chem.*, **1982**, *65*, 823–827.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Suspension + 100 mL MeOH:water 60:40, shake mechanically for 15 min, make up to 200 mL with MeOH:water 60:40, filter (0.45 μm silver membrane, Selas Corp.). Evaporate a 1 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL 200 μg/mL acetanilide in MeCN, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water 20:80
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: acetanilide (11)

OTHER SUBSTANCES
Simultaneous: sulfadiazine, sulfamerazine, sulfanilamide, sulfanilic acid
Noninterfering: erythromycin ethylsuccinate

KEY WORDS
oral suspensions; suspensions

REFERENCE
Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20

Detector: UV 270

CHROMATOGRAM
Retention time: 4.5
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfathiazole, sulfadiazine, sulfisoxazole, sulfadimethoxine

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 26.6
Limit of detection: 1.7 ppb
Limit of quantitation: 3.6 ppb

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamerazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 8.5
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole
Interfering: sulfamethizole (distinguish by MS)

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 5.56

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 PLRPS polymer-based (Polymer Laboratories)
Mobile phase: MeCN:buffer 22.5:80 (Buffer was 7.7 g ammonium acetate, 3 g tetraethylammonium chloride, and 1.8 g EDTA in 1 L water.)
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Simultaneous: sulfapyridine, N-acetylsulfapyridine, sulfasalazine

REFERENCE
Buggé,C.J.; Gautam,S.R.; Parke,L.E.; Mason,J.T.; Garcia,D.B. Simultaneous determination of sulfasalazine and its metabolites sulfapyridine and N-acetylsulfapyridine in human serum by ion-pair high-performance liquid chromatography using a polymer-based column, *J.Pharm.Sci.*, **1990**, *79*, 1095–1098.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfanilic acid, sulfathiazole, sulfisoxazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 8.51

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: ethylmorphine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 37.3

OTHER SUBSTANCES
Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadimethoxine, sulfadoxine, sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine, sulfisoxazole

REFERENCE
Nishikawa,M.; Takahashi,Y.; Ishihara,Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues, *J.Liq.Chromatogr.*, **1993**, *16*, 4031–4047.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine,

pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Sentry (Waters)
Column: 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: MeOH:water:glacial acetic acid 20:79:1
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7.3

OTHER SUBSTANCES

Simultaneous: sulfanilamide, sulfadiazine, sulfamerazine, sulfathiazole, succinylsulfathiazole

REFERENCE

Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: MeCN:50 mM acetic acid 10:90
Flow rate: 2
Detector: UV 266

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: metabolites, acetylsulfamethazine

REFERENCE

Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

SAMPLE
Matrix: solutions
Sample preparation: 1 mL 1 mg/mL Solution + 1 mL 100 mM pH 8 sodium bicarbonate + 2 mL 10 mM 1-fluorenylmethyl chloroformate in acetone, let stand for 30 min, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Micro Pak C18 TMS-capped
Mobile phase: Gradient. MeOH:buffer from 35:65 to 85:15 over 4 min (Waters medium concave gradient), maintain at 85:15. (Buffer was 50 mM NaH_2PO_4 adjusted to pH 3.5 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 264 or F ex 264 em 307

CHROMATOGRAM
Retention time: 12.5

KEY WORDS
derivatization

REFERENCE
Liang,G.S.; Zhang,Z.; Baker,W.L.; Cross,R.F. Formation and verification of the structure of the 1-fluorenylmethyl chloroformate derivative of sulfamethazine, *Anal.Chem.*, **1996**, *68*, 86–92.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim
Interfering: sulfamoxole

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 35

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim
Interfering: sulfamoxole

KEY WORDS

capillary HPLC

REFERENCE

Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE

Matrix: tissue
Sample preparation: Cut 20 g tissue into small pieces and add to 25 mL dichloromethane, let stand for 20-30 min with frequent stirring with a glass rod, filter through glass wool, repeat extraction twice more. Combine the dichloromethane layers and shake with 10 mL 1.5 M sulfuric acid for 15 min. Remove the aqueous phase and add it to 10 mL dichloromethane, shake for 10 min, discard the organic phase, repeat the wash, add 1 mL 10% K_2HPO_4 to the aqueous phase, add 3 mL 40% NaOH, mix well, allow to cool, adjust pH to 5-6 with 1.5 M sulfuric acid or 40% NaOH, add 5 mL dichloromethane, extract for 15 min, repeat the extraction. Pass the organic layers through anhydrous sodium sulfate, evaporate to 1 g on a hot plate at 60-70° (in a hood!), inject a 10 μL aliquot. For confirmation of sulfamethazine add 1 mL MeOH to the extract, add 2 drops acetic anhydride, heat at 80-90° on a hot plate until acetic acid fumes are no longer seen, reconstitute with 1 g dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 mm long MicroPak CN-10
Mobile phase: Isooctane:chloroform:MeOH:acetic acid 30.5:65:4:0.5
Flow rate: 0.33
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 4.34 , 5.34 (for acetylsulfamethazine)
Limit of detection: 20 ppb

OTHER SUBSTANCES

Simultaneous: sulfabromomethazine, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfaethoxypridazine
Noninterfering: sulfamerazine, sulfathiazole, sulfanilamide, sulfapyridine, sulfaquinoline

KEY WORDS

cow; liver; fat; kidney; muscle; derivatization

REFERENCE
Seymour,D.; Rupe,B.D. High-pressure liquid chromatographic determination of sulfamethazine residues in beef tissues, *J.Pharm.Sci.*, **1980**, *69*, 701–703.

SAMPLE
Matrix: tissue
Sample preparation: Blend (Waring blender) 10 g tissue and 150 mL chloroform:acetone 50:50 at low speed for 5 min, filter homogenate through 3-4 g Celite (previously washed with 25 mL acetone), rinse blender with acetone, add rinse to filter. Add 10 mL 1 M HCl to the filtrate and evaporate to 5-7 mL under reduced pressure at 40-45°, wash residue with 75 mL hexane. Extract hexane layer twice with 5 mL 1 M HCl. Combine all the aqueous layers and wash with 15 mL dichloromethane. Rinse apparatus with water and add the rinses to the aqueous layer, add 25 mL saturated trisodium citrate to the aqueous layer, adjust pH to 5.8-5.9 with 2 M NaOH, extract the aqueous layer four times with 15 mL portions of dichloromethane. Combine the extracts and evaporate them to dryness under reduced pressure at 40-45°, dissolve residue in 10 mL buffer, add to XAD-2 column, rinse flask three times with 15 mL portions of water, add rinses to column, rinse flask with 100 mL water, add rinse to column, rinse flask with 100 mL MeOH, add rinse to column, collect the MeOH eluate, remove residual MeOH with nitrogen pressure. Add 10 mL 1 M HCl to the eluate, evaporate to dryness under reduced pressure at 40-45°, reconstitute with 8 mL mobile phase, inject a 20 μL aliquot. (Buffer was 3.40 g KH_2PO_4 and 3.55 g Na_2HPO_4 in 1 L water, pH 6.8. Prepare XAD-2 column as follows. Wash (at 60-65 mL/min) 5 kg Amberlite XAD-2 resin in a 1200 × 110 column with water, 3 gallons acetone, 2 gallons MeOH, and 25 gallons water, remove fines by successive decantations, store in water. Prepare a 130 × 15 column of washed resin and wash with 250 mL water. Note that XAD-2 column step is not necessary for fat samples.)

HPLC VARIABLES
Guard column: RP-18
Column: 250 × 4.6 Zorbax ODS
Mobile phase: MeCN:water 25:75
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7-8
Limit of detection: 0.1 ppm

KEY WORDS
pig; liver; kidney; muscle; fat; SPE

REFERENCE
Cox,B.L.; Krzeminski,L.F. High pressure liquid chromatographic determination of sulfamethazine in pork tissue, *J.Assoc.Off.Anal.Chem.*, **1982**, *65*, 1311–1315.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 μL initial mobile phase, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18

Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 0.8
Injection volume: 50
Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 μL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 20
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

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KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Blend 3 g meat with 30 mL chloroform for 2 min in a Polytron homogenizer, shake for 10 min, centrifuge at 1600 g for 5 min, filter (5A filter paper). Add 5 mL filtrate to 1 mL 3 M HCl, shake for 10 min, centrifuge at 1600 g for 5 min. 250 μL Aqueous layer + 250 μL 3.5 M sodium acetate solution, vortex, add 100 μL 0.2% fluorescamine in acetone, vortex, let stand for 20 min at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:2% acetic acid 5:3
Column temperature: 55
Flow rate: 1
Injection volume: 10
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 0.005 ng/g

OTHER SUBSTANCES
Simultaneous: sulfisomidine, sulfamethoxazole, sulfamerazine, sulfadiazine, sulfamonomethoxine, sulfadimethoxine, sulfaquinoxaline

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KEY WORDS
cow; pig; chicken; ham; sausage; roast beef; derivatization

REFERENCE
Takeda,N.; Akiyama,Y. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products, *J.Chromatogr.*, **1991**, *558*, 175–180.

SAMPLE
Matrix: tissue

Sample preparation: Cut tissue into small pieces and homogenize in blender. 20 g Homogenized tissue + 200 μL 10 μg/mL methyl p-aminobenzoate in water + 60 mL acetone:chloroform 50:50, shake vigorously on a mechanical shaker for 10 min, centrifuge at 3000 g for 10 min, filter (Whatman No. 41 paper) supernatant, repeat extraction. Combine the extracts, if the extract is not clear centrifuge at 3000 g for 10 min and discard the aqueous layer, evaporate to an oily residue at 45° under reduced pressure, add 5 mL MeCN to flask, let stand for 10 min, remove MeCN layer, add 5 mL hexane and 5 mL MeCN, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer, add 5 mL MeCN to the hexane layer, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer. If hexane layer is not clear centrifuge at 3000 g for 10 min and remove the clear portion. Add 400 μL 15% trichloroacetic acid to the hexane layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Evaporate the MeCN layers, transfer the oily residue to a small flask with 3 mL hexane, add the aqueous trichloroacetic acid layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Discard the hexane layer, add 100 μL saturated aqueous sodium citrate solution to the aqueous layer, mix, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP 18 (Brownlee)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 1% aqueous acetic acid. B was MeCN:water 80:20. A:B from 90:10 to 60:40 over 20 min, return to initial conditions over 5 min, re-equilibrate for 5 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 7 m × 0.25 mm i.d. coil of stainless steel tubing to the detector. (Prepare reagent by dissolving 1 g p-dimethylaminobenzaldehyde in 30 mL MeCN, make up to 100 mL with 5% trichloroacetic acid in water.)

CHROMATOGRAM

Retention time: 14.4
Internal standard: methyl p-aminobenzoate (18.6)
Limit of detection: 20 ng/g

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfamerazine, sulfamethoxypyridazine, sulfapyridine, sulfaquinoxaline

KEY WORDS

chicken; liver; pig; kidney; sheep; cow; post-column reaction

REFERENCE

Bui,L.V. Liquid chromatographic determination of six sulfonamide residues in animal tissues using postcolumn derivatization, *J.AOAC Int.*, **1993**, *76*, 966–976.

SAMPLE

Matrix: tissue
Sample preparation: Extract with supercritical carbon dioxide into a MeOH solution.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:100 mM KH_2PO_4 adjusted to pH 4.5 with phosphoric acid 28:72
Flow rate: 0.5
Detector: UV 270

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Simultaneous: sulfamerazine, sulfamethoxazole, sulfamethizole, sulfamethoxypyridazine, N^4-acetylsulfamethoxazole

KEY WORDS

chicken; pig; liver; muscle; SFE

REFERENCE
Cross,R.F.; Ezzell,J.L.; Richter,B.E. The supercritical fluid extraction of polar drugs (sulphonamides) from inert matrices and meat animal products, *J.Chromatogr.Sci.*, **1993**, *31*, 162–169.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM
Retention time: 6
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole
Interfering: sulfamethoxypyridazine

KEY WORDS
post-column reaction; muscle; liver; kidney; SPE

REFERENCE
Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 μL 20 μg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the

extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min. Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72
Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM
Retention time: 6.0
Internal standard: sulfaethoxypyridazine (12.8)
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethoxazole, sulfamethoxypyridazine, sulfathiazole

KEY WORDS
cow; pig; muscle; liver; SPE

REFERENCE
Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 3 g ground tissue with 30 mL chloroform for 2 min, centrifuge at 3000 g for 5 min, filter (paper). Remove a 10 mL aliquot of the filtrate and add it to 1 mL 3 M HCl, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove a 250 μL aliquot of the aqueous layer and add it to 250 μL 3.8 M sodium acetate, add 100 μL 1 mg/mL fluorescamine in MeCN, vortex, let stand at room temperature for 20 min, inject a 20 μL aliquot.)Sodium acetate should be a highly pure grade.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:20 mM pH 4 NaH_2PO_4 34:66 containing 20 mM sodium octanesulfonate
Column temperature: 30
Flow rate: 1.2
Injection volume: 20
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 10
Limit of detection: 4 ng/g

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfadimethoxine, sulfaquinoxaline

KEY WORDS
derivatization; chicken; muscle

REFERENCE
Simeonidou,E.J.; Botsoglou,N.A.; Psomas,I.E.; Fletouris,D.J. Liquid chromatographic analysis of multiple sulfonamide residues in chicken muscle using pre-column derivatization and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2349–2364.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM
Retention time: 9.5
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 11

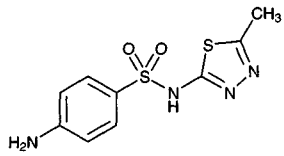
Internal standard: niacin (3.3)

OTHER SUBSTANCES
Extracted: sulfathiazole, sulfacetamide, sulfamethoxazole, sulfadiazine, sulfamerazine, sulfamonomethoxine

KEY WORDS
wastewater

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfamethizole



Molecular formula: $C_9H_{10}N_4O_2S_2$
Molecular weight: 270.34
CAS Registry No.: 144-82-1
Merck Index: 9084
Lednicer No.: 1 125

SAMPLE
Matrix: formulations
Sample preparation: Dissolve a capsule in 1 L 100 mM HCl, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax TMS
Mobile phase: DMF:water 10:90 containing 5 mM sodium ethylenediaminetetraacetate, 100 mM citric acid, 20 mM sodium citrate, and 50 mM potassium nitrate.
Flow rate: 1.8
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 6.0

OTHER SUBSTANCES
Simultaneous: tetracycline
Noninterfering: phenazopyridine

KEY WORDS
capsules

REFERENCE
Du Preez,J.L.; Botha,S.A.; Lötter,A.P. High-performance liquid chromatographic determination of phenazopyridine hydrochloride, tetracycline hydrochloride and sulphamethizole in combination, *J.Chromatogr.*, **1985**, *333*, 249–252.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 5
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfadiazine, sulfaguanidine, sulfamerazine, sulfamethoxazole, sulfanilamide, sulfathiazole
Noninterfering: benzocaine

KEY WORDS
tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE
Garcia-Alvarez-Coque,M.C.; Simo-Alfonso,E.F.; Ramis-Ramos,G.; Esteve-Romero,J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 21.9
Limit of detection: 1.8 ppb
Limit of quantitation: 3.9 ppb

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamerazine, sulfamethazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE

Matrix: milk

Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES

Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.

Flow rate: 1

Injection volume: 50-500

Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM

Retention time: 8.4

Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

Interfering: sulfamethazine (distinguish by MS)

KEY WORDS

cow; column-switching

REFERENCE

Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:water:acetic acid 12.5:86.5:1

Flow rate: 1.6

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 9.5

OTHER SUBSTANCES

Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE

Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: sulfanilic acid, sulfanilamide, sulfapyridine, sulfamerazine, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfisoxazole, sulfachlorpyridine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfanilic acid, sulfathiazole, sulfisoxazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 10.77

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 36

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 37

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS

capillary HPLC

REFERENCE

Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 µL initial mobile phase, centrifuge, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 5 µm LiChrospher 100 RP-18

Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 0.8

Injection volume: 50

Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 µL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM

Retention time: 18

Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES

Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS

post-column reaction; muscle; kidney; SPE

REFERENCE

Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Extract with supercritical carbon dioxide into a MeOH solution.

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:100 mM KH_2PO_4 adjusted to pH 4.5 with phosphoric acid 28:72
Flow rate: 0.5
Detector: UV 270

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethoxazole, sulfamethazine, sulfamethoxypyridazine, N^4-acetylsulfamethoxazole

KEY WORDS
chicken; pig; liver; muscle; SFE

REFERENCE
Cross,R.F.; Ezzell,J.L.; Richter,B.E. The supercritical fluid extraction of polar drugs (sulphonamides) from inert matrices and meat animal products, *J.Chromatogr.Sci.*, **1993**, *31*, 162–169.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 10 mL 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 µL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 µm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

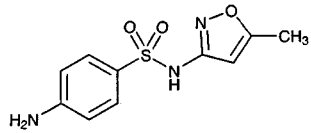
CHROMATOGRAM
Retention time: 5.3
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfaguanidine, sulfamethoxazole, sulfathiazole

KEY WORDS
derivatization

REFERENCE
Simó-Alfonso,E.F.; Ramis-Ramos,G.; García-Alvarez-Coque,M.C.; Esteve-Romero,J.S. Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography, *J.Chromatogr.B*, **1995**, *670*, 183–187.

Sulfamethoxazole



Molecular formula: $C_{10}H_{11}N_3O_3S$
Molecular weight: 253.28
CAS Registry No.: 723-46-6
Merck Index: 9086

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 µL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 µm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 10:90
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.42

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 10 mL 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 µL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 µm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

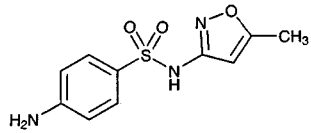
CHROMATOGRAM
Retention time: 5.3
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfaguanidine, sulfamethoxazole, sulfathiazole

KEY WORDS
derivatization

REFERENCE
Simó-Alfonso,E.F.; Ramis-Ramos,G.; García-Alvarez-Coque,M.C.; Esteve-Romero,J.S. Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography, *J.Chromatogr.B*, **1995**, *670*, 183–187.

Sulfamethoxazole



Molecular formula: $C_{10}H_{11}N_3O_3S$
Molecular weight: 253.28
CAS Registry No.: 723-46-6
Merck Index: 9086

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 µL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 µm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 10:90
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.42

OTHER SUBSTANCES
Extracted: ethosuximide, methamphetamine, primidone

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fritz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.445

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphet-

amine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 46

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 42

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 16
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS

fish; salmon; post-column reaction

REFERENCE

Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE

Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 7.7
Internal standard: niacin (3.3)

OTHER SUBSTANCES

Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamerazine, sulfamonomethoxine

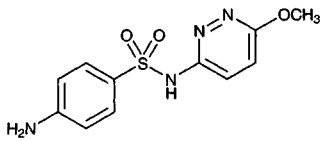
KEY WORDS

wastewater

REFERENCE

Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfamethoxypyridazine



Molecular formula: $C_{11}H_{12}N_4O_3S$
Molecular weight: 280.31
CAS Registry No.: 80-35-3, 2577-32-4 (sodium salt)
Merck Index: 9087

SAMPLE

Matrix: milk
Sample preparation: Mix 5 mL milk with 20 μL 12 M HCl, sonicate, add 25 mL ethyl acetate, extract using a rotary shaker (REAX 2, Heidolph) for 10 min. Centrifuge at 1500 g for 5 min, evaporate 20 mL of the ethyl acetate extract to dryness, dissolve the residue in 10 mL 1 HCl. Wash the aqueous phase with 10 mL dichloromethane, adjust to pH 5.5 with 900 μL 10 M NaOH and 5 mL 1 M pH 6.0 KH_2PO_4, extract with two 10 mL portions of dichloromethane. Evaporate the organic layer to dryness, dissolve the residue in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Novapak C18 (Waters)
Mobile phase: MeCN:10 mM pH 6.6 ammonium acetate 10:90
Flow rate: 1
Injection volume: 50
Detector: UV 265

CHROMATOGRAM

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Also analyzed: sulfadimethoxine (UV 271), sulfadimidine, sulfadoxine (UV 271)

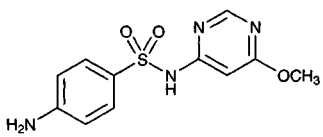
KEY WORDS

cow; milk

REFERENCE

Roudaut,B.; Moretain,J.P. Sulphonamide residues in milk of dairy cows following intravenous injection, *Food Addit.Contam.*, **1990**, *7*, 527–533.

Sulfamonomethoxine



Molecular formula: $C_{11}H_{12}N_4O_3S$
Molecular weight: 280.31
CAS Registry No.: 1220-83-3

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood. Filter serum through a 0.45 μm syringe filter with a cellulose acetate membrane, inject a 50 μL aliquot of the filtrate. Tissue. Add 1 mL MeCN:THF 95:5 to 1 g muscle, homogenize with a Pencil Mixer (Iuchi, Japan) for 2 min, centrifuge at 1500 g for 5 min, filter the supernatant through a syringe filter unit, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μL Hisep shielded hydrophobic phase precolumn (Supelco)
Column: 150 × 4.6 5 μL Hisep shielded hydrophobic phase (Supelco)
Mobile phase: MeCN:buffer 15:85 (Buffer was 50 mM citric acid:200 mM pH 2.5 Na_2HPO_4 buffer containing 10 mM tetra-*n*-butyl ammonium bromide 85:15.)

Flow rate: 1
Injection volume: 20-50
Detector: UV 265

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 50 ng/mL (serum), 100 ng/mL (muscle)

OTHER SUBSTANCES
Extracted: oxolinic acid, miloxacin

KEY WORDS
fish; muscle; serum

REFERENCE
Ueno,R.; Aoki,T. High-performance liquid chromatographic method for the rapid and simultaneous determination of sulfamonomethoxine, miloxacin and oxolinic acid in serum and muscle of cultured fish, *J.Chromatogr.B*, **1996**, *682*, 179–181.

SAMPLE
Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

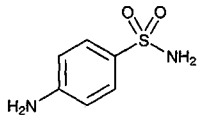
CHROMATOGRAM
Retention time: 9
Internal standard: niacin (3.3)

OTHER SUBSTANCES
Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamerazine, sulfamethoxazole

KEY WORDS
wastewater

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfanilamide



Molecular formula: $C_6H_8N_2O_2S$
Molecular weight: 172.21
CAS Registry No.: 63-74-1
Merck Index: 9094
Lednicer No.: 1 121

SAMPLE

Matrix: blood
Sample preparation: Keep tubes in crushed ice except when being processed throughout this procedure. 2 mL Plasma + 8 mL diethyl ether, vortex for 2 min, centrifuge at 4°. Remove ether layer and add it to 1 mL 100 mM NaOH, vortex for 2 min, centrifuge at 4°. Remove aqueous layer and add it to 1 mL 100 mM HCl and 500 μL 50 mM pH 7.4 sodium phosphate, add 8 mL ether, vortex for 2 min, centrifuge at 4°. Evaporate ether layer to dryness, reconstitute in 200 μL mobile phase, inject 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeCN:100 mM pH 3.6 sodium acetate buffer 43:57
Column temperature: 54
Flow rate: 1
Injection volume: 50
Detector: UV 241

CHROMATOGRAM

Retention time: 5.3
Internal standard: sulfanilamide

OTHER SUBSTANCES

Simultaneous: indapamide

KEY WORDS

plasma; sulfanilamide is IS

REFERENCE

Choi,R.L.; Rosenberg,M.; Grebow,P.E.; Huntley,T.E. High-performance liquid chromatographic analysis of indapamide (RHC 2555) in urine, plasma and blood, *J.Chromatogr.*, **1982**, *230*, 181–187.

SAMPLE

Matrix: blood, urine
Sample preparation: Keep tubes in crushed ice except when being processed throughout this procedure. Blood. 1 mL Blood + 4 mL diethyl ether, vortex for 2 min, centrifuge at 4°. Repeat extraction, combine ether layers, add 500 μL 10 mM NaOH, vortex for 2 min, centrifuge at 4°. Remove aqueous layer and add it to 500 μL 10 mM HCl and 250 μL 50 mM pH 7.4 sodium phosphate, add 4 mL ether, vortex for 2 min, centrifuge at 4°. Evaporate ether layer to dryness, reconstitute in 200 μL mobile phase, inject 50 μL aliquot. Urine. 1 mL Urine + 4 mL diethyl ether, vortex for 2 min, centrifuge at 4°. Repeat extraction, combine ether layers, add 500 μL 50 mM NaOH, vortex for 2 min, centrifuge at 4°. Remove aqueous layer and add it to 500 μL 50 mM HCl and 250 μL 50 mM pH 7.4 sodium phosphate, add 4 mL ether, vortex for 2 min, centrifuge at 4°. Evaporate ether layer to dryness, reconstitute in 200 μL mobile phase, inject 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 3.2 10 μm LiChrosorb C-18
Mobile phase: MeCN:100 mM pH 3.6 sodium acetate buffer 35:65
Column temperature: 54
Flow rate: 1.5
Injection volume: 50

Detector: UV 241

CHROMATOGRAM
Retention time: 2.5
Internal standard: sulfanilamide

OTHER SUBSTANCES
Simultaneous: indapamide

KEY WORDS
sulfanilamide is IS

REFERENCE
Choi,R.L.; Rosenberg,M.; Grebow,P.E.; Huntley,T.E. High-performance liquid chromatographic analysis of indapamide (RHC 2555) in urine, plasma and blood, *J.Chromatogr.*, **1982**, *230*, 181–187.

SAMPLE
Matrix: cell suspensions
Sample preparation: Cool cell suspension in an ice bath, centrifuge at 800 g at 4° for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water 20:80
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Also analyzed: sulfadiazine, sulfamethoxazole, sulfamerazine

REFERENCE
Climax,J.; Lenehan,T.J.; Lambe,R.; Kenny,M.; Caffrey,E.; Darragh,A. Interaction of antimicrobial agents with human peripheral blood leucocytes: uptake and intracellular localization of certain sulphonamides and trimethoprims, *J.Antimicrob.Chemother.*, **1986**, *17*, 489–498.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column

A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5

Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 1.0

Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfaquinoxaline, sulfathiazole, sulfatroxazole

Noninterfering: chloramphenicol, trimethoprim

Interfering: sulfacetamide, sulfaguanidine

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Homogenize 3 g milk and 500 μL 30% trichloroacetic acid, centrifuge at 5000 rpm for 5 min. Remove the aqueous phase and extract the residue with 4 mL 3% trichloroacetic acid. Combine the aqueous layers and make up to 10 mL with trichloroacetic acid, filter (0.45 μm), inject a 50 μL aliquot. Fish, eggs. Homogenize (Ultra-Turrax) 3 g fish or 4 g eggs with 4 mL 3% trichloroacetic acid, centrifuge at 5000 rpm for 5 min. Remove the aqueous phase and extract the residue with 4 mL 3% trichloroacetic acid. Combine the aqueous layers and make up to 10 mL with trichloroacetic acid, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm Spherisorb ODS-2

Column: 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: Gradient. MeCN:water 3:97 for 5 min, to 40:60 over 15 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (At the end of each day wash with MeCN:ethyl acetate 5:95.)

Flow rate: 0.5

Injection volume: 50

Detector: F ex 302 em 412 following post-column reaction. The column effluent mixed with reagent 1 pumped at 0.25 mL/min and with reagent 2 pumped at 0.25 mL/min and this mixture flowed through a 2.5 m × 0.8 mm i.d. PTFE coil at 40° to the detector. (Reagent 1 was 10 mM o-phthalaldehyde in EtOH:700 mM phosphoric acid 2:98. Reagent 2 was 20 mM β-mercaptoethanol in EtOH:700 mM phosphoric acid 2:98.)

CHROMATOGRAM

Retention time: 10

Limit of detection: 18 ng/mL

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfaguanidine, sulfamethoxazole, sulfapyridine

Noninterfering: sulfathiazole

KEY WORDS
post-column reaction; derivatization

REFERENCE
Viñas,P.; Erroz,C.L.; Campillo,N.; Hernández-Córdoba,M. Determination of sulphonamides in foods by liquid chromatography with postcolumn fluorescence derivatization, *J.Chromatogr.A*, **1996**, *726*, 125–131.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Suspension + 100 mL MeOH:water 60:40, shake mechanically for 15 min, make up to 200 mL with MeOH:water 60:40, filter (0.45 μm silver membrane, Selas Corp.). Evaporate a 1 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL 200 μg/mL acetanilide in MeCN, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water 20:80
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: acetanilide (11)

OTHER SUBSTANCES
Simultaneous: sulfadiazine, sulfamerazine, sulfamethazine, sulfanilic acid
Noninterfering: erythromycin ethylsuccinate

KEY WORDS
oral suspensions; suspensions

REFERENCE
Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE
Matrix: formulations
Sample preparation: Weigh capsule contents, dissolve in 50 mL MeOH, add 5 mL 0.75 mg/mL sulfanilamide in MeOH, make up to 100 mL with MeOH, filter (0.45 μm), inject a 5 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC8DB C8
Mobile phase: MeOH:1% acetic acid 40:60
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 0.8
Internal standard: sulfanilamide

OTHER SUBSTANCES
Simultaneous: temazepam

KEY WORDS
capsules; sulfanilamide is IS

REFERENCE
Fatmi,A.A.; Hickson,E.A. Determination of temazepam and related compounds in capsules by high-performance liquid chromatography, *J.Pharm.Sci.*, **1988**, 77, 87–89.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 11
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfadiazine, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfathiazole
Noninterfering: benzocaine

KEY WORDS
tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE
Garcia-Alvarez-Coque,M.C.; Simo-Alfonso,E.F.; Ramis-Ramos,G.; Esteve-Romero,J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245.

SAMPLE
Matrix: formulations, bulk
Sample preparation: Dilute with water to an idoxuridine concentration of 0.1%. Remove a 16 mL aliquot and add it to 2 mL 0.001% sulfanilamide in water, make up to 20 mL with water, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 4:96
Flow rate: 1.7
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: sulfanilamide

OTHER SUBSTANCES
Simultaneous: idoxuridine

KEY WORDS
eye drops; sulfanilamide is IS

REFERENCE
Carr,G.P.R. The development of British Pharmacopeia monographs for idoxuridine eye drops using high-pressure liquid chromatography for assay and for controlling related impurities, *J.Chromatogr.*, **1978**, *157*, 171–184.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 1.2
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfathiazole, sulfadiazine, sulfamethazine, sulfisoxazole, sulfadimethoxine

KEY WORDS
matrix solid-phase dispersion

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 3.1

Limit of detection: 4.9 ppb
Limit of quantitation: 9.1 ppb

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfapyridine, sulfathiazole

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100: 0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 20
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
cow

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: perfusate
Sample preparation: 1 mL Perfusate + 1 mL 1 M phosphoric acid + 10 mL ethyl acetate:isopropanol 90:10, vortex for 3 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 150 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 4 μm Novapak phenyl in a Z-module radial compression module
Mobile phase: MeOH:buffer 4:96 (Buffer was 25 mM K_2HPO_4 + 5 mM tetrabutylammonium + 5 mM triethylamine, pH adjusted to 6.8 with concentrated phosphoric acid.)
Flow rate: 3
Injection volume: 20

Detector: UV 264

CHROMATOGRAM
Internal standard: sulfanilamide

OTHER SUBSTANCES
Extracted: acipimox

KEY WORDS
sulfanilamide is IS

REFERENCE
Ghabrial,H.; Czuba,M.A.; Stead,C.K.; Smallwood,R.A.; Morgan,D.J. Transfer of acipimox across the isolated perfused human placenta, *Placenta.*, **1991**, *12*, 653–661.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfapyridine, sulfisoxazole

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: sulfanilic acid, sulfadiazine, sulfapyridine, sulfamerazine, sulfamethizole, sulfamethazine, sulfamethoxazole, sulfisoxazole, sulfachlorpyridine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 90 μL aliquot of a solution in MeOH:water 20:80.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:glacial acetic acid 10:89:1
Flow rate: 1.5
Injection volume: 90
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: sulfacetamide

REFERENCE
Hall,L.; Chadwick,V. Quantitative determination of sulfanilamide in sodium sulfacetamide raw material and ophthalmic solutions by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *478*, 438–445.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilic acid, sulfathiazole, sulfisoxazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 3.74

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 3.0

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-

diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Sentry (Waters)
Column: 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: MeOH:water:glacial acetic acid 20:79:1
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: sulfadiazine, sulfathiazole, sulfamerazine, sulfamethazine, succinylsulfathiazole

REFERENCE

Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 µL initial mobile phase, centrifuge, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 5 µm LiChrospher 100 RP-18

Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 0.8

Injection volume: 50

Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 µL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM

Retention time: 3.75

Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES

Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfapyridine, sulfathiazole

KEY WORDS

post-column reaction; muscle; kidney; SPE

REFERENCE

Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 µL 10 µg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 µL MeOH:water 50:50, filter (0.45 µm), inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM

Retention time: 3
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES

Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS

post-column reaction; muscle; liver; kidney; SPE

REFERENCE

Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 4

Limit of quantitation: 5 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine diluted 1:10 + 50 μL MeOH + 100 μL 0.5 M HCl + 100 μL 0.1% sodium nitrite in water, vortex, let stand for 10 min, add 100 μL 2% ammonium sulfamate in water, let stand for 15 min, add 100 μL 0.05% 2-aminoanthracene in MeCN (Caution! 2-Aminoanthracene causes cancer in experimental animals!), let stand for 15 min in the dark, add 5 mL diethyl ether, shake for 5 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 300 μL MeOH, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm YMC-Pack A-312 (YMC)
Mobile phase: MeOH:water:acetic acid 78:22:1
Flow rate: 1
Injection volume: 30
Detector: UV 279

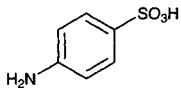
CHROMATOGRAM
Retention time: 8
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: p-aminobenzoic acid, 4-aminobenzoyl-β-alanine

KEY WORDS
derivatization

REFERENCE
Hayashi,T.; Amino,M.; Uchida,G.; Sato,M. High-performance liquid chromatographic determination of primary aromatic amines in urine after derivatization to an azo dye with 2-aminoanthracene, *J.Chromatogr.B*, **1995**, *665*, 209–212.

Sulfanilic acid



Molecular formula: $C_6H_7NO_3S$
Molecular weight: 173.19
CAS Registry No.: 121-57-3, 6101-32-2 (H2O), 6106-22-5 (sodium salt 2.H2O), 31884-76-1 (zinc salt 4.H2O)
Merck Index: 9096

SAMPLE
Matrix: solutions

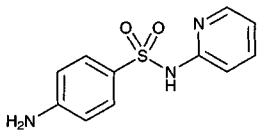
HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 67.5:32.5 (Prepare mobile phase by dissolving 784 mg K_2HPO_4 in 325 mL water. Dissolve 2.62 g hexadecyltrimethylammonium bromide in 350 mL MeOH. Combine solutions, add 325 mL MeOH, mix.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: nalidixic acid

REFERENCE
Walker,S.T. Liquid chromatographic determination of nalidixic acid in pharmaceutical preparations, *J.AOAC Int.*, **1996**, *79*, 431–433.

Sulfapyridine



Molecular formula: $C_{11}H_{11}N_3O_2S$
Molecular weight: 249.29
CAS Registry No.: 144-83-2, 127-57-1 (Na salt monohydrate)
Merck Index: 9108
Lednicer No.: 1 124

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (A) or MeCN:20 mM pH 6.9 phosphate buffer 20:80 (B)
Flow rate: 1
Injection volume: 20 (A), 5 (B)
Detector: UV 254

CHROMATOGRAM
Retention time: 14.5 (A), 2.06 (B)

OTHER SUBSTANCES
Extracted: acetaminophen (A), barbital (A), carbamazepine (A,B), phenobarbital (A), phenytoin (A), primidone (A)

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fritz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood

Sample preparation: Extract 250 μL plasma with 1 mL 1 M pH 4.7 sodium acetate and 5 mL chloroform (Caution! Chloroform is a carcinogen!), evaporate to dryness under nitrogen at 40°, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco LC-8
Mobile phase: MeOH:50 mM pH 7 sodium dihydrogen orthophosphate 16:84
Flow rate: 1.3
Detector: UV 260

CHROMATOGRAM
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Awni; W.M.; Braeckman; R.A.; Locke; Ch.S.; Dubé; L.M.; Granneman; G.R. The influence of multiple oral doses of zileuton on the steady-state pharmacokinetics of sulfasalazine and its metabolites, sulfapyridine and *N*-acetylsulfapyridine, *Clin.Pharmacokinet.*, **1995**, *29*, 98–104.

SAMPLE
Matrix: saliva
Sample preparation: 1 mL Saliva + 148 ng sulfadiazine + 1 mL MeCN + 400 mg potassium carbonate, vortex for 1 min, centrifuge at ≥1000 g for 10 min. Remove the upper MeCN layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm RP-18 (Brownlee)
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 mM $NaHPO_4$ (sic) containing 10 mM sodium 1-hexanesulfonate and 7.2 mM triethylamine, adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: F ex 395 em 470 following post-column reaction. The column effluent mixed with reagent pumped at 0.3 mL/min and the mixture flowed through a 4.8 m × 0.7 mm ID PTFE coil at 60° to the detector. (Prepare reagent by dissolving 400 mg fluorescamine in 250 mL MeOH, add 1 mL 2-mercaptoethanol, add 250 mL mobile phase.)

CHROMATOGRAM
Retention time: 7.32
Internal standard: sulfadiazine (5.66)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Noninterfering: N-acetylsulfapyridine, 2-amino-3-phenyl-1-propanol, 5-aminosalicylic acid, amphetamine, furosemide, levallorphan, metoprolol, riboflavin, salicylic acid, sulfasalazine, viloxazine

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KEY WORDS
post-column reaction

REFERENCE
Sista,H.S.; Dye,D.M.; Leonard,J. High-performance liquid chromatographic method for determination of sulfapyridine in human saliva using post-column, in-line derivatization with fluorescamine, *J.Chromatogr.*, **1983**, *273*, 464–468.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

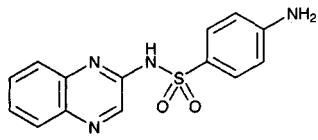
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Sulfaquinoxaline



Molecular formula: $C_{14}H_{12}N_4O_2$
Molecular weight: 300.34
CAS Registry No.: 59-40-5
Merck Index: 9109

SAMPLE

Matrix: blood, tissue
Sample preparation: Slurry 6 g alumina (alumina B Akt. I, ICN Biomedicals) in MeCN:MeOH 60:40, add to a 300 × 15 column, wash with 30 mL MeCN:MeOH 60:40. Homogenize (Niti-on Bio-mixer BM-2) 5 g chopped tissue or plasma with 25 mL MeCN for 2 min, wash twice with 20 mL portions of MeCN, filter (cotton plug), wash filter with 30 mL n-hexane saturated with MeCN, add 30 g anhydrous sodium sulfate to the filtrate, let stand at room temperature for 30 min, filter (cotton plug), add 30 mL isopropanol to the filtrate. Evaporate the filtrate to dryness at 35°, reconstitute with 5 mL MeCN:MeOH 60:40, sonicate, add to the column, wash with 35 mL MeCN:MeOH 60:40, elute with 35 mL MeOH:water 75:25. Add 10 mL isopropanol to the eluate and evaporate it to dryness at 40°, reconstitute with 1 μg/mL chloramphenicol in MeCN:200 mM KH_2PO_4 15:85 containing 5 mM sodium 1-hexanesulfonate, filter (Gelman Ekikurodisk 13 CR), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 L-column ODS (Chemicals Inspection and Testing Institute, Tokyo)
Mobile phase: MeCN:10 mM pH 5.0 phosphate buffer 21:79
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 20.7
Internal standard: chloramphenicol (18.8)
Limit of detection: 3 ng/g

OTHER SUBSTANCES

Extracted: N^4-acetyl sulfaquinoxaline
Simultaneous: acetylsulfadiazine, acetylsulfadimethoxine, acetylsulfamethazine, acetylsulfamethoxazole, acetylsulfamonomethoxine, amprolium, diaveridine, ethopabate, furazolidone, nalidixic acid, nitrofurazone, ormetoprim, oxolinic acid, pyrimethamine, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, thiamphenicol, trimethoprim

KEY WORDS

chicken; muscle; liver; kidney; skin; plasma; SPE

REFERENCE

Takahashi,Y.; Sekiya,T.; Nishikawa,M.; Endoh,Y.S. Simultaneous high-performance liquid chromatographic determination of amprolium, ethopabate, sulfaquinoxaline, and N4-acetylsulfaquinoxaline in chicken tissues, *J.Liq.Chromatogr.*, **1994**, *17*, 4489–4512.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Add 300 μL acetone slowly while vortexing to 100 μL plasma, centrifuge at 1700 g for 4 min. Remove 100 μL of the supernatant and evaporate it to dryness under a stream of nitrogen at 65°, reconstitute the residue in 200 μL mobile phase, inject a 20-100 μL aliquot. Urine. 500 μL Urine + 500 μL 3 M pH 6 acetate buffer, mix, extract with 4 mL dichloromethane. Remove 0.5-1.5 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 20-100 μL aliquot.

HPLC VARIABLES

Guard column: 40 × 3.2 30-40 μm Perisorb C18 (Merck)
Column: 250 × 4.6 10 μm Hibar II C18 (Merck)
Mobile phase: MeOH:200 mM pH 7 KH_2PO_4/Na_2HPO_4 35:65
Flow rate: 1.7
Injection volume: 20-100
Detector: UV 252 (plasma), UV 360 (urine)

CHROMATOGRAM

Retention time: 3.6
Limit of detection: 100 ng/mL (urine), 250 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites, N-acetylsulfaquinoxaline

KEY WORDS

rabbit; plasma; pharmacokinetics

REFERENCE

Eppel,J.G.; Thiessen,J.J. Liquid chromatographic analysis of sulfaquinoxaline and its application to pharmacokinetic studies in rabbits, *J.Pharm.Sci.*, **1984**, *73*, 1635–1638.

SAMPLE

Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 6.2
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfathiazole, sulfatroxazole

Noninterfering: chloramphenicol, trimethoprim

KEY WORDS
post-column reaction; meat; column-switching; dialysis

REFERENCE
Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 30:70
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 18.1
Limit of detection: 1.1 ppb
Limit of quantitation: 2.4 ppb

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadimethoxine, sulfamethazine

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing

100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 12.2
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfathiazole
Interfering: sulfadimethoxine (distinguish by MS)

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 13.09

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, , sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2

Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 18.05

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 56

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 65

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: tissue
Sample preparation: Blend 3 g meat with 30 mL chloroform for 2 min in a Polytron homogenizer, shake for 10 min, centrifuge at 1600 g for 5 min, filter (5A filter paper). Add 5 mL filtrate to 1 mL 3 M HCl, shake for 10 min, centrifuge at 1600 g for 5 min. 250 μL Aqueous layer + 250 μL 3.5 M sodium acetate solution, vortex, add 100 μL 0.2% fluorescamine in acetone, vortex, let stand for 20 min at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:2% acetic acid 5:3
Column temperature: 55
Flow rate: 1
Injection volume: 10
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 16
Limit of detection: 0.01 ng/g

OTHER SUBSTANCES
Simultaneous: sulfisomidine, sulfamethoxazole, sulfamerazine, sulfadiazine, sulfamonomethoxine, sulfamethazine (sulfadimidine), sulfadimethoxine

KEY WORDS
cow; pig; chicken; ham; sausage; roast beef; derivatization

REFERENCE
Takeda,N.; Akiyama,Y. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products, *J.Chromatogr.*, **1991**, *558*, 175–180.

SAMPLE
Matrix: tissue
Sample preparation: Cut tissue into small pieces and homogenize in blender. 20 g Homogenized tissue + 200 μL 10 μg/mL methyl p-aminobenzoate in water + 60 mL acetone:chloroform 50:50, shake vigorously on a mechanical shaker for 10 min, centrifuge at 3000 g for 10 min, filter (Whatman No. 41 paper) supernatant, repeat extraction. Combine the extracts, if the extract is not clear centrifuge at 3000 g for 10 min and discard the aqueous layer, evaporate to an oily residue at 45° under reduced pressure, add 5 mL MeCN to flask, let stand for 10 min, remove MeCN layer, add 5 mL hexane and 5 mL MeCN, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer, add 5 mL MeCN to the hexane layer, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer. If hexane layer is not clear centrifuge at 3000 g for 10 min and remove

the clear portion. Add 400 μL 15% trichloroacetic acid to the hexane layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Evaporate the MeCN layers, transfer the oily residue to a small flask with 3 mL hexane, add the aqueous trichloroacetic acid layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Discard the hexane layer, add 100 μL saturated aqueous sodium citrate solution to the aqueous layer, mix, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP 18 (Brownlee)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 1% aqueous acetic acid. B was MeCN:water 80:20. A:B from 90:10 to 60:40 over 20 min, return to initial conditions over 5 min, re-equilibrate for 5 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 7 m × 0.25 mm i.d. coil of stainless steel tubing to the detector. (Prepare reagent by dissolving 1 g p-dimethylaminobenzaldehyde in 30 mL MeCN, make up to 100 mL with 5% trichloroacetic acid in water.)

CHROMATOGRAM

Retention time: 26.0
Internal standard: methyl p-aminobenzoate (18.6)
Limit of detection: 30 ng/g

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfapyridine

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KEY WORDS

chicken; liver; pig; kidney; sheep; cow; post-column reaction

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REFERENCE

Bui,L.V. Liquid chromatographic determination of six sulfonamide residues in animal tissues using postcolumn derivatization, *J.AOAC Int.*, **1993**, *76*, 966–976.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The

effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM
Retention time: 17
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; liver; kidney; SPE

REFERENCE
Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 3 g ground tissue with 30 mL chloroform for 2 min, centrifuge at 3000 g for 5 min, filter (paper). Remove a 10 mL aliquot of the filtrate and add it to 1 mL 3 M HCl, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove a 250 μL aliquot of the aqueous layer and add it to 250 μL 3.8 M sodium acetate, add 100 μL 1 mg/mL fluorescamine in MeCN, vortex, let stand at room temperature for 20 min, inject a 20 μL aliquot. (Sodium acetate should be a highly pure grade.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:20 mM pH 4 NaH_2PO_4 34:66 containing 20 mM sodium octanesulfonate
Column temperature: 30
Flow rate: 1.2
Injection volume: 20
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 24
Limit of detection: 40 ng/g

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfadimethoxine, sulfamethazine

KEY WORDS
derivatization; chicken; muscle

REFERENCE
Simeonidou,E.J.; Botsoglou,N.A.; Psomas,I.E.; Fletouris,D.J. Liquid chromatographic analysis of multiple sulfonamide residues in chicken muscle using pre-column derivatization and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2349–2364.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL

water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

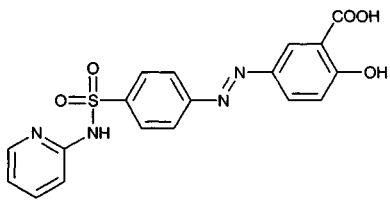
CHROMATOGRAM
Retention time: 24.5
Limit of quantitation: 5 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

Sulfasalazine



Molecular formula: $C_{18}H_{14}N_4O_5S$
Molecular weight: 398.40
CAS Registry No.: 599-79-1
Merck Index: 9112
Lednicer No.: 2 114

SAMPLE
Matrix: blood
Sample preparation: Mix 250 μL plasma with 500 μL 1 M HCl, extract with 4 mL ethyl acetate, evaporate to dryness under nitrogen at 40°, inject an aliquot.

HPLC VARIABLES
Column: 75 × 3.9 4 μm Nova-Pak LC-18
Mobile phase: MeOH:50 mM sodium acetate buffer 40:60
Flow rate: 0.5
Detector: UV 365

CHROMATOGRAM
Limit of quantitation: 100 ng/mL

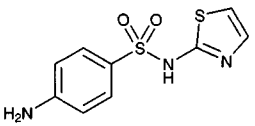
CHROMATOGRAM
Retention time: 6.5 (as the N-propionyl derivative of the metabolite and reduction product, 5-aminosalicylic acid)
Internal standard: N-propionyl-4-amino-2-hydroxybenzoic acid (12.5)
Limit of detection: 100 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; derivatization; SPE

REFERENCE
van Hogezand,R.A.; van Balen,H.C.J.G.; van Schaik,A.; Tangerman,A.; van Hees,P.A.M.; Zwanenburg,B.; van Tongeren,J.H.M. Determination of sodium azodisalicylate, salazosulphapyridine and their metabolites in serum, urine and faeces by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *305*, 470–476.

Sulfathiazole



Molecular formula: $C_9H_9N_3O_2S_2$
Molecular weight: 255.32
CAS Registry No.: 72-14-0, 144-74-1 (Na salt)
Merck Index: 9115
Lednicer No.: 1 124

SAMPLE
Matrix: blood, milk
Sample preparation: 1 mL Serum or milk + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL p-aminobenzoic acid in 0.01% trichloroacetic acid, centrifuge at 1000 g for 10 min. Remove a 500 μL aliquot of the clear layer and add it to 100 μL 1 mg/mL fluorescamine in acetone (prepared fresh each day), mix for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 6.7
Internal standard: p-aminobenzoic acid (5.5)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine

KEY WORDS
cow; serum; derivatization

REFERENCE
Tsai,C.-E.; Kondo,F. Liquid chromatographic determination of fluorescent derivatives of six sulfonamides in bovine serum and milk, *J.AOAC Int.*, **1995**, *78*, 674–678.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 9.022

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: eggs, honey, milk

Sample preparation: Honey. Dissolve 1 g honey in 10 mL water, homogenize, filter (0.45 μm), inject a 50 μL aliquot. Milk, eggs. 5 mL Milk or 0.4 g lyophilized eggs + 10 mL trichloroacetic acid solution (so as to give a final trichloroacetic acid concentration of 3%), homogenize, centrifuge at 5000 rpm for 5 min. Re-extract the residue with 10 mL 3% trichloroacetic acid. Combine the aqueous phases and make up to 25 mL with trichloroacetic acid solution, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: Gradient. MeCN:water 3:97 for 5 min, to 40:60 over 15 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (Wash column with MeCN:ethyl acetate 5:95 at the end of each day.)

Flow rate: 1

Injection volume: 50

Detector: UV 260

CHROMATOGRAM

Retention time: 12.5

Limit of detection: 70 ng/mL

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfaguanidine, sulfamethoxazole, sulfapyridine

REFERENCE

Viñas,P.; López Erroz,C.; Hernández Canals,A.; Hernández Córdoba,M. Liquid chromatographic analysis of sulfonamides in foods, *Chromatographia*, **1995**, *40*, 382–386.

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5

Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 3.5

Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline

Noninterfering: chloramphenicol, trimethoprim

Interfering: sulfamerazine, sulfatroxazole

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: feed

Sample preparation: 20 g Ground feed + 100 mL solvent + 5 mL 8 μg/mL sulfamerazine in diluent, shake for 1 h, chill an aliquot in an ice bath for 2 h, centrifuge at 1650 g for 5 min, filter (0.2 μm), inject a 200 μL aliquot of the filtrate. (Prepare solvent by mixing 250 mL MeOH, 300 mL water, and 25 mL HCl, mix, add 15 mL diethylamine, mix, make up to 1 L with water. Prepare diluent by mixing 250 mL MeOH, 300 mL water, and 12.5 mL HCl, mix, add 15 mL diethylamine, mix, make up to 1 L with water.)

HPLC VARIABLES

Guard column: C8 or C18

Column: 250 × 4.67 μm Lichrosorb RP-18
Mobile phase: MeCN:2% acetic acid 17:83
Flow rate: 1-1.3
Injection volume: 200
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.1-0.5 mL/min and the mixture flowed through a 3 m × 0.5 mm i.d. PTFE coil to the detector. (Prepare reagent by dissolving 1.5 g dimethylaminobenzaldehyde in 100 mL glacial acetic acid, add 60 mL MeOH, mix well, add 40 mL water, mix well, prepare fresh daily.)

CHROMATOGRAM
Retention time: 5.5
Internal standard: sulfamerazine (7)

OTHER SUBSTANCES
Extracted: sulfamethazine
Simultaneous: sulfadimethoxine, sulfaquinoxaline
Noninterfering: amino acids, amprolium, apramycin, arsanilic acid, bacitracin, hygromycin B, neomycin, nystatin, ormetoprim, procaine

KEY WORDS
post-column reaction

REFERENCE
Smallidge,R.L.; Kentzer,E.J.; Stringham,K.R.; Kim,E.H.; Lehe,C.; Stringham,R.W.; Mundell,E.C. Sulfamethazine and sulfathiazole determination at residue levels in swine feeds by reverse-phase liquid chromatography with post-column derivatization, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 710–717.

SAMPLE
Matrix: feed, premix
Sample preparation: Shake premix or ground feed with 150 mM HCl in MeOH:water 25:75 for 1 h, dilute with 150 mM HCl in MeOH:water 25:75 to achieve a sulfonamide concentration of 5.5 μg/mL, filter (glass fiber), inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2 30-40 μm Perisorb RP-18
Column: 250 × 4.6 10 μm Partisil ODS-3
Mobile phase: MeCN:2% acetic acid 18:82
Flow rate: 1
Injection volume: 200
Detector: UV 450 following post-column reaction. The column effluent mixed with reagent pumped at 0.5 mL/min and the mixture flowed through a 3 m × 0.5 mm ID coil of PTFE tubing to the detector. (Prepare reagent by dissolving 3 g dimethylaminobenzaldehyde in 100 mL glacial acetic acid, add 60 mL MeOH, add 40 mL water, mix well.)

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 1.65 μg/mL

KEY WORDS
post-column reaction

REFERENCE
Stringham,R.W.; Mundell,E.C.; Smallidge,R.L. Use of post-column derivatization in liquid chromatographic determination of sulfamethazine and sulfathiazole in feeds and feed premixes, *J.Assoc.Off.Anal.Chem.*, **1982**, *65*, 823–827.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium

dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 12
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfadiazine, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide
Noninterfering: benzocaine

KEY WORDS
tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE
Garcia-Alvarez-Coque,M.C.; Simo-Alfonso,E.F.; Ramis-Ramos,G.; Esteve-Romero,J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245.

SAMPLE
Matrix: formulations, bulk
Sample preparation: Dilute with water to an idoxuridine concentration of 0.1%. Remove a 15 mL aliquot and add it to 2 mL sulfathiazole solution, make up to 20 mL with water, inject a 10 μL aliquot. (Prepare sulfathiazole solution by dissolving 120 mg sulfathiazole in 10 mL EtOH, make up to 100 mL with water.)

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 13:87
Flow rate: 1.7
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12.5
Internal standard: sulfathiazole

OTHER SUBSTANCES
Simultaneous: idoxuridine

KEY WORDS
eye drops; sulfathiazole is IS

REFERENCE
Carr,G.P.R. The development of British Pharmacopeia monographs for idoxuridine eye drops using high-pressure liquid chromatography for assay and for controlling related impurities, *J.Chromatogr.*, **1978**, *157*, 171–184.

SAMPLE

Matrix: milk

Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES

Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM

Retention time: 10.3
Limit of detection: 1.0 ppb
Limit of quantitation: 2.2 ppb

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine

KEY WORDS

cow

REFERENCE

Dadgar,D.; Power,A. Applications of column-switching technique in biopharmaceutical analysis. I. High-performance liquid chromatographic determination of amitriptyline and its metabolites in human plasma, *J.Chromatogr.*, **1987**, *416*, 99–109.

SAMPLE

Matrix: milk

Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES

Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 2
Internal standard: sulfamerazine (3)

Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfadiazine, sulfamethazine, sulfisoxazole, sulfadimethoxine

KEY WORDS
matrix solid-phase dispersion

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 10.3
Limit of detection: 1.0 ppb
Limit of quantitation: 2.2 ppb

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing

100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 6.9
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfaquinoxaline

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 5.17

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 25-37 μm Co:Pell ODS
Column: 250 × 4.6 10 μm Partisil PXS ODS-2
Mobile phase: MeCN:MeOH 10:90
Flow rate: 0.7
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Simultaneous: oxybenzone, padimate-O, propyl paraben

REFERENCE
Tan,H.S.I.; Sih,R.; Moseley,S.E.; Lichtin,J.L. Assay of mixtures of padimate-O and oxybenzone in sunscreen formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *291*, 275–282.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.71

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfanilic acid, sulfisoxazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)

Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 7.97

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsphere C18 (Chrompack)
Mobile phase: MeCN:MeOH:THF:buffer 0.1:14:0.5:85.4 (Prepare buffer by dissolving 6.80 g KH_2PO_4 in 1 L water, adjust pH to 3.0 with concentrated phosphoric acid, add 4.15 mL triethylamine, add 10 mL glacial acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: k' 2.10

OTHER SUBSTANCES
Simultaneous: sulfachloropyridazine, sulfamerazine, sulfamethoxypyridazine, sulfapyridine, sulfisomidine

REFERENCE
Wieling,J.; Coenegracht,P.M.J.; Doornbos,D.A.; Jonkman,J.H.G. Robustness testing of an optimized reversed-phase high-performance liquid chromatographic system for the separation of six sulphonamides using the rules of error propagation, *J.Chromatogr.*, **1993**, *635*, 195–202.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Sentry (Waters)
Column: 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: MeOH:water:glacial acetic acid 20:79:1
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Simultaneous: sulfanilamide, sulfadiazine, sulfamerazine, sulfamethazine, succinylsulfathiazole

REFERENCE
Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 24

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfaquinoxaline, sulfisomidine, sulfisoxazole, trimethoprim
Interfering: sulfapyridine

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfaquinoxaline, sulfisomidine, sulfisoxazole, trimethoprim

Interfering: sulfapyridine

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 µL initial mobile phase, centrifuge, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 µm LiChrospher 100 RP-18
Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 0.8
Injection volume: 50
Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 µL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 15
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH

to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM

Retention time: 4.5
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES

Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide
Interfering: sulfapyridine

KEY WORDS

post-column reaction; muscle; liver; kidney; SPE

REFERENCE

Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 μL 20 μg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min. Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72

Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM
Retention time: 4.9
Internal standard: sulfaethoxypyridazine (12.8)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine

KEY WORDS
cow; pig; muscle; liver; SPE

REFERENCE
Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM
Retention time: 6.5
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 10 mL 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfaguanidine, sulfathiazole, sulfamethoxazole

KEY WORDS
derivatization

REFERENCE
Simó-Alfonso,E.F.; Ramis-Ramos,G.; García-Alvarez-Coque,M.C.; Esteve-Romero,J.S. Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography, *J.Chromatogr.B*, **1995**, *670*, 183–187.

SAMPLE
Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

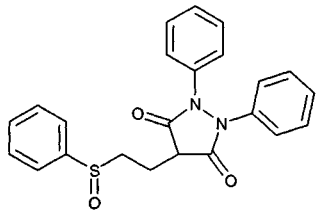
CHROMATOGRAM
Retention time: 5.5
Internal standard: niacin (3.3)

OTHER SUBSTANCES
Extracted: sulfamethazine, sulfacetamide, sulfamethoxazole, sulfadiazine, sulfamerazine, sulfamonomethoxine

KEY WORDS
wastewater

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfinpyrazone



Molecular formula: $C_{23}H_{20}N_2O_3S$
Molecular weight: 404.49
CAS Registry No.: 57-96-5
Merck Index: 9121
Lednicer No.: 1 238

SAMPLE
Matrix: bile, blood
Sample preparation: Plasma, urine, or bile + 50 μL 100 μg/mL fenbufen in MeOH + 2 mL 1 M HCl + 5 mL chlorobutane:1,2-dichloroethane 80:20, extract, centrifuge. Remove the organic layer and add it to 400 μL 100 mM NaOH, shake for 5 min, centrifuge, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:100 mM pH 5.6 phosphate buffer 50:50
Flow rate: 1.6
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Internal standard: fenbufen

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; urine; rabbit; pharmacokinetics

REFERENCE
Strong,H.A.; Renwick,A.G.; George,C.F. The site of reduction of sulphinpyrazone in the rabbit, *Xenobiotica*, **1984**, *14*, 815–826.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-pak C18 SPE cartridge with 3 mL MeOH and 3 mL 10 mM tetrabutylammonium phosphate. 1 mL Plasma + 1 mL 20 mM tetrabutylammonium phosphate in MeOH:water 50:50, agitate for 30 s, add to the SPE cartridge, wash with 1 mL 10 mM tetrabutylammonium phosphate MeCN:MeOH:water 15:15:70, elute with 1 mL MeOH, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 4 Bondapak C18
Mobile phase: MeOH:water 56:44 containing 5 mM tetrabutylammonium phosphate (PIC A)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 200 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Patel,N.J.; Kildsig,D.O.; Banker,G.S.; Mayer,P.R.; Gonzalez,M.A. Paired-ion high-performance liquid chromatographic assay for sulfinpyrazone in plasma, *J.Pharm.Sci.*, **1982**, *71*, 1413–1415.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 42 μg/mL naproxen + 500 μL MeCN, shake gently, centrifuge at 10000 g for 2 min. Remove the supernatant and dilute with an equal volume of water, inject a 50-200 μL aliquot.

HPLC VARIABLES
Guard column: Bondapak C18/Corasil
Column: 115 × 8 5 μm Radial-Pak C18 (Waters)
Mobile phase: MeCN:20 mM pH 7.0 phosphate buffer 26:74
Flow rate: 2
Injection volume: 50-200
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: naproxen (4.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: ibuprofen
Noninterfering: lidocaine, metoprolol, propranolol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Tam,Y.K.; Ferguson,S.M.; Yau,M.L.; Wyse,D.G. Simple and rapid high-performance liquid chromatographic method for the analysis of sulfinpyrazone and four of its metabolites in human plasma, *J.Chromatogr.*, **1984**, *310*, 438–444.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 10 μg naproxen + 1 mL 1 M HCl + 500 μL 20 mg/mL sodium sulfite + 4 mL dichloromethane:1-chlorobutane 75:25, shake for 15 min, centrifuge at 2000 g for 10 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL 2% sodium sulfite, vortex, inject a 10 μL aliquot. Urine. 500 μL Urine + 500 μL water + 25 μg naproxen + 1 mL 1 M HCl + 500 μL 20 mg/mL sodium sulfite + 4 mL dichloromethane:1-chlorobutane 75:25, shake for 15 min, centrifuge at 2000 g for 10 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL 2% sodium sulfite, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 4 35-50 μm Bondapak C18 Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was MeCN:100 mM ammonium acetate 22:78. B was MeCN. A:B from 0:100 to 100:0 over 15 min (Waters convex curve 7), re-equilibrate for 5 min.
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.86
Internal standard: naproxen (9.85)
Limit of detection: 500 ng/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: antipyrine, phenprocoumon
Noninterfering: ampicillin, aspirin, azlocillin, cimetidine, cotinine, digoxin, heparin, hippuric acid, lidocaine, methaqualone, metoprolol, mezlocillin, neostigmine, nicotine, pindolol, procainamide, propranolol, quinidine, salicylamide, salicylic acid, secobarbital, theobromine, theophylline, uric acid, ascorbic acid, vitamin B, warfarin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
de Vries,J.X.; Staiger,C.; Wang,N.S.; Schlicht,F. Analysis of sulfinpyrazone and its metabolites in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *277*, 408–413.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Evaporate 50-100 μL 100 μg/mL butyl 4-hydroxybenzoate in MeOH into the bottom of a glass tube using a stream of air at 30°, add 500 μL plasma, add 500 μL 600 mM HCl, add 6 mL 1-chlorobutane:chloroform 50:50, shake mechanically for 20 min, centrifuge at 1400 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 1 mL EtOH:water 50:50, inject a 10-20 μL aliquot. Urine. Evaporate 50-100 μL 100 μg/mL butyl 4-hydroxybenzoate in MeOH into the bottom of a glass tube using a stream of air at 30°, add 500 μL urine, add 500 μL 600 mM HCl, add 6 mL 1-chlorobutane:chloroform 50:50, shake mechanically for 20 min, centrifuge at 1400 g for 10 min. Remove the organic layer and add it to 500 μL 500 mM pH 4.5 citrate buffer, shake for 5 min, centrifuge at 1400 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 1 mL EtOH:water 50:50, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: EtOH:10 mM pH 2.5 citrate buffer 48:52
Column temperature: 40
Flow rate: 1
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: butyl 4-hydroxybenzoate (6)
Limit of quantitation: 100 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lentjes,E.G.W.M.; Tan,Y.; van Ginneken,C.A.M. Determination of sulfinpyrazone and four metabolites in plasma and urine by high pressure liquid chromatography, *Pharm.Weekbl.[Sci.]*, **1985**, *7*, 252–259.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in the mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Lichrosorb RP 18
Mobile phase: MeOH:water 50:50 containing 1% acetic acid
Flow rate: 1

Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8.94

OTHER SUBSTANCES
Simultaneous: kebuzone, oxyphenbutazone, phenylbutazone

REFERENCE
Nivaud-Guernet,E.; Guernet,M.; Ivanovic,D.; Medenica,M. Effect of eluent pH on the ionic and molecular forms of the non-steroidal anti-inflammatory agents in reversed-phase high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2343–2357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

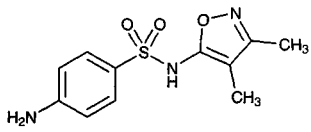
CHROMATOGRAM
Retention time: 5.71 (A), 5.33 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Sulfisoxazole



Molecular formula: $C_{12}H_{13}N_3O_3S$
Molecular weight: 267.31
CAS Registry No.: 127-69-5, 4299-60-9 (diolamine)
Merck Index: 9125
Lednicer No.: 1 124

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 400 μL 0.2 μg/mL N^4-acetylsulfamethoxazole in MeOH, vortex for 10 s, centrifuge at 2000 rpm for 10 min. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen, inject a 50 μL aliquot. Urine. 100 μL Urine + 200 μL 12 μg/mL N^4-acetylsulfamethoxazole in MeOH, vortex for 10 s, centrifuge at 2000 rpm for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-18 Hibar II
Mobile phase: MeOH:water:glacial acetic acid 32:68:0.06, pH adjusted to 4.7 with 4 M NaOH
Flow rate: 1.2
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: N^4-acetylsulfamethoxazole (11.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: acetylsulfisoxazole

KEY WORDS
plasma

REFERENCE
Jung,D.; Oie,S. "High-pressure" liquid chromatography of sulfisoxazole and N^4-acetylsulfisoxazole in body fluids, *Clin.Chem.*, **1980**, *26*, 51–54.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out ground tablets to contain 500 mg sulfisoxazole, add 25 mL MeOH, shake mechanically for 30 min, make up to 100 mL with MeOH, filter. Remove a 5 mL aliquot of the filtrate and add it to 15 mL 1 mg/mL sulfabenzamide in MeOH, mix, make up to 100 mL with MeOH, inject an aliquot. Injections, ophthalmic solutions. Measure out amount containing 200 mg sulfisoxazole, make up to 200 mL with MeOH. Remove a 25 mL aliquot and add it to 25 mL 1 mg/mL sulfabenzamide in MeOH, mix, make up to 100 mL with MeOH, inject an aliquot. Ointments. Weigh out ointment containing 50 mg sulfisoxazole, add 25 mL MeOH:water 50:50, wash twice with 50 mL portions of n-heptane. Extract the n-heptane layers three times with 25 mL portions of MeOH:water 50:50. Combine all the MeOH:water layers and add 50 mL 1 mg/mL sulfabenzamide in MeOH, mix, make up to 200 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:water:acetic acid 22.5:76.5:1
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: sulfabenzamide (8)

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine

KEY WORDS
tablets; injections; ophthalmic solutions; ointments

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: formulations
Sample preparation: Extract 1 mL suspension with three 15 mL aliquots of chloroform, combine the organic layers and make up to 50 mL with chloroform, filter (0.45 μm silver membrane, Selas Corp.). Evaporate a 2 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 5 mL 330 μg/mL benzanilide in MeCN, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water 40:60
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3
Internal standard: benzanilide (11)

OTHER SUBSTANCES
Simultaneous: acetylsulfisoxazole, sulfanilamide, sulfanilic acid
Noninterfering: erythromycin ethylsuccinate

KEY WORDS
oral suspensions; suspensions

REFERENCE
Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out ground tablets to contain 500 mg sulfisoxazole, add 25 mL MeOH, shake mechanically for 30 min, make up to 100 mL with MeOH, filter. Remove a 20 mL aliquot of the filtrate and make up to 100 mL with MeOH. Remove a 5 mL aliquot and add it to 5 mL 800 μg/mL sulfadimethoxine in MeOH, mix, make up to 100 mL with mobile phase, inject an aliquot. Injections, ophthalmic solutions. Measure out amount containing 200 mg sulfisoxazole, make up to 200 mL with MeOH. Remove a 5 mL aliquot and add it to 5 mL 800 μg/mL sulfadimethoxine in MeOH, mix, make up to 100 mL with mobile phase, inject an aliquot. Ointments. Weigh out ointment containing 50 mg sulfisoxazole, add 50 mL n-heptane, shake to disperse, extract three times with 25 mL portions of MeOH:water 2:1, wash each extract with 50 mL n-heptane. Combine all the MeOH:water layers and make up to 100 mL

with MeOH. Remove a 10 mL aliquot and add it to 5 mL 800 μg/mL sulfadimethoxine in MeOH, mix, make up to 100 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 30:69:1
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: sulfadimethoxine (5.5)

KEY WORDS
tablets; injections; ophthalmic solutions; ointments

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 1182–1185.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 8.8
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfathiazole, sulfadiazine, sulfamethazine, sulfadimethoxine

KEY WORDS
matrix solid-phase dispersion

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: sulfanilic acid, sulfanilamide, sulfapyridine, sulfamerazine, sulfadiazine, sulfamethizole, sulfamethazine, sulfamethoxazole, sulfachlorpyridine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5.6

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfanilic acid, sulfathiazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 15.57

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine

REFERENCE

Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 20.2

OTHER SUBSTANCES

Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadimethoxine, sulfadoxine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine

REFERENCE

Nishikawa,M.; Takahashi,Y.; Ishihara,Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues, *J.Liq.Chromatogr.*, **1993**, *16*, 4031–4047.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 51

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, trimethoprim

KEY WORDS

capillary HPLC

REFERENCE

Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE

Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 46

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

Sulfur

Molecular formula: S
Molecular weight: 32.06
CAS Registry No.: 7704-34-9
Merck Index: 9142

SAMPLE
Matrix: formulations
Sample preparation: Add a 200 mg tablet containing 6 mg captan and 5 mg sulfur to 5 mL carbon disulfide, extract the solid residue five times with 5 mL carbon disulfide, combine the extracts and evaporate to constant weight. Dissolve the residue in 2 mL carbon disulfide, make up to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Perkin-Elmer C8
Mobile phase: MeOH:water 90:10
Flow rate: 1
Injection volume: 20
Detector: UV 254

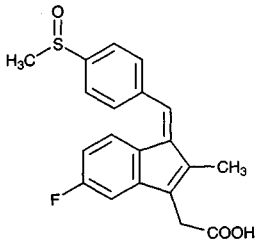
CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: captan

KEY WORDS
tablets

REFERENCE
Fedeli,G.; Moltrasio,D.; Aleotti,M.; Gazzani,G. High-performance liquid chromatographic determination of sulphur and captan in a mixture, *J.Chromatogr.*, **1988**, *447*, 263–267.

Sulindac



Molecular formula: $C_{20}H_{17}FO_3S$
Molecular weight: 356.42
CAS Registry No.: 38194-50-2
Merck Index: 9155
Lednicer No.: 2 210

SAMPLE
Matrix: bile, blood
Sample preparation: Plasma, urine, or bile + 2 mL 1 M HCl + 5 mL chlorobutane:1,2-dichloroethane 80:20, extract, centrifuge. Remove the organic layer and add it to 400 µL 100 mM NaOH, shake for 5 min, centrifuge, inject a 200 µL aliquot of the aqueous layer.

HPLC VARIABLES
Column: µBondapak
Mobile phase: MeCN:200 mM pH 3.5 ammonium phosphate buffer 50:50
Flow rate: 1.6
Injection volume: 200
Detector: UV 254

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; urine; rabbit

REFERENCE
Strong,H.A.; Renwick,A.G.; George,C.F. The site of reduction of sulphinpyrazone in the rabbit, *Xenobiotica*, **1984**, *14*, 815–826.

SAMPLE
Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: 100 µL Whole blood, bile, liver homogenate, urine, or gastric contents + 100 µL 1 mg/mL ketoprofen + 1 mL 3 M pH 1.5 phosphate buffer + 3 mL chloroform, vortex, rotate for 10 min, centrifuge for 10 min. Remove the organic layer and add it to 1 mL 50 mM NaOH, mix for 5 min, centrifuge for 5 min. Remove the aqueous layer and neutralize it with 1 mL 50 mM phosphoric acid, inject an aliquot.

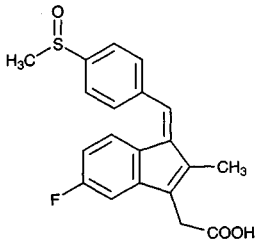
HPLC VARIABLES
Column: 50 mm long C18
Mobile phase: MeCN:350 mM acetic acid 35:65
Flow rate: 2
Detector: UV 313

CHROMATOGRAM
Internal standard: ketoprofen

KEY WORDS
whole blood; liver

REFERENCE
Fedeli,G.; Moltrasio,D.; Aleotti,M.; Gazzani,G. High-performance liquid chromatographic determination of sulphur and captan in a mixture, *J.Chromatogr.*, **1988**, *447*, 263–267.

Sulindac



Molecular formula: $C_{20}H_{17}FO_3S$
Molecular weight: 356.42
CAS Registry No.: 38194-50-2
Merck Index: 9155
Lednicer No.: 2 210

SAMPLE
Matrix: bile, blood
Sample preparation: Plasma, urine, or bile + 2 mL 1 M HCl + 5 mL chlorobutane:1,2-dichloroethane 80:20, extract, centrifuge. Remove the organic layer and add it to 400 µL 100 mM NaOH, shake for 5 min, centrifuge, inject a 200 µL aliquot of the aqueous layer.

HPLC VARIABLES
Column: µBondapak
Mobile phase: MeCN:200 mM pH 3.5 ammonium phosphate buffer 50:50
Flow rate: 1.6
Injection volume: 200
Detector: UV 254

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; urine; rabbit

REFERENCE
Strong,H.A.; Renwick,A.G.; George,C.F. The site of reduction of sulphinpyrazone in the rabbit, *Xenobiotica*, **1984**, *14*, 815–826.

SAMPLE
Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: 100 µL Whole blood, bile, liver homogenate, urine, or gastric contents + 100 µL 1 mg/mL ketoprofen + 1 mL 3 M pH 1.5 phosphate buffer + 3 mL chloroform, vortex, rotate for 10 min, centrifuge for 10 min. Remove the organic layer and add it to 1 mL 50 mM NaOH, mix for 5 min, centrifuge for 5 min. Remove the aqueous layer and neutralize it with 1 mL 50 mM phosphoric acid, inject an aliquot.

HPLC VARIABLES
Column: 50 mm long C18
Mobile phase: MeCN:350 mM acetic acid 35:65
Flow rate: 2
Detector: UV 313

CHROMATOGRAM
Internal standard: ketoprofen

KEY WORDS
whole blood; liver

REFERENCE

Singer,P.; Mozayani,A. An overdose fatality in a child involving disopyramide and sulindac, *J.Anal.Toxicol.*, **1995**, *19*, 529–530.

SAMPLE

Matrix: bile, blood, gastric contents, urine

Sample preparation: Plasma. 300 μL Plasma + 40 μL 300 μg/mL indomethacin in borate buffer + 1 mL MeCN, vortex for 30 s, centrifuge at 3500 rpm. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen at 50°, inject a 20 μL aliquot. Urine, bile, gastric fluid. 300 μL Urine, bile, or gastric fluid + 100 μL 5 M NaOH, let stand at room temperature for 15 min, adjust the pH with 100 μL 28.3% phosphoric acid, add 40 (urine), 25 (bile), or 6 (gastric fluid) μL 300 μg/mL indomethacin in borate buffer, add 1 (urine, bile) or 1.5 (gastric fluid) mL MeCN, vortex for 30 s, centrifuge at 3500 rpm. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen at 50°, inject a 20 (urine), 35 (bile), or 40 (gastric fluid) μL aliquot. (Borate buffer was 12.4 g boric acid and 10 mL 1 M NaOH made up to 1 L with water, pH 7.2.)

HPLC VARIABLES

Guard column: Microguard reverse-phase (Bio-Rad)

Column: 100 × 8 Radial-PAK C18 in a radial compression module

Mobile phase: MeCN:buffer 70:30 (Buffer was 6.8 g/L KH_2PO_4 adjusted to pH 3.0 with 85% phosphoric acid.)

Flow rate: 2

Injection volume: 20-40

Detector: UV 340

CHROMATOGRAM

Retention time: 6

Internal standard: indomethacin (13)

Limit of quantitation: 500 ng/mL (urine, bile, gastric fluid), 250 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Musson,D.G.; Vincek,W.C.; Constanzer,M.L.; Detty,T.E. Analytical methods for the determination of sulindac and metabolites in plasma, urine, bile, and gastric fluid by liquid chromatography using ultraviolet detection, *J.Pharm.Sci.*, **1984**, *73*, 1270–1273.

SAMPLE

Matrix: blood

Sample preparation: Mix 500 μL serum with 100 μL 20 mg/mL IS in MeOH:50 mM pH 3.0 sodium phosphate buffer 50:50, vortex for 5 s. Add 1 mL MeCN, vortex for 1 min. Centrifuge the mixture at 14000 rpm for 5 min, decant the clear upper layer. Add 500 μL MeCN to the pellet, mix, centrifuge at 14000 rpm for 5 min. Combine the upper layers and evaporate at 40°. Reconstitute the residue with 300 μL MeOH:50 mM pH 3.0 sodium phosphate buffer 40:60 containing 0.5% sodium metabisulfate (sic). Vortex for 30 s and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Techsil C18 (HPLC Technology, Macclesfield)

Mobile phase: MeCN:50 mM phosphate buffer 46:63, adjusted to pH 3.0 with NaOH

Flow rate: 1.2

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 4.17

Internal standard: indomethacin (7.17)

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics

REFERENCE
Kanfer,I.; Brown,C.; Koninigs,M.; Swarbrick,J. Absorption of sulindac from a novel (Pro-SorbTM) liquid formulation, *Biopharm.Drug Dispos.*, **1996**, *17*, 209–221.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 250 μL 1 M sulfuric acid + 5 mL 1.7 μg/mL diphenylacetic acid in dichloromethane, vortex for 10 s, centrifuge at 500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax ODS
Mobile phase: Gradient. MeOH:100 mM pH 5 acetate buffer from 51:49 to 80:20 over 3 min, maintain at 80:20 for 6 min.
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 17.42
Internal standard: diphenylacetic acid (6.97)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: caffeine, carbamazepine, ethosuximide, fenoprofen, ibuprofen, indomethacin, naproxen, phenobarbital, phenytoin, primidone, quinidine, theophylline, tolmetin
Noninterfering: acetaminophen, salicylic acid, valproic acid

KEY WORDS
plasma

REFERENCE
Shimek,J.L.; Rao,N.G.S.; Wahba Khalil,S.K. High performance liquid chromatographic analysis of tolmetin, indomethacin and sulindac in plasma, *J.Liq.Chromatogr.*, **1981**, *4*, 1987–2013.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + indomethacin + 100 μL pH 2 dilute sulfuric acid + 1 mL MeCN, vortex for 30 s, centrifuge at 2500 rpm for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:4% aqueous acetic acid 45:55
Flow rate: 1
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 4
Internal standard: indomethacin (6)
Limit of quantitation: 630 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Clark,C.R.; McMillian,C.L.; Hoke,J.F.; Campagna,K.D.; Ravis,W.R. Liquid chromatographic determination of sulindac and metabolites in serum, *J.Chromatogr.Sci.*, **1987**, *25*, 247–251.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 250 μL 2.5 M phosphoric acid + 6 mL 100 ng/mL phenprocoumon in dichloromethane, vortex for 1 min, centrifuge at 1500 g for 10 min. Remove the lower organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL 50 mM NaOH, vortex for 1 min, inject a 75-150 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm phenyl (Waters)
Mobile phase: MeCN:water:glacial acetic acid 42:57:1 containing 10 mM sodium acetate, pH 4.2
Flow rate: 2
Injection volume: 75-150
Detector: UV 315

CHROMATOGRAM
Retention time: 4.2
Internal standard: phenprocoumon (8.6)
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Grgurinovich,N. High-performance liquid chromatography of sulindac and its sulphone and sulphide metabolites in plasma, *J.Chromatogr.*, **1987**, *414*, 211–216.

SAMPLE
Matrix: blood
Sample preparation: Wash a Sep-Pak C18 cartridge with 2 mL MeOH, 5 mL water, and 1 mL 0.25 mM pH 3.0 ammonium phosphate buffer. 20-200 μL Plasma + 100 μL MeOH + 20 μL 50 μg/mL indomethacin in MeOH + 100 μL 0.25 mM pH 3.0 ammonium phosphate buffer + 100 μL water, vortex for 2 min, centrifuge at 1800 g for 10 min. Add the supernatant to the cartridge, wash with 5 mL water, elute twice with 5 mL portions of MeOH. Evaporate eluate to dryness under vacuum, dissolve the residue in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee RP18
Mobile phase: MeOH:buffer 75:25 (Buffer prepared by diluting 0.25 mM ammonium phosphate buffer adjusted to pH 3.0 with orthophosphoric acid.)
Injection volume: 20
Detector: E ESA Coulochem Model 5100 A, + 0.9 V

CHROMATOGRAM
Retention time: 7.6
Internal standard: indomethacin (14.6)
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Also analyzed: naproxen, piroxicam, diflunisal

KEY WORDS
plasma; SPE

REFERENCE
Kazemifard,A.G.; Moore,D.E. Liquid chromatography with amperometric detection for the determination of non-steroidal anti-inflammatory drugs in plasma, *J.Chromatogr.*, **1990**, *533*, 125–132.

SAMPLE
Matrix: blood
Sample preparation: Prepare a Sep-Pak C18 SPE cartridge by washing with 2 mL MeOH, 5 mL water, and 1 mL buffer. 20-200 μL Plasma + 100 μL MeOH + 20 μL 50 μg/mL indomethacin in MeOH + 100 μL buffer + 100 μL water, vortex for 2 min, centrifuge at 1800 g for 10 min, apply supernatant to the SPE cartridge, wash with 5 mL water, elute with two 5 mL portions of MeOH, evaporate eluate and take up residue in 1 mL mobile phase. (Buffer was 250 mM ammonium phosphate buffer adjusted to pH 3.0 with orthophosphoric acid.)

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee RP18
Mobile phase: MeOH:25 mM pH 3.0 phosphate buffer 75:25 (Prepare buffer by diluting a 250 mM ammonium phosphate buffer adjusted to pH 3.0 with orthophosphoric acid.)
Injection volume: 20
Detector: E, ESA Coulochem Model 5100 A, +0.9 V

CHROMATOGRAM
Retention time: 7.6
Internal standard: indomethacin (14.6)
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Simultaneous: naproxen
Interfering: diflunisal

KEY WORDS
plasma; SPE

REFERENCE
Kazemifard,A.G.; Moore,D.E. Liquid chromatography with amperometric detection for the determination of non-steroidal anti-inflammatory drugs in plasma, *J.Chromatogr.*, **1990**, *533*, 125–132.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C2 SPE cartridge with 1 mL MeOH and 1 mL 100 mM HCl just before use. 500 μL Plasma + 500 μL 100 mM HCl, vortex briefly, add to SPE cartridge, wash with 1 mL 100 mM HCl, elute with 400 μL MeOH:MeCN 60:40, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 RCSS Guard Pak
Column: 100 × 8 4 μm Nova Pak C18 Radial Pak
Mobile phase: MeCN:buffer 42:58 (Buffer was 50 mM sodium citrate adjusted to pH 4.3 with 50 mM HCl.)
Flow rate: 2.3
Injection volume: 20

Detector: UV 342

CHROMATOGRAM
Retention time: 3.07
Internal standard: sulindac

OTHER SUBSTANCES
Extracted: rifampin

KEY WORDS
plasma; sulindac is IS; SPE

REFERENCE
Swart,K.J.; Papgis,M. Automated high-performance liquid chromatographic method for the determination of rifampicin in plasma, *J.Chromatogr.*, **1992**, *593*, 21–24.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C2 SPE cartridge with 1 mL MeOH and 1 mL mobile phase. 1 mL Serum + 75 μL 100 μg/mL indomethacin in MeOH + 1 drop saturated ammonium sulfate solution + 1 drop 1 M HCl, vortex for 3 min, add to the SPE cartridge, wash with six 500 μL portions of wash solvent, elute with four 250 μL aliquots of mobile phase, combine the eluates, vortex, inject a 100 μL aliquot. (Wash solvent was MeCN:water adjusted to pH 3.0 with phosphoric acid 20:80.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:68 mM pH 2.5 phosphate buffer 55:45
Flow rate: 0.5
Injection volume: 100
Detector: F ex 232 em 335 (filter) following post-column photolysis. The effluent from the column flowed through a 7.9 m × 0.3 mm i.d. coil of PTFE irradiated by an SC3-9 UV lamp (UVP) (cooled with air) to the detector.

CHROMATOGRAM
Retention time: 7
Internal standard: indomethacin (12)
Limit of detection: 10 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; post-column photochemical derivatization; SPE

REFERENCE
Siluveru,M.; Stewart,J.T. Determination of sulindac and its metabolites in human serum by reversed-phase high-performance liquid chromatography using on-line post-column ultraviolet irradiation and fluorescence detection, *J.Chromatogr.B*, **1995**, *673*, 91–96.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 227

CHROMATOGRAM

Retention time: 4.11
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C2 SPE cartridge with 1 mL MeOH and 1 mL water. 500 μL Plasma + 100 μL 100 mM HCl, vortex briefly, centrifuge at 630 rpm for 5 min, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeOH, add the eluate to 500 μL 3 mg/mL ascorbic acid, inject a 150 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-8 (Brownlee, ABI)
Column: 250 × 4.6 5 μm Zorbax RX C8
Mobile phase: MeCN:50 mM KH_2PO_4 45:55
Flow rate: 1
Injection volume: 150
Detector: UV 340

CHROMATOGRAM
Retention time: 7.8
Internal standard: sulindac

OTHER SUBSTANCES
Extracted: rifampin
Simultaneous: atevirdine, delavirdine, U-89,255, U-96,183

KEY WORDS
sulindac is IS; plasma; protect from light; SPE

REFERENCE
Lau,Y.Y.; Hanson,G.D.; Carel,B.J. Determination of rifampin in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1996**, *676*, 147–152.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C8 SPE cartridge with 1 mL MeOH and 1 mL water. Centrifuge 1 mL plasma at 630 rpm for 5 min, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 RP-8 (Brownlee, ABI)
Column: 250 × 4.6 5 μm Zorbax RX C8
Mobile phase: MeCN:buffer 47:53 (Buffer was 50 mM KH_2PO_4 containing 50 mM sodium acetate, pH adjusted to 4.0 with acetic acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM
Retention time: 6.9
Internal standard: sulindac

OTHER SUBSTANCES
Extracted: rifabutin
Simultaneous: atevirdine, delavirdine, U-89,255, U-96,183

KEY WORDS
plasma; SPE; sulindac is IS

REFERENCE
Lau,Y.Y.; Hanson,G.D.; Carel,B.J. Determination of rifabutin in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1996**, *676*, 125–130.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 100 μL MeCN:water 80:20 + 100 μL 40 μg/mL indomethacin in MeCN:water 80:20 + 1 mL MeCN, vortex for 10 s, centrifuge at 2000 g for 10 min. Remove the supernatant and add it to 1 mL water, inject a 30 μL aliquot. Urine. 500 μL Urine + 100 μL MeCN:water 80:20 + 100 μL 125 μg/mL indomethacin in MeCN:water 80:20

+ 250 μL Glusulase solution, heat at 37° for 1 h, inject a 25 μL aliquot. (Glusulase solution was 250 μL Glusulase and 500 μL 1 M pH 5.2 sodium acetate in 25 mL water.)

HPLC VARIABLES
Column: 50 × 4.6 3 μm Sepralyte C18 (Analytichem)
Mobile phase: Gradient. MeCN:buffer 34:66 for 3 min, to 70:30 over 2 min, maintain at 70:30 for 1 min, re-equilibrate at initial conditions for 3 min.
Column temperature: 50
Flow rate: 1.5
Injection volume: 25-30
Detector: UV 340

CHROMATOGRAM
Retention time: 4 (plasma), 2.3 (urine)
Internal standard: indomethacin (5.9)
Limit of detection: 200 ng/mL (urine), 100 ng/mL (plasma)

KEY WORDS
plasma

REFERENCE
Stubbs,R.J.; Ng,L.L.; Entwistle,L.A.; Bayne,W.F. Analysis of sulindac and metabolites in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 171–180.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 300 μL Plasma + 1 mL 6 μg/mL indomethacin in MeCN, vortex for 30 s, centrifuge for 5 min. Remove the supernatant and evaporate it to 200 μL under a stream of nitrogen, add 400 μL MeCN:50 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 40:60, vortex, inject an aliquot. Urine. 300 μL Urine + 100 μL 5 M NaOH, vortex, let stand for 15 min at room temperature, add 100 μL phosphoric acid, vortex, add 1 mL 6 μg/mL indomethacin in MeCN, vortex for 30 s, centrifuge for 5 min. Remove the supernatant and evaporate it to 200 μL under a stream of nitrogen, add 400 μL MeCN:50 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 40:60, vortex, inject an aliquot.

HPLC VARIABLES
Column: reverse phase
Mobile phase: Gradient. MeCN:50 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid from 40:60 to 60:40 over 10 min.
Detector: UV

CHROMATOGRAM
Retention time: 3.0
Internal standard: indomethacin (6.8)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Brandli,D.W.; Sarkissian,E.; Ng,S.C.; Paulus,H.E. Individual variability in concentrations of urinary sulindac sulfide, *Clin.Pharmacol.Ther.*, **1991**, *50*, 650–655.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.627

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cytosol incubations
Sample preparation: 2 mL Incubation + 2 mL 2 M + 15 μg sulfinpyrazone sulfide + 5 mL chlorobutane:1,2-dichloroethane 80:20, shake for 15 min, centrifuge at 4000 g for 10 min. Remove the upper organic layer and add it to 400 μL 100 mM NaOH, shake for 10 min, inject a 50-80 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:200 mM pH 3.5 ammonium phosphate 47:53
Flow rate: 2.2
Injection volume: 50-80
Detector: UV 254

CHROMATOGRAM
Internal standard: sulfinpyrazone sulfide

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; rabbit

REFERENCE
Lee,S.C.; Renwick,A.G. Sulphoxide reduction by rat and rabbit tissues *in vitro*, *Biochem.Pharmacol.*, **1995**, *49*, 1557–1565.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablet, add 100 mL MeOH, stir for 5 min. Remove a 1 mL aliquot and add it to 5 mL 200 μg/mL propyl paraben in MeOH, make up to 25 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL ODS
Mobile phase: MeOH:50 mM pH 6.0 acetate buffer 50:50
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 3.5
Internal standard: propyl paraben (7.8)

KEY WORDS
stability-indicating; tablets

REFERENCE
Jalal,I.M.; Khalil,H.S.; Jawhari,D. Stability-indicating assay for sulindac in tablet formulations by reverse-phase HPLC, *J.Liq.Chromatogr.*, **1989**, *12*, 3087–3102.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 6-10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 2.69
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetanilide, amobarbital, barbital, butabarbital, butalbital, caffeine, carbamazepine, chloramphenicol, cyheptamide, diazoxide, diflunisal, disopyramide, ethchlorvynol, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, mephobarbital, methaqualone, methsuximide, methsuximide, methyl salicylate, methyprylon, naproxen, nirvanol, oxphenylbutazone, pentobarbital, phenacetin, phenobarbital, phensuximide, phenytoin, salicylamide, secobarbital, theophylline, thiopental, tolmetin
Noninterfering: N-acetylcysteine, N-acetylprocainamide, amikacin, ampicillin, aspirin, chlorpropamide, codeine, diphylline, gentamicin, gentisic acid, meprobamate, morphine, netilmicin, procainamide, quinidine, salicylic acid, sulfamethoxazole, tetracycline, tobramycin, trimethoprim, valproic acid, vancomycin
Interfering: ethosuximide, cimetidine, primidone, phenylbutazone

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 3 Ecocart LiChrospher 100 RP-18
Mobile phase: Isopropanol:100 mM KH_2PO_4:formic acid 54:100:0.1
Flow rate: 0.6
Detector: UV 254

CHROMATOGRAM
Retention time: 3.2
Limit of quantitation: 200-500 ng/mL

OTHER SUBSTANCES
Simultaneous: acemetacin; diclofenac; flurbiprofen; indomethacin; lonazolac; ketoprofen; naproxen; piroxicam; tenoxicam

REFERENCE
Baeyens,W.R.G.; Van Der Weken,G.; Van Overbeke,A.; Zhang,Z.D. Preliminary results on the LC-separation of non-steroidal anti-inflammatory agents in conventional and narrow-bore RP set-ups applying columns with different internal diameters, *Biomed.Chromatogr.*, **1995**, *9*, 261–262.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 6.17 (A), 5.99 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 µL 20 µg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 µL 1 M pH 5.0 acetate buffer, vortex. Add 250 µL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL mobile phase, inject a 10-30 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230, UV 320

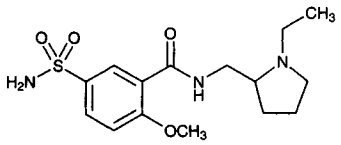
CHROMATOGRAM
Retention time: 4
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, fenbufen, flurbiprofen, ketoprofen, loxoprofen, mefenamic acid, naproxen, piroxicam

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

Sulpiride



Molecular formula: $C_{15}H_{23}N_3O_4S$
Molecular weight: 341.43
CAS Registry No.: 15676-16-1
Merck Index: 9163
Lednicer No.: 2 94

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 292

CHROMATOGRAM

Retention time: 3.26
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

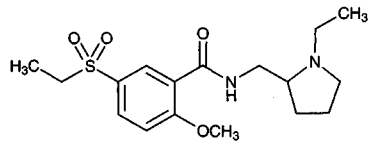
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 212.2

CHROMATOGRAM
Retention time: 3.858

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Sultopride



Molecular formula: $C_{17}H_{26}N_2O_4S$
Molecular weight: 354.47
CAS Registry No.: 53583-79-2, 23694-17-9 (HCl)
Merck Index: 9168

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 4 mL 500 mM NaOH, 2 g NaCl and 1 micro.L 100 μg/mL tiapride in MeOH. Add 10 mL MTBE, shake for 10 min and centrifuge at 850 g for 10 min. Remove the solvent layer, mix with 2.5 mL 100 mM HCl shake and centrifuge at 850 g for 10 min. Remove the aqueous layer, add it to 1 mL 500 mM NaOH, 1.5 g NaCl, and 2 mL MTBE, shake for 10 min and centrifuge. Remove the solvent layer and evaporate to dryness under a stream of nitrogen. Reconstitute the residue in 50 μL MeCN and inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 5 μm Hypersil silica
Mobile phase: MeCN:100 mM ammonium acetate 94:6
Column temperature: 40
Flow rate: 0.4
Injection volume: 10
Detector: UV 240; MS, Hewlett-Packard Model 59980A, particle beam nebulizer helium 35 psi, solvation chamber 60°, Model 5989A, negative ion chemical ionization mode, reagent gas methane at 1 torr, source 250°, m/z 339

CHROMATOGRAM
Retention time: 12.9
Internal standard: tiapride (11.0)
Limit of quantitation: 5 ng/mL (UV), 10 ng/ml (MS)

KEY WORDS
plasma; pharmacokinetics

REFERENCE

Jitsufuchi,N.; Kudo,K.; Tokunaga,H.; Imamura,T. Selective determination of sultopride in human plasma using high-performance liquid chromatography with ultraviolet detection and particle beam mass spectrometry, *J.Chromatogr.B*, **1997**, *690*, 153–159.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 235

CHROMATOGRAM

Retention time: 3.47

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

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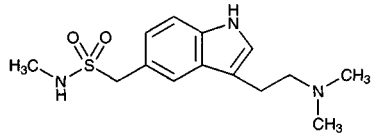
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 212.2

CHROMATOGRAM
Retention time: 13.012

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Sumatriptan



Molecular formula: $C_{14}H_{21}N_3O_2S$
Molecular weight: 295.41
CAS Registry No.: 103628-46-2, 103628-48-4 (succinate)
Merck Index: 9172
Lednicer No.: 5 108

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Isolute CBA SPE cartridge (Hengoed, UK) with full column volumes of MeOH and water. Apply 1 mL plasma to SPE cartridge, wash with two column volumes of water. Dry SPE cartridge under vacuum. Elute with one column volume of 1% ammonia in MeOH. Evaporate eluate to dryness under vacuum at 50°, reconstitute the residue in 150 μL mobile phase, vortex, inject an aliquot.

HPLC VARIABLES
Guard column: Brownlee Newguard C2 (Anachem, UK)
Column: 100 × 4 5 μm cyano-propyl column (Capitol HPLC, UK)

Mobile phase: MeOH:40 mM pH 5.3 potassium phosphate buffer 60:40
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem 2, Model 5020 guard cell, Model 5011 analytical cell, detector 1 + 450 mV, detector 2 + 850 mV, guard cell + 900 mV

CHROMATOGRAM
Retention time: 6.1
Internal standard: sumatriptan succinate

OTHER SUBSTANCES
Extracted: naloxone

KEY WORDS
plasma; SPE; sumatriptan is IS

REFERENCE
Franklin,M.; Odontiadis,J. Determination of naloxone in human plasma by high-performance liquid chromatography with coulometric determination, *J.Chromatogr.B*, **1996**, *679*, 199–203.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Lichrolut C18-Select B SPE cartridge with 3 mL MeOH and 3 mL water. Mix 100 or 300 μL plasma with 500 μL 40 ng/mL IS in MeOH:water 50:50, add 800 μL MeOH, vortex, centrifuge at 15000 rpm for 10 min. Evaporate the supernatant to dryness under a stream of nitrogen at 40°, reconstitute the sample in 1 mL MeOH:water 10:90. Add 1 mL of the sample to the SPE cartridge, wash with 5 mL water, wash with 3 mL MeOH:water 20:80, elute with 2 mL MeCN containing 10% 1 M HCl, evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 300 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 2 4 μm Nova-Pak C8
Mobile phase: Gradient. A was MeCN:20 mM ammonium acetate 10:90. B was MeCN:20 mM ammonium acetate 80:20. A:B from 80:20 to 20:80 over 20 min, re-equilibrate at initial conditions for 10 min
Column temperature: 35
Flow rate: 0.5
Injection volume: 30
Detector: MS, Finnigan MAT SSQ-700 or TSQ-7000, API, ESI, 4.8 kV needle, +5.8 V to the capillary, +44.6 V to the tube lens, source 230°, m/z 339

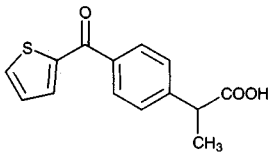
CHROMATOGRAM
Internal standard: MDL 74,967

OTHER SUBSTANCES
Extracted: MDL 74,721, naratriptan

KEY WORDS
plasma; rabbit; SPE; pharmacokinetics

REFERENCE
Duléry,B.D.; Petty,M.A.; Schoun,J.; David,M.; Huebert,N.D. A method using a liquid chromatographic-electrospray-mass spectrometric assay for the determination of antimigraine compounds: preliminary pharmacokinetics of MDL 74,721, sumatriptan and naratriptan, in rabbit, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1009–1020.

Suprofen



Molecular formula: $C_{14}H_{12}O_3S$
Molecular weight: 260.31
CAS Registry No.: 40828-46-4
Merck Index: 9180
Lednicer No.: 2 65

SAMPLE

Matrix: blood
Sample preparation: Adjust pH of plasma to 3 with phosphoric acid. 100 µL Plasma + 300 µL IS in MeCN, mix, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 µL MeCN:water 25:75, inject an aliquot.

HPLC VARIABLES

Guard column: 15 × 2.1 C18 (Brownlee)
Column: 150 × 4 5 µm Axxiom C18
Mobile phase: MeOH:10 mM pH 5.1 sodium acetate 37.5:62.5
Flow rate: 1
Detector: UV 295

CHROMATOGRAM

Retention time: 12.9
Internal standard: 5-(4-methoxybenzoyl)-1,4-dimethyl-1H-pyrrole-2-acetic acid (MCN 2967, R.W. Johnson) (11.1)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, glucuronides

KEY WORDS

cow; plasma

REFERENCE

Smith,P.C.; Liu,J.H. Covalent binding of suprofen acyl glucuronide to albumin *in vitro*, *Xenobiotica*, **1993**, *23*, 337–348.

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 400 µg/mL solution in MeCN:water 30:70, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Cyclobond I β-cyclodextrin (Advanced Separation Technologies)
Mobile phase: MeCN:buffer 30:70 (Buffer was 1 mL/L triethylamine in water adjusted to pH 4.5 ± 0.1 with glacial acetic acid.)
Column temperature: 35
Flow rate: 2
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Limit of detection: 0.04% (of major isomer)

OTHER SUBSTANCES

Simultaneous: positional isomers

REFERENCE

Marziani,F.C.; Sisco,W.R. Liquid chromatographic separation of positional isomers of suprofen on a cyclodextrin-bonded phase, *J.Chromatogr.*, **1989**, *465*, 422–428.

SAMPLE

Matrix: cell suspensions

Sample preparation: Centrifuge cell suspension at 2000 g for 4 min. Remove a 2 mL aliquot of the supernatant and add it to 200 μL 200 μg/mL IS in DMF, mix, add 200 μL 5 M HCl, extract twice with 3 mL portions of toluene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, add 20 μL 10 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 300 μL 10 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 300 μL 10 mg/mL (-)-(S)-α-methylbenzylamine in dichloromethane, let stand for 30 min, evaporate to dryness, reconstitute with 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 10 mm long Techsphere ODS (HPLC Technology, Macclesfield UK)
Column: 250 × 5 5 μm Techsphere ODS (HPLC Technology, Macclesfield UK)
Mobile phase: MeCN:7.5 mM NaH_2PO_4 50:50, containing 5 mM sodium pentanesulfonate, pH adjusted to 2.8 with phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: k' 5.65, 6.40 (enantiomers)
Internal standard: (S)-naproxen (k' 7.45)
Limit of detection: 500 ng/mL

KEY WORDS

derivatization; chiral

REFERENCE

Thomason,M.J.; Hung,Y.-F.; Rhys-Williams,W.; Hanlon,G.W.; Lloyd,A.W. Indirect enantiomeric separation of 2-arylpropionic acids and structurally related compounds by reversed phase HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1765–1774.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.5 5 μm Ultrasphere octyl
Mobile phase: MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, pH 4.35
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 3.22
Internal standard: naproxen (3.89)

OTHER SUBSTANCES

Simultaneous: bacitracin, diazepam, diclofenac, flurbiprofen, hydrocortisone acetate, imipramine, indomethacin, ketoprofen, ketorolac tromethamine, levobunolol, meclofenamic acid, metipranolol, neomycin, proparacaine, propranolol, salicylic acid, sulfacetamide
Noninterfering: acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide
Interfering: cortisone acetate, fluorometholone, prednisolone acetate

REFERENCE

Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)
Mobile phase: 50 mM pH 5.5 KH_2PO_4
Flow rate: 0.8
Injection volume: 20
Detector: UV

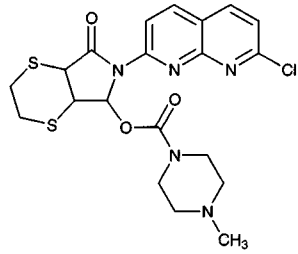
CHROMATOGRAM
Retention time: k' 8.27

OTHER SUBSTANCES
Simultaneous: flurbiprofen, isradipine, ketoprofen, nimodipine

KEY WORDS
chiral; α = 1.08

REFERENCE
Massolini,G.; De Lorenzi,E.; Ponci,M.C.; Gandini,C.; Caccialanza,G.; Monaco,H.L. Egg yolk riboflavin binding protein as a new chiral stationary phase in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 55–65.

Suriclone



Molecular formula: $C_{20}H_{20}ClN_5O_3S_2$
Molecular weight: 478.00
CAS Registry No.: 53813-83-5
Merck Index: 9182

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 244

CHROMATOGRAM

Retention time: 6.74
Limit of detection: <120 ng/mL

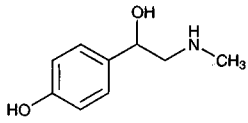
KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Synephrine



Molecular formula: $C_9H_{13}NO_2$
Molecular weight: 167.21
CAS Registry No.: 94-07-5, 5985-28-4 (HCl), 6414-49-9 (tartaric acid monoester), 16589-24-5 (tartrate)
Merck Index: 9189

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.02

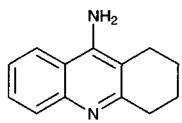
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tacrine

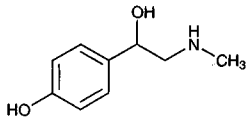


Molecular formula: $C_{13}H_{14}N_2$
Molecular weight: 198.27
CAS Registry No.: 321-64-2, 1684-40-8 (HCl)
Merck Index: 9199
Lednicer No.: 5 166

SAMPLE

Matrix: bile, dialysate
Sample preparation: Bile. 100 µL Bile + 50 µL 500 mM NaOH, vortex for 1 min, add 500 µL ethyl acetate, vortex for 2 min, centrifuge at 12000 g for 30 s. Remove the organic layer and evaporate it to dryness under a stream of argon at 55°, reconstitute the residue in 100 µL

Synephrine



Molecular formula: $C_9H_{13}NO_2$
Molecular weight: 167.21
CAS Registry No.: 94-07-5, 5985-28-4 (HCl), 6414-49-9 (tartaric acid monoester), 16589-24-5 (tartrate)
Merck Index: 9189

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.02

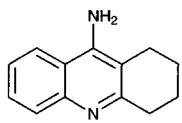
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tacrine



Molecular formula: $C_{13}H_{14}N_2$
Molecular weight: 198.27
CAS Registry No.: 321-64-2, 1684-40-8 (HCl)
Merck Index: 9199
Lednicer No.: 5 166

SAMPLE

Matrix: bile, dialysate
Sample preparation: Bile. 100 µL Bile + 50 µL 500 mM NaOH, vortex for 1 min, add 500 µL ethyl acetate, vortex for 2 min, centrifuge at 12000 g for 30 s. Remove the organic layer and evaporate it to dryness under a stream of argon at 55°, reconstitute the residue in 100 µL

Ringer's solution, inject a 5 μL aliquot. Dialysate. Inject a 5 μL aliquot of dialysate directly. (The dialysis solution was Ringer's solution that contained 155 mM NaCl, 5.5 mM KCl, and 2.3 mM $CaCl_2$.)

HPLC VARIABLES
Column: 100 × 1 5 μm Spherisorb phenyl
Mobile phase: MeCN:MeOH:50 mM pH 2.5 ammonium phosphate buffer 5:10:85
Flow rate: 0.045
Injection volume: 5
Detector: F ex 330 em 365

CHROMATOGRAM
Retention time: 11
Limit of detection: 0.25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
microbore; rat; pharmacokinetics

REFERENCE
Hadwiger,M.E.; Telting-Diaz,M.; Lunte,C.E. Liquid chromatographic determination of tacrine and its metabolites in rat bile microdialysates, *J.Chromatogr.B*, **1994**, *655*, 235–241.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 500 mM NaOH + 5 mL dichloromethane, shake gently for 15 min, centrifuge at 500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:20 mM pH 2.7 phosphate buffer 20:80
Flow rate: 1.1
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Ekman,L.; Lindström,B.; Roxin,P. Determination of tacrine and its 1-hydroxy metabolite in plasma using column liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1989**, *494*, 397–402.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 250 μL picric acid (1:50 dilution of saturated picric acid solution) + 250 μL water + 2.5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 μL MeCN:water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 μPorasil
Mobile phase: MeOH:0.1 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100
Detector: UV 210

CHROMATOGRAM
Retention time: 5
Internal standard: tacrine

OTHER SUBSTANCES
Simultaneous: laudanosine

KEY WORDS
plasma; tacrine is IS

REFERENCE
Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 ng IS + 500 μL 500 mM NaOH, vortex briefly, add 5 mL cyclohexane:ethyl acetate 50:50, shake for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Hypersil phenyl
Mobile phase: MeCN:20 mM pH 2.75 ammonium formate buffer 70:30
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: 15
Internal standard: N-methoxy-1,2,3,4-tetrahydroacridin-9(10H)-imine (18)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Hsu,R.S.; DiLeo,E.M.; Chesson,S.M. High-performance liquid chromatography for the determination of tacrine and its metabolites in plasma, *J.Chromatogr.*, **1990**, *530*, 170–176.

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. Add 25 μL 10 μM IS , 250 μL 500 mM sodium hydroxide, and 5 mL ethyl acetate to 500 μL plasma, shake for 10 min, centrifuge at 1100 g for 10 min. Keep at -30° until the aqueous phase is frozen. Evaporate the organic phase to dryness under a stream of nitrogen at 40° for 45 min, reconstitute in 300 μL mobile phase, vortex for 30 s, centrifuge at 1100 g for 1 min and at 13000 g for 15 min. Take a 100 μL sample from the middle and inject. Urine. Add 25 μL 100 μM IS, 250 μL 500 mM sodium hydroxide, and 5 mL ethyl acetate to 500 μL plasma, shake for 10 min and centrifuge at 1100 g for 10 min. Keep at -30° until the aqueous phase is frozen. Evaporate the organic phase to dryness under a

stream of nitrogen at 40° for 45 min, reconstitute in 900 μL mobile phase and vortex for 30 s. Inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: LiChrospher 60 RP-select B
Column: 250 × 4 5 μm LiChrospher 60 RP-select B
Mobile phase: MeCN:200 mM pH 4.0 acetate buffer 13:87 (Buffer was 16.406 g sodium acetate in 1 L water, pH adjusted to 4.0 with 60% perchloric acid.)
Column temperature: 30
Flow rate: 1.25 for 16 min, 2.5 for 24 min
Injection volume: 100
Detector: F ex 330 em 365

CHROMATOGRAM
Retention time: 32.93 (blood), 31.71 (urine)
Internal standard: 1,2,3,4,-tetrahydro-9-acridanone (Aldrich) (30.39 (blood), 29.30 (urine))
Limit of detection: 2 nM (blood), 80 nM (urine)
Limit of quantitation: 2 nM (blood), 120 nM (urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Hansen,L.L.; Larsen,J.T.; Brosen,K. Determination of tacrine and its metabolites in human plasma and urine by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.B*, **1998**, *712*, 183–191.

SAMPLE
Matrix: diffusate, tissue
Sample preparation: Homogenize (Polytron PCU-2) 150-200 mg skin and diazepam with 4 mL chloroform, repeat homogenization, filter (phase-separating paper) extracts. Make the residue alkaline with 2 mL 10% NaOH, extract twice with 4 mL portions of chloroform, wash the extracts twice with 2 mL portions of water, filter (phase-separating paper) the organic layer. Combine all the chloroform layers and evaporate them to dryness under a stream of air, reconstitute the residue in 1 mL mobile phase, filter (microfilter), inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 4 40 μm ODS (Valco)
Column: 150 × 4.6 5 μm Spherisorb ODS-I
Mobile phase: MeCN:water 52:48 containing 10 mM octanesulfonic acid and 1% acetic acid, pH 3.5
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.7
Internal standard: diazepam (6.0)
Limit of detection: 5 μg/g

OTHER SUBSTANCES
Extracted: physostigmine

KEY WORDS
skin; pharmacokinetics; stability-indicating

REFERENCE

Lau,S.W.J.; Chow,D.; Feldman,S. Simultaneous determination of physostigmine and tetrahydroaminoacridine in a transdermal permeation study by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *526*, 87–95.

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 5 mL ice-cold MeCN, let stand overnight at 4°, centrifuge at 1000 g for 15 min. Evaporate the supernatant to dryness, reconstitute in MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: present but not specified
Column: 250 mm long Ultracarb 5 C8 (Phenomenex)
Mobile phase: MeCN:100 mM pH 3.9 ammonium acetate buffer 12:88
Flow rate: 1.25
Detector: UV 254

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; rat; mouse; dog; hamster; rabbit; liver

REFERENCE

Madden,S.; Spaldin,V.; Hayes,R.N.; Woolf,T.F.; Pool,W.F.; Park,B.K. Species variation in the bioactivation of tacrine by hepatic microsomes, *Xenobiotica*, **1995**, *25*, 103–116.

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 5 mL MeCN, let stand at 4° overnight, centrifuge at 1000 g. Remove the supernatant and evaporate it to dryness, reconstitute the residue in a small volume of MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.2 Nucleosil 5 C8
Mobile phase: Gradient. MeCN:100 mM pH 3.8 ammonium acetate buffer 10:90 for 15 min, to 15:85 over 5 min, to 20:80 over 10 min.
Flow rate: 1.25
Detector: UV 254

CHROMATOGRAM

Retention time: 32

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; liver

REFERENCE

Spaldin,V.; Madden,S.; Adams,D.A.; Edwards,R.J.; Davies,D.S.; Park,B.K. Determination of human hepatic cytochrome P4501A2 activity *in vitro*. Use of tacrine as an isoenzyme-specific probe, *Drug Metab.Dispos.*, **1995**, *23*, 929–934.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: urine
Sample preparation: 0.5-1 mL Urine + 1 μg IS + 1 mL 1 M NaOH, vortex briefly, add 5 mL cyclohexane:ethyl acetate 50:50, shake for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, inject an aliquot. (Hydrolyze conjugates by heating 0.5-1 mL urine, 2 mL 100 mM pH 5 acetate buffer, and 1000 units β-glucuronidase (type H-1, Sigma) at 37° for 4 h, proceed as above.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Hypersil phenyl
Mobile phase: MeCN:50 mM ammonium formate buffer 70:30, pH 3.1
Flow rate: 1.5
Detector: UV 325

CHROMATOGRAM
Internal standard: N-methoxy-1,2,3,4-tetrahydroacridin-9(10H)-imine

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; pharmacokinetics

REFERENCE
Hsu,R.S.; Shutske,G.M.; DiLeo,E.M.; Chesson,S.M.; Linville,A.R.; Allen,R.C. Identification of the urinary metabolites of tacrine in the rat, *Drug Metab.Dispos.*, **1990**, *18*, 779–783.

SAMPLE
Matrix: urine
Sample preparation: Evaporate 5 μg of IS dissolved in MeOH into the bottom of a tube using a stream of nitrogen, add 1 mL urine, add 1 mL water, add 500 μL 5 M NaOH, add 5 mL chloroform, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL mobile phase, inject the whole amount. (To hydrolyze conjugates heat 1 mL urine containing 5 μg IS and 1 mL enzyme solution at 37° for 16 h, add 500 μL 5 M NaOH, proceed as above. Prepare enzyme solution by diluting 400 μL 100000 U/mL β-glucuronidase and 3000 U/mL sulfatase (Type H2, Sigma) to 20 mL with 100 mM pH 5.0 acetate buffer.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: Hexane:isopropanol:diethylamine 70:30:0.1
Flow rate: 1
Injection volume: 100
Detector: UV 254

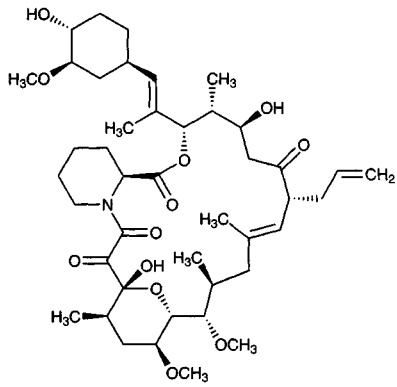
CHROMATOGRAM
Retention time: 27
Internal standard: 1-amino-4-nitronaphthalene (9)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Hooper,W.D.; Pool,W.F.; Woolf,T.F.; Gal,J. Stereoselective hydroxylation of tacrine in rats and humans, *Drug Metab.Dispos.*, **1994**, *22*, 719–724.

Tacrolimus

Molecular formula: $C_{44}H_{69}NO_{12}$
Molecular weight: 804.03
CAS Registry No.: 104987-11-3
Merck Index: 9200



SAMPLE

Matrix: blood
Sample preparation: Add 100 µL 50 ng/mL IS in MeOH + 1.0 mL water + 5 mL dichloromethane:cyclohexane 40:60 to 500 µL whole blood. Mix using reciprocating shaker at low speed for 1 hour, centrifuge at 3000 rpm for 10 min. Remove the organic phase (leaving at least 0.5 cm behind) and evaporate it to dryness. Reconstitute the residue with 50 µL MeOH. Inject a 5 µL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 5 µm ODS-Hypersil
Mobile phase: MeOH:5 mM ammonium acetate adjusted to pH 9.0 with 28-30% ammonium hydroxide solution 99:1
Flow rate: 0.3
Injection volume: 5
Detector: MS, PE SCIEX (Perkin-Elmer Sciex) API III MS/MS, nebulizer 400° interface 60°, auxiliary flow (nitrogen) 6.0 L/min, nebulizer flow (nitrogen) 0.6 L/min, curtain gas (nitrogen) 1.2 L/min, collision energy 40 V, (m/z 802.5)

CHROMATOGRAM

Retention time: 0.97
Internal standard: FR900520 (Fujisawa Pharmaceutical, Japan) (0.95) (m/z 790.6)
Limit of detection: 100 pg/mL

KEY WORDS

whole blood; pharmacokinetics

REFERENCE

Alak,A.M.; Moy,S.; Cook,M.; Lizak,P.; Niggebiugge,A.; Menard,S.; Chilton,A. An HPLC/MS/MS assay for tacrolimus in patient blood samples. Correlation with results of an ELISA assay, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 7–13.

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL blood containing IS with and MeCN:MeOH:zinc sulfate 20:30:50, mix, centrifuge. Add the supernatant to a C18 SPE cartridge. Evaporate the eluate to dryness at 40°. Reconstitute the residue with 100 µL MeCN:water 50:50. Inject a 75 µL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 3 µm Hypersil BCS CPS
Mobile phase: MeCN:water 50:50
Column temperature: 68
Flow rate: 1
Injection volume: 75
Detector: MS, tandem mass, negative API mode, m/z 802.4-560.5

CHROMATOGRAM
Limit of quantitation: 25 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Hill,H.M.; Clarke,S.D.; Bentley,L.; Noctor,T.A.G.; Iwasaki,K.; Shiraga,T.; Hata,T.; Undre,N. A high sensitivity assay for tacrolimus (FK506) in human blood (Abstract 2125), *Pharm.Res.*, **1997**, *14*, S261.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 500 μL 2% saponin in water, vortex for 10 s, after 5 min add 2 mL 180 mM HCl and 6 mL diethyl ether, shake at 60 rpm for 15 rpm, centrifuge at 4000 rpm for 10 min. Remove the organic layer and add it to 2 mL 95 mM NaOH, shake at 60 rpm for 15 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL MeCN, vortex for 1 min, add 100 μL 1% trifluoroacetic acid in MeCN, add 100 μL 0.6 mg/mL dansylhydrazine in MeCN, vortex for 1 min, evaporate to dryness under a stream of nitrogen at 37°, store in the dark at 4°. Just before analysis reconstitute in 100 μL MeCN, inject a 25 μL aliquot onto column A with mobile phase A, elute column A with mobile phase A for 3 min, after 3 min elute contents of column A onto column B with mobile phase B, after 30 s remove column A from circuit, elute column B with mobile phase B for 6.5 min, elute tacrolimus fraction with mobile phase B from column B onto column C for 2 min, elute column C with mobile phase C and monitor the effluent. (Flush column A with MeOH for 5 min then re-equilibrate with mobile phase A for 4 min before next injection.)

HPLC VARIABLES
Column: A 15 × 3.9 25-40 μm C18 (Applied Biosystems); B 150 × 3.9 4 μm Novapack C18; C 100 × 3.2 Hypercarb Ph graphite
Mobile phase: A MeOH:water 50:50; B MeCN:water 80:20; C MeOH:dichloromethane 50:50
Flow rate: A 1; B 1; C 1.5
Injection volume: 25
Detector: F ex 338 em 520 (or 430 nm cut-off filter)

CHROMATOGRAM
Retention time: 3.5 (after the start of the elution of column C with mobile phase C)
Limit of quantitation: 3 ng/mL

KEY WORDS
whole blood; column-switching; heart-cut

REFERENCE
Beysens,A.J.; Beuman,G.H.; van der Heijden,J.J.; Hoogtanders,K.E.J.; Steijger,O.M.; Lingeman,H. Determination of tacrolimus (FK 506) in whole blood using liquid chromatography and fluorescence detection, *Chromatographia*, **1994**, *39*, 490–496.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Whole blood + 400 μL MeCN, agitate for 15 min, centrifuge at 9500 g for 5 min. Evaporate the supernatant to dryness at 40°, reconstitute with 70 μL isopropanol, agitate for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 mm long 3 μm Spherisorb CN
Mobile phase: Hexane:isopropanol 72:28
Column temperature: 40
Flow rate: 1.8

Injection volume: 50
Detector: Immunoassay (Evaporate fraction eluting between 4.1 and 4.9 min, reconstitute with 100 μL drug-free whole blood, treat with 200 μL reagent (Abbott IMx Tacrolimus).)

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites, acetaminophen, N-acetylprocainamide, amikacin, azathioprine, carbamazepine, cyclosporine, digitoxin, digoxin, diltiazem, disopyramide, erythromycin, ethosuximide, flecainide, gentamicin, lidocaine, methylprednisolone, netilmicin, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
whole blood

REFERENCE
Firdaous,I.; Hassoun,A.; Otte,J.B.; Reding,R.; Squifflet,J.P.; Besse,T.; Wallemacq,P.E. HPLC-microparticle enzyme immunoassay specific for tacrolimus in whole blood of hepatic and renal transplant patients, *Clin.Chem.*, **1995**, *41*, 1292–1296.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 5 mL ethyl acetate, shake for 30 min, centrifuge at 1660 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL THF, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 TSKgel ODS-80TM (TOSOH)
Mobile phase: MeCN:water 60:40
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 220

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
whole blood

REFERENCE
Namiki,Y.; Fujiwara,A.; Kihara,N.; Koda,S.; Hane,K.; Yasuda,T. Determination of the immunosuppressive drug tacrolimus in its dosage forms by high-performance liquid chromatography, *Chromatographia*, **1995**, *40*, 253–258.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 3 mL Recipe C18 SPE cartridge with 2 mL MeCN and 2 mL water adjusted to pH 3.0 with sulfuric acid. 1 mL Blood or urine + 10 μL 1 μg/mL IS in MeCN:water 70:30 (pH 3.0) + 2 mL MeOH:1 M zinc sulfate 70:30, vortex for 20 s, centrifuge at 2000 g for 2 min, add the supernatant to the SPE cartridge, wash with 2 mL water, dry cartridge, elute with 400 μL MeCN:pH 3.0 water 90:10, inject a 99 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Spherical C18 (Waters)
Mobile phase: MeOH:water 90:10
Flow rate: 0.4
Injection volume: 99

Detector: MS, Hewlett-Packard 5989 A quadrupole, type 59980 B particle-beam interface, chemical ionization, reagent gas methane, electron multiplier 2646 V, particle-beam interface 55°, ionization source 250°, quadrupole 100

CHROMATOGRAM
Retention time: 5.2
Internal standard: 32-O-acetyltacrolimus (6.4)
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Gonschior,A.-K.; Christians,U.; Winkler,M.; Schiebel,H.M.; Sewing,K.-F. Simplified high-performance liquid chromatography-mass spectrometry assay for measurement of tacrolimus and its metabolites and cross-validation with microparticle enzyme immunoassay, *Ther.Drug Monit.*, **1995**, *17*, 504–510.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Dissolve 25 mg tacrolimus in 300 μg/mL heptyl p-hydroxybenzoate in EtOH + 25 mL water, let stand for 6 h at room temperature, inject a 10 μL aliquot. Capsules. Extract 10 capsules with 300 μg/mL heptyl p-hydroxybenzoate in EtOH, centrifuge at 1660 g for 10 min, dilute an aliquot of the supernatant with an equal volume of water, let stand for 6 h at room temperature, inject a 10 μL aliquot. Ampules. 2.5 mL Ampule solution + 22.5 mL 300 μg/mL heptyl p-hydroxybenzoate in EtOH + 25 mL water, let stand for 6 h at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 TSKgel ODS-80TM (TOSOH)
Mobile phase: THF:isopropanol:water 2:2:5
Column temperature: 50
Flow rate: 0.8
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 10
Internal standard: heptyl p-hydroxybenzoate (14.5)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
ampules; capsules

REFERENCE
Namiki,Y.; Fujiwara,A.; Kihara,N.; Koda,S.; Hane,K.; Yasuda,T. Determination of the immunosuppressive drug tacrolimus in its dosage forms by high-performance liquid chromatography, *Chromatographia*, **1995**, *40*, 253–258.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 60:40 containing 2 mM sodium 1-hexanesulfonate

Column temperature: 70
Flow rate: 1.5
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 12.6

OTHER SUBSTANCES
Simultaneous: cimetidine

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Ku,Y.-M.; Min,D.I.; Kumar,V.; Noormohamed,S.E. Compatibility of tacrolimus injection with cimetidine hydrochloride injection in 0.9% sodium chloride injection, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2024–2025.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 50 μL 1 M HCl + 3.5 mL ethyl acetate, shake for 10 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen below 40°, reconstitute the residue in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 Inertsil ODS-2
Column: 250 × 4.6 Inertsil ODS-2
Mobile phase: Gradient. MeCN:0.1% phosphoric acid from 36:64 to 54:46 over 20 min, to 100:0 over 7 min, return to initial conditions over 2.1 min
Column temperature: 50
Flow rate: from 1.2 to 2.5 over 27.1 min
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 28.4

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat

REFERENCE
Iwasaki,K.; Shiraga,T.; Matsuda,H.; Nagase,K.; Tokuma,Y.; Hata,T.; Fujii,Y.; Sakuma,S.; Fujitsu,T.; Fujikawa,A.; Shimatani,K.; Sato,A.; Fujioka,M. Further metabolism of FK506 (Tacrolimus). Identification and biological activities of the metabolites oxidized at multiple sites of FK506, *Drug Metab.Dispos.*, **1995**, *23*, 28–34.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 3 mL glass 25-40 μm C8 SPE cartridge with 3 mL MeCN and 3 mL pH 3.0 sulfuric acid. 1.5 mL Microsomal incubation + 500 μL MeCN, mix, centrifuge at 2500 g for 2 min, add the supernatant to the SPE cartridge, wash with 3 mL MeOH:pH 3.0 sulfuric acid 50:50, wash with 500 μL hexane, pull air through the cartridge for 3 min, elute with 1.5 mL dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 56°, reconstitute with 300 μL MeCN:pH 3.0 sulfuric acid 70:30, wash this solution with 500 μL hexane, inject a 125 μL aliquot of the MeCN layer.

HPLC VARIABLES
Column: 250 × 4 3 μm Hypersil C8

Mobile phase: Gradient. MeCN:pH 3.0 sulfuric acid from 42:58 to 48:52 over 20 min, to 57:43 over 15 min, to 75:25 over 10 min (concave gradient), wash with 95:5 for 5 min, re-equilibrate at initial conditions for 7 min.
Column temperature: 75
Flow rate: 0.7
Injection volume: 125
Detector: UV 205

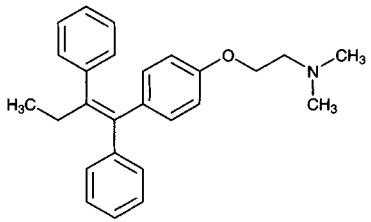
CHROMATOGRAM
Retention time: 26
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rat; pig; intestines; SPE

REFERENCE
Lampen,A.; Christians,U.; Guengerich,F.P.; Watkins,P.B.; Kolars,J.C.; Bader,A.; Gonschior,A.-K.; Dralle,H.; Hackbarth,I.; Sewing,K.-F. Metabolism of the immunosuppressant tacrolimus in the small intestine: Cytochrome P450, drug interactions, and interindividual variability, *Drug Metab.Dispos.*, **1995**, *23*, 1315–1324.

Tamoxifen



Molecular formula: $C_{26}H_{29}NO$
Molecular weight: 371.52
CAS Registry No.: 10540-29-1, 54965-24-1 (citrate)
Merck Index: 9216
Lednicer No.: 2 127; 3 70; 4 65

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond-elut C2 SPE cartridge with 2 mL MeOH and 2 mL water. Apply 1 mL plasma to the cartridge, wash with 1 mL water, wash with 1 mL MeOH: water 50:50, wash with 1 mL MeCN, elute with 2 mL 1 M NaCl:MeOH 5:95. Dry the eluate under vacuum, resuspend in 200 μL MeOH, inject a 20 μL aliquot. GC 10 mm long C18 (Waters)

HPLC VARIABLES
Column: 30 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:1% pH 8.0 triethylamine 89:11
Flow rate: 1.2
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 10.45
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma

REFERENCE
MacCallum,J.; Cummings,J.; Dixon,J.M.; Miller,W.R. Solid-phase extraction and high-performance liquid chromatographic determination of tamoxifen and its major metabolites in plasma, *J.Chromatogr.B*, **1996**, *678*, 317–323.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL cyano-bonded silica (J.T.Baker) SPE cartridge with 1 mL MeOH and three 1 mL portions of water. Mix 100 μL serum with 500 μL water. Add to the SPE cartridge and allow to pass through by gravity. Wash three times with 1 mL potions of water and with 1 mL MeOH:water 50:50. Elute with 1 mL MeOH containing 1 mL/L triethylamine. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax cyano bonded silica
Mobile phase: MeCN:MeOH:phosphate buffer 23:37:40, adjusted to pH 3.5
Column temperature: 45
Flow rate: 0.8
Injection volume: 20
Detector: UV 241

CHROMATOGRAM
Retention time: 5.57
Internal standard: tamoxifen

OTHER SUBSTANCES
Extracted: amiodarone, desethylamiodarone, bepridil, L8040 (Sanofi Recherche), trifluoperazine
Simultaneous: aprindine, bromocriptine, captopril, carbamazepine, chlorpromazine, diltiazem, dimefline, dipyridamole, disopyramide, flecainide, flurazepam, furosemide, imipramine, labetalol, miconazole, nifedipine, norverapamil, procainamide, propafenone, propranolol, quinidine, tocainide, trifluoropromazine, verapamil, warfarin

KEY WORDS
tamoxifen is IS; serum; SPE

REFERENCE
Pollak,P.T. A systematic review and critical comparison of internal standards for the routine liquid chromatographic assay of amiodarone and desethylamiodarone, *Ther.Drug Monit.*, **1996**, *18*, 168–178.

SAMPLE
Matrix: blood
Sample preparation: Extract 2 mL serum with two 20 mL portions of diethyl ether, evaporate to dryness using compressed air, reconstitute the residue in 1 mL MeOH, centrifuge at 3000 rpm for 3 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hichrom ODS 1 (Anachem, Luton, UK)
Mobile phase: MeCN:MeOH:water:trichloroacetic acid 31:50:18.9:0.1, adjusted to pH 2.9
Flow rate: 1.8
Injection volume: 20
Detector: F ex 254 em 360 following post-column reaction. The column effluent flowed through a 200 cm × 0.2 mm ID coil of PTFE tubing irradiated by a mercury UV lamp to the detector.

CHROMATOGRAM
Retention time: 8.3 (E), 9.2 (Z)
Limit of detection: 100 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; chiral; post-column photochemical derivatization; post-column reaction

REFERENCE
Manns,J.E.; Hanks,S.; Brown,J.E. Optimised separation of E- and Z-isomers of tamoxifen, and its principal metabolites using reversed-phase high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 847–852.

SAMPLE
Matrix: blood
Sample preparation: Vortex serum with 10 volumes hexane:butanol 98:2 for 15 s, centrifuge for 10 min. Remove an aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 50-100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm ODS-2 (Whatman)
Mobile phase: MeOH containing 0.04% diethylamine acetate
Flow rate: 2
Detector: F ex 220 or 254 em 360 following post-column reaction. The column effluent flowed through a 70 cm × 0.2 mm ID quartz coil irradiated with two Mineralite short-wave UV lamps to the detector.

CHROMATOGRAM
Retention time: 5.4
Limit of detection: 0.2 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
post-column reaction; post-column photochemical derivatization; serum; protect from light

REFERENCE
Brown,R.R.; Bain,R.; Jordan,V.C. Determination of tamoxifen and metabolites in human serum by high-performance liquid chromatography with post-column fluorescence activation, *J.Chromatogr.*, **1983**, *272*, 351–358.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 2 μg clomiphene + 2 mL diethyl ether, extract, centrifuge at 2000 rpm at 4° for 10 min, repeat extraction. Combine the organic phases and evaporate them to dryness under a stream of nitrogen at 37°. Reconstitute the residue in 250 μL MeOH, centrifuge at 2000 rpm at 4° for 10 min, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeCN:10 mM KH_2PO_4:300 mM phosphoric acid :water 42:20:10:28
Flow rate: 2.8
Injection volume: 10-100
Detector: F ex 258 em 378 following post-column reaction. The column effluent flowed through a 6.5 m × 0.35 mm × 1.5 mm o.d. PTFE tube irradiated with a Philips HPK 125 watt high pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 9
Internal standard: clomiphene (12)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; post-column photochemical derivatization

REFERENCE
Milano,G.; Etienne,M.C.; Frenay,M.; Khater,R.; Formento,J.L.; Renee,N.; Moll,J.L.; Francoual,M.; Berto,M.; Namer,M. Optimised analysis of tamoxifen and its main metabolites in the plasma and cytosol of mammary tumours, *Br.J.Cancer*, **1987**, *55*, 509–512.

SAMPLE
Matrix: blood
Sample preparation: 800 μL Plasma + 200 μL 100 mM HCl, centrifuge at 12000 g for 2 min. Inject the following solutions onto column A; 500 μL MeOH, 700 μL water, 300 μL 100 mM HCl, 500 μL supernatant, and 1 mL water. Flush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 35 × 2 40 μm Sepralyte CN-propyl modified silica (Analytichem); B 110 × 4.6 Partisil Si
Mobile phase: MeOH:5 mM ammonium acetate 90:10
Flow rate: 1.4
Injection volume: 500
Detector: F ex 256 em 380 following post-column reaction. The column effluent flowed through a 15 m × 0.25 mm ID crocheted PTFE tube irradiated with a Sylvania G8 UV lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 3
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; post-column photochemical derivatization; column-switching

REFERENCE
Kikuta,C.; Schmid,R. Specific high-performance liquid chromatographic analysis of tamoxifen and its major metabolites by "on-line" extraction and post-column photochemical reaction, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 329–337.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL phenyl SPE cartridge with five 1 mL portions of MeCN, 1 mL water, and 1 mL 2.5 mL/L triethylamine in 50 mM pH 3.0 phosphate buffer. 980 μL Plasma + 30 μL MeOH:water 50:50 + 1 mL MeCN, vortex for 1 min, centrifuge at -10° at 2500 g for 1 h. Remove a 1.6 mL aliquot of the supernatant and add it to 400 μL 2% heptanesulfonic acid in 50 mM pH 3.0 KH_2PO_4/phosphoric acid buffer, add to the SPE cartridge, wash with two 100 μL portions of MeCN:buffer 80:20, wash with 50 μL 25 mM sulfuric acid, elute with five 100 μL portions of MeCN:buffer 80:20. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL MeCN:buffer 70:30, inject a 10-30 μL aliquot. (Buffer was 5 mM heptanesulfonic acid in 50 mM pH 3.0 phosphate buffer.)

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Si-100-S Phenyl (BST, Budapest)
Column: 250 × 4.6 10 μm Si-100-S Phenyl (BST, Budapest)
Mobile phase: MeCN:buffer 75:25 (Buffer was 50 mM pH 3.0 KH_2PO_4/phosphoric acid buffer containing 5 mM heptanesulfonic acid and 300 μL/L triethylamine. Temperature of MeCN was 60° and temperature of buffer was 80°.)
Flow rate: 1.2
Injection volume: 10-30

Detector: F ex 257 em 378 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID knitted PTFE coil irradiated by a mercury lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 7.03
Internal standard: tamoxifen

OTHER SUBSTANCES
Extracted: panomifene

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma; pharmacokinetics; SPE; tamoxifen is IS

REFERENCE
Erdélyi-Tóth,V.; Pap,E.; Kralovánszky,J.; Bojti,E.; Klebovich,I. Determination of panomifene in human plasma by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *668*, 419–425.

SAMPLE
Matrix: blood
Sample preparation: 150 μL Plasma + 150 μL MeCN, vortex for 2 min, centrifuge at 13000 g for 5 min, inject 50 μL supernatant onto column A with mobile phase A, elute column A to waste for 2 min with mobile phase A, elute column A to waste for another 2 min with mobile phase B, elute column A onto column B with mobile phase B, analyze effluent from column B. (Single pump used. Switch from mobile phase A to mobile phase B by switching solvent reservoirs.)

HPLC VARIABLES
Column: A SPS CN guard column (Regis); B Regis C18 guard column + 250 × 4.6 5 μm Regis Rexchrom CN
Mobile phase: A water; B MeCN: 20 mM pH 3.1 K_2HPO_4 35:65
Flow rate: A 1; B 1
Injection volume: 50
Detector: F ex 250 em 370 (cut-off filter) preceded by a photochemical reactor, ICT Beam Boost, 254 nm UV lamp, 5 m reaction coil.

CHROMATOGRAM
Retention time: 47
Limit of detection: 8 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, 4-hydroxytamoxifen, N-desdimethyltamoxifen, N-desmethyltamoxifen, tamoxifen-ol

KEY WORDS
plasma; rugged; post-column photochemical derivatization

REFERENCE
Fried,K.M.; Wainer,I.W. Direct determination of tamoxifen and its four major metabolites in plasma using coupled column high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *655*, 261–268.

SAMPLE
Matrix: blood
Sample preparation: Extract 2 mL plasma twice with 3 mL portions of hexane:EtOH 98:2. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeCN, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 LC-18 (Supelco)
Mobile phase: Gradient. MeCN:20 mM ammonium acetate 20:80 for 4 min, to 40:60 over 20 min, to 65:35 over 16 min

Flow rate: 0.75
Injection volume: 10-20
Detector: UV 280 or MS, Finnigan TSQ triple quadrupole, electrospray, atmospheric pressure ionization, capillary 220°, electrode -4.5 kV, drying gas nitrogen, 50% of column effluent directed into MS, SIM 372

CHROMATOGRAM
Retention time: 42.33

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; LC-MS

REFERENCE
Poon,G.K.; Walter,B.; Lonning,P.E.; Horton,M.N.; McCague,R. Identification of tamoxifen metabolites in human Hep G2 cell line, human liver homogenate, and patients on long-term therapy for breast cancer, *Drug Metab.Dispos.*, **1995**, *23*, 377–382.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 50% urea + 5 mL diethyl ether, extract. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:67 mM pH 2.2 phosphate buffer 50:50
Flow rate: 1
Injection volume: 50
Detector: F ex 260 em 375 following post-column reaction. The column effluent flowed though a 7 m × 0.3 mm ID knitted PTFE coil irradiated by a Sylvana G8T6 UV lamp to the detector.

CHROMATOGRAM
Limit of quantitation: 0.51 ng/mL

KEY WORDS
post-column reaction; post-column photochemical derivatization; pharmacokinetics; plasma

REFERENCE
Fuchs,W.S.; Leary,W.P.; Van der Meer,M.J.; Gay,S.; Witschital,K.; von Nieciecki,A. Pharmacokinetics and bioavailability of tamoxifen in postmenopausal healthy women, *Arzneimittelforschung*, **1996**, *46*, 418–422.

SAMPLE
Matrix: blood
Sample preparation: 30 μL Plasma + 12 μL 15 μg/mL quinine bisulfate in MeOH, mix, make up to 90 μL with 600 mM orthophosphoric acid in MeCN, illuminate in a shortwave UV transilluminator (UVP, San Gabriel CA) with 0.25 J/min for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: CN Resolve (Waters)
Column: 100 × 8 10 μm Radial Pak CN radial compression (Waters)
Mobile phase: MeOH:buffer 70:30 (Buffer was 100 mM sodium acetate containing 1 mM tetrabutylammonium phosphate, adjusted to pH 6 with orthophosphoric acid.)
Flow rate: 4
Injection volume: 50
Detector: F ex 258 em 378

CHROMATOGRAM
Retention time: 10.13

Internal standard: quinine bisulfate (4.36)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, aspirin, azathioprine, carmustine, chlorambucil, cytarabine, dacarbazine, N-desmethyltamoxifen, etoposide, 4-hydroxytamoxifen, indomethacin, lomustine, methotrexate, procarbazine, salicylic acid, tenoposide, thioguanine
Noninterfering: cyclophosphamide, doxorubicin, ifosfamide, mitomycin C, prednisone, taxol, vincristine

KEY WORDS
derivatization; plasma

REFERENCE
el-Yazigi,A.; Legayada,E. Direct liquid chromatographic micro-measurement of tamoxifen in plasma of cancer patients, *J.Chromatogr.B*, **1997**, *691*, 457–462.

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. 300 μL Serum + 10 μL 10 μg/mL enclomiphene in EtOH + 1 mL hexane:amyl alcohol 98:2, mix vigorously for 5 min, freeze aqueous layer in dry ice/acetone, remove organic layer, repeat extraction two more times. Combine the extracts and evaporate them to dryness, resuspend the residue in 100 μL mobile phase, inject an aliquot. Tissue. 10 mg Liver or 15 mg uteri or 20 mg muscle + enclomiphene + 1.5 mL MeOH:acetic acid 98:2, homogenize in a 7.5 mL Potter-Elvehjem ground glass-ground glass homogenizer, centrifuge at 2000 g. Remove the organic layer and evaporate it to dryness. Extract the resulting residue with 1 mL acetone with vigorous mixing for 5 min. Centrifuge at 2000 g, remove the acetone extract and evaporate it to dryness, resuspend in 1 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm silica (Scientific Glass Engineering)
Mobile phase: Isooctane:EtOH:isopropanol:diethylamine:acetic acid 75:23.5:1.5:0.05:0.05
Flow rate: 1
Detector: F ex 257 em 383 preceded by a post-column in-line UV reactor

CHROMATOGRAM
Retention time: 3
Internal standard: enclomiphene (2)
Limit of detection: 0.7 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; liver; muscle; uterus; human; rat; mouse; normal phase; post-column photochemical derivatization

REFERENCE
Robinson,S.P.; Langan-Fahey,S.M.; Johnson,D.A.; Jordan,V.C. Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient, *Drug Metab.Dispos.*, **1991**, *19*, 36–43.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 20.652

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 5 mL chilled chloroform to microsomal mixture, vortex, adjust aqueous phase to pH 9, extract with 5 mL chloroform. Combine the organic phases and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeOH:water 85:15, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-Select-B C8
Mobile phase: MeOH:water:triethylamine 80:20:0.01
Flow rate: 0.8
Detector: UV 238, or UV 277, or F ex 258 em 318 preceded by an on-line Knauer UV photoreactor

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Simultaneous: metabolites
Also analyzed: toremifene

KEY WORDS
post-column photochemical derivatization

REFERENCE
Berthou,F.; Dréano,Y. High-performance liquid chromatographic analysis of tamoxifen, toremifene and their major human metabolites, *J.Chromatogr.*, **1993**, *616*, 117–127.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 2 mL Microsomal incubation + 4 mL MeOH:DMSO 80:20, vortex, centrifuge at 2000 g for 20 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Res Elute-BD (Varian)

Mobile phase: MeOH:500 mM ammonium acetate 70:30
Flow rate: 1
Injection volume: 100
Detector: UV 280, MS, VG Quattro BQ tandem quadrupole, API electrospray, capillary 380 V, high voltage electrode 3.78 kV, source 150°, lens 1 and lens 2 80-85 V, positive ion mode, column flow split 1:6 before entering MS, m/z 372

CHROMATOGRAM
Retention time: 51
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; liver

REFERENCE
Jones,R.M.; Yuan,Z.-X.; Lamb,J.H.; Lim,C.K. On-line high-performance liquid chromatographic-electrospray ionization mass spectrometric method for the study of tamoxifen metabolism, *J.Chromatogr.A*, **1996**, *722*, 249–255.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic

acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 3 mL Bond-Elut C2 SPE cartridge with 2 mL MeOH and 2 mL water. Dismembrate (Mikro-dismembrator, Braun, Germany) 50 mg tissue in liquid nitrogen, re-suspend in 1 mL DMSO. Centrifuge at 3000 rpm for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL water, 1 mL MeOH:water, and 1 mL MeCN. Elute with 1 mL MeOH:1 M NaCl 95:5. Dry eluate under a stream of nitrogen, reconstitute with 400 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 mm C18 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:1% pH 9.0 triethylamine 89:11
Flow rate: 1.2
Injection volume: 20
Detector: UV 265

CHROMATOGRAM

Retention time: 11.00
Limit of detection: 20 ng/g

OTHER SUBSTANCES

Extracted: metabolites, cis-tamoxifen

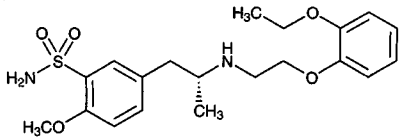
KEY WORDS

breast; tumor; SPE

REFERENCE

MacCallum,J.; Cummings,J.; Dixon,J.M.; Miller,W.R. Solid-phase extraction and high-performance liquid chromatographic determination of tamoxifen and its major metabolites in breast tumour tissues, *J.Chromatogr.B*, **1997**, *698*, 269–275.

Tamsulosin



Molecular formula: $C_{20}H_{28}N_2O_5S$
Molecular weight: 408.52
CAS Registry No.: 106133-20-4, 80223-99-0 ((±) HCl), 106463-17-6 ((R), HCl), 106463-19-8 ((S) HCl)
Merck Index: 9217

SAMPLE

Matrix: bile, microsomal incubations, urine
Sample preparation: Bile, urine. Inject a 50-200 μL aliquot bile or urine. Microsomal incubations. Mix 1 mL microsomal incubation with 5 mL ethyl acetate, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.0 5 μm Nucleosil C18
Mobile phase: Gradient. A was 100 mM pH 4.5 KH_2PO_4. B was MeCN:100 mM pH 4.9 KH_2PO_4 20:80. A:B 100:0 for 5 min, to 0:100 over 60 min, maintain at 0:100 for 60 min
Column temperature: 27
Flow rate: 1
Detector: UV 275; Radioactivity

CHROMATOGRAM

Retention time: 80

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; dog; liver; kidney; intestine; radiolabeled

REFERENCE

Soeishi,Y.; Matsushima,H.; Teraya,Y.; Watanabe,T.; Higuchi,S.; Kaniwa,H. Metabolism of tamsulodin in rat and dog, *Xenobiotica*, **1996**, *26*, 355–365.

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL saturated sodium bicarbonate solution and 100 μL 2.5 μg/mL IS in water to 1.5 mL plasma, extract with 5 mL ethyl acetate. Remove the organic layer and add it to 2.5 mL 400 mM HCl. Shake, centrifuge, and discard the organic layer. Add 2 mL saturated sodium bicarbonate solution to the aqueous layer and extract again with 5 mL ethyl acetate. Evaporate the organic layer to dryness under reduced pressure, reconstitute the residue with 50 μL 100 mM sodium bicarbonate. Add 100 μL 5 mg/mL dansyl chloride in acetone. Heat at 35° for 90 min. Add 5 mL distilled water and extract with 5 mL diethyl ether. Evaporate the organic layer to dryness at 45°, reconstitute the residue with 60 μL mobile phase. Inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 Nucleosil SI100-5 (Chemco, Japan)
Mobile phase: MeOH:benzene 1:100 (Caution! Benzene is a carcinogen!)
Injection volume: 20-50
Detector: F ex 352 em 500

CHROMATOGRAM

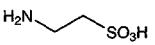
Internal standard: amosulalol
Limit of quantitation: 500 pg/mL (human), 1.0 ng/mL (rat, dog)

KEY WORDS

plasma; human; rat; dog; pharmacokinetics; normal phase; derivatization

REFERENCE
Matsushima,H.; Kamimura,H.; Soeishi,Y.; Watanabe,T.; Higuchi,S.; Tsunoo,M. Pharmacokinetics and plasma protein binding of tamsulosin hydrochloride in rats, dogs, and humans, *Drug Metab.Dispos.*, **1998**, *26*, 240–245.

Taurine



Molecular formula: $C_2H_7NO_3S$
Molecular weight: 125.15
CAS Registry No.: 107-35-7
Merck Index: 9241

SAMPLE
Matrix: amniotic fluid
Sample preparation: 200 μL Amniotic fluid + 800 μL MeOH, mix, centrifuge. 200 μL Supernatant + 80 μL pH 9.5 sodium borate + 60 μL reagent, mix, let stand for 3.5 min, add 25 μL 0.5 M HCl, mix, dilute 1:4 with 50 mM pH 7.0 sodium acetate buffer, inject a 20 μL aliquot. (Prepare reagent by dissolving 50 mg o-phthaldialdehyde in 4.5 mL MeOH, add 500 μL pH 9.5 sodium borate, add 50 μL 2-mercaptoethanol.)

HPLC VARIABLES
Guard column: 10-20 × 4 C18
Column: 300 × 3.9 5 μm NovaPak C18
Mobile phase: Gradient. MeOH:50 mM pH 7.0 sodium acetate buffer from 15:85 to 20:80 over 30 min, to 35:65 over 15 min, to 75:25 over 25 min, maintain at 75:25 for 5 min, return to initial conditions over 5 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 330 em 450

CHROMATOGRAM
Retention time: 50

OTHER SUBSTANCES
Extracted: alanine, 2-aminoadipic acid, 2-aminobutyric acid, 3-aminobutyric acid, 4-aminobutyric acid, arginine, asparagine, aspartic acid, citruline, glutamine, glutathione, glutatic acid, glycine, histidine, homoserine, 5-hydroxylysine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, saccharopine, serine, threonine, tryptophan, tyrosine, valine

KEY WORDS
derivatization

REFERENCE
Klein,B.H.; Dudenhausen,J.W. Ion-exchange chromatography and ion-pair chromatography. Complementation of HPLC analysis of amino acids in body fluids by pre-column derivatization using ortho-phthaldialdehyde, *J.Liq.Chromatogr.*, **1995**, *18*, 4007–4028.

SAMPLE
Matrix: amniotic fluid, blood, CSF, urine
Sample preparation: Plasma. Condition a 100 mg Bond Elut SCX (propylbenzenesulfonic acid, H^+ form) SPE cartridge with 1 mL 50 mM HCl, 1 mL MeOH, 2 mL water, and 1 mL 50 mM HCl. 100 μL Plasma + 100 μL 250 μM norleucine in 100 mM HCl + 10 mg solid sulfosalicylic acid + 800 μL acetone or MeOH, mix, centrifuge, add a 50 μL aliquot to the SPE cartridge, wash with 2 mL water, elute with two 500 μL portions of MeOH:water:triethylamine 40:40:20, dry the eluate under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness

under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Dried blood. Add 25 μL 250 μM norleucine in 100 mM HCl to a 6 mm filter paper disc containing dried blood, add 100 μL MeCN, let stand for 30 min, centrifuge, remove a 75 μL aliquot of the supernatant, evaporate to dryness under reduced pressure, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 2 min, evaporate to dryness under vacuum, reconstitute with 50 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Amniotic fluid, CSF. Mix amniotic fluid or CSF with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 50 (CSF) or 100 (amniotic fluid) μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Urine. Dilute urine with water to a creatinine concentration of 1 mM, mix an aliquot with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Pico-Tag amino acid column (Waters)
Mobile phase: Gradient. A was MeCN:70 mM pH 6.55 sodium acetate 2.5:97.5. B was MeCN:MeOH:water 45:15:40. A:B 100:0 for 13.5 min, to 97:3 (step gradient), to 94:6 over 10.5 min (Waters curve 8 (slightly concave)), to 91:9 over 6 min (Waters curve 5 (slightly convex)), to 66:34 over 20 min, maintain at 66:34 for 12 min, to 0:100 over 0.5 min, maintain at 0:100 for 4 min, return to initial conditions over 0.5 min.
Column temperature: 46
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 13.82
Internal standard: norleucine (55.07)

OTHER SUBSTANCES

Extracted: α-alanine, alanine, alloisoleucine, β-aminoadipic acid, 4-aminobenzoic acid, gamma-aminobutyric acid, β-amino-n-butyric acid, gamma-amino-n-butyric acid, 4-aminohippuric acid, β-aminoisobutyric acid, 4-aminophenylacetic acid, α-aminophenylacetic acid, 3-amino-3-phenylpropionic acid, δ-amino-n-valeric acid, ammonia, anserine, arginine, asparagine, aspartic acid, aspartylglucosamine, carnosine, citrulline, cystathionine, cysteic acid, cysteine, cysteine-homocysteine (mixed disulfide), cystine, ethanolamine, ethionine, ethylamine, galactosamine, glucosamine, glutamic acid, glutamine, glutathionine (oxidized), glycine, glycylglycine, glycylhistidine, glycylleucine, glycylphenylalanine, glycyltyrosine, histidine, homoarginine, homocitrulline, homoserine, homocystine, 3-hydroxyanthranilic acid, 3-hydroxykynurenine, hydroxyproline, isoleucine, kynurenine, leucine, levodopa, lysine, methionine sulfone, methionine, 3-methylhistidine, 1-methylhistidine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, sarcosine, serine, serotonin, threonine, tromethamine, tryptophan, tyrosine, valine
Noninterfering: cadaverine, 2-phenylethylamine

KEY WORDS

derivatization; SPE; ultrafiltrate; plasma; dried blood

REFERENCE

Davey,J.F.; Ersser,R.S. Amino acid analysis of physiological fluids by high-performance liquid chromatography with phenylisothiocyanate derivatization and comparison with ion-exchange chromatography, *J.Chromatogr.*, **1990**, *528*, 9–23.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with an equal volume of 5% trichloroacetic acid, centrifuge at 12000 rpm for 5 min. Evaporate the supernatant to dryness, reconstitute with 2-methoxyethanol:0.15 N pH 2.65 sodium citrate 7:93, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4 ISC-07/S1504 Li type (strongly acidic cation-exchange resin of styrene-divinylbenzene copolymer with 10% crosslinkage) (Shimadzu)
Mobile phase: Gradient. A was 2-methoxyethanol:0.15 N pH 2.65 sodium citrate 7:93. B was 0.3 N pH 10.0 sodium citrate. C was 200 mM NaOH. A:B:C from 100:0:0 to 95:5:0 over 50 min, to 85:15:0 over 20 min, to 75:25:0 over 10 min, to 55:45:0 (step gradient), maintain at 55:45:0 for 15 min, to 50:50:0 over 10 min, to 40:60:0 (step gradient), to 30:70:0 over 20 min, to 10:90:0 (step gradient), maintain at 10:90:0 for 15 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 5 min. (Parameters are approximate.)
Column temperature: 38° for 40 min, 52° for 65 min, 55° for 20 min, 58° for 10 min, 38° for 50 min
Flow rate: 0.4
Detector: F ex 348 em 450 following post-column reaction. The column effluent mixed with the reagent solution pumped at 0.2 mL/min and the mixture flowed through a 200 × 0.5 stainless steel or PTFE coil at 55°. The effluent from the coil mixed with the fluorescence solution pumped at 0.2 mL/min and flowed through a 2 m × 0.5 mm stainless steel or PTFE coil at 55° to the detector. (Prepare reagent solution by adding 400 μL NaOCl solution (chlorine concentration 10%) to 1 L buffer, discard after 2 weeks. Prepare fluorescence solution by adding 15 mL EtOH containing 1.6 g o-phthalaldehyde and 2.0 g N-acetyl-L-cysteine and 4 mL 10% Brij 35 in water to 980 mL buffer, discard after 1 month. Buffer contained 384 mM sodium carbonate, 216 mM boric acid, and 108 mM potassium sulfate, pH 10.0.)

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 10 pmole

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
post-column reaction; plasma

REFERENCE
Fujiwara,M.; Ishida,Y.; Nimura,N.; Toyama,A.; Kinoshita,T. Postcolumn fluorometric detection system for liquid chromatographic analysis of amino and imino acids using o-phthalaldehyde/N-acetyl-L-cysteine reagent, *Anal.Biochem.*, **1987**, *166*, 72–78.

SAMPLE
Matrix: blood
Sample preparation: Subject whole blood to two freeze-thaw cycles. 50 μL Plasma or 10 μL whole blood + 200 μL MeCN:MeOH:triethylamine:water 25:22:3:50, vortex for 15 s, filter (Centricon-10 10000 MW exclusion filter) while centrifuging at 2677 g for 15 min. Remove a 20 μL aliquot of the ultrafiltrate and add it to 10 μL 10 mg/mL dansyl chloride in MeCN (prepare fresh daily), vortex, let stand in the dark for 30 min, add 10 μL water:ethylamine 96.5:3.5, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 Spheri-5 ODS
Mobile phase: MeOH:water:acetic acid:triethylamine 33:66.5:0.5:0.025, after 6 min purge column with MeOH:water 90:10 for 4 min, re-equilibrate for 5 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 10
Detector: F ex 329 em 530

CHROMATOGRAM
Retention time: 7
Limit of detection: 2.5 ng

KEY WORDS
cat; plasma; derivatization; ultrafiltrate; whole blood

REFERENCE
Amiss,T.J.; Tyczkowska,K.L.; Aucoin,D.P. Analysis of taurine in feline plasma and whole blood by liquid chromatography with fluorimetric detection and confirmation by thermospray mass spectrometry, *J.Chromatogr.*, **1990**, *526*, 375–382.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 5 μL 2.5 M homoserine, make up to 500 μL with 2 μL/mL 2-mercaptoethanol in MeCN, vortex, centrifuge for 4 min (Beckman microfuge). Remove a 40 μL aliquot of the supernatant and add it to 40 μL reagent and 20 μL 3.7% iodoacetic acid in 400 mM pH 9.5 sodium borate buffer, mix, let stand for 1 min, make up to 200 μL with 100 mM pH 4 potassium phosphate buffer, mix, inject a 20 μL aliquot. (Reagent was 50 mg o-phthaldialdehyde in 1 mL MeOH added to 11 mL 400 mM pH 9.5 sodium borate buffer, 50 μL 2-mercaptoethanol, and 10 mg nitrilotriacetic acid. Filter (0.2 μm), store in the dark at 4°, add 20 μL 2-mercaptoethanol each week to maintain the level of this reagent.

HPLC VARIABLES
Guard column: 5 μm LiChrospher 100 RP-18
Column: 150 × 4.6 5 μm Dynamax Microsorb C18 (Rainin)
Mobile phase: Gradient. A was MeOH:100 mM pH 6.8 sodium acetate buffer 95:5. B was MeOH:100 mM pH 6.8 sodium acetate buffer 5:95. A:B from 15:85 to 30:70 over 15.5 min, to 55:45 over 9 min, to 60:40 over 2 min, to 100:0 over 8 min, maintain at 100:0 for 3 min, to 0:100 over 4 min, maintain at 0:100 for 3 min, to 15:85 over 1 min, stay at 15:85 for 2 min.
Column temperature: 35
Flow rate: 1.5 for 37.5 min, 1 for 10 min
Injection volume: 20
Detector: F ex 338 em 425

CHROMATOGRAM
Retention time: 22.60
Internal standard: homoserine (16.31)
Limit of quantitation: 31 μM

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
plasma; derivatization

REFERENCE
Uhe,A.M.; Collier,G.R.; McLennan,E.A.; Tucker,D.J.; O'Dea,K. Quantitation of tryptophan and other plasma amino acids by automated pre-column o-phthaldialdehyde derivatization high-performance liquid chromatography: improved sample preparation, *J.Chromatogr.*, **1991**, *564*, 81–91.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 5% perchloric acid, centrifuge at 3000 rpm for 10 min. Remove the supernatant and neutralize it with 3 M potassium carbonate, centrifuge at 3000 rpm for 5 min, adjust the volume to 2 mL. (Alternatively, filter (Amicon CF-50) 4 mL plasma while centrifuging at 2500 rpm for 10 min.) 10 μL Perchloric acid extract or ultrafiltrate + 10 μL 100 mM pH 9.0 sodium bicarbonate + 40 μL freshly prepared 4 mM 4-dimethylaminoazobenzene-4'-sulfonyl chloride in MeCN, heat at 70° for 10 min, cool, make up to 500 μL with EtOH:water 70:30, centrifuge at 14000 rpm for 3 min, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-18 T
Column: 150 × 4.6 3 μm Supelcosil LC-18 T
Mobile phase: Gradient. A was 25 mM pH 6.8 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 80:20 for 1 min, to 77:23 over 4 min, maintain at 77:23 for 7 min, to 73:27 over 11 min, to 70:30 over 7 min, to 40:60 over 9 min, to 30:70 over 1 min, maintain at 30:70 for 5 min, return to initial conditions over 1 min, re-equilibrate for 6 min.
Flow rate: 1.5
Injection volume: 5
Detector: UV 436

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
plasma; ultrafiltrate; derivatization

REFERENCE
Stocchi,V.; Palma,F.; Piccoli,G.; Biagarelli,B.; Cucchiarini,L.; Magnani,M. HPLC analysis of taurine in human plasma sample using the DABS-Cl reagent with sensitivity at picomole level, *J.Liq.Chromatogr.*, **1994**, *17*, 347–357.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 200 mg/mL 5-sulfosalicylic acid in EtOH to a 1 mL tube, evaporate EtOH at 50° overnight, add 200-500 μL plasma, vortex, freeze in liquid nitrogen, store at -70°, thaw, centrifuge at 4° at 3000 g. 5 μL Supernatant + 20 μL water + 5 μL 1 mM norvaline in water + 90 μL reagent, mix thoroughly, incubate at room temperature for 3 min, add 50 μL neutralizing buffer, inject a 3 μL aliquot. (Prepare reagent stock solution by dissolving 25 mg o-phthalaldehyde in 500 μL MeOH, add 4.5 mL 100 mM pH 10.0 borate buffer, add 25 μL 3-mercaptopropionic acid. At the start of each day prepare reagent by diluting 1 part of stock solution with 20 parts 100 mM pH 10.0 borate buffer. Neutralizing buffer was 400 mM KH_2PO_4 containing 10 mL/L triethylamine.)

HPLC VARIABLES
Guard column: 10 × 2 Chrompack reverse phase
Column: 100 × 4.6 3 μm Microsphere C18 (Chrompack)
Mobile phase: Gradient. A was buffer:water:THF 50:50:0.2. B was MeOH:MeCN:buffer 35:15:50. A:B from 98:2 to 75:25 over 3.5 min, to 56:44 over 1.7 min, to 48:52 over 1.7 min, to 0:100 over 3.1 min, reset to initial conditions over 1 min.
Flow rate: 1.5
Injection volume: 3
Detector: F ex 230 em 389 (cut-off filter)

CHROMATOGRAM
Retention time: 7.6
Internal standard: norvaline (10.3)
Limit of quantitation: 5000 nM

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
plasma; derivatization

REFERENCE
Teerlink,T.; Van Leeuwen,P.A.M.; Houdijk,A. Plasma amino acids determined by liquid chromatography within 17 minutes, *Clin.Chem.*, **1994**, *40*, 245–249.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 150 μL MeCN, vortex, centrifuge at 5800 g for 10 min. Remove the supernatant and add it to 50 μL buffer, add 50 μL 5 mM fluorescamine in MeCN, vortex, inject an aliquot. (Prepare buffer by adjusting the pH of 100 mM disodium tetraborate to 9.2 with 10 mM boric acid

HPLC VARIABLES
Guard column: C8
Column: 300 × 3.9 10 μm Bondclone C18
Mobile phase: MeCN:THF:buffer 24:4:72 (Prepare buffer by adjusting the pH of 15 mM KH_2PO_4 to 3.5 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 385

CHROMATOGRAM
Limit of quantitation: 5 μg/mL

KEY WORDS
derivatization; plasma

REFERENCE
McMahon,G.P.; O'Kennedy,R.; Kelly,M.T. High-performance liquid chromatographic determination of taurine in human plasma using pre-column extraction and derivatization, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1287–1294.

SAMPLE
Matrix: blood, CSF
Sample preparation: Plasma. For each volume of plasma add 4 volumes of MeOH, centrifuge at 11600 g for 5 min. Remove a 10 μL aliquot and add it to 5 μL phthaldialdehyde/β-mercaptoethanol derivatizing reagent (Fluoraldehyde, Pierce) (use fresh reagent), allow to react at room temperature for 1 min, add 100 μL THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid, inject a 20 μL aliquot. CSF. Add an equal volume of MeOH to the CSF, centrifuge at 11600 g for 5 min. Remove a 10 μL aliquot and add it to 5 μL phthaldialdehyde, allow to react at room temperature for 1 min, add 100 μL THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Spherisorb 5 ODS
Column: 50 × 4.6 Spherisorb 5 ODS
Mobile phase: Gradient. A was THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid. B was MeOH:THF 95:5. A:B from 10:90 to 0:100 over 13 min (sic), maintain at 0:100 for 4 min, return to initial conditions over 1 min.
Column temperature: 43
Flow rate: 1.5
Injection volume: 20
Detector: F (wavelengths not specified)

CHROMATOGRAM
Retention time: 0.5 (Asp), 0.7 (Glu), 1.2 (Taurine), 1.5 (Ser), 1.8 (Gln), 2.2 (His), 2.3 (Gly), 3.0 (Thr), 3.1 (Asn), 3.2 (Ala), 3.3 (Arg), 3.8 (Tyr), 5.2 (Met), 5.4 (Val), 5.8 (Trp), 6.0 (Phe), 5.5 (Ile), 5.7 (Leu), 8.3 (Orn), 8.5 (Lys)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tryptophan, tyrosine, valine

KEY WORDS
plasma; Thr; Gly; His co-elute; derivatization

REFERENCE
Begley,D.J.; Reichel,A.; Ermisch,A. Simple high-performance liquid chromatographic analysis of free primary amino acid concentrations in rat plasma and cisternal cerebrospinal fluid, *J.Chromatogr.B*, **1994**, *657*, 185–191.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Prepare a SPE column by placing a 2 cm layer of 200-400 mesh Dowex 2x8 Cl^-form in a column, on top of this place a 2 cm layer of 100-200 mesh Dowex 50 W-x8 H^+form. 1 mL Serum, CSF, or urine + 100 μL 4 M perchloric acid, centrifuge, add the supernatant to the SPE column, elute with three 1 mL portions of water, collect all the effluent from the column. Remove a 1 mL aliquot and add it to 500 μL 1 M pH 9.0 borate buffer, vortex while adding 500 μL 300 μg/mL fluorescamine in MeCN, let stand for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 mm long LiChrosorb RP-18-10A
Mobile phase: MeCN:15 mM pH 6.0 phosphate buffer 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 395 em 455

CHROMATOGRAM
Retention time: 3
Limit of detection: 0.25 pmole

OTHER SUBSTANCES
Noninterfering: amino acids, hypotaurine, phosphoethanolamine

KEY WORDS
serum; derivatization; SPE

REFERENCE
Stabler,T.V.; Siegel,A.L. Rapid liquid-chromatographic/fluorometric method for taurine in biological fluids, involving pre-derivatization with fluorescamine, *Clin.Chem.*, **1981**, *27*, 1771–1771.

SAMPLE
Matrix: blood, food, peptides, plants, tissue
Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.

Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM
Retention time: 32.31
Internal standard: norleucine (40.90), norvaline (35.06)

OTHER SUBSTANCES
Extracted: amino acids dopamine, epinephrine, histamine, norepinephrine

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 9 volumes of plasma with 1 volume of 35% 5-sulfosalicylic acid, centrifuge at 2000 g for 10 min. Neutralize the supernatant with 10 M KOH, dilute with 2 volumes of water. Mix an aliquot with an equal volume of reagent, inject a 20 μL aliquot within 1 min. Tissue. Homogenize tissue with four volumes 5% 5-sulfosalicylic acid, centrifuge at 5000 g for 10 min, neutralize the supernatant with 10 M KOH. Mix an aliquot with an equal volume of reagent, inject a 20 μL aliquot within 1 min. (Prepare reagent each day by dissolving 35 mg o-phthalaldehyde in 500 μL 95% EtOH and adding this mixture to 50 mL 100 mM pH 10.4 borate buffer, add 100 μL 2-mercaptoethanol.)

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/Corasil
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was THF:water 3:97 containing 100 mM potassium phosphate, pH 7.0. B was THF:MeCN:water 3:40:57 containing 100 mM potassium phosphate, pH 7.0. A:B 97:3 for 1.5 min, to 68:32 over 17 min (Waters curve profile 3), to 0:100 over 2 min, maintain at 0:100 for 4.5 min, return to initial conditions over 2 min, re-equilibrate for 8 min.
Column temperature: 41
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 455

CHROMATOGRAM
Retention time: 21.5

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
plasma; human; rat; liver; kidney; heart; brain; derivatization

REFERENCE
Hirschberger,L.L.; De La Rosa,J.; Stipanuk,M.H. Determination of cysteinesulfinate, hypotaurine and taurine in physiological samples by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 303–313.

SAMPLE
Matrix: blood, tissue, urine

Sample preparation: Urine. Adjust urine to pH 7 with 2 M NaOH or 2 M HCl, dilute 5 (human) or 100 (rat) fold with water. Remove a 1 mL aliquot and add 10 μL triethylamine and 20 mg freshly powdered 3,5-dinitrobenzoyl chloride, shake vigorously on a mechanical shaker for 10 min, add 100 μL 2 M HCl, centrifuge at 1200 g for 5 min. Remove a 200 μL aliquot of the supernatant and dilute it to 5 mL with water, inject a 10-100 μL aliquot. Whole blood. Add 7 volumes of water to whole blood, mix, add 1 volume of 10% sodium tungstate dihydrate solution, mix, add with shaking 1 volume of 333 mM sulfuric acid, shake vigorously (J. Biol. Chem. 1919, 38, 81), centrifuge at 1200 g for 10 min. Remove a 1 mL aliquot of the supernatant and add 10 μL triethylamine and 20 mg freshly powdered 3,5-dinitrobenzoyl chloride, shake vigorously on a mechanical shaker for 10 min, add 100 μL 2 M HCl, centrifuge at 1200 g for 5 min. Remove a 200 μL aliquot of the supernatant and dilute it to 5 mL with water, inject a 10-100 μL aliquot. Tissue. Liver or heart + 7 volumes of water + 1 volume 10% sodium tungstate + 1 volume 330 mM sulfuric acid, homogenize (Potter-Elvehjem, glass pestle), centrifuge at 1200 g for 10 min, dilute 2 (liver) or 5 heart) fold with water. Remove a 1 mL aliquot and add 10 μL triethylamine and 20 mg freshly powdered 3,5-dinitrobenzoyl chloride, shake vigorously on a mechanical shaker for 10 min, add 100 μL 2 M HCl, centrifuge at 1200 g for 5 min. Remove a 200 μL aliquot of the supernatant and dilute it to 5 mL with water, inject a 10-100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 TSK gel ODS-80Ts C18 (Tosoh)
Mobile phase: MeCN:100 mM pH 3.7 ammonium acetate buffer 16:84
Flow rate: 0.8
Injection volume: 10-100
Detector: UV 254

CHROMATOGRAM

Retention time: 18
Limit of detection: 500 nM

OTHER SUBSTANCES

Extracted: amino acids (some), hypotaurine

KEY WORDS

derivatization; human; rat; whole blood; liver; heart

REFERENCE

Masuoka,N.; Yao,K.; Kinuta,M.; Ohta,J.; Wakimoto,M.; Ubuka,T. High-performance liquid chromatographic determination of taurine and hypotaurine using 3,5-dinitrobenzoyl chloride as derivatizing reagent, *J.Chromatogr.B*, **1994**, *660*, 31–35.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Urine. Centrifuge at 4° at 2500 g for 10 min, add 25-100 μL urine to the SPE column, discard the first 25-100 μL eluate, elute with 4 mL water, add 100 μL 100 μM homoserine to the eluate, add an equal volume of the reagent, let stand for 1.5 min, inject a 5-10 μL aliquot. Liver. Homogenize (polytron) 350-450 mg frozen liver in 4 mL 200 mM sulfosalicylic acid at 4°, centrifuge at 4° at 2500 g for 10 min, add a 25 μL aliquot to the SPE column, elute with 4 mL water, add 100 μL 100 μM homoserine to the eluate, add an equal volume of the reagent, let stand for 1.5 min, inject a 5-10 μL aliquot. Serum. 100 μL Serum + 100 μL 200 mM sulfosalicylic acid, mix at 4°, centrifuge at 4° at 11500 g for 2 min, add a 50 μL aliquot of the supernatant to the SPE column, discard the first 50 μL eluate, elute with 4 mL water, add 100 μL 100 μM homoserine to the eluate, add an equal volume of the reagent, let stand for 1.5 min, inject a 5-10 μL aliquot. Hepatocytes. 500 μL Cell suspension + 500 μL 200 mM sulfosalicylic acid, mix at 4°, centrifuge at 4° at 11500 g for 2 min, add a 200 μL aliquot of the supernatant (or 100 μL of the centrifuged medium) to the SPE column, elute with eight 500 μL aliquots of water, add 100 μL 40 μM homoserine to the eluate, add an equal volume of the reagent, let stand for 1.5 min, inject a 5-10 μL aliquot. (Prepare SPE columns as follows. Wash 100 g 100-200 mesh Dowex 1-X4 (anion-exchange, Cl- form) with three volumes of water to remove fines then with 250 mL 1 M HCl until pH was above 2.5. Wash 100 g 100-200 mesh Dowex 50W-X8 (cation-exhange, H+ form) with three volumes of water to remove fines then with 500 mL 4 M HCl in three washings then with 250 mL 1 M HCl. Prepare a column in a Pasteur pipette with 0.5 mL Dowex 1-X4 on top of 1.5 mL Dowex 50W-X8, wash

with 12 mL water. After use regenerate with 12 mL 1 M HCl. Prepare reagent by adding 40 mg o-phthalaldehyde in 800 μL EtOH and 40 μL 2-mercaptoethanol to 10 mL buffer, dilute the mixture with an equal volume of water. Buffer was 3.1 g boric acid in 90 mL water adjusted to pH 10.4 with 5 M NaOH and made up to 100 mL.)

HPLC VARIABLES
Guard column: 4 × 4 LiChrospher
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 43:57 containing 50 mM NaH_2PO_4, pH 5.4
Flow rate: 2
Injection volume: 5-10
Detector: F ex 305-395 (filter) em 420-650 (filter)

CHROMATOGRAM
Retention time: 3.7
Internal standard: homoserine (3)
Limit of detection: 0.5 pmole

KEY WORDS
rat; liver; serum; dog; human; SPE; derivatization; hepatocytes

REFERENCE
Waterfield,C.J. Determination of taurine in biological samples and isolated hepatocytes by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1994**, *657*, 37–45.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 10 mL water. 500 μL Plasma + 500 μL 10% trichloroacetic acid, mix, centrifuge at 4° at 9000 g for 10 min. 1 mL Urine + 1 mL 10% trichloroacetic acid, mix, centrifuge at 4° at 9000 g for 10 min. Add a 1 mL aliquot of the supernatant to the SPE cartridge, elute with 2 mL 5% trichloroacetic acid. Collect all the effluent from the SPE cartridge and make up to 10 mL with 5% trichloroacetic acid. Remove a 50 μL aliquot and add it to 150 μL 400 mM pH 9.0 sodium borate buffer, mix, add 50 μL MeOH, add 100 μL 30 mM 7-chloro-4-nitrobenz-2-oxa-1,3-diazole in MeOH, heat at 60° in the dark for 40 min, make up to 1 mL with cold mobile phase, inject a 25-100 μL aliquot.

HPLC VARIABLES
Guard column: CN Guard-PAK (Waters)
Column: 250 × 4 10 μm Partisil SAX
Mobile phase: MeCN:25 mM citric acid 10:90, pH adjusted to 2.9 with 1 M NaOH
Flow rate: 1.3
Injection volume: 25-100
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 10
Limit of detection: 5 pmole

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Palmerini,C.A.; Fini,C.; Cantelmi,M.G.; Floridi,A. Assessment of taurine in plasma and urine by anion-exchange high-performance liquid chromatography with pre-column derivatization, *J.Chromatogr.*, **1987**, *423*, 292–296.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or 50 μL urine + 200 μL water + 200 μL 167 mM sulfuric acid + 300 μL 76 mM sodium tungstate(VI), shake mechanically, let stand for 10 min, centrifuge at 2000 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 50 × 8 Shodex Ionpac C-811P sulfonic acid-type polystyrene porous polymer (Showa Denko, Tokyo)
Column: 300 × 8 Shodex Ionpac KC-811 sulfonic acid-type polystyrene porous polymer (Showa Denko, Tokyo)
Mobile phase: 10 mM Phosphoric acid
Column temperature: 45
Flow rate: 0.8
Injection volume: 20
Detector: F ex 370 em 440 following post-column reaction. The column effluent mixed with the oxidant pumped at 0.3 mL/min and this mixture flowed through a 3 m × 0.5 mm ID PTFE coil at 65°. The effluent from this coil mixed with the reagent pumped at 0.3 mL/min and this mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 65° and a 1 m × 0.5 mm ID PTFE cooling coil to the detector. (Prepare oxidant by adding 60 mL 1 M NaOH and 4 mL 25% Brij-35 to 6 mL 10% sodium hypochlorite solution, make up to 1 L with 100 mM pH 7.0 phosphate buffer, final pH 12.0. Prepare reagent by dissolving 22.8 g sodium nitrite and 200 mg thiamine hydrochloride in 100 mM pH 7.0 phosphate buffer, adjust to pH 7.0 with 100 mM Na_2HPO_4 and 100 mM NaH_2PO_4, make up to 1 L with 100 mM pH 7.0 phosphate buffer. Thiamine is oxidized to the fluorescent thiochrome.)

CHROMATOGRAM

Retention time: 14
Limit of detection: 6 ng

KEY WORDS

post-column reaction; serum

REFERENCE

Yokoyama,T.; Kinoshita,T. High-performance liquid chromatographic determination of taurine in biological fluids by post-column fluorescence reaction with thiamine, *J.Chromatogr.*, **1991**, *568*, 212–218.

SAMPLE

Matrix: formula, media, tissue
Sample preparation: Boil 500 g tuna or squid meat in 1 L water for 2 h, centrifuge an aliquot at 15000 g for 3 min. Dilute 1 g infant formula with 1 mL water, centrifuge at 15000 g for 3 min. Centrifuge an aliquot of liquid media containing bacteria at 15000 g for 3 min. 100 μL Supernatant + 100 μL 4% NaOH, mix, add 20 μL 10% 2,4-dinitrofluorobenzene in acetone, shake vigorously, let stand for 15 min, add 100 μL 10% orthophosphoric acid, add 500 μL chloroform, shake, centrifuge at 15000 g for 15 s. Remove 200 μL of the upper aqueous layer, add 500 μL chloroform, shake, centrifuge at 15000 g for 15 s, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 50 × 4 10 μm Silosorb C18 (Elsico, Moscow)
Mobile phase: MeOH:water:glacial acetic acid:triethylamine 25:75:1:0.1
Flow rate: 5
Injection volume: 1
Detector: UV 350

CHROMATOGRAM

Retention time: 0.2
Limit of detection: 10 pmole

OTHER SUBSTANCES

Extracted: cysteic acid

KEY WORDS

fish; tuna; squid; derivatization

REFERENCE

Polanuer,B.; Ivanov,S.; Sholin,A. Rapid assay of dinitrophenyl derivative of taurine by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 81–85.

SAMPLE
Matrix: formula, milk
Sample preparation: 3 g Milk or formula + 80 mL water, heat at 50-60° with periodic agitation for 10 min, cool to room temperature, add 1 mL 150 mg/mL potassium ferrocyanide trihydrate in water, swirl, add 1 mL 300 mg/mL zinc acetate dihydrate in water, mix, let stand with periodic inversion for 20 min, make up to 100 mL with water, mix thoroughly, filter, discard the first 3-5 mL. Mix 1 mL filtrate with 1 mL buffer, add 1 mL 1.5 mg/mL dansyl chloride in MeCN, mix by inversion, let stand in the dark (with mixing after 1 h) at room temperature for 2 h, add 100 μL 20 mg/mL methylamine hydrochloride in water, vortex, filter (0.45 μm), inject a 20 μL aliquot of the filtrate. (Buffer was 80 mM sodium carbonate adjusted to pH 9.5 with 1 M HCl.)

HPLC VARIABLES
Guard column: C18
Column: 5 μm Resolve (Waters)
Mobile phase: MeCN:buffer 16:84
Flow rate: 1
Injection volume: 20
Detector: UV 254, F ex 330 em 530

CHROMATOGRAM
Retention time: 4-6
Limit of detection: 50 μg/g

KEY WORDS
derivatization

REFERENCE
Woollard,D.C.; Indyk,H.E. Taurine analysis in milk and infant formulae by liquid chromatography: Collaborative study, *J.AOAC Int.*, **1997**, *80*, 860–865.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeCN:MeOH:139 mM Na_2HPO_4 3.1:25:71.9 adjusted to pH 6.8 with phosphoric acid
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 450 mV

CHROMATOGRAM
Retention time: 18.99
Limit of detection: 0.75 ng/mL

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
rat; pharmacokinetics; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: solutions
Sample preparation: Mix sample:50 (?) mM NaCN in 50 mM pH 9.3 borate buffer:25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was 50 mM pH 7.0 sodium phosphate buffer. B was MeOH:THF:water 50:20:30. A:B from 25:75 to 0:100 over 75 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM
Retention time: 40

OTHER SUBSTANCES
Simultaneous: amino acids

KEY WORDS
derivatization

REFERENCE
Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Kontes micro-ultrasonic cell disrupter) 1-5 mg rat pineal tissue in 200 μL water, add 200 μL 2% picric acid in water (Caution! Picric acid is toxic and explosive when dry!), let stand at room temperature for 5 min, add to the column, rinse the container with three 200 μL portions of water, add the rinses to the column, add 1 mL water to the column, lyophilize the eluate, reconstitute with 100 μL water. Mix an aliquot of this solution with an equal volume of the reagent, let stand for 1 min, inject a 2-6 μL aliquot. (Prepare the column by making a 25 mm layer of Bio-Rad 200-400 mesh 50W-X8 (hydrogen form)ion-exchange resin in a 5 mm diameter column, add a 25 mm layer of 100-200 mesh Bio-Rad AG 1-X8 (chloride form) ion-exchange resin on top of this, wash with 10 mL water just before use. Prepare the reagent by dissolving 20 mg o-phthalaldehyde in 400 μL EtOH, adding 20 μL 2-mercaptoethanol, and adding 10 mL 500 mM pH 10.3 borate buffer. Dilute 1:10 with water before use.)

HPLC VARIABLES
Column: 300 × 3.9 μBondapak alkyl phenyl
Mobile phase: MeOH:buffer 42.75:57.25 (Prepare by mixing A:B in the ratio 43:57. A was prepared by dissolving 6.9 g $NaH_2PO_4.H_2O$ in water, adjusting pH to 5.3 with 5 M NaOH, and making up to 1 L with water. B was prepared by dissolving 6.9 g $NaH_2PO_4.H_2O$ in 250 mL water and making up to 1 L with MeOH.)
Flow rate: 2
Injection volume: 2-6
Detector: F ex 360 em 455

CHROMATOGRAM
Retention time: 4
Limit of quantitation: 600 pg

OTHER SUBSTANCES
Noninterfering: cysteic acid, hypotaurine, phosphoethanolamine

KEY WORDS
rat; pineal; SPE; derivatization

REFERENCE
Larsen,B.R.; Grosso,D.S.; Chang,S.Y. A rapid method for taurine quantitation using high performance liquid chromatography, *J.Chromatogr.Sci.*, **1980**, *18*, 233–236.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron for >10 mg; Kontes micro-ultrasonic cell disrupter for <10 mg) tissue with 40 volumes 25 μg/mL β-aminoisobutyric acid in EtOH:water:glacial acetic acid 75:20:5, centrifuge at 4° at 25000 g for 20 min. Remove a 50 μL aliquot of the supernatant and evaporate it to dryness under reduced pressure, suspend the residue in 100 μL 100 mM sodium bicarbonate by sonicating or vortexing, add 200 μL 1.25 mg/mL dansyl chloride in acetone, vortex, heat at 90° for 30 min, centrifuge at 5000 g for 20 min, inject a 4 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:water:phosphoric acid 13:87:0.15
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.26
Internal standard: β-aminoisobutyric acid (k' 9.25)
Limit of quantitation: 10 pmole

OTHER SUBSTANCES
Extracted: amino acids, urea
Interfering: asparagine, methionine

KEY WORDS
rat; brain; derivatization

REFERENCE
Saller,C.F.; Czupryna,M.J. γ-Aminobutyric acid, glutamate, glycine and taurine analysis using reversed-phase high-performance liquid chromatography and ultraviolet detection of dansyl chloride derivatives, *J.Chromatogr.*, **1989**, *487*, 167–172.

SAMPLE
Matrix: tissue, dialysate
Sample preparation: Homogenize (Kontes micro-ultrasonic cell disrupter) rat brain with 100 μL 50 mM ice-cold perchloric acid and 10 ng homoserine for 5 s, centrifuge at 4° at 13000 g for 5 min, filter (0.2 μm) the supernatant. Mix 25 μL of the filtrate from the tissue or dialysate (Ringer's) with 50 (tissue) or 12.5 (dialysate) μL working reagent, let stand for 2 min, inject an aliquot. (Prepare the reagent stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate, discard after 5 days. Prepare the working reagent by diluting 1 mL stock solution with 3 mL 100 mM sodium tetraborate, let stand for 24 h before use.)

HPLC VARIABLES
Column: 80 × 4.6 3 μm C18 HR-80 (ESA)
Mobile phase: MeOH:water 28:72 containing 100 mM Na_2HPO_4 and 0.13 mM disodium EDTA adjusted to pH 6.00 (tissue) or pH 6.40 (dialysate) with phosphoric acid. (Prepare by dissolving 14.2 g Na_2HPO_4 and 50 mg disodium EDTA in 720 mL water, add 280 mL MeOH, adjust pH. Recycle mobile phase.)

Flow rate: 1.2
Injection volume: 20
Detector: E, ESA Model 5100A coulometric, model 5011 dual electrode analytical cell preceded by a 0.2 μm carbon filter at -0.4 V and +0.6 V

CHROMATOGRAM
Retention time: 9
Internal standard: homoserine (3.5)
Limit of detection: 100-200 pg

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
rat; brain; derivatization

REFERENCE
Donzanti,B.A.; Yamamoto,B.K. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates, *Life Sci.*, **1988**, *43*, 913–922.

SAMPLE
Matrix: urine
Sample preparation: 10 μL Urine + 50 μL 5 mM S-carboxymethyl-L-cysteine in water + 500 μL 100 mM pH 9.0 sodium bicarbonate buffer + 50 μL acetone + 1 mL 650 μg/mL dabsyl chloride in acetone, heat at 40° for 30 min, add 1.5 mL EtOH, let stand for 30 min, centrifuge, filter (0.2 μm) the supernatant, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 15 × 3.2 5 μm TSKguardgel ODS-80Ts
Column: 150 × 4.6 5 μm TSKgel ODS-80Ts
Mobile phase: Gradient. MeCN:50 mM pH 4.00 sodium acetate buffer from 28:72 to 31:69 over 20 min, to 32:68 over 20 min, wash with 95:5 for 5 min, re-equilibrate at initial conditions for 20 min.
Column temperature: 16
Injection volume: 10
Detector: UV 430

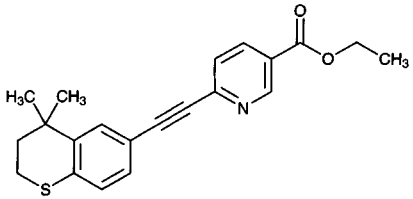
CHROMATOGRAM
Retention time: 27
Internal standard: S-carboxymethyl-L-cysteine (23)
Limit of detection: 4 pmole

OTHER SUBSTANCES
Extracted: hypotaurine

KEY WORDS
rat; derivatization

REFERENCE
Futani,S.; Ubuka,T.; Abe,T. High-performance liquid chromatographic determination of hypotaurine and taurine after conversion to 4-dimethylaminoazobenzene-4'-sulfonyl derivatives and its application to the urine of cysteine-administered rats, *J.Chromatogr.B*, **1994**, *660*, 164–169.

Tazarotene



Molecular formula: $C_{21}H_{21}NO_2S$
Molecular weight: 351.47
CAS Registry No.: 118292-40-3
Merck Index: 9249

SAMPLE

Matrix: blood, microsomal incubations
Sample preparation: Blood. 500 µL Whole blood + 2.5 mL MeCN:1-butanol 50:50 + 200 µL 1 µg/mL IS, vortex for 30 s, centrifuge at 1500 g for 5 min. Evaporate supernatant to dryness with nitrogen. Microsomal incubations. 1 mL Microsomal incubation + 2.5 mL MeCN:1-butanol 50:50, centrifuge at 1500 g for 5 min. Decant the supernatant, dry it with nitrogen and reconstitute in 300 µL mobile phase. Inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: dry packed C18
Column: 150 × 4.6 5 µm Ultrasphere C18
Mobile phase: MeCN:water:acetic acid 65:34.5:0.5
Flow rate: 1.2
Injection volume: 100
Detector: UV 345

CHROMATOGRAM

Retention time: 17
Internal standard: AGN 190252 (7)

OTHER SUBSTANCES

Extracted: metabolites

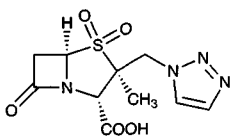
KEY WORDS

liver; human; rat; whole blood

REFERENCE

Madhu,C.; Duff,S.; Baumgarten,V.; Rix,P.; Small.D.,; Tang-Liu,D. Metabolic deesterification of tazarotene in human blood and rat and human liver microsomes, *J.Pharm.Sci.*, **1997**, *86*, 972–974.

Tazobactam



Molecular formula: $C_{10}H_{12}N_4O_5S$
Molecular weight: 300.30
CAS Registry No.: 89786-04-9
Merck Index: 9251
Lednicer No.: 5 156

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Plasma, serum. 200 µL Plasma or serum + 200 µL 25 µg/mL penicillin G in 50 mM pH 6.0 sodium phosphate buffer + 800 µL MeCN, vortex for 30 s, centrifuge at 3000 g for 10 min. Remove the clear supernatant and add it to 2 mL dichloromethane, mix for 30 s, centrifuge at 3000 g for 10 min, inject a 25 µL aliquot of the upper aqueous layer. Bile. 200 µL Bile + 400 µL 50 mM pH 7.0 sodium phosphate buffer + 2 mL MeCN, vortex for 30 s, centrifuge at 3000 g for 10 min. Remove the clear supernatant and add it to 2 mL dichloromethane, mix for 30 s, centrifuge at 3000 g for 10 min, inject a 25 µL aliquot of the upper

aqueous layer. Urine. 100 μL Urine + 50 μL 5 mg/mL penicillin G in water, vortex for 30 s, make up to 10 mL with 50 mM pH 6.0 sodium phosphate buffer, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Brownlee C 18 guard column
Column: 250 × 4.6 5 μm Hypersil ODS (Keystone)
Mobile phase: Gradient. A was MeCN:10 mM sodium phosphate adjusted to pH 2.7 with concentrated phosphoric acid 3:97. B was MeCN:10 mM sodium phosphate adjusted to pH 2.7 with concentrated phosphoric acid 90:10. A:B from 95:5 to 50:50 over 9 min and then to 95:5 over 1 min.
Flow rate: 1.5
Injection volume: 25
Detector: UV 220

CHROMATOGRAM
Retention time: 5.7
Internal standard: penicillin G (12.5)
Limit of quantitation: 50000 ng/mL (urine), 1000 ng/mL (plasma, serum, bile)

OTHER SUBSTANCES
Extracted: piperacillin
Simultaneous: amoxicillin, ampicillin, cefoperazine, cefometazole, cefotaxime, cefotetan, cefuroxime, mezlocillin

KEY WORDS
plasma; serum

REFERENCE
Ocampo,A.P.; Hoyt,K.D.; Wadgaonkar,N.; Carver,A.H.; Puglisi,C.V. Determination of tazobactam and piperacillin in human plasma, serum, bile and urine by gradient elution reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *496*, 167–179.

SAMPLE
Matrix: blood
Sample preparation: Homogenize tissue in water at 4° for 30 s, centrifuge at 15000 rpm (Sorvall centrifuge) for 10 min. 250 μL Plasma or tissue homogenate + 500 μL 10 μg/mL cefpodoxime in MeCN, mix, centrifuge at 15000 rpm (Sorvall centrifuge), extract with 1 mL dichloromethane, inject 120 μL of the aqueous phase onto column A with mobile phase A, elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 40 × 4.6 10 μm Lichrosorb RP 2; B 250 × 4.6 5 μm Spherisorb ODS II
Mobile phase: A MeCN:100 mM NaH_2PO_4 and 5 mM tetrabutylammonium hydrogen sulfate 5:95, pH adjusted to 6.5; B MeCN:100 mM NaH_2PO_4 and 5 mM tetrabutylammonium hydrogen sulfate 10:90, pH adjusted to 6.5
Column temperature: 25
Flow rate: A 1; B 1.5
Injection volume: 120
Detector: UV 210 (UV 300 for IS)

CHROMATOGRAM
Retention time: 18.6
Internal standard: cefpodoxime (24.9)
Limit of detection: 96 ng/mL

KEY WORDS
plasma; column-switching

REFERENCE
Kinzig,M.; Sörgel,F.; Brismar,B.; Nord,C.E. Pharmacokinetics and tissue penetration of tazobactam and piperacillin in patients undergoing colorectal surgery, *Antimicrob.Agents Chemother.*, **1992**, *36*, 1997–2004.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 400 μL 10 mg/mL zinc sulfate containing 350 μg/mL benzoic acid, vortex for 30 s, centrifuge at 5500 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.3 5 μm Nova Pak
Mobile phase: MeOH:pH 6.30 phosphate buffer 5:95
Column temperature: 45
Flow rate: 2
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 2.4
Internal standard: benzoic acid (1.9)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Extracted: sulbactam

KEY WORDS
serum

REFERENCE
Guillaume,Y.; Peyrin,E.; Guinchard,C. Rapid determination of sulbactam and tazobactam in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 363–371.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum or hemofiltrate + 100 μL 3 M sulfuric acid + 4 mL diethyl ether, vortex for 1 min, centrifuge at 5500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL reagent and 10 μL 1% mercuric chloride in water, heat at 37° for 30 min, inject a 20 μL aliquot. (Prepare reagent by dissolving 4 g 1,2,4-triazole in water, adjusting the pH to 9.0 with 10 M NaOH, and making up to 20 mL with water.)

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher RP (18)
Mobile phase: MeOH:$(NH_4)_2HPO_4$ buffer 0.5:99.5, adjusted to pH 6.00 with phosphoric acid (Buffer concentration not given.)
Flow rate: 2.2
Injection volume: 20
Detector: UV 325

CHROMATOGRAM
Retention time: 8
Limit of detection: 50 ng/mL

KEY WORDS
serum; hemofiltrate; derivatization

REFERENCE
Peyrin,E.; Guillaume,Y.; Guinchard,C. High-performance liquid chromatographic determination of tazobactam by precolumn derivatization, *J.Chromatogr.B*, **1995**, *672*, 160–164.

SAMPLE
Matrix: blood, tissue

Sample preparation: Plasma. 250 μL Plasma + 500 μL 10 μg/mL cefpodoxime in MeCN, mix, centrifuge at 15000 rpm (Sorvall centrifuge). Remove the supernatant and add it to 1 mL dichloromethane, extract, inject a 120 μL aliquot of the aqueous phase. Tissue. Blot tissue, homogenize with two volumes of water (IKA-Ultra-Turrax) at 4° for 30 s, centrifuge at 15000 rpm (Sorvall centrifuge) for 10 min, remove 250 μL of the supernatant, add 500 μL 10 μg/mL cefpodoxime in MeCN, mix, centrifuge at 15000 rpm (Sorvall centrifuge). Remove the supernatant and add it to 1 mL dichloromethane, extract, inject a 120 μL aliquot of the aqueous phase. (A column-switching technique is used but no details are given in the paper.)

HPLC VARIABLES
Column: A 40 × 4.6 10 μm RP-2 (Bischoff Chromatography)); B 250 × 4.6 5 μm Spherisorb ODS II
Mobile phase: A MeCN:100 mM NaH_2PO_4 + 5 mM tetrabutylammonium hydrogen sulfate 5:95, pH 6.5; B MeCN:100 mM NaH_2PO_4 + 5 mM tetrabutylammonium hydrogen sulfate 10:90, pH 6.5
Column temperature: 25
Flow rate: A 1; B 1.5
Injection volume: 120
Detector: UV 210 for tazobactam, UV 300 for cefpodoxime

CHROMATOGRAM
Retention time: 18.6
Internal standard: cefpodoxime (24.9)
Limit of quantitation: 76 ng/g (tissue), 96 ng/mL (plasma)

KEY WORDS
plasma; column-switching; pharmacokinetics; fat; muscle; skin; intestinal mucosa; appendix

REFERENCE
Kinzig,M.; Sörgel,F.; Brismar,B.; Nord,C.E. Pharmacokinetics and tissue penetration of tazobactam and piperacillin in patients undergoing colorectal surgery, *Antimicrob.Agents Chemother.*, **1992**, *36*, 1997–2004.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 18:1 with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere
Mobile phase: MeCN:10 mM NaH_2PO_4 7:93, pH adjusted to 2.7 with 85% phosphoric acid
Flow rate: 1.2
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 5.0

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Choi,J.-S.; Burm,J.-P.; Jhee,S.S.; Chin,A.; Ulrich,R.W.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium and ranitidine hydrochloride in 0.9% sodium chloride injection during simulated Y-site administration, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2273–2276.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 8-fold to 20-fold with saline, filter (0.2 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere C18

Mobile phase: MeCN:100 mM sodium phosphate buffer 7:93 adjusted to pH 2.7 with 85% phosphoric acid
Flow rate: 1.2
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 7-8

KEY WORDS
saline; injections; stability-indicating

REFERENCE
Chung,K.C.; Moon,Y.S.K.; Chin,A.; Ulrich,R.W.; Gill,M.A. Compatibility of ondansetron hydrochloride and piperacillin sodium tazobactam sodium during simulated Y-site administration, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1554–1556.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 25-50 μL sample with 2 mL mobile phase, filter (0.2 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: MeCN:10 mM sodium phosphate 7:93 adjusted to pH 2.7 with 85% phosphoric acid
Flow rate: 1.2
Injection volume: 20
Detector: UV 215

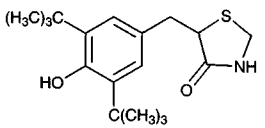
CHROMATOGRAM
Retention time: 6.40

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; saline; 5% dextrose

REFERENCE
Moon,Y.S.K.; Chung,K.C.; Chin,A.; Gill,M.A. Stability of piperacillin sodium-tazobactam sodium in polypropylene syringes and polyvinyl chloride minibags, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 999–1001.

Tazofelone



Molecular formula: $C_{18}H_{27}NO_2S$
Molecular weight: 321.49
CAS Registry No.: 136433-51-7

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 1 mL cold MeCN to 250 μL microsomal incubation, centrifuge at 13000 rpm for 3 min, evaporate the supernatant under nitrogen, reconstitute the residue in 100 μL MeCN:water 40:60, inject 60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Prodigy C 18 (Phenomenex)

Mobile phase: MeCN:water 60:40
Column temperature: 40
Flow rate: 1
Injection volume: 60
Detector: UV 214

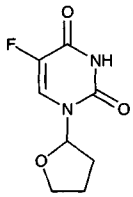
CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Surapaneni,S.S.; Clay,M.P.; Spangle,L.A.; Paschal,J.W.; Lindstrom,T.D. In vitro biotransformation and identification of human cytochrome P450 isozyme-dependent metabolism of tazofelone, *Drug Metab.Dispos.*, **1997**, *25*, 1383–1388.

Tegafur



Molecular formula: $C_8H_9FN_2O_3$
Molecular weight: 200.17
CAS Registry No.: 17902-23-7
Merck Index: 9267

SAMPLE
Matrix: blood
Sample preparation: Mix 20 μL plasma with 50 μL 500 mM pH 8.0 phosphate buffer, 100 μL 500 ng/mL 5-chlorouracil in water, and 7 mL ethyl acetate. Shake for 30 min and centrifuge at 2200 g for 10 min. Remove the ethyl acetate layer and evaporate it. Reconstitute the residue in 500 μL n-hexane:mobile phase 40:60 with sonication for 10 min. Inject the whole amount.

HPLC VARIABLES
Column: 100 × 4.6 Develosil 60-3 (Nomura Chemical, Japan)
Mobile phase: n-Hexane:ethyl acetate:formic acid:water 50:0.5:0.5:0.3
Flow rate: 0.9
Injection volume: 500
Detector: UV 264

CHROMATOGRAM
Internal standard: 5-chlorouracil
Limit of detection: 50 ng/mL

KEY WORDS
plasma; rat; normal phase

REFERENCE
Fuse,E.; Takai,K.; Okuno,K.; Kobayashi,S. Hepatic extraction ratio of 5-fluorouracil in rats. Dose dependence and effect of uracil and interleukin-2, *Biochem.Pharmacol.*, **1996**, *52*, 561–568.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute 250 μL plasma or urine 1:10 with water, add 100 μL 200 mM pH 7.0 phosphate buffer, add 100 μL 10 μg/mL β-hydroxyethyltheophylline, add 4 mL dichloromethane, shake for 10 min, centrifuge at 2000 g for 5 min, transfer the organic layer to another

tube, repeat the extraction. Evaporate the combined organic layers to dryness under a stream of nitrogen at 40°, dissolve the residue in 200 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (GL Sciences)
Mobile phase: MeOH:10 mM pH 5.5 phosphate buffer 15:85
Flow rate: 1
Injection volume: 40
Detector: UV 270

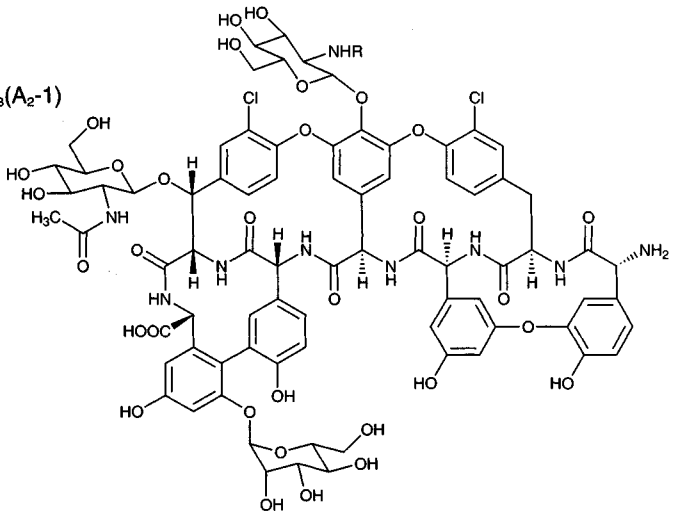
CHROMATOGRAM
Retention time: 6.95
Internal standard: β-hydroxyethyltheophylline (10.53)
Limit of quantitation: 10 ng/mL (plasma), 100 ng/mL (urine)

KEY WORDS
plasma

REFERENCE
Matsushima,E.; Yoshida,K.; Kitamura,R.; Yoshida,K.-. Determination of S-1 (combined drug of tegafur, 5-chloro-2,4-dihydroxypyridine and potassium oxonate) and 5-fluorouracil in human plasma and urine using high-performance liquid chromatography and gas-chromatography-negative ion chemical ionization mass spectrometry, *J.Chromatogr.B*, **1997**, *691*, 95–104.

Teicoplanin

Molecular formula: $C_{88}H_{95}Cl_2N_9O_{33}$($A_2$-1)
Molecular weight: 1877.68 (A_2-1)
CAS Registry No.: 61036-64-4, 61036-62-2
Merck Index: 9269



Teicoplanin A_2-1 (Z)-4-decanoic acid
A_2-2 8-methylnonanoic acid
A_2-3 n-decanoic acid
A_2-4 8-methyldecanoic acid
A_2-5 9-methyldecanoic acid

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL MeCN to 500 μL plasma, vortex briefly, centrifuge at 2000 g for 3 min, add 2 mL chloroform (Caution! Chloroform is a carcinogen!) to the supernatant, vortex, inject an aliquot of the aqueous supernatant layer.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrocart RP8

Mobile phase: MeCN:20 mM pH 4.4 ammonium acetate buffer 26:74
Flow rate: 1.3
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 30 ng/mL
Limit of quantitation: 90 ng/mL

KEY WORDS
plasma

REFERENCE
Cociglio,M.; Peyrière,H.; Hillaire-Buys,D.; Alric,R. Application of a standardized coextractive cleanup procedure to routine high-performance liquid chromatography assays of teicoplanin and ganciclovir in plasma, *J.Chromatogr.B*, **1998**, *705*, 79–85.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SAX SPE cartridge with 4 mL MeOH, 1 mL water, and 1 mL 10 mM n-heptanesulfonic acid. 1 mL Plasma + 1 mL 10 mM n-heptanesulfonic acid, vortex, centrifuge at 4000 g for 2 min, add the supernatant to the SPE cartridge, wash with 3 mL 10 mM heptanesulfonic acid, elute with 1 mL MeOH, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 10 × 4.2 10 μm LiChrosorb RP-8
Column: 150 × 4.2 5 μm LiChrosorb RP-8
Mobile phase: MeOH:water 5:95 containing 10 mM disodium n-heptanesulfonate, adjusted to pH 4.0 with 2 g/L sodium acetate and glacial acetic acid
Column temperature: 30
Flow rate: 0.8
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 200-400 ng/mL

KEY WORDS
SPE; plasma

REFERENCE
Georgopoulos,A.; Czejka,M.J.; Starzengruber,N.; Jäger,W.; Lackner,H. High-performance liquid chromatographic determination of teicoplanin in plasma: comparison with a microbiological assay, *J.Chromatogr.*, **1989**, *494*, 340–346.

SAMPLE
Matrix: blood
Sample preparation: Add plasma to a Bond Elut C8 SPE cartridge, wash with water, elute with MeCN:pH 6 buffer 20:80, inject a 50 μL aliquot of the eluate into a 500 μL sample lop filled with water, inject 200 μL water into the sample loop, operate the valve.

HPLC VARIABLES
Column: 100 × 1 5 μm Hypersil ODS
Mobile phase: MeCN:25 mM pH 6.0 NaH_2PO_4 20:80
Flow rate: 0.05
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 3 (TA3-1), 10 (TA2-1), 13 (TA2-2), 16 (TA2-3), 30 (TA2-4), 33 (TA2-5)

Limit of detection: 50 ng/mL

KEY WORDS
SPE; plasma

REFERENCE
Taylor,R.B.; Reid,R.G.; Gould,I.M. Determination of teicoplanin in plasma using microbore high-performance liquid chromatography and injection-generated gradients, *J.Chromatogr.*, **1991**, *563*, 451–457.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1-10 mL plasma or urine to the affinity column, wash with 1 mL buffer, elute with three 500 μL portions of 1.5% ammonium hydroxide. Add 100 μL 18% HCl to the eluate, inject a 170 μL aliquot. (Affinity columns consisted of 0.5 mL D-alanyl-D-alanine-ε-aminocaproyl Sepharose CL-6B stored in 100 mM pH 8.5 Tris buffer containing 0.004% sodium merthiolate, equilibrate with buffer before use. After use wash with 5 mL buffer containing 0.004% sodium merthiolate, store at 4°. Buffer was 50 mM NaH_2PO_4 containing 200 mM NaCl adjusted to pH 7.5 with 1 M NaOH. Prepare the column material by coupling D-alanyl-D-alanine to activated CH-Sepharose 4B using ε-aminocaproic acid according to the manufacturer's instructions. 30 mg D-Alanyl-D-alanine in 5 mL 100 mM sodium bicarbonate buffer containing 500 mM NaCl (pH 8) was coupled to 3 mL gel in 1 h, block unreacted ester groups with 1 M ethanolamine hydrochloride at pH 9 for 1 h, wash repeatedly with 100 mM pH 4 sodium acetate buffer containing 500 mM NaCl and 100 mM pH 8 tris-HCl buffer containing 500 mM NaCl (Appl. Biochem. Biotechnol. 1985, 11, 101.).)

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Nucleosil C18
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. A was 10 mM pH 4.9 NaH_2PO_4. B was MeCN:10 mM pH 4.9 NaH_2PO_4 50:50. A:B 78:22 of 0.5 min, to 47:53 over 44 min, maintain at 47:53 for 2 min, to 0:100 over 1.5 min, maintain at 0:100 for 5 min, return to initial conditions over 2 min, re-equilibrate for 5 min.
Flow rate: 1.3
Injection volume: 170
Detector: UV 240

CHROMATOGRAM
Retention time: 9.1 (A3), 28.8 (A2-1), 31.6 (A2-2), 32.8 (A2-3), 37.1 (A2-4), 38.0 (A2-5)
Limit of detection: 100 ng/mL (A2-2)

OTHER SUBSTANCES
Noninterfering: acebutolol, acetaminophen, aspirin, atenolol, benfluorex, chlorazepam, clofibrate, diazepam, furosemide, hydrochlorothiazide, nitrazepam, oxprenolol

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Riva,E.; Ferry,N.; Cometti,A.; Cuisinaud,G.; Gallo,G.G.; Sassard,J. Determination of teicoplanin in human plasma and urine by affinity and reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *421*, 99–110.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 1 mL urine to 7.4, add 1 mL of a homogeneous suspension of D-alanyl-D-alanine-epsilon-aminocaproyl-Sepharose resin:50 mM pH 7.4 phosphate buffer containing 200 mM NaCl 50:50, let stand at 4° overnight, place on top of a column containing 6 mL of a homogeneous suspension of D-alanyl-D-alanine-epsilon-aminocaproyl-Sepharose resin:50 mM pH 7.4 phosphate buffer containing 200 mM NaCl 50:50, wash with 6 mL 50 mM pH 7.4 phosphate buffer containing 200 mM NaCl, elute with four 3 mL portions of 1.5% aqueous ammonia, immediately neutralize the eluate with 100 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 25 mM pH 6.0 sodium phosphate buffer. B was MeCN:water 90:10. A:B from 84:16 to 55:45 over 2 h, wash with 49:51 for 5 min.
Column temperature: 20
Flow rate: 1.5
Detector: UV 254

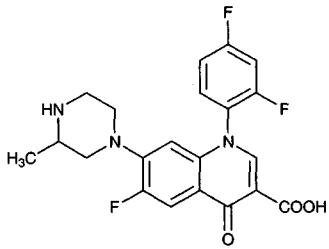
CHROMATOGRAM
Retention time: 8 (A3), 19 (A2-1), 20 (A2-2), 22 (A2-3,3a), 27 (A2-4), 28 (A2-5,5a)
Limit of detection: 0.5-5 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; SPE

REFERENCE
Zerilli,L.F.; Cavenaghi,L.; Bernareggi,A.; Assandri,A. Teicoplanin metabolism in rats, *Antimicrob.Agents Chemother.*, **1989**, *33*, 1791–1794.

Temafloxacin



Molecular formula: $C_{21}H_{18}F_3N_3O_3$
Molecular weight: 417.39
CAS Registry No.: 108319-06-8, 105784-61-0 (HCl)
Merck Index: 9284
Lednicer No.: 5 125

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Dilute urine 1:20. Dilute bile 1:10. 500 μL Serum, diluted urine, or diluted bile + 3.2 mL dichloromethane, vortex, rotate at 20 rpm for 10 min, centrifuge at 1000 g for 10 min. Remove 3 mL of the lower organic phase and add it to 200 μL 100 mM NaOH, rotate at 20 rpm for 10 min, centrifuge at 1000 g for 10 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:buffer 19:81, pH adjusted to 2 with 14.6 M phosphoric acid (Buffer was 10 mM NaH_2PO_4 containing 5 mM tetrabutylammonium bromide.)
Flow rate: 2
Injection volume: 20
Detector: F ex 275 em 450

CHROMATOGRAM
Retention time: 3
Limit of detection: 100 ng/mL (bile), 200 ng/mL (urine), 10 ng/mL (serum)

OTHER SUBSTANCES
Noninterfering: amikacin, aztreonam, carbamazepine, cephalosporins, ciprofloxacin, clavulanic acid, difloxacin, digitoxin, digoxin, fleroxacin, fosfomycin, furosemide, gentamycin, imipenem, lidocaine, netilmicin, norfloxacin, ofloxacin, pefloxacin, penicillins, phenobarbital, phenytoin, primidone, procainamide, quinidine, rifampin, salicylic acid, teicoplanin, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
serum; human; rabbit

REFERENCE
Koechlin,C.; Jehl,F.; Linger,L.; Monteil,H. High-performance liquid chromatography for the determination of three new fluoroquinolones, fleroxacin, temafloxacin and A-64730, in biological fluids, *J.Chromatogr.*, **1989**, *491*, 379–387.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize (Ultra-Turrax T25) mouse lung in 1-3 mL pH 6.8 Soerensen phosphate buffer, centrifuge. Add 1 μg sparfloxacin to serum or lung homogenate supernatant, extract using a Bond Elut C2 SPE cartridge, inject a 100 μL aliquot of the extract.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrabase C8 (SFCC, Neuilly Plaisance, France)
Mobile phase: MeCN:MeOH:5% acetic acid 15:10:75
Flow rate: 1
Injection volume: 100
Detector: UV 280

CHROMATOGRAM
Retention time: 6
Internal standard: sparfloxacin
Limit of detection: 15 ng

KEY WORDS
serum; lung; mouse; pharmacokinetics; SPE

REFERENCE
Vallée,E.; Azoulay-Dupuis,E.; Bauchet,J.; Pocidalo,J.-J. Kinetic disposition of temafloxacin and ciprofloxacin in a murine model of pneumococcal pneumonia. Relevance for drug efficacy, *J.Pharmacol.Exp.Ther.*, **1992**, *262*, 1203–1208.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Mix equal volumes of plasma and 100-500 ng/mL IS in MeCN: 75 mM pH 7.4 phosphate buffer containing 0.5% sodium dodecyl sulfate 30:70, filter (Amicon Centrifree with YMT membrane) while centrifuging at less than 1000 g for 20 min, inject an aliquot of the ultrafiltrate. Alternatively, mix 400 μL plasma and 400 μL 500 mM pH 7 phosphate buffer, add 6 mL dichloromethane:EtOH 90:10, shake slowly horizontally for 10 min, centrifuge in a refrigerated centrifuge at 900 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 50-60°, reconstitute the residue in mobile phase, inject an aliquot. Urine. Dilute urine 20-100 fold with mobile phase containing IS, inject an aliquot. Hydrolyze conjugates by heating urine in 1 M NaOH at 60° for 30 min, neutralize, dilute 20-100 fold with mobile phase containing IS, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Adsorbosphere HS C18
Mobile phase: MeCN:water 53:47 containing 40 mM phosphoric acid, 10 mM NaH_2PO_4, 0.2% sodium dodecyl sulfate, and 5 mM N-acetylhydroxamic acid
Flow rate: 1.5
Injection volume: 50
Detector: F ex 280 em 389 (or UV 280, Antimicrob. Agents Chemother. 1991, 35, 436)

CHROMATOGRAM
Retention time: 7.5
Internal standard: 1-(4-bromophenyl)-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-oxo-3-quinoline carboxylic acid (A57084) (8.5)
Limit of quantitation: 1 ng/mL (extraction), 10 ng/mL (ultrafiltrate)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

REFERENCE
Granneman,G.R.; Varga,L.L. High-performance liquid chromatographic procedures for the determination of temafloxacin in biological matrices, *J.Chromatogr.*, **1991**, *568*, 197–206.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum or 500 μL urine + 500 μL 1 M ammonium acetate + 5 mL dichloromethane, shake vigorously for 10 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL MeOH:thionyl chloride 90:10, heat at 60° for 30 min, evaporate to dryness under a stream of nitrogen at 40°, add 500 μL 500 mM sulfuric acid, add 1 mL hexane, shake vigorously for 10 min, centrifuge for 5 min. Remove the aqueous layer and add it to 1 mL 2 M sodium carbonate and 3 mL ether, shake vigorously for 10 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 20 μL 2 mg/mL (S)-(-)-N-1-(2-naphthylsulfonyl)-2- pyrrolidinecarbonyl chloride in dichloromethane, add 5 μL triethylamine, let stand at room temperature for 10 min. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL dichloromethane, inject a 50 μL aliquot. (No details of synthesis of chiral reagent given in paper.)

HPLC VARIABLES
Column: 150 × 4.6 Zorbax SIL
Mobile phase: Hexane:methyl acetate:MeOH:aqueous ammonia 150:100:10:1
Flow rate: 0.8
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 18.5 (S), 19.5 (R)
Limit of detection: 5 ng/mL

KEY WORDS
serum; chiral; pharmacokinetics; derivatization; normal phase

REFERENCE
Matsuoka,M.; Banno,K.; Sato,T. Analytical chiral separation of a new quinolone compound in biological fluids by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *676*, 117–124.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 100 mg bulk drug in 25 mL MeCN:water 50:50 containing 1 mL 1 M NaOH, add 15 mL 20 mM KH_2PO_4 adjusted to pH 2.4 with orthophosphoric acid, make up to 50 mL with MeCN:water 50:50, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:THF:buffer from 5:5:90 to 5:60:35 over 50 min, maintain at 5:60:35 for 10 min, return to initial conditions over 5 min, re-equilibrate for 15 min. (Buffer was 20 mM KH_2PO_4 adjusted to pH 2.4 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 325

CHROMATOGRAM
Retention time: 11
Limit of detection: 0.05% (of temafloxacin)

OTHER SUBSTANCES
Simultaneous: degradation products, impurities

REFERENCE
Elrod,L.,Jr.; Linton,C.L.; Shelat,B.P.; Wong,C.F. Determination of minor impurities in temafloxacin hydrochloride by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *519*, 125–136.

SAMPLE
Matrix: cells
Sample preparation: Incubate cells in 2 mL 100 mM pH 3.0 glycine-HCl buffer for 2 h at room temperature, centrifuge at 5600 g for 5 min, inject an aliquot.

HPLC VARIABLES
Column: Bondapak C18
Mobile phase: MeCN:25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 25:75
Flow rate: 1.5
Detector: F ex 340 em 425

OTHER SUBSTANCES
Also analyzed: ciprofloxacin, fleroxacin, lomefloxacin, norfloxacin, ofloxacin

REFERENCE
Pascual,A.; Garcia,I.; Conejo,M.C.; Perea,E.J. Fluorometric and high-performance liquid chromatographic measurement of quinolone uptake by human neutrophils, *Eur.J.Clin.Microbiol.Infect.Dis.*, **1991**, *10*, 969–971.

SAMPLE
Matrix: hair
Sample preparation: Hair. Cut 10 hairs into 2 mm sections, weigh, add 1 mL 1 M NaOH, heat at 60° for 1 h, cool, add 500 μL 2 M HCl, add 1 mL 200 mM pH 7.0 phosphate buffer, add 500 μL 4-20 ng/mL IS in MeOH, add 5 mL chloroform, shake for 30 min, centrifuge at 2000 rpm for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness by aspiration at 40° for 30 min, reconstitute the residue in 200-500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 TSKgel ODS-80TM (Tosoh)
Mobile phase: MeCN:50 mM citric acid:1 M ammonium acetate 22:78:1
Flow rate: 1
Injection volume: 100
Detector: F ex 280 em 406

CHROMATOGRAM
Retention time: 12
Internal standard: 1-(4-bromophenyl)-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-oxo-3-quinoline carboxylic acid (A57084) (14)
Limit of detection: 0.5 ng/mL

REFERENCE
Uematsu,T.; Kondo,K.; Yano,S.; Yamaguchi,T.; Umemura,K.; Nakashima,M. Measurement of temafloxacin in human scalp hair as an index of drug exposure, *J.Pharm.Sci.*, **1994**, *83*, 42–45.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 450 μg/mL solution in MeCN:water 50:50. 5 mL Solution + 5 mL THF + 200 molar excess of acetic anhydride + 3 molar excess of 1 M NaOH, sonicate for 15 min, add 15 mL mobile phase, sonicate for 15 min, cool to room temperature, make up to 50 mL with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:buffer 35:65 (Buffer was prepared by mixing equal volumes of 20 mM citric acid and 20 mM sodium citrate, pH adjusted to 2.4 with perchloric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 280

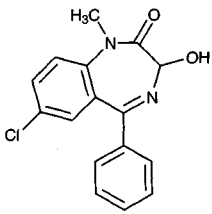
CHROMATOGRAM
Retention time: 21.4

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, norfloxacin, sarafloxacin

KEY WORDS
derivatization

REFERENCE
Morley,J.A.; Elrod,L.,Jr. Determination of fluoroquinolone antibacterials as N-Acyl derivatives, *Chromatographia*, **1993**, *37*, 295–299.

Temazepam



Molecular formula: $C_{16}H_{13}ClN_2O_2$
Molecular weight: 300.74
CAS Registry No.: 846-50-4
Merck Index: 9285
Lednicer No.: 2 402

SAMPLE
Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 10 μL 1 mg/mL prazepam and 1 mL pH 7.4 phosphate buffer, vortex briefly, add 4 mL diethyl ether and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL diethyl ether to extraction sample, mix. Evaporate combined organic layers to dryness under a stream of dry air at 50°. Purify extracts by partition between 1 mL MeCN and 2 mL heptane, separate MeCN layer, evaporate it to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot of the solution.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II ODS
Mobile phase: MeCN:MeOH:10 mM phosphoric acid:10 mM Na_2HPO_4 40:20:36:4
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 4.8
Internal standard: prazepam (14.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: diazepam, nitrazepam, oxazepam

KEY WORDS
liver; lung; muscle; urine; pericardial fluid

REFERENCE
Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE
Matrix: blood
Sample preparation: 200 mg Extrelut + 400 µL blood + 100 µL 50 µg/mL IS in MeOH, mix, let dry at room temperature for 1-2 h. Add to a 30 × 4.6 stainless steel extraction column, extract with carbon dioxide:ethyl acetate 95:5 at 2 mL/min, 65°, and 300 psi. for 10 min, collect by expansion into MeOH. Dry the collected extract at 65° under nitrogen. Reconstitute the residue in 50 µL mobile phase. Inject a 20 µL aliquot. Condition ca. 10 g Extrelut in a 10 mL plastic syringe with dichloromethane. Add 250 µL 5% ammonia to the top. Mix 900 µL blood with 100 µL 50 µg/mL IS in MeOH. Add 1 mL pH 4 phosphate buffer and 250 µL 5% ammonia solution, mix thoroughly, add to the extraction column. After 5 min elute with diethyl ether under the influence of gravity. Collect 8 mL eluate, evaporate to dryness at 65° under nitrogen. Reconstitute the residue in 180 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 µm Hypersil ODS
Column: 250 × 4.6 5 µm Hypersil ODS
Mobile phase: MeOH:Na_2HPO_4 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3
Internal standard: prazepam (11.5)

OTHER SUBSTANCES
Also analyzed: diazepam, chlordiazepoxide, nordiazepam, oxazepam

KEY WORDS
SFE; SPE; whole blood

REFERENCE
Scott,K.S.; Oliver,J.S. Development of a supercritical fluid extraction method for the determination of temazepam in whole blood, *J.Anal.Toxicol.*, **1997**, *21*, 297–300.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

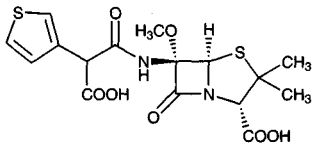
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 18.562

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Temocillin



Molecular formula: $C_{16}H_{18}N_2O_7S_2$
Molecular weight: 414.46
CAS Registry No.: 66148-78-5
Merck Index: 9288
Lednicer No.: 4 178

SAMPLE
Matrix: blood
Sample preparation: 350 μL Serum + 150 μL water + 250 μL 400 mM HCl + 3.5 mL chloroform: n-amyl alcohol (3:1), mix for 5 min, centrifuge for 5 min. Remove the organic layer and add it to 350 μL 10 mM pH 7.0 phosphate buffer, mix for 5 min, centrifuge for 5 min, inject a 20 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 15:85 (Buffer was 100 mM ammonium acetate adjusted to pH 4.0 with glacial acetic acid.)
Flow rate: 1.8
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 5.4
Internal standard: temocillin

OTHER SUBSTANCES
Extracted: cefoxitin, cefuroxime, cephalothin, ticarcillin
Noninterfering: acetaminophen, acetazolamide, allopurinol, amikacin, ampicillin, azlocillin, caffeine, cefamandole, cefoperazone, cefotaxime, cefsulodin, ceftazidime, ceftizoxime, chloramphenicol, chlorpromazine, clindamycin, dicloxacillin, 5-fluorocytosine, flurazepam, gentamicin, methicillin, metronidazole, mezlocillin, moxalactam, nafcillin, penicillin, phenobarbital, piperacillin, procainamide, rifampin, sulfamethoxazole, theophylline, thienamycin, tobramycin, trimethoprim, vancomycin

KEY WORDS
serum; temocillin is IS

REFERENCE
Shull,V.H.; Dick,J.D. Determination of ticarcillin levels in serum by high-pressure liquid chromatography, *Antimicrob.Agents Chemother.*, **1985**, *28*, 597–600.

SAMPLE
Matrix: bulk

Sample preparation: Prepare a 300 μg/mL solution in 100 mM pH 7.0 phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 15.6 g $NaH_2PO_4.2H_2O$ in 900 mL water, adjust pH to 7.0 with NaOH, make up to 1 L with water.)
Flow rate: 2
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 6.0 (R), 7.5 (S)

OTHER SUBSTANCES
Simultaneous: isomers, diastereomers, impurities, degradation products, ticarcillin

REFERENCE
Bird,A.E.; Charsley,C.H.; Jennings,K.R.; Marshall,A.C. High-performance liquid chromatographic assay of temocillin and epimerisation of its diastereoisomers, *Analyst*, **1984**, *109*, 1209–1212.

SAMPLE
Matrix: solutions

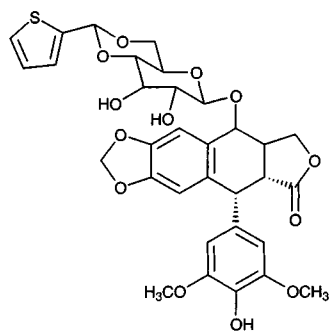
HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:100 mM pH 6.5 potassium phosphate 10:90
Flow rate: 2
Injection volume: 50
Detector: UV 254

KEY WORDS
radiolabelled compounds

REFERENCE
Morecombe,D.J. High-efficiency preparative-scale reversed-phase high-performance liquid chromatographic purification of 14C-labelled antibiotics, *J.Chromatogr.*, **1987**, *389*, 389–395.

Teniposide

Molecular formula: $C_{32}H_{32}O_{13}S$
Molecular weight: 656.66
CAS Registry No.: 29767-20-2
Merck Index: 9291



SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma (Centrifree micropartition device, molecular mass cut-off 30000, Amicon, USA) using a 33° fixed angle centrifuge (Beckman ModelG56R) at 2000 g and 25° for 30 min. Add 1 mL chloroform to the ultrafiltrate (Caution! Chloroform is a carcinogen!), agitate slowly for 20 min, centrifuge at 1000 g for 5 min. Evaporate the organic phase to dryness under vacuum at 40°, dissolve the dry extract in 50 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm 125 Å μBondapak Phenyl (Waters)
Mobile phase: MeCN:water:glacial acid 35:64:1
Flow rate: 1
Injection volume: 25
Detector: F ex 288 em 328

CHROMATOGRAM
Retention time: 18
Internal standard: teniposide

OTHER SUBSTANCES
Extracted: etoposide
Noninterfering: alizapride, doxorubicin, furosemide, idarubicin, ranitidine, vinblastine, vinorelbine

KEY WORDS
plasma; ultrafiltrate; teniposide is IS

REFERENCE
Robieux,I.; Aita,P.; Sorio,R.; Toffoli,G.; Boiocchi,M. Determination of unbound etoposide concentration in ultrafiltered plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1996**, *686*, 35–41.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 1 mg/mL etoposide in MeOH + 5 mL chloroform, rock gently for 15 min, centrifuge. Remove 4.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, dissolve residue in 50 μL MeOH, vortex, centrifuge for 5-10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 60:40
Flow rate: 1-1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: etoposide (5)
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Strife,R.J.; Jardine,I.; Colvin,M. Analysis of the anticancer drugs VP 16-213 and VM 26 and their metabolites by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 211–220.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 100 μg/mL etoposide in MeOH + 5 mL chloroform, rock gently for 15 min, centrifuge. Remove 4.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, dissolve residue in 50 μL MeOH, vortex, centrifuge for 5-10 min, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 30 μm Co:Pell (Whatman)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 60:40

Flow rate: 1
Injection volume: 25
Detector: F ex 215 em 328

CHROMATOGRAM
Retention time: 8
Internal standard: etoposide (5.5)
Limit of detection: 25 ng/mL
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma

REFERENCE
Strife,R.J.; Jardine,I.; Colvin,M. Analysis of the anticancer drugs etoposide (VP 16-213) and teniposide (VM 26) by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1981**, *224*, 168–174.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma or serum with an equal volume of proteinase K, let stand for 10 min. (Alternatively, heat serum or plasma with an equal volume of 1 mg/mL subtilisin A at 50° for 15 min.) Inject 1.6 mL hydrolyzed blood or filtered serum on to column A with mobile phase A at 1 mL/min, backflush column A with mobile phase A to waste for 2 min at 2 mL/min, backflush the contents of column A on to column B with mobile phase B, after 30 s remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Clean column A by backflushing with MeOH at 2 mL/min for 3 min then forward flush with water at 1 mL/min for 2 min.)

HPLC VARIABLES
Column: A 2 × 4.6 10 μm PRP-1 divinylbenzene-styrene copolymer (Hamilton); B 125 × 4 10 μm LiChrosorb RP-18
Mobile phase: A water; B MeOH:water 55:45
Flow rate: A 1-2; B 1
Injection volume: 1600
Detector: F ex 230 em 328 following post-column extraction. The column effluent mixed with dichloroethane pumped at 0.6 mL/min, the mixture flowed through a 2 mm i.d. glass reactor (Technicon) to a phase separator (Technicon (J.Chromatogr. 1979, 185, 473)) with a PTFE insert and 0.3 mL/min of the organic phase flowed through the detector.

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: etoposide

KEY WORDS
column-switching; post-column extraction; plasma; serum

REFERENCE
Werkhoven-Goewie,C.E.; Brinkman,U.A.T.; Frei,R.W.; de Ruiter,C.; de Vries,J. Automated liquid chromatographic analysis of the anti-tumorigenic drugs etoposide (VP 16-213) and teniposide (VM 26), *J.Chromatogr.*, **1983**, *276*, 349–357.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 5 μg etoposide + 3 mL chloroform, shake for 10 min, centrifuge at 1500 g for 10 min, repeat extraction. Combine the organic layers and evaporate a 5 mL aliquot to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:250 mM ammonium acetate:acetic acid 54:45:1
Flow rate: 1.5
Injection volume: 20
Detector: E, Bioanalytical Systems LC4, TL5 glassy carbon electrode, + 900 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8.3
Internal standard: etoposide (4.1)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Canal,P.; Michel,C.; Bugat,R.; Soula,G.; Carton,M. Quantification of teniposide in human serum by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1986**, *375*, 451–456.

SAMPLE
Matrix: blood
Sample preparation: 450 μL Plasma + 50 μL 380 mM sodium dodecyl sulfate in 59 mM pH 7 sodium phosphate buffer + 5 μL 500 ng/mL etoposide in MeOH, sonicate for 5 min. Inject a 100 μL aliquot onto column A with mobile phase A, elute with mobile phase A for 7.5 min, elute the contents of column A onto column B with mobile phase B for 1 min. After 1 min remove column A from the circuit and re-equilibrate it with mobile phase A for 1.5 min, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 2.1 40 μm Chromsep C18 (Chrompack); B 300 × 4.6 10 μm μBondapak phenyl
Mobile phase: A 10 mM pH 7.0 sodium phosphate; B MeOH:10 mM pH 7.0 sodium phosphate buffer 55:45
Flow rate: A 0.4; B 1
Injection volume: 100
Detector: E, +500 mV vs Ag/AgCl or UV 254

CHROMATOGRAM
Retention time: 6.4
Internal standard: etoposide (4.2)
Limit of detection: 20 ng/mL (electrochemical), 150 ng/mL (UV)

KEY WORDS
plasma; column-switching

REFERENCE
van Opstal,M.A.J.; van der Horst,F.A.L.; Holthuis,J.J.M.; Van Bennekom,W.P.; Bult,A. Automated reversed-phase chromatographic analysis of etoposide and teniposide in plasma by using on-line surfactant-mediated sample clean-up and column-switching, *J.Chromatogr.*, **1989**, *495*, 139–151.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 100 μg/mL etoposide in MeOH, vortex, add 2 mL dichloroethane, shake thoroughly for 1 min, centrifuge at 3000 g for 5 min. Remove 1. 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 150 μL MeOH:water 70:30, sonicate for 6 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm LiChrosorb C18
Column: 100 × 4.6 10 μm Novapak phenyl

Mobile phase: MeOH:10 mM pH 7.0 phosphate buffer 55:45
Flow rate: 0.7
Injection volume: 10
Detector: E, Metrohm Model 641 VA, EA 286/1 glassy carbon electrode + 500 mV, stainless-steel auxiliary electrode, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7
Internal standard: etoposide (4.5)
Limit of detection: 10 ng/mL

KEY WORDS
plasma

REFERENCE
van der Horst,F.A.L.; van Opstal,M.A.J.; Teeuwsen,J.; Post,M.H.; Holthuis,J.J.M.; Brinkman,U.A.T. Comparative study on the determination of the anti-neoplastic drug teniposide in plasma using micellar liquid chromatography and surfactant-mediated plasma clean-up, *J.Chromatogr.*, **1991**, *567*, 161–174.

SAMPLE
Matrix: blood
Sample preparation: Sonicate 50 million leukemic cells in 1 mL phosphate buffered saline. 500 μL Plasma or 1 mL sonicated cells + 2 mL chloroform, mix. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeOH: water 50:50, sonicate for 5 min, inject a 100 μL aliquot. To measure non-protein-bound etoposide filter (Amicon Centrifree) while centrifuging at 20°, inject a 100-200 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeOH:water:acetic acid 45:54:1
Flow rate: 1
Injection volume: 100-200
Detector: F ex 220 em 330

CHROMATOGRAM
Retention time: 9.3
Internal standard: teniposide

OTHER SUBSTANCES
Extracted: etoposide, cis-etoposide

KEY WORDS
plasma; cells; ultrafiltrate; teniposide is IS

REFERENCE
Liliemark,E.; Petterson,B.; Peterson,C.; Liliemark,J. High-performance liquid chromatography with fluorometric detection for monitoring of etoposide and its *cis*-isomer in plasma and leukaemic cells, *J.Chromatogr.B*, **1995**, *669*, 311–317.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 1.6 mL diethyl ether, vortex, centrifuge at 1000 g for 2 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:MeOH:buffer 30:20:50 (Buffer was 20 g/L NaH_2PO_4 containing 0.8 g/L heptanesulfonic acid, pH adjusted to 3.0 with orthophosphoric acid.)
Flow rate: 1

Injection volume: 100
Detector: E, Environmental Sciences Coulochem 5100 A, guard cell +0.90 V (before injector), clean-up cell +0.40 V, detection cell +0.90 V

CHROMATOGRAM
Retention time: 10.6
Internal standard: teniposide

OTHER SUBSTANCES
Extracted: vinorelbine

KEY WORDS
plasma; rabbit; teniposide is IS

REFERENCE
Mouchard-Delmas,C.; Gourdier,B.; Vistelle,R. Determination of vinorelbine in rabbit plasma by high-performance liquid chromatography with coulometric detection, *J.Chromatogr.B*, **1995**, *663*, 390–394.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: 500 μL Plasma, urine, or CSF + 500 μL saturated ammonium sulfate + 4 mL ethyl acetate + 10 μL 100 μg/mL etoposide, vortex for 5 min, centrifuge at 3000 rpm for 15 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm μBondapak phenyl
Column: 250 × 4.6 10 μm μBondapak phenyl
Mobile phase: MeCN:water:acetic acid 30:68:2
Flow rate: 1
Injection volume: 50
Detector: UV 284 or E, Bioanalytical Systems LC-4A, 0.75 V

CHROMATOGRAM
Retention time: 18
Internal standard: etoposide (7)
Limit of detection: 20 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Sinkule,J.A.; Evans,W.E. High-performance liquid chromatographic analysis of the semisynthetic epipodophyllotoxins teniposide and etoposide using electrochemical detection, *J.Pharm.Sci.*, **1984**, *73*, 164–168.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water:650 mM pH 4.0 sodium citrate 40:60:0.4, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3.9 10 μm μBondapak phenyl
Column: 150 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:water:650 mM pH 4.0 sodium citrate 35:57.3:7.7. When run is over wash with MeCN:water:650 mM pH 4.0 sodium citrate 70:27.3:7.7 for 4 min, re-equilibrate with initial mobile phase for 9 min.

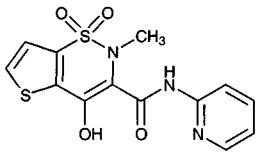
Flow rate: 2
Injection volume: 30
Detector: E, ESA 5100A detector, Model 5020 guard cell between pump and autosampler +0.7 V, Model 5011 dual electrode analytical cell, upstream (screening) electrode +0.2 V, downstream electrode +0.45 V against Ag/AgCl

CHROMATOGRAM
Retention time: 5.8

OTHER SUBSTANCES
Simultaneous: etoposide

REFERENCE
Eisenberg,E.J.; Eickhoff,W.M. Determination of etoposide in blood by liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *621*, 110–114.

Tenoxicam



Molecular formula: $C_{13}H_{11}N_3O_4S_2$
Molecular weight: 337.38
CAS Registry No.: 59804-37-4
Merck Index: 9293
Lednicer No.: 4 173

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL pH 2 phosphate buffer + 10 mL diethyl ether, vortex for 1 min, centrifuge at 1300 g for 5 min. Evaporate the organic layer to dryness under a stream of nitrogen. Reconstitute the residue in 100 μL 10 mM HCl in MeOH, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak ODS
Mobile phase: MeOH:10 mM pH 2 phosphate buffer 45:55
Flow rate: 1.5
Injection volume: 40
Detector: UV 361

CHROMATOGRAM
Retention time: 5.81
Internal standard: tenoxicam

KEY WORDS
plasma; rat; tenoxicam is IS

REFERENCE
Amanlou,M.; Dehpour,A.R. Rapid method for the determination of piroxicam in rat plasma using high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *696*, 317–319.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with 800 μL 5 mM pH 4 phosphate buffer. Centrifuge at 2500 rpm for 5 min. Add the supernatant to Extrelut-1 cartridge (Merck, Darmstadt, Germany). Elute with 10 mL dichloromethane, dry eluate under a stream of nitrogen at 35°, dissolve the residue in 300 μL mobile phase, centrifuge and inject 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:50 mM pH 6 phosphate buffer 50:50
Column temperature: 35
Flow rate: 1.3
Injection volume: 100
Detector: UV 371

CHROMATOGRAM
Retention time: 2.2
Internal standard: tenoxicam

KEY WORDS
plasma; tenoxicam is IS

REFERENCE
Bareggi,S.R.; Gambaro,V.; Valenti,M.; Benvenuti,C. Absorption of oral lornoxicam in healthy volunteers using a granular formulation in comparison with standard tablets, *Arzneimittelforschung*, **1997**, *47*, 755–757.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M HCl + 1 mL water + 100 μL 2 μg/mL piroxicam + 100 μL MeOH + 5 mL dichloromethane, mix for 5 min, centrifuge at 1800 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:100 mM pH 7.4 phosphate buffer 40:60
Flow rate: 1.1
Injection volume: 40
Detector: UV 355

CHROMATOGRAM
Retention time: 3.4
Internal standard: piroxicam (4.5)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma

REFERENCE
Múnera-Jaramillo,M.I.; Botero-Garcés,S. Determination of tenoxicam in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *616*, 349–352.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μg ketorolac in water + 100 μL 5% zinc sulfate in water, vortex for 2 min, add 440 μL buffer, vortex for 1 min, centrifuge at 2000 g for 10 min, inject a 100 μL aliquot of the supernatant. (Buffer was 100 mM NaH_2PO_4 and 10 mM sodium lauryl sulfate, pH adjusted to 2.8 with phosphoric acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:water 35:65 containing 10 mM NaH_2PO_4 and 1 mM sodium lauryl sulfate, pH adjusted to 2.8 with phosphoric acid
Flow rate: 1.5
Injection volume: 100
Detector: UV 355

CHROMATOGRAM
Retention time: 4.6
Internal standard: ketorolac (10.3)
Limit of detection: 40 ng/mL

KEY WORDS
plasma; protect from light; pharmacokinetics

REFERENCE
Mason,J.L.; Hobbs,G.J. Simple method for the analysis of tenoxicam in human plasma using high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 410–415.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 376

CHROMATOGRAM
Retention time: 2.99
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; metha-

done; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 150 μL Plasma + 300 μL 1 mg/mL piroxicam in MeCN, mix, centrifuge at 3500 rpm for 20 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 Nucleosil C18
Mobile phase: MeCN:water:acetic acid 58:38:4
Flow rate: 1
Injection volume: 10
Detector: UV 365

CHROMATOGRAM

Internal standard: piroxicam
Limit of detection: 200 ng/mL

KEY WORDS

rat; pharmacokinetics; plasma

REFERENCE

Troconiz,I.F.; Lopez-Bustamente,L.G.; Fos,D. Tenoxicam pharmacokinetics in rats: A population model, *J.Pharm.Sci.*, **1995**, *84*, 1482–1487.

SAMPLE

Matrix: blood, dialysate

Sample preparation: Blood, plasma. 150 μL Blood or plasma + 300 μL 1 mg/mL piroxicam in MeCN, centrifuge at 3500 rpm for 20 min, inject a 10 μL aliquot of the supernatant. Dialysate. 100 μL Dialysate + 200 μL 1 mg/mL piroxicam in MeCN, centrifuge at 3500 rpm for 20 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 Nucleosil C18
Mobile phase: MeCN:water:acetic acid 55:38:4
Flow rate: 1
Injection volume: 10
Detector: UV 365

CHROMATOGRAM

Retention time: 1.5
Internal standard: piroxicam (2.2)
Limit of detection: 200 ng/mL

KEY WORDS

rat; whole blood; plasma; pharmacokinetics

REFERENCE

Lopez-Bustamante,L.G.; Troconiz,J.I.; Fos,D. Tenoxicam: acute dose-dependent disposition studies in rats, *J.Pharm.Sci.*, **1993**, *82*, 851–853.

SAMPLE
Matrix: blood, synovial fluid
Sample preparation: Plasma. 500 μL Plasma + 500 μL 500 mM pH 4 phosphate buffer, mix, homogenize, centrifuge at 900 g. Add 1 mL of the mixture to an Extrelut-1 SPE cartridge. Elute with two 5 mL portions of dichloromethane. Evaporate the eluate under a stream of nitrogen at 35°. Dissolve the residue in 120 μL mobile phase by vortexing, inject an aliquot. Plasma, synovial fluid. Condition a 100 mg C18 SPE cartridge (Phenomenex) with 1 mL MeOH, 1 mL water, and 500 μL 500 mM pH 2 phosphate buffer. 500 μL Plasma or synovial fluid + 500 μL 500 mM pH 2 phosphate buffer. Mix, homogenize, centrifuge at 2500 rpm. Add 1 mL of the clean supernatant to the SPE cartridge, wash with 1 mL water, dry with 2 mL air, elute with 1.25 mL MeCN:25% ammonia 90:10. Evaporate under reduced pressure at 35°. Reconstitute the residue in 100 μL MeOH:100 mM pH 8 phosphate buffer 50:50, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:100 mM pH 6 sodium dihydrogenphosphate buffer 50:50
Flow rate: 1.5
Injection volume: 50-100
Detector: UV 372

CHROMATOGRAM
Retention time: 2.6-2.8
Internal standard: tenoxicam

OTHER SUBSTANCES
Extracted: lornoxicam

KEY WORDS
plasma; mouse; rat; rabbit; dog; monkey; human; SPE; lornoxicam is IS

REFERENCE
Radhofer-Welte,S.; Dittrich,P. Determination of the novel non-steroidal anti-inflammatory drug lornoxicam and its main metabolite in plasma and synovial fluid, *J.Chromatogr.B*, **1998**, *707*, 151–159.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.733

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

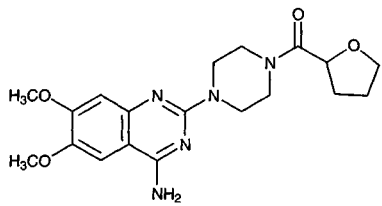
HPLC VARIABLES
Column: 125 × 3 Ecocart LiChrospher 100 RP-18
Mobile phase: Isopropanol:100 mM KH_2PO_4:formic acid 54:100:0.1
Flow rate: 0.6
Detector: UV 254

CHROMATOGRAM
Retention time: 1.5
Limit of quantitation: 200-500 ng/mL

OTHER SUBSTANCES
Simultaneous: acemetacin; diclofenac; flurbiprofen; indomethacin; lonazolac; ketoprofen; naproxen; piroxicam; sulindac

REFERENCE
Baeyens,W.R.G.; Van Der Weken,G.; Van Overbeke,A.; Zhang,Z.D. Preliminary results on the LC-separation of non-steroidal anti-inflammatory agents in conventional and narrow-bore RP set-ups applying columns with different internal diameters, *Biomed.Chromatogr.*, **1995**, *9*, 261–262.

Terazosin



Molecular formula: $C_{19}H_{25}N_5O_4$
Molecular weight: 387.44
CAS Registry No.: 65390-64-7, 70024-40-7 (HCl dihydrate), 63074-08-8 (HCl)
Merck Index: 9297
Lednicer No.: 3 194

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 100 ng/mL IS in water, vortex briefly, add 1 mL 0.9% NaCl and 100 μL 2 M NaOH, vortex briefly. Add 5 mL pentane:dichloromethane 50:50, mix at 40 rpm for 20 min, centrifuge at 500 g for 10 min, freeze the lower aqueous layer in a acetone-dry ice bath, evaporate the organic layer to dryness under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase, vortex vigorously for 15 s, inject a 65 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralpak AD (Chiral Technologies, USA)
Mobile phase: Hexane:2-propanol:diethylamine 70:30:0.1
Column temperature: 30
Flow rate: 1
Injection volume: 65
Detector: F em 238 ex 370

CHROMATOGRAM
Internal standard: (+)-glaucine
Limit of quantitation: 500 pg/mL

OTHER SUBSTANCES
Interfering: prazosin

KEY WORDS
plasma; chiral

REFERENCE
Zavitsanos,A.P.; Alebic-Kolbah,T. Enantioselective determination of terazosin in human plasma by normal phase high-performance liquid chromatography--electrospray mass spectrometry, *J.Chromatogr.A*, **1998**, *794*, 45–56.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in water, vortex briefly, add 1 mL 0.9% NaCl and 100 μL 2 M NaOH, vortex briefly. Add 5 mL pentane:dichloromethane 50:50, mix at 40 rpm for 20 min, centrifuge at 500 g for 10 min, freeze the lower aqueous layer in a acetone-dry ice bath, evaporate the organic layer to dryness under a stream of nitrogen. Reconstitute the residue in 70 μL hexane:2-propanol 90:10, vortex vigorously for 15 s, inject a 20 μL aliquot.

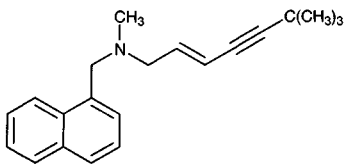
HPLC VARIABLES
Column: 100 × 2.1 10 μm Chiralpak AD (Chiral Technologies, USA)
Mobile phase: Hexane:2-propanol containing 0.05% diethylamine 65:35
Flow rate: 0.15
Injection volume: 20
Detector: MS, HP 1100 electrospray, positive ion mode, drying gas nitrogen 310°, 10 L/min, nebulizer pressure 60 p.s.i.g., quadropole temperature 100°, capillary voltage 4500 V, SIM, m/z 388.2, post-column solvent addition of isopropanol:5 mM ammonium acetate 75:25

CHROMATOGRAM
Retention time: 4.8, 5.8 (enantiomers)
Internal standard: prazosin (4.8)
Limit of quantitation: 62.5 pg/mL

KEY WORDS
plasma; chiral

REFERENCE
Zavitsanos,A.P.; Alebic-Kolbah,T. Enantioselective determination of terazosin in human plasma by normal phase high-performance liquid chromatography--electrospray mass spectrometry, *J.Chromatogr.A*, **1998**, *794*, 45–56.

Terbinafine



Molecular formula: $C_{21}H_{25}N$
Molecular weight: 291.44
CAS Registry No.: 91161-71-6
Merck Index: 9299
Lednicer No.: 4 55

SAMPLE
Matrix: blood, tissue
Sample preparation: Adjust pH of 100 μL plasma or 500 μg tissue to 9.0, add IS, extract with hexane. Extract the organic layer with sulfuric acid:isopropanol, inject an aliquot of this solution.

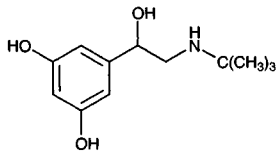
HPLC VARIABLES
Column: 150 × 2 Ultrasphere C18
Mobile phase: MeCN:pH 4.0 phosphate buffer 40:60
Detector: UV 224

CHROMATOGRAM
Limit of detection: 1.1 ng/g (fetal tissue), 2.23 ng/mL (plasma)

KEY WORDS
rabbit; plasma; placenta; fetus

REFERENCE
Gries,W.J.; Wan,W.; Matos,F.J.; de Meireles,J.C.; Pimplaskar,H.K.; Sileno,A.P.; Romeo,V.D.; Xia,W.J.; Behl,C.R. A specific and sensitive method for quantitating buprenorphine hydrochloride in a nasal solution (Abstract 2517), *Pharm.Res.*, **1997**, *14*, S381.

Terbutaline



Molecular formula: $C_{12}H_{19}NO_3$
Molecular weight: 225.29
CAS Registry No.: 23031-25-6, 23031-32-5 (sulfate)
Merck Index: 9302

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with MeCN and water. 1 mL Plasma + 1 mL 20 mM pH 9.0 Na_2HPO_4, mix, add to the SPE cartridge, wash with 5 column volumes of water, dry the SPE cartridge for 5 min, wash with 3 mL dichloromethane:n-butanol 97:3, elute with two 1 mL portions of 0.09% HCl in MeCN, evaporate the eluate to dryness under a stream of nitrogen at 37°, dissolve the residue in 300 μL mobile phase, inject a 200 μL aliquot. (To hydrolyze 3-O-metaproterenol sulfate mix 1 mL plasma and 200 μL 2 μg/mL terbutaline sulfate, add 1 mL 6% trichloroacetic acid, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and add it to 200 μL 2 M HCl, heat at 65° for 90 min, cool, adjust pH to 10 with 400 μL 2 M carbonate buffer, proceed as above.)

HPLC VARIABLES
Column: 250 × 4.9 5 μm Spherisorb C8
Mobile phase: MeCN:buffer:water 4:1.5:94.5
Flow rate: 1.8
Injection volume: 200
Detector: F ex 200 em 300 (cut-off filter)

CHROMATOGRAM
Retention time: 14.8
Internal standard: terbutaline

OTHER SUBSTANCES
Extracted: metaproterenol

KEY WORDS
SPE; plasma; terbutaline is IS

REFERENCE
Selinger,K.; Hill,H.M.; Matheou,D.; Dehelean,L. Determination of free and total metaproterenol in human plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1989**, *493*, 230–238.

SAMPLE
Matrix: blood
Sample preparation: Inject 1 mL plasma onto column A with mobile phase A, wash with mobile phase A for 1.7 min, backflush contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 1.5 37 μm Corasil C18; B C18 guard column + 250 × 4.6 10 μm Spherisorb ODS
Mobile phase: A water; B MeOH:67 mM pH 5 phosphate buffer:40 g/L sodium dodecyl sulfate: diethylamine 55:45:0.5:0.02 (before use condition column with 55:45:5:0.05)
Flow rate: 1
Injection volume: 1000
Detector: E, EG & G Princeton Applied Research Model 400 EC, carbon fiber working electrode + 1.3 V, silver phosphate reference electrode (construction details given)

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.8 ng/mL

KEY WORDS
plasma; column-switching

REFERENCE
Sagar,K.A.; Kelly,M.T.; Smyth,M.R. Analysis of terbutaline in human plasma by high-performance liquid chromatography with electrochemical detection using a micro-electrochemical flow cell, *J.Chromatogr.*, **1992**, *577*, 109–116.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut Si SPE cartridge by washing twice with 1 mL MeOH, twice with 1 mL water, and once with 1 mL 100 mM pH 9.2 K_2HPO_4. Add 1 mL plasma + 100 μL 500 ng/mL atenolol in water, wash twice with 1 mL water, centrifuge at 1000 g for 5 min, elute with 1 mL MeOH. Evaporate MeOH to dryness at 40° under a stream of air and dissolve residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S5 SCX
Mobile phase: MeOH:MeCN:water 40:40:20 containing 0.2% perchloric acid (apparent pH 1.7)
Flow rate: 1.5
Injection volume: 100
Detector: F ex 200 no emission filter

CHROMATOGRAM
Retention time: 7
Internal standard: atenolol (13)
Limit of detection: 2500 ng/mL

OTHER SUBSTANCES
Extracted: albuterol
Noninterfering: aminophylline, beclomethasone, cloprednol, dexamethasone, fenoterol, ipratropium bromide, methylprednisolone, orciprenaline, prednisolone, reproterol, rimiterol, salmeterol, sodium cromoglycate, theophylline

KEY WORDS
plasma; SPE

REFERENCE
McCarthy,P.T.; Atwal,S.; Sykes,A.P.; Ayres,J.G. Measurement of terbutaline and salbutamol in plasma by high performance liquid chromatography with fluorescence detection, *Biomed.Chromatogr.*, **1993**, *7*, 25–28.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 225

CHROMATOGRAM

Retention time: 3.36

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.683

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Tablets, capsules. Mix tablets or capsules with 10 mL water, sonicate 30 min, centrifuge, inject an aliquot. Liquid formulations. Dilute liquid formulations with water, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18 endcapped
Mobile phase: MeOH:water 40:60 containing 2 mM KOH + 10 mM hexanoic acid
Flow rate: 0.4
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Limit of detection: 1030 ng/mL

OTHER SUBSTANCES
Also analyzed: albuterol, fenoterol

KEY WORDS
tablets; capsules; liquid formulations

REFERENCE
Ackermans,M.T.; Beckers,J.L.; Everaerts,F.M.; Seelen,I.G. Comparison of isotachophoresis, capillary zone electrophoresis and high-performance liquid chromatography for the determination of salbutamol, terbutaline sulphate and fenoterol hydrobromide in pharmaceutical dosage forms, *J.Chromatogr.*, **1992**, *590*, 341–353.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with three 3 mL portions of EtOH, two 3 mL portions of water, and with 3 mL 10 mM pH 7.5 phosphate buffer. Add the incubation mixture to the SPE cartridge, wash with two 3 mL portions of water, elute with two 1 mL portions of EtOH:50 mM pH 8.5 ammonium chloride buffer 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 500 μL of the initial mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 5 Nucleosil 10SA
Mobile phase: Gradient. A was 250 mM pH 4.6 ammonium acetate buffer. B was MeCN:500 mM pH 4.6 ammonium acetate buffer 50:50. From A:B 90:10 to 10:90 over 20 min.
Flow rate: 1
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 9.5

OTHER SUBSTANCES
Extracted: bambuterol

KEY WORDS
rat; SPE

REFERENCE
Lindberg,C.; Roos,C.; Tunek,A.; Svensson,L.Å. Metabolism of bambuterol in rat liver microsomes: identification of hydroxylated and demethylated products by liquid chromatography mass spectrometry, *Drug Metab.Dispos.*, **1989**, *17*, 311–322.

SAMPLE
Matrix: perfusate, tissue
Sample preparation: Perfusate. 400 μL Lung perfusate + 400 μL 5% perchloric acid, mix, centrifuge, inject a 200 μL aliquot of the supernatant. Tissue. Homogenize lung in 2 volumes of water (Polytron Homogenizer), mix 400 μL homogenate with 400 μL 5% perchloric acid, mix, centrifuge, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 5 Nucleosil 10SA
Mobile phase: Gradient. A was 19.3 g ammonium acetate and 14.4 mL acetic acid in 1 L water. B was 19.3 g ammonium acetate and 14.4 mL acetic acid in 1 L MeCN:water 50:50. A:B from 90:10 to 10:90 over 20 min, stay at 10:90 for 3 min, return to initial conditions over 3 min, re-equilibrate for 7 min.
Flow rate: 1
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: metabolites, bambuterol

KEY WORDS
lung; guinea pig

REFERENCE
Ryrfeldt,Å.; Nilsson,E.; Tunek,A.; Svensson,L.-Å. Bambuterol: uptake and metabolism in guinea pig isolated lungs, *Pharm.Res.*, **1988**, *5*, 151–155.

SAMPLE
Matrix: solutions
Sample preparation: Dilute with 5% dextrose, inject a 40 μL aliquot.

HPLC VARIABLES
Column: Waters microparticulate C18
Mobile phase: MeOH:350 mM acetic acid and 5 mM sodium heptanesulfonate 35:65
Flow rate: 1.6-2.0
Injection volume: 40
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 5.33

OTHER SUBSTANCES
Simultaneous: theophylline, methyldopate, isoproterenol

REFERENCE
Williams,D.A.; Fung,E.Y.Y.; Newton,D.W. Ion-pair high-performance liquid chromatography of terbutaline and catecholamines with aminophylline in intravenous solutions, *J.Pharm.Sci.*, **1982**, *71*, 956–958.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine,

phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 278

KEY WORDS

chiral; α = 1.28 for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES

Column: 100 × 4.7 7 μm Hypercarb (Shandon)
Mobile phase: MeOH containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH
Column temperature: 17
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: k' 3.6 (first enantiomer)

KEY WORDS

chiral; α = 1.17

REFERENCE

Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonylglyclyl-L-proline as counter ion in methanol, *J.Chromatogr.A*, **1995**, *705*, 275–287.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.62 (A), 3.27 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of an 8 μg/mL solution.

HPLC VARIABLES
Column: 250 × 4 4 μm Superspher 100 RP-18
Mobile phase: Buffer containing 10 mM β-cyclodextrin substituted with 2-hydroxy-3-trimethylammoniumpropyl groups (Roquette Frères, Lestrem, France) (Buffer was 0.8% triethylamine adjusted to pH 5.9 with acetic acid.)
Column temperature: 22.5
Flow rate: 0.8
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 18.02, 19.88 (enantiomers)

KEY WORDS
chiral

REFERENCE
Roussel,C.; Favrou,A. Cationic β-cyclodextrin: a new versatile chiral additive for separation of drug enantiomers by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 67–74.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.39

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Zorbax phenyl
Mobile phase: MeCN:water 15:85 containing 10 mM KH_2PO_4, adjusted to pH 3.1 with phosphoric acid
Flow rate: 1.5
Detector: UV 212

CHROMATOGRAM
Retention time: 2.2

REFERENCE
Liu,P.; Bergstrom,T.K. Quantitative evaluation of aqueous isopropyl alcohol enhancement on skin flux of terbutaline (sulfate). 2. Permeability contributions of equilibrated drug species across human skin in vitro, *J.Pharm.Sci.*, **1996**, *85*, 320–325.

SAMPLE
Matrix: tissue
Sample preparation: 300 mg Skin + propranolol, homogenize with 5 mL MeOH four times, combine the homogenates, filter. Evaporate the filtrate to dryness, reconstitute in mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb cyano
Mobile phase: MeCN:pH 5.6 buffer 30:70
Flow rate: 1.4
Injection volume: 5
Detector: UV 225

CHROMATOGRAM
Retention time: 3.3
Internal standard: propranolol hydrochloride (7.9)
Limit of detection: 100 ng/mL

KEY WORDS
stability-indicating; skin

REFERENCE
Tenjarla,S.N.; Allen,R.; Mitchell,B. High-performance liquid chromatographic assay of terbutaline for preformulation studies, *J.Liq.Chromatogr.*, **1995**, *18*, 1603–1615.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 volumes of MeOH and 2 volumes of water, dry under vacuum. Add 500 μL urine to the SPE cartridge, wash with 5 volumes of water, elute with 200 μL MeOH:50 mM pH 6 potassium phosphate buffer 50:50, add 50 μL 50 mM Na_3PO_4 to the eluate, pass argon through the mixture, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 mm long μBondapak phenyl
Mobile phase: MeCN:50 mM pH 5 phosphate buffer 6:94
Flow rate: 2.8
Injection volume: 25
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 4.1
Internal standard: terbutaline

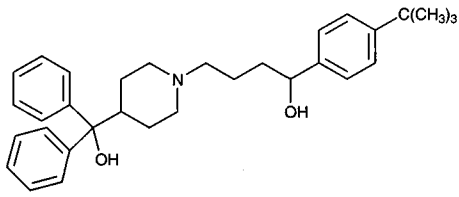
OTHER SUBSTANCES
Extracted: metaproterenol

KEY WORDS
SPE; protect from light; terbutaline is IS

REFERENCE
MacGregor,T.R.; Nastasi,L.; Farina,P.R.; Keirns,J.J. Isolation and characterization of metaproterenol-3-O-sulfate: a conjugate of metaproterenol in human urine, *Drug Metab.Dispos.*, **1983**, *11*, 568–573.

Terfenadine

Molecular formula: $C_{32}H_{41}NO_2$
Molecular weight: 471.68
CAS Registry No.: 50679-08-8
Merck Index: 9307
Lednicer No.: 4 48, 104



SAMPLE
Matrix: blood

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb cyano
Mobile phase: MeCN:pH 5.6 buffer 30:70
Flow rate: 1.4
Injection volume: 5
Detector: UV 225

CHROMATOGRAM
Retention time: 3.3
Internal standard: propranolol hydrochloride (7.9)
Limit of detection: 100 ng/mL

KEY WORDS
stability-indicating; skin

REFERENCE
Tenjarla,S.N.; Allen,R.; Mitchell,B. High-performance liquid chromatographic assay of terbutaline for preformulation studies, *J.Liq.Chromatogr.*, **1995**, *18*, 1603–1615.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 volumes of MeOH and 2 volumes of water, dry under vacuum. Add 500 μL urine to the SPE cartridge, wash with 5 volumes of water, elute with 200 μL MeOH:50 mM pH 6 potassium phosphate buffer 50:50, add 50 μL 50 mM Na_3PO_4 to the eluate, pass argon through the mixture, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 mm long μBondapak phenyl
Mobile phase: MeCN:50 mM pH 5 phosphate buffer 6:94
Flow rate: 2.8
Injection volume: 25
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 4.1
Internal standard: terbutaline

OTHER SUBSTANCES
Extracted: metaproterenol

KEY WORDS
SPE; protect from light; terbutaline is IS

REFERENCE
MacGregor,T.R.; Nastasi,L.; Farina,P.R.; Keirns,J.J. Isolation and characterization of metaproterenol-3-O-sulfate: a conjugate of metaproterenol in human urine, *Drug Metab.Dispos.*, **1983**, *11*, 568–573.

Terfenadine

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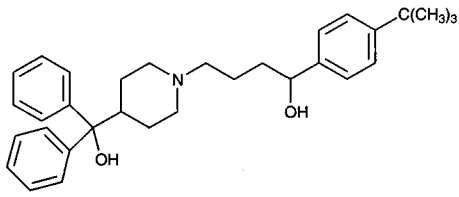


SAMPLE
Matrix: blood

Sample preparation: 500 μL Plasma + 50 μL 250 ng/mL IS + 5 mL MTBE:dichloromethane:n-butyl chloride 3:2:1, vortex for 1 min, centrifuge at 2500 rpm for 1 min. Froze in an acetone/dry ice bath, transfer organic layer to another tube and extract plasma layer with 5 mL MTBE:dichloromethane:n-butyl chloride 3:2:1 for a second time. Evaporate combined organic layers to dryness under a stream of nitrogen at 40°. Reconstitute in 100 μL MeCN:20 mM pH 3.5 ammonium acetate 50:50, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.0 5 μm TSK gel ODS-80TS (Tosho, Japan)
Mobile phase: MeCN:1% formic acid:10 mM pH 4.0 ammonium acetate 85:13:2
Flow rate: 0.2
Injection volume: 10
Detector: MS, Fisons, VG Quattro I triple quadrupole, electrospray, source 200°, cone voltage 35 V, collision energy 30 eV, parent/daughter ions 472.2/436 for terfenadine, 470.2/203.2 for IS

CHROMATOGRAM
Retention time: 2.5
Internal standard: α-[4-(1,1-dimethylethyl)phenyl]-4-(hydroxydiphenylmethyl)-1-piperidinebutanone (2.8)
Limit of quantitation: 200 pg/mL

KEY WORDS
plasma

REFERENCE
Lau,Y.Y.; Anderson,P.H.; Talaat,R. High sensitivity high performance liquid chromatography electrospray tandem mass spectrometry determination of terfenadine in human plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2669–2679.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 20 ng/mL terfenadine-d_{10} to 1 mL heparinized plasma, vortex briefly, add 50 μL 1 M ammonia solution and 4 mL hexane, shake for 5 min on a horizontal shaker, centrifuge at 2500 rpm for 5 min, freeze the aqueous layer in a dry ice-acetone bath, evaporate the organic phase to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 5 μm BDS Hypersil C18
Column: 50 × 3 3 μm BDS Hypersil C18
Mobile phase: MeCN:MeOH:10 mM ammonium acetate 54.4:32.6:13
Flow rate: 0.8
Injection volume: 30
Detector: MS, Perkin Elmer Sciex API-III, APCI interface, nebulizer 480°, nitrogen flow 1.2 L/min, interface heater 55°, multiplier 4 kV, m/z 472.7, 437

CHROMATOGRAM
Retention time: 1.10-1.15
Internal standard: terfenadine-d_{10} (1.1)
Limit of quantitation: 100 pg/mL

KEY WORDS
plasma

REFERENCE
Xu,A.; Linderholm,K.; Peng,L.; Hulse,J. Development and validation of an LC-MS-MS method for the determination of terfenadine in human plasma, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1675–1680.

SAMPLE
Matrix: blood, tissue

Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 7 phosphate buffer, add 100 μL MeCN and 8 mL diethyl ether, extract. Evaporate the organic layer, dissolve the residue in 400 μL mobile phase. Inject 200 μL aliquot. Tissue. Homogenize the brain with 2 fold the weight of 1 M glycine-5 M NaOH pH 10 buffer. Extract 1500 μL brain homogenate with 22 mL n-hexane. Evaporate the organic layer and dissolve the residue in 400-500 μL mobile phase. Inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Intersil PH
Mobile phase: MeCN:0.018% TFA 32:68 (plasma), MeCN:0.018% TFA 37.5:62.5 (tissue)
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 10 ng/mL (plasma), 25 ng/mL (brain)

KEY WORDS
brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE
Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 19.118

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations

Sample preparation: 500 μL Microsomal incubation + 3 mL dichloromethane, add 100 μL buffer containing 1 M sodium carbonate, 10 mM EDTA, and 2 M NaCl, vortex for 5 min, centrifuge at 2000 g for 10 min. Evaporate the organic phase to dryness under a stream of nitrogen. Reconstitute the residue with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco cyano
Mobile phase: MeCN:MeOH:12 mM pH 4.3 ammonium acetate buffer 30:30:40
Flow rate: 1.3
Detector: F ex 230 em 280

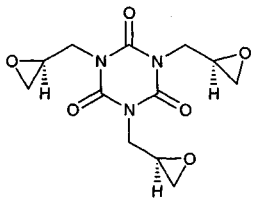
CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
Caco-2-TC7 cells; intestine; liver; pharmacokinetics

REFERENCE
Raeissi,S.D.; Guo,Z.; Dobson,G.L.; Artursson,P.; Hidalgo,I.J. Comparison of CYP3A activities in a subclone of Caco-2 cells (TC7) and human intestine, *Pharm.Res.*, **1997**, *14*, 1019–1025.

Teroxirone



Molecular formula: $C_{12}H_{15}N_2O_6$
Molecular weight: 297.27
CAS Registry No.: 59653-73-5
Lednicer No.: 4 122

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Whole blood, plasma, or urine + 500 μL 10 mM pH 7.4 phosphate buffer + 5 mL chloroform, shake mechanically for 15 min, centrifuge at 3000 g. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 10 mM pH 7.4 phosphate buffer, add 500 μL 5% diethyldithiocarbamate in water (freshly prepared), let stand at room temperature for 1 h, add 5 mL chloroform, shake, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50-100 μL toluene, inject an aliquot.

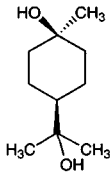
HPLC VARIABLES
Column: 10 μm PAC-CN (Whatman)
Mobile phase: Gradient. Heptane:EtOH 60:40 for 10 min, to 10:90 over 6 min.
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 7.7
Limit of detection: 15 ng/mL

KEY WORDS
derivatization; whole blood; plasma; normal phase; human; rabbit; pharmacokinetics

REFERENCE
Ames,M.M.; Kovach,J.S.; Rubin,J. Pharmacological characterization of teroxirone, a triepoxide antitumor agent, in rats, rabbits, and humans, *Cancer Res.*, **1984**, *44*, 4151–4156.

Terpin



Molecular formula: $C_{10}H_{20}O_2$
Molecular weight: 172.27
CAS Registry No.: 80-53-5
Merck Index: 9314

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 312.8

CHROMATOGRAM

Retention time: 13.868

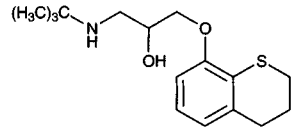
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tertatolol



Molecular formula: $C_{16}H_{25}NO_2S$
Molecular weight: 295.45
CAS Registry No.: 34784-64-0
Merck Index: 9318

SAMPLE

Matrix: blood, urine
Sample preparation: Evaporate 25 μL 1 μg/mL (-)-alprenolol hydrochloride in EtOH into the bottom of a tube, add 1 mL plasma or urine, add 100 μL 1 M NaOH, vortex for 1 min, add to an Extrelut SPE cartridge, elute with two 4 mL portions of diethyl ether. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL dichloromethane, add 10 μL 0.1% S-(+)-naphthylethylisocyanate in dichloromethane, shake for 1 min, let stand

at room temperature for 12 h, add 10 μL tert-butylamine, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 20 μL MeCN, vortex for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XLODS
Mobile phase: MeCN:water 40:60
Flow rate: 2
Injection volume: 20
Detector: F ex 220 em 320

CHROMATOGRAM
Retention time: 15 (-), 17 (+)
Internal standard: (-)-alprenolol (13)
Limit of quantitation: 6 ng/mL

KEY WORDS
SPE; derivatization; chiral; plasma; pharmacokinetics

REFERENCE
Lave,T.; Efthymiopoulos,C.; Koffel,J.C.; Jung,L. Determination of tertatolol enantiomers in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *572*, 203–210.

SAMPLE
Matrix: saliva
Sample preparation: Condition a 100 mg 1 mL Bond-Elut C2 SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL pH 9.0 borate buffer. Centrifuge a cotton roll soaked with saliva at 1000 g for 5 min, remove the liquid supernatant. Add 1 mL supernatant to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL mobile phase, mix for 15 s, inject a 40 μL aliquot. (Acidified MeOH was 50 mL MeOH + 300 μL 96% acetic acid.)

HPLC VARIABLES
Guard column: RCSS silica guard-pack (Waters)
Column: 250 × 4.6 Chiralcel OD-H
Mobile phase: n-Hexane:EtOH:diethylamine 50:50:1
Flow rate: 1
Injection volume: 40
Detector: F ex 225 em 290 cut-off filter

CHROMATOGRAM
Internal standard: (R,S)-tertatolol

OTHER SUBSTANCES
Extracted: atenolol, pindolol

KEY WORDS
SPE; chiral; tertatolol is IS

REFERENCE
Höld,K.M.; de Boer,D.; Zuidema,J.; Maes,R.A.A. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva, *J.Chromatogr.B*, **1995**, *663*, 103–110.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 4 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)

Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 3.3

OTHER SUBSTANCES
Also analyzed: alprenolol, betaxolol, bopindolol, propranolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

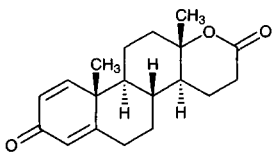
HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 40:60:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: k' 0.32, 1.69 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

Testolactone



Molecular formula: $C_{19}H_{24}O_3$
Molecular weight: 300.40
CAS Registry No.: 968-93-4
Merck Index: 9321
Lednicer No.: 1 160

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 25 μL 10 μg/mL testosterone in MeOH + 4 mL dichloromethane, shake for 15 min, centrifuge at 2000 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 150-300 (plasma) or 300-500 (urine) μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 3 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: MeCN:0.1% acetic acid 40:60
Flow rate: 1.2

Injection volume: 100
Detector: UV 242

CHROMATOGRAM
Retention time: 9.2
Internal standard: testosterone (13.6)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Pascucci,V.L.; Yeager,R.L.; Sherins,R.J.; Clark,R.V.; Gallelli,J.F.; Chatterji,D.C. Quantitation of testolactone and 4,5-dihydrotestolactone in plasma and urine using high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *277*, 79–85.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out finely-powdered tablets equivalent to about 50 mg testolactone, add 30 mL MeOH, shake vigorously for 30 min, make up to 50 mL with water, mix well, filter. Remove a 20 mL aliquot of the filtrate and mix it with 20 mL 1 mg/mL propylparaben in MeOH, make up to 100 mL with MeOH, mix, inject a 10 μL aliquot. Suspensions. Measure out an amount of suspension equivalent to about 100 mg of testolactone, add 60 mL MeOH, make up to 100 mL with water, mix well, filter (if necessary). Remove a 20 mL aliquot and mix it with 20 mL 1 mg/mL propylparaben in MeOH, make up to 100 mL with MeOH, mix, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm RP-8 (Brownlee)
Mobile phase: MeOH:water 55:45
Flow rate: 1.75
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: k' 1.89
Internal standard: propylparaben (k' 4.78)
Limit of detection: 0.1% (of testolactone)

OTHER SUBSTANCES
Simultaneous: impurities, degradation products, benzyl alcohol, 11-deoxycortisol, progesterone, testosterone

KEY WORDS
tablets; suspensions

REFERENCE
Carignan,G.; Lodge,B.A.; Skakum,W. Analysis of testolactone and its formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *206*, 174–176.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

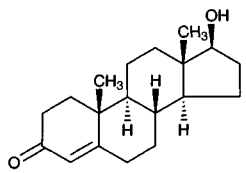
CHROMATOGRAM
Retention time: 2.9
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate
Interfering: aspirin, caffeine, formebolone, benzyl alcohol, cortisone

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

Testosterone



Molecular formula: $C_{19}H_{28}O_2$
Molecular weight: 288.43
CAS Registry No.: 58-22-0, 53608-96-1 (17-chloral hemiacetal), 58-20-8 (17β-cypionate), 315-37-7 (enanthate), 668-56-4 (nicotinate), 5704-03-0 (phenylacetate), 57-85-2 (propionate), 5874-98-6 (ketolaurate)
Merck Index: 9322
Lednicer No.: 1 172

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with MeOH and water. Add 500 μL plasma to the SPE cartridge, wash with 2 mL water, wash with 2 mL MeOH:water 20:80, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 1 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 25:25:50
Flow rate: 0.1
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 6
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: androstenedione, 20α-hydroxy-4-pregnen-3-one, 17α-hydroxyprogesterone, norethindrone, progesterone

KEY WORDS
microbore; rat; plasma; SPE

REFERENCE
Taylor,R.B.; Kendle,K.E.; Reid,R.G.; Hung,C.T. Chromatography of progesterone and its major metabolites in rat plasma using microbore high-performance liquid chromatography columns with conventional injection and detection systems, *J.Chromatogr.*, **1987**, *385*, 383–392.

SAMPLE
Matrix: blood
Sample preparation: Extract 1 mL serum twice with 5 volumes ether by vortexing for 2 min, evaporate extracts to dryness under a stream of nitrogen at 35°, reconstitute in 100 μL MeOH.

HPLC VARIABLES
Column: 240 × 4.5 Bio-Rad ODS-5S
Mobile phase: Gradient. MeOH:MeCN:water at 20:60:20 for 3 min then to 5:85:10 over 26 min
Flow rate: 1
Injection volume: 50
Detector: UV 230

OTHER SUBSTANCES
Simultaneous: estradiol, androstenedione, progesterone

KEY WORDS
serum

REFERENCE
Yu,F.H.; Yun,Y.W.; Yuen,B.H.; Moon,Y.S. Effects of hydroxyflutamide on rats treated with a superovulatory dose of pregnant mare serum gonadotropin, *Can.J.Physiol.Pharmacol.*, **1991**, *69*, 185–190.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL 95% EtOH and 2 mL MeCN:water 15:85. 200 μL Plasma or whole blood + 50 μL MeOH + 3 mL MeCN:water 15:85, vortex for 30 s, add to the SPE cartridge, wash with 9 mL MeCN:water 30:70, dry, elute with 200 μL 95% EtOH, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-8DB
Mobile phase: MeOH:buffer 72.5:27.5 (Buffer was 25 mM K_2HPO_4 adjusted to pH 3 with 670 mM phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 3.60 (testosterone propionate)
Internal standard: testosterone propionate

OTHER SUBSTANCES
Extracted: clotrimazole, doxepin, itraconazole
Noninterfering: acetaminophen, N-acetylprocainamide, amitriptyline, aspirin, barbituric acid, brompheniramine, caffeine, carbamazepine, chloramphenicol, chlorpheniramine, clonazepam, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, ethosuximide, felbamate, gentamicin, ibuprofen, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phen-

obarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, procainamide, protriptyline, quinidine, theophylline, tobramycin, trimethadione, valproic acid, vancomycin

KEY WORDS
testosterone propionate is IS; plasma; SPE; whole blood

REFERENCE
Rifai,N.; Sakamoto,M.; Law,T.; Platt,O.; Mikati,M.; Armsby,C.C.; Brugnara,C. HPLC measurement, blood distribution, and pharmacokinetics of oral clotrimazole, potentially useful antisickling agent, *Clin.Chem.*, **1995**, *41*, 387–391.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 4 mL dichloromethane, shake for 15 min, centrifuge at 2000 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 150-300 (plasma) or 300-500 (urine) μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 3 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: MeCN:0.1% acetic acid 40:60
Flow rate: 1.2
Injection volume: 100
Detector: UV 242

CHROMATOGRAM
Retention time: 13.6
Internal standard: testosterone

OTHER SUBSTANCES
Extracted: testolactone

KEY WORDS
plasma; testosterone is IS

REFERENCE
Pascucci,V.L.; Yeager,R.L.; Sherins,R.J.; Clark,R.V.; Gallelli,J.F.; Chatterji,D.C. Quantitation of testolactone and 4,5-dihydrotestolactone in plasma and urine using high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *277*, 79–85.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with two 500 μL portions of MeOH and two 500 μL portions of water. 1 mL Plasma or urine + 1 mL water, add to the SPE cartridge, let stand for 2 min, wash with two 1 mL portions of water, wash with two 1 mL portions of MeOH:water 10:90, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 50 μL dry benzene (Caution! Benzene is a carcinogen!), add 5 mg potassium carbonate, add 50 μL 200 mM salicylic acid chloride in dry benzene, add 50 μL 250 mM 18-crown-6 in dry benzene, shake, heat at 70° for 1 h, cool, centrifuge, evaporate to dryness under a stream of nitrogen, reconstitute with 100 μL mobile phase, inject a 20 μL aliquot on to column A and elute to waste with mobile phase A, after 8 min elute the contents of column A on to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B. (Prepare salicylic acid chloride by stirring 27.5 g freshly distilled thionyl chloride in 30 mL dry benzene at 0° (Caution! Benzene is a carcinogen!), protect the reaction with a calcium chloride drying tube and a nitrogen atmosphere, add 25 g sodium salicylate, stir at 0° for 1 h, remove solvent by vacuum distillation, take up the residue in 50 mL dry petroleum ether, stir for 15 min, centrifuge. Remove the petroleum ether layer and evaporate it to give salicylic acid chloride.)

HPLC VARIABLES
Column: A 5 × 4 35-40 μm RP8 Perisorb (Merck); B 100 × 4.6 Spheri 5 RP8

Mobile phase: A MeOH:water 30:70; B MeOH:water 70:30 containing 2 g/L lithium perchlorate trihydrate and 2 mL/L glacial acetic acid
Flow rate: A 0.8; B 1
Injection volume: 20
Detector: E, LKB (Bromma) 2143, glassy carbon electrode +1.0 V, palladium reference electrode

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: androsterone

KEY WORDS
derivatization; plasma; SPE; column-switching

REFERENCE
Wintersteiger,R.; Sepulveda,M.J. Electrochemical detection of anabolics in human plasma and urine, *Anal.Chim.Acta*, **1993**, *273*, 383–390.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Injection + 30 mL MeOH:water 90:10, shake for 15 min, centrifuge, remove the MeOH layer, repeat the extraction three times. Combine the extracts, make up to 200 mL with MeOH:water 90:10, cool to -8° for 1 h, filter an aliquot immediately. Remove an aliquot equivalent to 5 mg testosterone, add 5 mL 2 mg/mL 1,2,4,5-tetrachlorobenzene in MeOH, make up to 50 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 3.2 RP8 Express Series (Altex)
Mobile phase: MeOH:THF:water 57:11:32
Flow rate: 1
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: k' 0.60 (testosterone), k' 1.80 (testosterone acetate), k' 2.68 (testosterone propionate), k' 5.00 (testosterone benzoate), k' 6.20 (testosterone phenylpropionate), k' 12.81 (testosterone enanthate), k' 13.67 (testosterone cypionate)
Internal standard: 1,2,4,5-tetrachlorobenzene (k' 4.20)

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, sesamin (from sesame oil), sesamolin (from sesame oil)

KEY WORDS
oils; injections

REFERENCE
Carignan,G.; Lodge,B.A.; Skakum,W. High-performance liquid chromatographic analysis of testosterone esters in oily solution, *J.Pharm.Sci.*, **1980**, *69*, 1214–1217.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 6.3 (testosterone), 10.8 (testosterone acetate), 25.6 (testosterone propionate)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone
Interfering: nandrolone, norgestrel

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 9.2 (testosterone), 16.2 (testosterone acetate), 24.2 (testosterone cypionate), 23.9 (testosterone enanthate), 20.0 (testosterone isobutyrate), 17.3 (testosterone propionate), 29.3 (testosterone undecanoate)

OTHER SUBSTANCES
Simultaneous: boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, danazol (UV 280), fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, stanozolol
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE
Lurie,I.S.rling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 1 mL dichloromethane, extract, centrifuge, remove organic layer and evaporate it under vacuum, dissolve residue in 30 μL MeCN:water 50:50, centrifuge for 3 min, inject supernatant. After each run wash column with MeCN for 1 min, re-equilibrate for 1 min.

HPLC VARIABLES
Column: 50 × 4.6 3 μm Spherisorb ODS-2
Mobile phase: MeCN:water 50:50
Column temperature: 60
Flow rate: 2
Injection volume: 30
Detector: UV 200

CHROMATOGRAM
Retention time: 0.9
Limit of detection: <0.1 nmol/mL

OTHER SUBSTANCES
Simultaneous: estradiol
Interfering: estrone, androstenedione

KEY WORDS
human; placenta

REFERENCE
Taniguchi,H.; Feldmann,H.R.; Kaufmann,M.; Pyerin,W. Fast liquid chromatographic assay of androgen aromatase activity, *Anal.Biochem.*, **1989**, *181*, 167–171.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 11β-hydroxytestosterone to microsomal incubation, extract with 5 volumes dichloromethane, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5 C18
Mobile phase: MeOH:water 50:50
Flow rate: 0.8
Detector: UV 254

CHROMATOGRAM
Internal standard: 11β-hydroxytestosterone

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Anderson,C.D.; Wang,J.; Kumar,G.N.; McMillan,J.M.; Walle,U.K.; Walle,T. Dexamethasone induction of taxol metabolism in the rat, *Drug Metab.Dispos.*, **1995**, *23*, 1286–1290.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 4 mL Microsomal incubation + 100 μL 10 μg/mL 11β-hydroxytestosterone + 6 mL dichloromethane, rotate for 30 min, centrifuge at 2000 g for 5 min. Remove the organic

layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL MeOH:water 25:75, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Hypersil C18
Column: 150 × 3.9 4 μm Novapack C18
Mobile phase: Gradient. A was MeOH:water 25:75. B was MeCN:MeOH:water 1.5:63.5:35. A:B 75:25 for 10 min, to 49.5:50.5 over 8.5 min, to 30:70 over 6.5 min, to 0:100 over 10 min, maintain at 0:100 for 15 min, return to initial conditions over 10 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 29.5
Internal standard: 11β-hydroxytestosterone (20.8)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Ekins,S.; Murray,G.I.; Burke,M.D.; Williams,J.A.; Marchant,N.C.; Hawksworth,G.M. Quantitative differences in phase I and II metabolism between rat precision-cut liver slices and isolated hepatocytes, *Drug Metab.Dispos.*, **1995**, *23*, 1274–1279.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 2.5 mL cold ethyl acetate (4°), mix, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm C18 (Supelco)
Mobile phase: MeCN:MeOH:water 1.3:37.8:60.9
Flow rate: 1.3
Detector: UV 240

OTHER SUBSTANCES
Extracted: metabolites, phenacetin

KEY WORDS
rat; liver

REFERENCE
Lin,J.H.; Chiba,M.; Chen,I.-W.; Vastag,K.J.; Nishime,J.A.; Dorsey,B.D.; Michelson,S.R.; McDaniel,S.L. Time- and dose-dependent pharmacokinetics of L-754,394, an HIV protease inhibitor, in rats, dogs and monkeys, *J.Pharmacol.Exp.Ther.*, **1995**, *274*, 264–269.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 200 μL Microsomal incubation + 200 μL ice-cold MeOH containing 3 nmoles corticosterone, centrifuge at 1500 g for 5 min, inject a 200 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 6 × 4 10 μm μBondapak C18 Guard-Pak
Column: 250 × 4.6 5 μm Ultrasphere IP
Mobile phase: MeOH:THF:water 35:10:55 adjusted to pH 4.0 with glacial acetic acid

Flow rate: 1
Injection volume: 200
Detector: UV 245

CHROMATOGRAM
Retention time: 26.1
Internal standard: corticosterone (15.1)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Sanwald,P.; Blankson,E.A.; Duléry,B.D.; Schoun,J.; Huebert,N.D.; Dow,J. Isocratic high-performance liquid chromatographic method for the separation of testosterone metabolites, *J.Chromatogr.B*, **1995**, *672*, 207–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S5-ODS2 (A), 125 × 4.5 μm LiChrospher ODS-3 (B), 250 × 4.6 10 μm Partisil 10 ODS-3 (C)
Mobile phase: MeCN:water 65:35 (A), MeCN:MeOH:water 40:30:30 (B and C)
Flow rate: 1(A), 1.5 (B), 2 (C)
Injection volume: 20 (A), 100 (B and C)
Detector: UV 270

CHROMATOGRAM
Internal standard: testosterone propionate

OTHER SUBSTANCES
Simultaneous: danazol

REFERENCE
Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

SAMPLE
Matrix: solutions
Sample preparation: Prepare solutions in MeCN:water 50:50, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 4 μm Sentry guard column (Waters)
Column: 150 × 3.9 4 μm mean pore diameter 60 Å Nova-Pak Phenyl
Mobile phase: THF:water 21.5:78.5
Flow rate: 1
Injection volume: 30
Detector: UV 250

CHROMATOGRAM
Retention time: 22.5

OTHER SUBSTANCES
Simultaneous: spironolactone

REFERENCE

Kaukonen,A.M.; Vuorela,P.; Vuorela,H.; Mannermaa,J.-P. High-performance liquid chromatography methods for the separation and quantitation of spironolactone and its degradation products in aqueous formulations and of its metabolites in rat serum, *J.Chromatogr.A*, **1998**, *797*, 271–281.

SAMPLE

Matrix: solutions

Sample preparation: Prepare solutions in MeCN, dilute to an appropriate concentration with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 120 × 4.6 5 μm octadecyl Bakerbond

Mobile phase: MeCN:water 30:70 containing 16 mM β-cyclodextrin

Column temperature: 5

Flow rate: 1

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 0.5

OTHER SUBSTANCES

Simultaneous: hydrocortisone, prednisone, cortisone, 17α-methyltestosterone, 17α-hydroxyprogesterone

REFERENCE

Zarzycki,P.K.; Wierzbowska,M.; Lamparczyk,H. The influence of temperature on the high performance liquid chromatographic separation of steroids using mobile phases modified with β-cyclodextrin, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1305–1311.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μPorasil

Mobile phase: Butyl chloride:water-saturated butyl chloride:THF:glacial acetic acid 55:55:3:2

Flow rate: 2-3

Detector: UV 254

CHROMATOGRAM

Retention time: 8

Internal standard: testosterone acetate

OTHER SUBSTANCES

Simultaneous: iodochlorhydroxyquin

KEY WORDS

normal phase; testosterone acetate is IS

REFERENCE

Kubiak,E.J.; Munson,J.W. Analysis of iodochlorhydroxyquin in cream formulations and bulk drugs by high-performance liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 872–875.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 80:1.5:0.5:18
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 0.52 (testosterone), k' 8.07 (testosterone cypionate), k' 7.12 (testosterone enanthate), k' 1.95 (testosterone propionate)

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: Radial-PAK μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 2
Injection volume: 100
Detector: UV 254 or 214

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: estrone, estriol, progesterone
Interfering: estradiol

REFERENCE
Erkoc,F.U.; Özsar,S.; Güven,B.; Kalkandelen,G.; Ugrar,E. High-performance liquid chromatographic analysis of steroid hormones, *J.Chromatogr.Sci.*, **1989**, *27*, 86–90.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher CH-18
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 75:25 containing 2.5 mM cethexonium bromide (Rinse with 100 mL MeOH:EtOH:water 50:25:25 at the end of the day.)
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: k' 1.3

OTHER SUBSTANCES
Extracted: glucuronides

REFERENCE
Liu,H.-F.; Leroy,P.; Nicolas,A.; Magdalou,J.; Siest,G. Evaluation of a versatile reversed-phase high-performance liquid chromatographic system using cethexonium bromide as ion-pairing reagent for the analysis of glucuronic acid conjugates, *J.Chromatogr.*, **1989**, *493*, 137–147.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 Bondex C18 (Phenomenex)
Mobile phase: MeOH:water 85:15
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5 (testosterone), 6 (testosterone acetate), 8 (testosterone propionate), 13 (testosterone benzoate), 19 (testosterone enanthate), 21 (testosterone cypionate)

OTHER SUBSTANCES
Simultaneous: boldenone acetate, nandrolone propionate, boldenone benzoate, nandrolone phenylpropionate

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and mass spectral analysis of the anabolic 17-hydroxy steroid esters, *J.Chromatogr.Sci.*, **1990**, *28*, 263–268.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: methyltestosterone, nandrolone, methandrostenolone, boldenone, danazol, fluoxymesterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: MeOH:THF:water 26:18:56
Column temperature: 48
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: cortisone, estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, 17α-hydroxyprogesterone, dexamethasone acetate, estradiol, estrone, progesterone

REFERENCE
Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, *4*, 34–38.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 22.9 (testosterone), 30.7 (testosterone propionate)

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, doxapram, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, tranylcypromine, tripelennamine
Interfering: (with testosterone) diflunisal, estrone

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine,

chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 25 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 ODS-1
Mobile phase: MeOH:water 55:45
Column temperature: 40
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM

Retention time: k' 4.146

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone acetate (UV 240), prednisolone (UV 240), prednisone (UV 240), pregnenolone (UV 210), progesterone (UV 240)

REFERENCE
Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES
Column: 250 × 4 7 μm LichroCART RP-8 (Merck)
Mobile phase: MeCN:MeOH:water 32:37:31
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 16 (testosterone propionate)

OTHER SUBSTANCES
Simultaneous: fluoxymesterone, medrogestone, mestranol, norethindrone, progesterone

REFERENCE
Gau,Y.S.; Sun,S.W.; Chem,R.R.-L. Optimization of high-performance liquid chromatographic separation for progestogenic, estrogenic, and androgenic steroids using factorial design, *J.Liq.Chromatogr.*, **1995**, *18*, 2373–2382.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil phenyl
Mobile phase: Gradient. Carbon dioxide:MeOH from 98:2 to 78:22 over 40 min
Column temperature: 50
Flow rate: 2
Detector: UV

CHROMATOGRAM
Retention time: 9.2 (testosterone), 5.6 (testosterone enanthate)

OTHER SUBSTANCES
Simultaneous: estradiol, hydrocortisone, norethisterone, hydroxyprogesterone, estriol, other steroids

KEY WORDS
SFC; 200 bar

REFERENCE
Hanson,M. Aspects of retention behaviour of steroids in packed column supercritical fluid chromatography, *Chromatographia*, **1995**, *40*, 58–68.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: Gradient. A was hexane. B was ethyl acetate. C was 0.1% formic acid in MeCN. D was 0.1% formic acid in water. A:B:C:D 100:0:0:0 for 5 min, to 0:100:0:0 over 15 min, maintain

at 0:100:0:0 for 5 min, to 0:0:100:0 over 5 min, maintain at 0:0:100:0 for 5 min; to 0:0:0:100 over 25 min, maintain at 0:0:0:100 for 5 min.
Flow rate: 0.9
Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM
Retention time: 17.71

OTHER SUBSTANCES
Simultaneous: acetylcholine, cholesterol, choline, cortisone, dextrose, estradiol, glycine, phenylalanine, sodium

REFERENCE
Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.31

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: tissue
Sample preparation: Extract 70-125 mg tissue four times with 5 mL portions of ether:chloroform 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 80 mm long 10 μm octadecylsilane radial compression (Radial-Pak) (Waters)
Mobile phase: Gradient. A was MeOH:water 50:50. B was MeOH. A:B form 100:0 to 70:30 over 20 min, to 0:100 over 20 min
Flow rate: 2

Detector: UV 254

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Extracted: androstenedione, deoxycortisol, hydrocortisone, 17-hydroxyprogesterone
Simultaneous: estriol, estradiol, pregnenolone, progesterone, testosterone enanthate, testosterone propionate

KEY WORDS
tumor

REFERENCE
Kessler,M.J. Analysis of steroids from normal and tumor tissue by HPLC, *Clin.Chim.Acta*, **1982**, *125*, 21–30.

SAMPLE
Matrix: tissue
Sample preparation: 1 g Tissue + 10 mL Chloroform:MeOH 2:1, homogenize for 1 min (Polytron setting 5), filter, rinse tube with an additional 10 mL chloroform:MeOH, filter, combine filtrates, add 4 mL water, vortex for 1 min, centrifuge at 600 g for 10 min. Remove organic layer and dry it under air at 40°. Reconstitute with 200 μL MeOH, add to an activated Sep-Pak C18 cartridge, wash tube onto cartridge with 200 μL MeOH, elute with 5 mL each 0, 25, 50, 75, 100% MeOH, collect 5 mL fractions, inject a 100 μL aliquot of each fraction. (Elutes in 75% MeOH fraction.)

HPLC VARIABLES
Guard column: in line guard column
Column: 80 mm long 10 μm μBondapak C18 radially compressed
Mobile phase: MeCN:water 45:55
Flow rate: 3
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 5.05
Limit of quantitation: 1000 ng/g

OTHER SUBSTANCES
Simultaneous: methyltestosterone

KEY WORDS
fish; muscle; tilapia aurea; SPE

REFERENCE
Goudie,C.A. Extraction of a synthetic androgen from fish muscle and quantitation by high performance liquid chromatography, *Steroids*, **1984**, *44*, 241–252.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH: water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, add 1 mL MeOH to column, collect all eluates from column,

evaporate to dryness under nitrogen at 40°, take up in 200 μL MeOH:water 50:50, add 25 μL 10 μg/mL p-chlorophenol, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: Gradient. MeCN:water from 40:60 to 65:35 in 30 min
Flow rate: 1.2
Injection volume: 50
Detector: UV 242

CHROMATOGRAM
Retention time: 11
Internal standard: p-chlorophenol (7)
Limit of detection: 1 ng/g

OTHER SUBSTANCES
Simultaneous: trenbolone, progesterone

KEY WORDS
muscle; liver; chicken; ox; SPE

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: urine
Sample preparation: Add 10 mL urine to a Supelclean LC-18 SPE tube at a flow rate of 2 mL/min, wash with 4 mL 25 mM sodium borate buffer, wash with 4 mL 40% MeOH, wash with 4 mL 20% acetone, elute with two 500 μL aliquots of 73% MeOH, evaporate under nitrogen at 40°, reconstitute with 1 mL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb silica
Mobile phase: Cyclohexane:ethyl acetate 40:60
Injection volume: 200
Detector: F ex 247 em 547, after post-column reaction with 30 mM $Tb(NO_3)_3$ in ethyl acetate using a 50 cm tightly coiled capillary tube to ensure mixing

CHROMATOGRAM
Retention time: 10 (testosterone acetate), 17 (testosterone)
Limit of detection: 130 pg/mL (testosterone acetate), 85 pg/mL (testosterone)

OTHER SUBSTANCES
Extracted: progesterone, bolasterone
Simultaneous: 17-methyltestosterone

KEY WORDS
SPE; normal phase; post-column reaction

REFERENCE
Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by senstized terbium fluorescence, *Anal.Chem.*, **1993**, *65*, 2346–2351.

SAMPLE
Matrix: urine
Sample preparation: Inject 200 μL directly.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Rainin C18

Mobile phase: MeCN:100 mM sodium dodecyl sulfate + 10 mM $Tb(NO_3)_3$ 20:80 (Sodium dodecyl sulfate was 99.99%.)
Column temperature: 40
Injection volume: 200
Detector: F ex 247 em 547

CHROMATOGRAM
Retention time: 8 (testosterone), 27 (testosterone acetate)
Limit of detection: 50 ng/mL (testosterone), 10 ng/mL (testosterone acetate)

OTHER SUBSTANCES
Extracted: progesterone, 17-methyltestosterone, bolasterone

KEY WORDS
micellar chromatography

REFERENCE
Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by senstized terbium fluorescence, *Anal.Chem.*, **1993**, *65*, 2346–2351.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 10 μL 100 μg/mL methyltestosterone + 1 mL 200 mM pH 7.0 sodium phosphate buffer + 50 μL β-glucuronidase (E. coli K12, Boehringer Mannheim), heat at 55° for 1 h, cool to room temperature, add 1 g sodium bicarbonate:potassium carbonate 1:2, add 1 g sodium sulfate, add 5 mL n-pentane, shake for 20 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot.

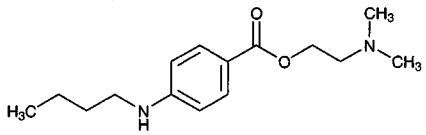
HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:1 mM phosphoric acid from 25:75 to 30:70 over 10 min, to 35:65 over 6.5 min, maintain at 35:65 for 11.5 min.
Column temperature: 40
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 20
Internal standard: methyltestosterone (25)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: epitestosterone

REFERENCE
Navajas,R.; Imaz,C.; Carreras,D.; García,M.; Pérez,M.; Rodríguez,C.; Rodríguez,A.F.; Cortés,R. Determination of epitestosterone and testosterone in urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *673*, 159–164.

Tetracaine



Molecular formula: $C_{15}H_{24}N_2O_2$
Molecular weight: 264.37
CAS Registry No.: 94-24-6, 136-47-0 (HCl)
Merck Index: 9330
Lednicer No.: 1 110

SAMPLE
Matrix: blood
Sample preparation: Collect 10 mL blood in a bottle containing 200 µL 10% sodium metabisulfite and 200 µL 2 M NaOH, centrifuge to separate plasma. 2 mL Plasma + salicylic acid + propiophenone + 8 mL diethyl ether:dichloromethane 10:8, shake for 10 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness at 50°, reconstitute the residue in 200 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Column: µBondapak C18
Mobile phase: MeCN:MeOH:water 20:20:60 containing 600 µL/L sulfuric acid, 5 g/L sodium sulfate, and 200 mg/L sodium heptanesulfonate, pH 2.6
Flow rate: 2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 5
Internal standard: salicylic acid (6), propiophenone (10)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Mazumdar,B.; Tomlinson,A.A.; Faulder,G.C. Preliminary study to assay plasma amethocaine concentrations after topical application of a new local anaesthetic cream containing amethocaine, *Br.J.Anaesth.*, **1991**, *67*, 432–436.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 µL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 µg sodium acetate, inject a 40 µL aliquot. (The sodium acetate was measured out by adding 50 µL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES
Column: 250 × 4 10 µm µBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 35:65, adjusted to pH 2.1
Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM
Retention time: 8

Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: pramocaine

KEY WORDS
plasma; rabbit

REFERENCE
Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL propentofylline + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:MeOH:0.5 mM phosphoric acid 23:20:57
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Internal standard: propentofylline

KEY WORDS
plasma; rat

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 313

CHROMATOGRAM
Retention time: 6.16
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Adjust pH of urine to 5 before freezing. Adjust pH of 5 mL urine to 9.5 with borax buffer, extract twice with 7 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL MeOH, inject a 20-100 μL aliquot. Blood. Adjust pH of 4 mL plasma or whole blood to 9.5 with borax buffer, extract twice with 7 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 0.2-2 mL MeOH, inject a 125 μL aliquot.

HPLC VARIABLES

Column: 10 μm Radial Pak C18

Mobile phase: MeCN:16.5 mM triethylamine 85:15, pH adjusted to 3 with concentrated phosphoric acid

Flow rate: 2

Injection volume: 20-125

Detector: UV 288

CHROMATOGRAM

Internal standard: tetracaine

Limit of detection: 10 ng/mL (urine), 1 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: procaine

KEY WORDS
horse; plasma; whole blood; pharmacokinetics; tetracaine is IS

REFERENCE
Stevenson,A.J.; Weber,M.P.; Todi,F.; Mendonca,M.; Fenwick,J.D.; Young,L.; Kwong,E.; Chen,F.; Beaumier,P.; Timmings,S.; Woodard,W.; Kacew,S. Determination of procaine in equine plasma and urine by high-performance liquid chromatography, *J.Anal.Toxicol.*, **1992**, *16*, 93–96.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 50 μL 10 μg/mL butacaine, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: 14
Internal standard: butacaine (10)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: procaine, p-aminobenzoic acid
Also analyzed: articaine, prilocaine, o-toluidine, lidocaine, bupivacaine, etidocaine, dibucaine, caffeine, amphetamine, ephedrine, epinephrine, morphine, monoacetylmorphine, diamorphine, ethylmorphine, codeine, acetylcodeine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 312.8

CHROMATOGRAM
Retention time: 13.69

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: 5 mL Injection + 20 mL 10 mg/mL salicylic acid in MeOH:water 50:50 + 5 mL 10 mg/mL propiophenone in MeOH:water 50:50, make up to 50 mL with MeOH:water 50:50, homogenize (if necesary), inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6
Flow rate: 2
Injection volume: 5
Detector: UV 305

CHROMATOGRAM
Retention time: 6
Internal standard: salicylic acid (4), propiophenone (8)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; saline

REFERENCE
Menon,G.N.; Norris,B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 569–570.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 1.25 g ground pastilles, add 50 mL mobile phase, stir mechanically until dissolved, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM pH 5 potassium phosphate buffer 25:75, containing 5.9 g/L NaCl and 30 mM tetrabutylammonium hydrogen sulfate
Flow rate: 2
Injection volume: 50
Detector: UV 294

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: degradation products, n-butyl p-aminobenzoic acid, p-chloroaniline, chlorhexidine

KEY WORDS
pastilles

REFERENCE
Bauer,M.; Degude,C.; Mailhe,L. Simultaneous determination of chlorhexidine, tetracaine and their degradation products by ion-pair liquid chromatography, *J.Chromatogr.*, **1984**, *315*, 457–464.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Brownlee silica (Applied Biosystems)
Mobile phase: MeOH:10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 13.3
Limit of detection: 334 ng/mL

OTHER SUBSTANCES
Simultaneous: morphine, hydromorphone

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher100RP-18
Column: 125 × 4 5 μm Spherisorb ODS 2
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM sodium acetate containing 0.28% triethylamine, adjusted to pH 4.5 with acetic acid.)
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: k' 2.85

OTHER SUBSTANCES
Simultaneous: 4-butylaminobenzoic acid

REFERENCE
Yang,H.; Thyrion,F.C. Determination of six pharmaceuticals and their degradation products in reversed-phase high performance liquid chromatography by using amine additives, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1347–1357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 45 × 4.6 5 μm Ultrasphere ODS
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:water containing 2.5 mM hexanesulfonic acid 35:40:25, adjusted to pH 6.0 with 100 mM acetic acid
Flow rate: 2
Detector: UV 310

CHROMATOGRAM
Retention time: 4.5
Internal standard: procaine (2.6)
Limit of detection: 800 pg
Limit of quantitation: 5 ng

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Asavapichayont,P.; Hu,J.; Foldvari,M. Development of an HPLC method for simultaneous analysis of tetracaine and its metabolite in dosage forms and biological fluids, with comparison to capillary electrophoresis method (Abstract 3307), *Pharm.Res.*, **1997**, *14*, S565.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine,

mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 14

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in EtOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4.1 10 μm Versapack C18
Mobile phase: MeCN:250 mM pH 2.7 potassium phosphate buffer 30:70
Flow rate: 2
Injection volume: 20
Detector: UV 235

CHROMATOGRAM

Retention time: 4.9

OTHER SUBSTANCES

Simultaneous: desmethylsertraline, sertraline

REFERENCE

Wiener,H.L.; Kramer,H.K.; Reith,M.E.A. Separation and determination of sertraline and its metabolite, desmethylsertraline, in mouse cerebral cortex by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 467–472.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 222, UV309

CHROMATOGRAM

Retention time: 2.4
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 Lichrospher 100 RP-18
Column: 125 × 4 Lichrospher 100 RP-18
Mobile phase: MeOH:buffer 75:25 (Buffer was 320 mL 20 mM K_2HPO_4 + 680 mL 20 mM KH_2PO_4, pH 7.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 235

CHROMATOGRAM

Retention time: 9.40

OTHER SUBSTANCES

Simultaneous: benzoylecgonine, cocaine

REFERENCE

Fernández,P.; Rodríguez,P.; Bermejo,A.M.; López-Rivadulla,M.; Cruz,A. Simultaneous determination of cocaine and benzoylecgonine in vitreous humor by HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 883–890.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6

Injection volume: 25
Detector: UV 229

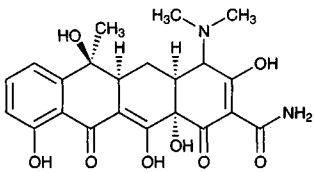
CHROMATOGRAM
Retention time: 10.81 (A), 5.44 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Tetracycline



Molecular formula: $C_{22}H_{24}N_2O_8$
Molecular weight: 444.44
CAS Registry No.: 60-54-8, 6416-04-2 (trihydrate), 64-75-5 (HCl), 1336-20-5 (phosphate)
Merck Index: 9337
Lednicer No.: 1 212

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

Injection volume: 25
Detector: UV 229

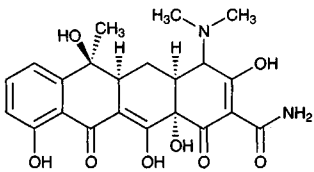
CHROMATOGRAM
Retention time: 10.81 (A), 5.44 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Tetracycline



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CAS Registry No.: 60-54-8, 6416-04-2 (trihydrate), 64-75-5 (HCl), 1336-20-5 (phosphate)
Merck Index: 9337
Lednicer No.: 1 212

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 274.8

CHROMATOGRAM
Retention time: 9.888

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Prepare a metal chelate affinity chromatography (MCAC) column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH:water 20:80 (Pharmacia) to a 150 × 10 glass column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Condition an SBD-RPS extraction membrane (3M Company, St. Paul, MN) with 2 mL MeOH and 2 mL 100 mM HCl. Add 20 mL 100 mM pH 4.0 sodium succinate buffer to 3 g pig kidney, pig muscle, cow liver, or whole chicken egg, vortex for 1 min and shake for 10 min on a horizontal shaker. Add 20 mL MeOH, sonicate for 5 min and centrifuge at 2666 g for 10 min at 4°. Filter the supernatant through a Whatman 541 filter paper. Add the clear supernatant to the MCAC column. Wash sequentially with 2 mL 100 mM sodium succinate buffer, 2 mL water, 2 mL MeOH, 2 mL water, and with 500 μL McIlvaine-EDTA-NaCl buffer. Elute with 3 mL McIlvaine-EDTA-NaCl buffer and adjust the eluate to pH 1.3 with 400 μL 4 M HCl. Add the eluate directly to the extraction membrane to prevent crystallization of EDTA. Wash the membrane with 1 mL 100 mM HCl and elute with four 250 μL portions of MeOH:25% ammonia 97:3, evaporate the eluate to dryness under the nitrogen at 40°. Reconstitute the dry residue with 250 μL 10 mM oxalic acid in water, vortex, sonicate. Inject a 100 μL aliquot. (The sodium succinate buffer was 100 mM succinic acid, pH adjusted to 4.0 with 10 M NaOH. Prepare the McIlvaine buffer by dissolving 12.9 g citric acid monohydrate and 10.9 g Na_2HPO_4 in 1 L water. The McIlvaine-EDTA-NaCl buffer was 100 mM EDTA and 500 mM NaCl in McIlvaine buffer. Protect all solutions from light.)

HPLC VARIABLES
Guard column: 5 × 3.0 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 8 μ PLRP-S (Polymer Laboratories)
Mobile phase: Gradient. A was 10 mM oxalic acid in water adjusted to pH 2.0 with 4 M HCl. B was MeCN. A:B from 85:15 to 60:40 over 16 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water stored at 4°.)

CHROMATOGRAM
Retention time: 13.5
Limit of detection: 1.20 ng/g (pig kidney), 0.49 ng/g (pig muscle), 1.01 ng/g (cow liver), 0.23 ng/g (chicken egg)
Limit of quantitation: 3 ng/g (pig kidney)

OTHER SUBSTANCES
Extracted: chlortetracycline, doxycycline, oxytetracycline
Also analyzed: demeclocycline

KEY WORDS
cow; liver; pig; kidney; muscle; chicken; metal chelate affinity chromatography; MCAC; SPE

REFERENCE
Croubels,S.M.; Vanoosthuyze,K.E.I.; Van Peteghem,C.H. Use of metal chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg, *J.Chromatogr.B*, **1997**, *690*, 173–179.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Condition an Anagel-TSK Chelate-SPW column with 25 μL 50 mg/mL copper sulfate in water and 500 μL. Homogenize 2 g sliced chicken liver with 1.2 mL 1 M pH 4 citrate buffer and 12 mL ethyl acetate for 1 min. Homogenize 2 g sliced tissue with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 1 min. Shake 2 g blended egg with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 15 min. Centrifuge the mixture at 11000 rpm for 10 min, decant the supernatant, reextract the residue with two 12 mL portions of ethyl acetate. Add 10 g anhydrous sodium sulfate to the combined supernatants, swirl, let stand for 5-10 min, filter (Whatman 1PS phase-separating filter paper). Evaporate the filtrate to dryness or to an oily residue on a rotary evaporator under reduced pressure at 40°, reconstitute the residue in 2 mL MeOH by vortexing, filter (0.2 μm syringe filter). Add 1.5 mL of the filtrate to the Anagel column at 0.36 mL/min, wash with 500 μL water, 500 μL MeOH, and 500 μL water. Elute the contents of the Anagel column onto the analytical column with mobile phase A, after 11 min remove the Anagel column from the circuit, elute column B using gradient elution of mobile phase A:B, monitor the effluent from column B. (Prepare 1 M pH 4 or 5 citrate buffer as follows: dissolve 192 g citric acid in approximately 800 mL water, adjust pH value with 1 M NaOH and make up to 1 L with water.)

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S
Column: 150 × 4.6 5 μm Polymer Labs PLRP-S
Mobile phase: Gradient. A:B 100:0 for 11 min, to 0:100 in 10 min, maintain at 0:100 for 10 min. A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid, and 10 mM EDTA).
Flow rate: 1
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM
Retention time: 25.2
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: oxytetracycline, chlortetracycline, demeclocycline

KEY WORDS
chicken; egg; metal chelate affinity chromatography; muscle; liver; salmon; trout; venison; SPE

REFERENCE
Cooper,A.D.; Stubbings,G.W.F.; Kelly,M.; Tarbin,J.A.; Farrington,W.H.H.; Shearer,G. Improved method for the on-line metal chelate affinity chromatography-high-performance liquid chromatographic determination of tetracycline antibiotics in animal products, *J.Chromatogr.A*, **1998**, *812*, 321–326.

SAMPLE

Matrix: milk

Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with 20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES

Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)

Mobile phase: MeOH:5 mM oxalic acid 58:42

Flow rate: 0.5

Injection volume: 100

Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM

Retention time: 5.12

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline, doxycycline, minocycline, oxytetracycline

KEY WORDS

metal chelate affinity chromatography; cow; SPE

REFERENCE

Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE

Matrix: milk, tissue

Sample preparation: Wash column A with 20 mL 5 mg/mL aqueous copper sulfate and column B with 100 mL acetone, 100 mL MeOH, and 100 mL water. Sonicate 10 mL milk or 10 g sliced tissue with 40 mL pH 4.0 succinate buffer for 3 min. (Buffer was 5 g succinic anhydride in 1 L water adjusted to pH 4.0 with 1 M NaOH.) Homogenize for 2 min (Ultra-Turrax), centrifuge at 12000 and 24000 rpm for 5 min, filter the supernatant through a Whatman 541 filter paper. Repeat extraction with 40 mL and 20 mL portions of succinate buffer, load the combined filtrates onto column A. Wash column A with 10 mL water, 30 mL MeOH, and two 10 mL portions of water. Elute tetracycline fraction with 40 mL pH 4.0 succinate buffer containing 100 mM disodium EDTA. After elution wash column A with 10 mL pH 4.0 succinate buffer containing 100 mM disodium EDTA. Load 50 mL combined eluate from column A onto column B, wash with two 100 mL portions of water and elute with 100 mL redistilled MeOH, discard the first 10 mL eluate. Evaporate eluate to a small volume on rotary evaporator at 40° and transfer to pear-shaped flask with three 2 mL portions of redistilled MeOH. Add 100 μL 5% β-mercaptopropionic acid in MeOH, evaporate MeOH under reduced pressure at 40°, reconstitute the residue in 500 μL mobile phase, vortex for 15 s and sonicate for 30 s, inject a 10 μL aliquot. (Column A was a 200 × 20 chelating Sepharose column. Prepare column as follows. Thoroughly mix 5 mL chelating Sepharose suspension (Pharmacia AB), place it in a 200 × 20 glass column, let settle to a 15 mm bed height. Remove liquid excess and load the column by passing 20 mL 5 mg/mL copper sulfate through it. Vortex the column after first 10 mL to remove bubbles, then pass 15 mL pH 4.0 succinate buffer through the column. Wash the column with 20 mL water after use, store in MeOH:water 20:80 at 4°. Column B was a 200 × 20 Amberlite XAD-

2 resin column. Prepare as follows. Wash Amberlite resin sequentially with 100 mL MeOH and 100 mL water, place the resin in a 200 × 2 0 glass column to 100 mm bed height.)

HPLC VARIABLES
Guard column: 10 × 2.1 30-40 μm Lichrosorb RP8
Column: 200 × 3 Lichrosorb RP8
Mobile phase: MeCN:10 mM oxalic acid 50:50
Flow rate: 0.4
Injection volume: 10
Detector: UV 350

CHROMATOGRAM
Retention time: 7.5
Limit of quantitation: 10 ng/g

OTHER SUBSTANCES
Extracted: chlortetracycline, oxytetracycline

KEY WORDS
cow; kidney; milk; sheep; pig; muscle; trout; SPE

REFERENCE
Farrington,W.H.; Tarbin,J.; Bygrave,J.; Shearer,G. Analysis of trace residues of tetracyclines in animal tissues and fluids using metal chelate affinity chromatography/HPLC, *Food Addit.Contam.*, **1991**, *8*, 55–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil SAS or 150 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Mobile phase was 340 mL 100 mM citric acid, 5 mL 100 mM trisodium citrate, and 5 mL 100 mM Na_2EDTA made up to 500 mL with MeCN.)
Flow rate: 2
Injection volume: 100
Detector: UV 370

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Simultaneous: furazolidone, oxytetracycline, chlortetracycline

REFERENCE
Murray,J.; McGill,A.S.; Hardy,R. Development of a method for the determination of oxytetracycline in trout, *Food Addit.Contam.*, **1987**, *5*, 77–83.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond-Elut C8 SPE cartridge with 6 mL MeOH, 6 mL water, and 2 mL buffer A. Condition a 6 mL SPE cartridge containing 3 g wet XAD-2 resin with 10 mL MeOH, 10 mL water, and 2 mL buffer B. Homogenize (Ultra-Turrax) 2 g tissue with 20 mL succinate buffer for 1 min, centrifuge at 30 897 g for 15 min, filter (Whatman No. 1 paper) the supernatant, dilute 12 mL filtrate with 6 mL buffer B. For sheep liver add the diluted filtrate to the C8 SPE cartridge, wash with 10 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. For cow kidney add the diluted filtrate to the XAD-2 cartridge, wash with 14 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. Inject 25 μL 50 mg/mL copper sulfate and 500 μL water onto column A then load 1.5 mL of the eluate from the SPE cartridge at 0.36 mL/min onto column A. Wash to waste with 500 μL water, 500 μL MeOH, and 500 μL water then elute the contents of column A onto column B with mobile phase A. After 11 min remove column A from the circuit and elute column B with a linear gradient of A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 10 min, re-equilibrate to 100:0.

Monitor the effluent from column B. (Buffer A was 100 mM KH_2PO_4 containing 3 g/L pentanesulfonic acid. Succinate buffer was 60 g succinic acid in 1 L water adjusted to pH 4.0 with 1 M NaOH. Buffer B was 37.2 g disodium EDTA and 3 g pentanesulfonic acid in 1 L succinate buffer.)

HPLC VARIABLES
Column: A 10 × 6 10 μm Anagel-TSK-Chelate-SPW (Anachem); B 5 × 3 5 μm Polymer Labs. PLRP-S + 150 × 4.6 5 μm Polymer Labs. PLRP-S
Mobile phase: A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid and 10 mM EDTA.)
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM
Retention time: 23
Limit of detection: 10 μg/kg

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline, oxytetracycline

KEY WORDS
SPE; sheep; cow; liver; kidney; column-switching

REFERENCE
Stubbings,G.; Tarbin,J.A.; Shearer,G. On-line metal chelate affinity chromatography clean-up for the high-performance liquid chromatographic determination of tetracycline antibiotics in animal tissues, *J.Chromatogr.B*, **1996**, *679*, 137–145.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg C18 SPE cartridge with 20 mL MeOH and 20 mL water. Mix 5 g tissue with 20 mL buffer, homogenize for 30 s, rinse probe twice with 2 mL buffer into the centrifuge tube. Shake for 10 min at high speed and centrifuge at 2500 g for 10 min. Remove the supernatant. Add 20 mL buffer to the tissue plug, shake for 10 min at high speed and centrifuge at 2500 g for 10 min. Remove the supernatant and repeat all steps as described above with 10 mL buffer. Combine the supernatants from all three extractions, centrifuge at 2500 g for 20 min, filter (GF/B paper). Rinse the centrifuge tube twice with 2 mL portions of buffer and filter. Add the filtrate to the SPE cartridge, rinse the flask twice with buffer and add the rinses to the SPE cartridge, wash with 20 mL water, dry the cartridge by drawing air through it, elute with 6 mL 1.26 g/L oxalic acid dihydrate in MeOH, dilute the eluate to 10 mL with water, filter, inject a 60 μL aliquot. (Prepare the buffer (McIlvaine-EDTA buffer) as follows. Mix 1 L 21.0 g/L citric acid monohydrate with 625 mL 28.4 g/L disodium hydrogen phosphate, adjust pH to 4.0 with 100 mM HCl or 100 mM NaOH, add 60.5 g disodium EDTA dihydrate, mix until the solid dissolves.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C8
Mobile phase: MeCN:MeOH:10 mM oxalic acid 15:20:65
Flow rate: 1.5
Injection volume: 10-60
Detector: UV 350

CHROMATOGRAM
Retention time: 5.9
Limit of detection: 1.5 ng

OTHER SUBSTANCES
Extracted: chlortetracycline, tetracycline

KEY WORDS
SPE; pig; muscle; cow

REFERENCE
MacNeil,J.D.; Martz,V.K.; Korsrud,G.O.; Salisbury,C..C.; Oka,H.; Epstein,R.L.; Barnes,C.J. Chlortetracycline, oxytetracycline, and tetracycline in edible animal tissues, liquid chromatographic method: Collaborative study, *J.AOAC Int.*, **1996**, *79*, 405–417.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Bond Elut C18 SPE cartridge with saturated aqueous disodium EDTA. Blend 5 g tissue with two 20 mL portions and one 10 mL portion of 100 mM pH 4.0 disodium EDTA-McIlvaine buffer at high speed, centrifuge at 850 g for 5 min each time. Combine the supernatants, centrifuge at 850 g for 15 min, filter. Add the filtrate to the SPE cartridge, wash with 20 mL water, air-dry by aspiration for 5 min, elute with 10 mL ethyl acetate followed by 20 mL MeOH:ethyl acetate 5:95, evaporate the eluate to dryness under reduced pressure at 30°, dissolve the residue in 100 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK Gel Super Octyl (Tosoh)
Mobile phase: MeCN:0.05% aqueous trifluoroacetic acid 20:80
Flow rate: 0.5
Injection volume: 50
Detector: MS, Finnigan MAT TSQ 7000 Triple-Stage Quadrupole, electrospray voltage 4.5 kV, gas sheath flow 483 kPa nitrogen, collision gas argon, collision offset -25 V, m/z 445

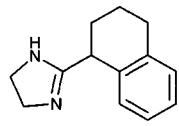
CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Extracted: chlortetracycline, doxycycline, oxytetracycline

KEY WORDS
cow; kidney; liver; muscle; SPE

REFERENCE
Oka,H.; Ikai,Y.; Ito,Y.; Hayakawa,J.; Harada,K.-.; Suzuki,M.; Odani,H.; Maeda,K. Improvement of chemical analysis of antibiotics. XXIII. Identification of residual tetracyclines in bovine tissues by electrospray high-performance liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *693*, 337–344.

Tetrahydrozoline



Molecular formula: $C_{13}H_{16}N_2$
Molecular weight: 200.28
CAS Registry No.: 84-22-0, 522-48-5 (HCl)
Merck Index: 9358
Lednicer No.: 1 242

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 6 5 μm Capcell Pak C8 (Shiseido, Japan)
Mobile phase: MeOH:50 mM KH_2PO_4 containing 5 mM tetra-n-butylammonium phosphate 15:85, adjusted to pH 2.6 with 5% orthophosphoric acid (After one week of use, wash the column with water and MeOH:water 70:30 at 1 mL/min for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10-20
Detector: UV 215

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, dipotassium glycyrrizate, fumaric acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, maleic acid, neostigmine methylsulfate, pyridoxine, vitamin B12
Noninterfering: chondroitin sulfate, lysozyme

KEY WORDS
ophthalmic solutions; ion-pair agents

REFERENCE
Yamato,S.; Nakajima,M.; Shimada,K. Simultaneous determination of chlorpheniramine and maleate by high-performance liquid chromatography using tetra-n-butylammonium phosphate as an ion-pair reagent, *J.Chromatogr.A*, **1996**, *731*, 346–350.

SAMPLE
Matrix: formulations
Sample preparation: 5 mL Ophthalmic solution + 5 mL 40 μg/mL naphazoline in water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:buffer 30:70 (Buffer was 6 g sodium citrate dihydrate and 4 g anhydrous citric acid in 700 mL water, add 7 mL perchloric acid, adjust pH to 2.2 ± 0.2 with perchloric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 5.31
Internal standard: naphazoline (4.37)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
ophthalmic solutions; stability-indicating

REFERENCE
Bauer,J.; Krogh,S. High-performance liquid chromatographic stability-indicating assay for naphazoline and tetrahydrozoline in ophthalmic preparations, *J.Pharm.Sci.*, **1983**, *72*, 1347–1349.

SAMPLE
Matrix: formulations
Sample preparation: Dilute ophthalmic solution with mobile phase to a tetrahydrozoline concentration of 50 μg/mL, filter, mix an aliquot of the filtrate with 100 μg/mL dimethylaminobenzaldehyde in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil C8
Mobile phase: MeCN:MeOH:buffer 5:5:90 (Buffer was 5 mM Na_2HPO_4 containing 5 mM sodium octanesulfonate adjusted to pH 7 with HCl.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 222

CHROMATOGRAM
Retention time: 7

Internal standard: dimethylaminobenzaldehyde (1.3)
Limit of quantitation: 250 ng

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline

KEY WORDS
ophthalmic solutions

REFERENCE
Puglisi,G.; Sciuto,S.; Chillemi,R.; Mangiafico,S. Simultaneous high-performance liquid chromatographic determination of antazoline phosphate and tetrahydrozoline hydrochloride in ophthalmic solution, *J.Chromatogr.*, **1986**, *369*, 165–170.

SAMPLE
Matrix: formulations
Sample preparation: Mix 5 mL nasal solution and 10 mL 500 μg/mL tolazoline hydrochloride in MeOH:water 40:60, make up to 50 mL with MeOH:water 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 RSIL C18 (RSL, Eke, Belgium)
Mobile phase: MeOH:water 40:60 containing 20 mM sodium 1-octanesulfonate and 10 mM N,N-dimethyloctylamine, pH adjusted to 3.0 with orthophosphoric acid
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 3.5
Internal standard: tolazoline (2.5)

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline, coumazoline, lidocaine, naphazoline, oxymetazoline, prednisolone, sulfadimidine, sulfanilamide, sulfathiazole, tenaphtoxaline, tramazoline, xylometazoline

KEY WORDS
nasal solutions; stability-indicating

REFERENCE
De Schutter,J.A.; Van den Bossche,W.; De Moerloose,P. Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography, *J.Chromatogr.*, **1987**, *391*, 303–308.

SAMPLE
Matrix: formulations
Sample preparation: 2 mL Sample + 1 mL 200 μg/mL emetine hydrochloride in water, make up to 10 mL with mobile phase, filter (0.45 μm), inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Technosphere RP C-8 (HPLC Technology)
Mobile phase: MeCN:40 mM tetramethylammonium bromide:1 M acetic acid 80:15:5 (apparent pH 4.5)
Flow rate: 1.5
Injection volume: 50-100
Detector: UV 260

CHROMATOGRAM
Retention time: 1.43

Internal standard: emetine (1.83)
Limit of quantitation: 50 μg/mL

OTHER SUBSTANCES
Simultaneous: benzalkonium (C12, C14, C16)
Interfering: naphazoline

KEY WORDS
nasal; ophthalmic solutions

REFERENCE
Santoni,G.; Medica,A.; Gratteri,P.; Furlanetto,S.; Pinzauti,S. High-performance liquid chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions, *Farmaco*, **1994**, *49*, 751–754.

SAMPLE
Matrix: solutions

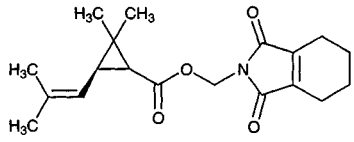
HPLC VARIABLES
Column: 250 × 4.6 Chirex 3017 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 50:35:15 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 10.5, 12.5 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Tetramethrin



Molecular formula: $C_{19}H_{25}NO_4$
Molecular weight: 331.41
CAS Registry No.: 7696-12-0
Merck Index: 9362

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 3.233

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: fruit, vegetables
Sample preparation: Prepare a cleanup column by placing 4 g Florisil, 1 g activated charcoal, and a 20 mm layer of anhydrous sodium sulfate in a 400 × 10 glass column, wash with 40 mL toluene, wash with 40 mL toluene:MeCN 99:1. Homogenize 25 g chopped fruit or vegetable with 70 mL MeOH at high speed for 3 min, filter, homogenize solid with 30 mL MeOH, filter. Combine the filtrates and add them to 60 mL toluene and 300 mL 10% NaCl in water, shake well for 3 min, let layers separate. Dry the organic layer by passing it through 20 g anhydrous sodium sulfate in a 20 mm diameter column, concentrate to about 5 mL under reduced pressure at 80°, add to the cleanup column, elute with 40 mL toluene:MeCN 99:1. Evaporate the eluate just to dryness under reduced pressure at 80°, reconstitute with 1 mL MeOH, inject an aliquot. (Reflux activated charcoal (20-40 mesh) with 1 M HCl for 4 h, wash with water until the washings are neutral, dry at 95-100° (J.Assoc.Off.Anal.Chem. 1983, 66, 1013). Heat 60-100 mesh Florisil at 200° for 24 h, cool, add 4% water, mix thoroughly, store in a sealed jar (J.Assoc.Off.Anal.Chem. 1983, 66, 1003).)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:water from 62:38 to 78:22 over 32 min (Waters curve 6).
Column temperature: 50
Flow rate: 1.5
Detector: UV 206

CHROMATOGRAM
Retention time: 13.08
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Simultaneous: allethrin, biphenthrin, cypermethrin, fenpropathrin, fenvalerate, flucythrinate, methothrin, permethrin, Py-115

KEY WORDS
cucumber; tomato; cabbage; apple; pear; peach; SPE

REFERENCE
Pang,G.-F.; Chao,Y.-Z.; Liu,X.-S.; Fan,C.-L. Multiresidue liquid chromatographic method for simultaneous determination of pyrethroid insecticides in fruits and vegetables, *J.AOAC Int.*, **1995**, *78*, 1474–1480.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm silica (IBM)
Mobile phase: Hexane:isopropanol 98:2
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.90 (cis), k' 4.15 (trans)

OTHER SUBSTANCES
Also analyzed: allethrin, chrysanthemol, dimethrin, ethyl chrysanthemate, cyfluthrin (baythroid), permethrin, phenothrin, resmethrin, RU-11679

KEY WORDS
normal phase

REFERENCE
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm β-cyclodextrin-bonded silica (7.4 mequivalents cyclodextrin per gram of silica) (Advanced Separation Technologies)
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 10.0 (+, cis), k' 10.4 (-, cis), k' 17.8 (+, trans), k' 20.4 (-, trans)

OTHER SUBSTANCES
Also analyzed: allethrin, cyfluthrin (baythroid), chrysanthemol, dimethrin, ethyl chrysanthemate, permethrin, phenothrin, resmethrin, RU-11679

KEY WORDS
chiral

REFERENCE
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

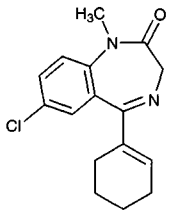
HPLC VARIABLES
Guard column: 5 μm Spherisorb NH2
Column: 250 × 4.6 Pirkle ionic type 1-A column (Technicol)
Mobile phase: Hexane:isopropanol 99.85:0.15
Flow rate: 2.5
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: allethrin, cypermethrin, fenpropathrin, fenvalerate

KEY WORDS
chiral

REFERENCE
Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

Tetrazepam



Molecular formula: $C_{16}H_{17}ClN_2O$
Molecular weight: 288.78
CAS Registry No.: 10379-14-3
Merck Index: 9379

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 227

CHROMATOGRAM
Retention time: 8.08
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazo-

lam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 226.3

CHROMATOGRAM

Retention time: 22.378

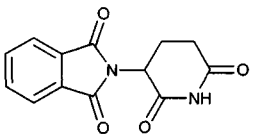
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Thalidomide



Molecular formula: $C_{13}H_{10}N_2O_4$
Molecular weight: 258.23
CAS Registry No.: 50-35-1
Merck Index: 9390
Lednicer No.: 1 257

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: chiral protein (avidin) column
Mobile phase: 2-propanol:1 M pH 4.0 phosphate buffer 2:98
Injection volume: 50
Detector: UV 300

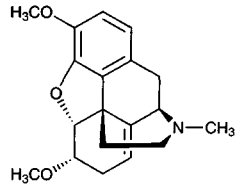
CHROMATOGRAM
Internal standard: labetalol
Limit of detection: 50 ng/ml
Limit of quantitation: 100 ng/ml

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
chiral

REFERENCE
Tata,P.N.V.; Bramer,S.L. Enantiomeric assay of grepafloxacin in plasma (Abstract 4162), *Pharm.Res.*, **1997**, *14*, S684.

Thebaine



Molecular formula: $C_{19}H_{21}NO_3$
Molecular weight: 311.38
CAS Registry No.: 115-37-7
Merck Index: 9411

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

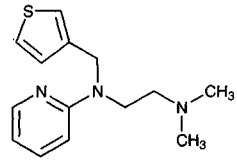
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphet-

amine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Thenyldiamine



Molecular formula: $C_{14}H_{19}N_3S$
Molecular weight: 261.39
CAS Registry No.: 91-79-2
Merck Index: 9416

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

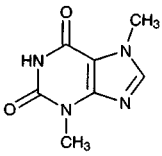
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Theobromine



Molecular formula: $C_7H_8N_4O_2$
Molecular weight: 180.17
CAS Registry No.: 83-67-0
Merck Index: 9418
Lednicer No.: 1 423

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 3.79

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

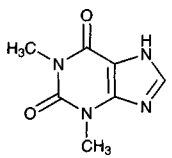
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide,

hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Theophylline



Molecular formula: $C_7H_8N_4O_2$

Molecular weight: 180.17

CAS Registry No.: 58-55-9, 32156-80-2 (diethanolamine), 573-41-1 (ethanolamine), 5600-19-1 (isopropanolamine), 8002-89-9 (sodium acetate), 8000-10-1 (sodium glycinate), 5967-84-0 monohydrate

Merck Index: 9421

Lednicer No.: 1 423; 2 464; 3 230; 4 165, 168, 213

SAMPLE

Matrix: blood

Sample preparation: Mix 50 μL plasma with 50 μL 20 μg/mL IS in water, vortex for 10 s, add 20 μL 20% perchloric acid, vortex for 10 s, centrifuge at 2000 g for 5 min, inject a 50 μL of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb C18
Mobile phase: MeCN:THF:concentrated acetic acid:water 2:2:0.5:95.5
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: UV 273

CHROMATOGRAM

Retention time: 5
Internal standard: 7-(β-hydroxypropyl)theophylline (9.2)
Limit of detection: 100 ng/mL

Limit of quantitation: 2 μg/mL

OTHER SUBSTANCES
Extracted: caffeine
Simultaneous: β-hydroxyethyltheophylline, 8-chlorotheophylline, theobromine

KEY WORDS
plasma

REFERENCE
Schreiber-Deturmeny,E.; Bruguerolle,B. Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma, *J.Chromatogr.B*, **1996**, *677*, 305–312.

SAMPLE
Matrix: blood
Sample preparation: 190 μL Plasma + 200 μL 10% trichloroacetic acid solution, vortex for 30 s, centrifuge at 3000 g for 10 min. Inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:water adjusted to pH 4 9:91
Flow rate: 1.2
Injection volume: 25-50
Detector: UV 273

CHROMATOGRAM
Limit of detection: 300 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Contreras,J.; Pérez,N.; González,R.; Ontivero,E.; López,M. Single dose study of the bioequivalence of two sustained-release theophylline formulations, *Arzneimittelforschung*, **1998**, *48*, 259–262.

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 5:95
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 2.33

OTHER SUBSTANCES
Extracted: acetaminophen

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, saliva
Sample preparation: Add 500 μL plasma, serum, or saliva to 200 mg ammonium sulfate, add 50 μL 15 μg/mL IS in water, add 500 μL 200 mM pH 4.5 sodium acetate buffer, vortex briefly, add 3 mL dichloromethane, shake at 85 cycles/min for 10 min, centrifuge at 2000 g for 10min. Evaporate the organic layer to dryness at 40° under a stream of nitrogen, reconstitute in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18
Column: 100 × 4.6 3 μm Microsorb MV C18
Mobile phase: MeOH:THF:100 mM pH 4.5 sodium acetate:water 6.5:1.4:10:82.1
Flow rate: 0.8
Injection volume: 50
Detector: UV 274

CHROMATOGRAM
Retention time: 5.9
Internal standard: β-hydroxyethyltheophylline (6.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: caffeine, paraxanthine, theobromine
Noninterfering: chlorzoxazone, dapsone

KEY WORDS
plasma; serum

REFERENCE
Frye,R.F.; Stiff,D.D.; Branch,R.A. A sensitive method for the simultaneous determination of caffeine and its dimethylxanthine metabolites in human plasma: Application to CYP1A2 phenotyping, *J.Liq. Chromatogr.Rel.Technol.*, **1998**, *21*, 1161–1171.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 4.877

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weight out powdered tablets, dissolve in 50 mM sodium dodecyl sulfate in an ultrasonic bath. Filter (no.4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve capsules in 50 mM sodium dodecyl sulfate in an ultrasonic bath. Drops. Dilute drops with 50 mM sodium dodecyl sulfate. Inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 5 μmC18 (Scharlau, Spain)
Column: 120 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Propanol:50 mM sodium dodecyl sulfate 3:97, adjusted to pH 7 with 10 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 4

KEY WORDS
tablets; capsules; drops

REFERENCE
Perez-Martinez,I.; Sagrado,S.; Medina-Hernández,M.J. Determination of theophylline in pharmaceuticals by micellar liquid chromatography and spectrophotometric detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1957–1966.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 18:82
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 275

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm 3-aminopropylsilyl silica gel with the amino group derivatized with 1,8-naphthalic anhydride (Bunseki Kagaku 1993, 42, 817)
Mobile phase: MeOH:buffer 50:50 (Prepare buffer by dissolving 6.183 g boric acid and 1.461 g NaCl in 500 mL water, adjust pH to 6.4 with sodium borate solution.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 13.5
Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)

OTHER SUBSTANCES
Simultaneous: caffeine, hypoxanthine, pentoxifylline, propentofylline, theobromine, uric acid, xanthine

REFERENCE
Nakashima,K.; Inoue,K.; Mayahara,K.; Kuroda,N.; Hamachi,Y.; Akiyama,S. Use of 3-(1,8-naphthalimido)propyl-modified silyl silica gel as a stationary phase for the high-performance liquid chromatographic separation of purine derivatives, *J.Chromatogr.A*, **1996**, *722*, 107–113.

SAMPLE
Matrix: urine
Sample preparation: Add 100-120 mg NaCl, 50 μL 100 μg/mL β-hydroxyethyltheophylline, and 100 μL ammonia buffer to 1 mL urine. Extract with 5 mL MeOH:dichloromethane 10:90 for 10 min, centrifuge at 150 g for 5 min, remove the upper aqueous layer and evaporate the organic layer under nitrogen at 40°. Dissolve the residue in 200 μL mobile phase, inject a 20 μL aliquot. (Prepare the pH 9.5 ammonia buffer by adding ammonia to a saturated ammonium chloride solution.)

HPLC VARIABLES
Guard column: 10 × 2 40 μm C18
Column: 100 × 3 5 μm Hypersil 5 ODS (Chrompack)
Mobile phase: THF:water 1:100
Flow rate: 1
Injection volume: 20
Detector: UV 275

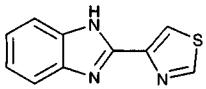
CHROMATOGRAM
Retention time: 3.6
Internal standard: β-hydroxyethyltheophylline (4.5)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Extracted: caffeine, theobromine, paraxanthine

KEY WORDS
horse; human; urine

REFERENCE
Delbeke,F.T.; De Backer,P. Threshold level for theophylline in doping analysis, *J.Chromatogr.B*, **1996**, *687*, 247–252.

Thiabendazole



Molecular formula: $C_{10}H_7N_3S$
Molecular weight: 201.25
CAS Registry No.: 148-79-8
Merck Index: 9426
Lednicer No.: 1 325

SAMPLE
Matrix: abomasal fluid, blood, duodenal fluid, rumen fluid
Sample preparation: 4 mL Plasma, rumen fluid, abomasal fluid, or duodenal fluid + 4 mL pH 7.4 phosphate buffer + 20 mL ether, shake on a rotary mixer for 10 min, remove 16 mL of the

ether layer, add 20 mL ether, shake on a rotary mixer for 10 min, remove 20 mL of the ether layer. Combine the ether layers and evaporate them under a stream of nitrogen at 60° to dryness, reconstitute in 50 μL MeOH, sonicate, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 ODS Hypersil 10
Mobile phase: MeOH:50 mM ammonium carbonate 65:35
Flow rate: 1.5
Injection volume: 5
Detector: UV 292

CHROMATOGRAM
Retention time: 3.8
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: albendazole, oxfendazole, cambendazole, mebendazole, oxibendazole, fenbendazole, parbendazole

KEY WORDS
plasma; sheep

REFERENCE
Bogan,J.A.; Marriner,S. Analysis of benzimidazoles in body fluids by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 422–423.

SAMPLE
Matrix: food
Sample preparation: Blend 10 g food and 20 mL MeOH in a Polytron at high speed for 3 min. Centrifuge at 5000 g for 10 min, inject a 50 μL aliquot. Alternatively, shake 5 or 10 g food, 20 mL MeOH, and 5 ball bearings for 10 min. Remove a 1 mL aliquot, centrifuge at 5000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: Ultracarb 30 ODS
Mobile phase: MeCN:MeOH:water:monoethanolamine 36.4:13.6:50:0.02 (A) or 31.32:8.44:60.24:0.01 (B)
Flow rate: 1.0
Injection volume: 50
Detector: F ex 305 em 345

CHROMATOGRAM
Retention time: 3.5 (A), 5.7 (B)
Limit of quantitation: 6 ppb

KEY WORDS
fruits; vegetables

REFERENCE
Rushway,B.J.; Perkins,L.B.; Larkin,K.L.; Fan,T.S. A modified high performance liquid chromatographic analysis of thiabendazole in fruits and vegetables with ELISA confirmation, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1217–1226.

SAMPLE
Matrix: food
Sample preparation: Place 25 g homogenized potato (washed or peeled) in a separating funnel, add 20 mL saturated aqueous NaCl solution and extract with three 50 mL portions of dichloromethane. Dry organic layers over anhydrous sodium sulfate and evaporate to dryness under reduced pressure. Reconstitute the residue in 1 mL dichloromethane and spot four 400 μL portions of this solution on a 200 × 200 mm × 250 μm thick silica 60 TLC plate (Merck). Develop with chloroform:triethylamine 100:5 then with MeOH:chloroform 5:100 (Caution!

Chloroform is a carcinogen!), remove the area containing thiabendazole and shake with 400 μL MeOH, centrifuge. Dilute solution to 2.5 mL with MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC18
Mobile phase: MeOH:100 mM pH 6.6 phosphate buffer 60:40
Flow rate: 2
Injection volume: 6
Detector: UV 248

CHROMATOGRAM
Retention time: 1.8
Limit of detection: 500 pg/g
Limit of quantitation: 1.7 ng/g

OTHER SUBSTANCES
Extracted: propham, chlorpropham

KEY WORDS
comparison with HPTLC; potato

REFERENCE
Corti,P.; Dreassi,E.; Politi,N.; Aprea,C. Comparison of an HPTLC and an HPLC procedure for the determination of chlorpropham, propham and thiabendazole residues in potatoes, *Food Addit.Contam.*, **1991**, *8*, 607–615.

SAMPLE
Matrix: food
Sample preparation: Raw potatoes. Weigh out 23.0-25.5 g potato peelings or flesh macerated in a domestic food processor (Magimix 4000) for 1 min, homogenize with 60 mL dichloromethane and 70 g anhydrous sodium sulfate (Silverson mixer-emulsifier) for 1 min. Decant the supernatant through glass fiber filter (Whatman GF/A), repeat extraction with three 40 mL portions of fresh dichloromethane, filter homogenate through a suction Buchner funnel, wash with two 15 mL portions of dichloromethane. Dry the combined filtrate and washings with anhydrous sodium sulfate, re-filter and evaporate to near dryness on a rotary evaporator at 35°. Dissolve the concentrate in 100-200 mL MeOH (peelings), or 5-25 mL MeOH (flesh), filter the extract through a 0.45 μm nylon membrane (Micron Separation Inc.) and inject an aliquot. Baked potatoes. Mix 29.89-51.85 g baked peelings or 50 g baked flesh with 80-100 g anhydrous sodium sulfate before maceration for 2 min. Homogenize with 80 mL dichloromethane for 2 min, decant the supernatant through a glass fiber filter (Whatman GF/A), repeat extraction with two 70 mL portions of fresh dichloromethane, filter homogenate through a suction Buchner funnel, wash with two 15 mL portions of dichloromethane. Dry the combined filtrate and washings with anhydrous sodium sulfate, re-filter and evaporate to near dryness on a rotary evaporator at 35°. Dissolve the concentrate in 100-200 mL MeOH (peelings), or 5-25 mL MeOH (flesh), filter extract through a 0.45 μm nylon membrane (Micron Separation Inc.) and inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 7 μm LiChrosorb RP Select B (Merck)
Column: 125 × 4 7 μm LiChrosorb RP Select B (Merck)
Mobile phase: MeCN:ammonia solution (d 0.88):water 55:0.27:45
Flow rate: 1
Injection volume: 20
Detector: UV 303, F ex 296 em 351

CHROMATOGRAM
Retention time: 1.68 (UV), 1.94 (F)

KEY WORDS
potatoes

REFERENCE
Friar,P.M.; Reynolds,S.L. The effects of microwave-baking and oven-baking on thiabendazole residues in potatoes, *Food Addit.Contam.*, **1991**, *8*, 617–626.

SAMPLE
Matrix: food
Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac Silica SPE cartridge with 10 mL MeOH and 10 mL ethyl acetate. Condition a 3 mL 300 mg Bond Elut PRS cartridge with 10 mL water and 10 mL MeOH. Place the Sep Pak Vac Silica cartridge on top of the Bond Elut PRS cartridge. Citrus fruit. Slice and homogenize citrus fruit with a mixer. 5 g Aliquot of sample + 20 g anhydrous sodium sulfate + 1.5 g anhydrous sodium hydrogen phosphate + 30 mL ethyl acetate, blend at high-speed, centrifuge at 3100 rpm for 8 min, remove the supernatant. Re-extract with 20 mL ethyl acetate, combine the supernatants. Add the crude extract to the double SPE cartridge, allow to pass at ca. 1 mL/min, wash with 5 mL ethyl acetate, remove the Sep-Pak Vac Silica cartridge. Wash the second SPE cartridge with 10 mL MeOH and 10 mL 100 mM NaCl, elute with 10 mL mobile phase, inject a 20 μL aliquot. Banana. 10 g Homogenized sample + 40 g anhydrous sodium sulfate + 50 mL ethyl acetate, extract as described above. Re-extract with 30 mL ethyl acetate, combine the supernatants. Add the crude extract to the double SPE cartridge, allow to pass at ca. 1 mL/min, wash with 5 mL ethyl acetate, remove the Sep-Pak Vac Silica cartridge. Wash the second SPE cartridge with 10 mL MeOH and 10 mL 100 mM NaCl, elute with 10 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel ODS-80Ts (TOSOH, Japan)
Mobile phase: MeCN:MeOH:water 40:30:30 containing 10 mM sodium 1-tridecanesulfonate, adjusted to pH 2.5 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 100 ng/g (citrus fruit), 50 ng/g (banana)

OTHER SUBSTANCES
Extracted: enilconazole

KEY WORDS
citrus fruit; banana; SPE

REFERENCE
Ito,Y.; Ikai,Y.; Oka,H.; Hayakawa,J.; Kagami,T. Application of ion-exchange cartridge clean-up in food analysis. I. Simultaneous determination of thiabendazole and imazalil in citrus fruit and banana using high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.A*, **1998**, *810*, 81–87.

SAMPLE
Matrix: fruit
Sample preparation: Condition a 10 mL 500 mg Bond-Elut PRS LRC cartridge with 10 mL MeOH:water 80:20 containing 1% phosphoric acid, 3 mL MeOH, and 5 mL ethyl acetate. Homogenize unwashed, divided citrus fruit with dry ice. Mix 5 g citrus homogenate with 5 mL pH 8 buffer (Fisher Scientific). Adjust the pH to about 8.0 with 200 mM NaOH. Add 25 mL ethyl acetate, shake for 15 min, centrifuge at 2500 rpm for 15 min. Mix a 15-20 mL aliquot of the ethyl acetate extract with 4 g anhydrous sodium sulfate, shake manually for 5 s, add more sodium sulfate if the ethyl acetate is not clear. Add 10 mL of the dry extract to the SPE cartridge, wash with 5 mL ethyl acetate, air dry the SPE cartridge under vacuum, elute with 9.8 mL elution solution, dilute the eluate to 10 mL with elution solution, inject a 50 μL aliquot. (Elution solution was 100 mM KH_2PO_4 in MeCN:water 70:30.)

HPLC VARIABLES
Column: 125 × 4.6 5 μm PartiSphere SCX (Whatman)

Mobile phase: MeOH:buffer 25:75 (Prepare buffer as follows. Dissolve 13.6 g KH_2PO_4 in 1 L water, add 500 mL MeCN, shake, dilute to 2 L with water. Adjust pH to about 3.4 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 305 em 380

CHROMATOGRAM
Retention time: 4.3
Limit of quantitation: 0.05 ppm

KEY WORDS
SPE; citrus

REFERENCE
Arenas,R.V.; Rahman,H.; Johnson,N.A. Determination of thiabendazole residues in whole citrus fruits by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1996**, *79*, 579–582.

SAMPLE
Matrix: fruit, juice, vegetables
Sample preparation: 5 g Fruit, juice, or vegetables or 2 g bulk juice or 0.5 g dried sample + 20 mL solvent + 20 mL dichloromethane, homogenize (Brinkmann polytron) at medium speed for 2 min, shake rapidly by hand for 3 min, centrifuge at 5000 g for 3 min. Remove the dichloromethane layer and dry it over 0.5 g anhydrous sodium sulfate. Remove a 10 mL aliquot and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, centrifuge at 10000 g for 5 min, inject a 10 μL aliquot. (Solvent was 5 mL EtOH + 15 mL 2 M ammonium chloride adjusted to pH 9.5 with 14.5 M ammonium hydroxide.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultracarb 30 ODS (Phenomenex)
Mobile phase: MeCN:MeOH:water:monoethanolamine 26:7:50:0.01
Flow rate: 1
Injection volume: 10
Detector: F ex 305 em 345

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 0.04 ng

KEY WORDS
banana; orange; grapefruit; pear; apple; potato; raspberry; cranberry; grape; lime

REFERENCE
Bushway,R.J.; Li,L.; Paradis,L.R.; Perkins,L.B. Determination of thiabendazole in potatoes, fruits, and their processed products by liquid chromatography, *J.AOAC Int.*, **1995**, *78*, 815–820.

SAMPLE
Matrix: fruit, vegetables
Sample preparation: 50 g Homogenized sample + 100 mL MeOH, shake mechanically for 10 min, filter (shark skin paper), rinse solid with 50 mL MeOH. Add 10 mL 1 M HCl and 100 mL 1% NaCl to the filtrate, add 100 mL dichloromethane, shake for 1 min, repeat extraction. Combine the extracts and evaporate just to dryness under reduced pressure below 30°, reconstitute the residue in 4 mL MeOH, add 6 mL buffer, mix, filter (0.45 μm), inject a 25 μL aliquot (minor part). Rinse aqueous/MeOH layer into beaker with two 10 mL portions of water, adjust pH to 7.5-8 with NaOH or HCl, extract twice with 100 mL portions of dichloromethane. Combine the extracts and evaporate just to dryness under reduced pressure below 30°, reconstitute the residue in 4 mL MeOH, add 6 mL buffer, mix, filter (0.45 μm), inject a 25 μL aliquot (major part). (Buffer was 1 g sodium 1-decanesulfonate in 200 mL water, 7 mL phosphoric acid, and 10 mL triethylamine, make up to 1 L with water.)

HPLC VARIABLES
Guard column: Supelguard LC-18-DB (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-18-DB or Ultrasphere C-18 IP
Mobile phase: MeOH:buffer 35:65 (Buffer was 1 g sodium 1-decanesulfonate in 200 mL water, 7 mL phosphoric acid, and 10 mL triethylamine, make up to 1 L with water.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 25
Detector: UV 305 or F ex 305 em 345

CHROMATOGRAM
Retention time: 20
Limit of quantitation: 10 ppb (F)

OTHER SUBSTANCES
Extracted: allophanate, benomyl, thiophanate methyl

KEY WORDS
apples; bananas; lemons; peaches; pineapples; peas; rice

REFERENCE
Gilvydis,D.M.; Walters,S.M. Ion-pairing liquid chromatographic determination of benzimidazole fungicides in foods, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 753–761.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Milk + 5 mL 1 M sodium carbonate, mix, add 150 mL ethyl acetate, add 1 mL 10 mg/mL BHT in ethyl acetate, blend (tissuemizer) at high speed for 5 min, add 10 g anhydrous sodium sulfate, blend for 1 min, let settle for 2-3 min, filter (No. 41 paper), add another 150 mL ethyl acetate to the sodium sulfate, blend for 2 min, filter. Combine the filtrates and evaporate them to dryness under vacuum. Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid. Combine rinses, shake vigorously for 2 min, extract the hexane layer with two 10 mL portions of 1 mL phosphoric acid. Combine all the aqueous layers and wash them with 5 mL hexane, adjust the pH to 8-9 by slowly adding about 9 mL 10 M KOH (use an ice bath), add 50 mL ethyl acetate, shake vigorously for 2 min, repeat extraction. Filter the organic layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the organic layers, add 200 μL 10 mg/mL BHT in ethyl acetate, evaporate to dryness under vacuum. Take up the residue in two 3 mL portions of dichloromethane and add them to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under nitrogen, reconstitute in 1 mL mobile phase, vortex, filter (0.2 μm), inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 pellicular C18 (Alltech)
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 53:47 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 298

CHROMATOGRAM
Retention time: 17
Limit of detection: 0.5 ppb

OTHER SUBSTANCES
Extracted: oxfendazole

KEY WORDS
cow; SPE

REFERENCE
Tai,S.S.; Cargile,N.; Barnes,C.J. Determination of thiabendazole, 5-hydroxythiabendazole, fenbendazole, and oxfendazole in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 368–373.

SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg 2.8 mL Regular Bond Elut PRS (propylsulfonic acid) SPE cartridge with 10 mL 1% phosphoric acid in MeOH:water 80:20, with 3 mL MeOH, and with 5 mL ethyl acetate. Heat 5 g milk + 2.5 mL concentrated HCl at 85-90° for 4 h, cool to room temperature, add 5 mL 6 M NaOH, shake, cool to room temperature, adjust the pH to 8.0 with 6 M and 0.2 M NaOH, add 5 mL buffer, add 20 mL ethyl acetate, shake on a reciprocating shaker for 15 min, centrifuge at 3200 g for 5 min, add the ethyl acetate layer to the SPE cartridge, repeat the extraction, add this ethyl acetate layer to the SPE cartridge, wash with 5 mL ethyl acetate. Air dry the SPE cartridge under vacuum, elute with 9.5 mL 100 mM KH_2PO_4 in MeCN:water 30:70, collect the eluate and make up to 10 mL with 100 mM KH_2PO_4 in MeCN:water 30:70, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm PartiSphere SCX benzenesulfonic acid (Whatman)
Mobile phase: MeCN:8.5 g/L KH_2PO_4 20:80, adjust the pH to 3.8 with phosphoric acid
Column temperature: 25
Flow rate: 1
Injection volume: 50
Detector: F ex 305 em 380

CHROMATOGRAM
Retention time: 9.0
Limit of quantitation: 50 ppb

OTHER SUBSTANCES
Extracted: metabolites, 5-hydroxythiabendazole (F ex 318 em 525)

KEY WORDS
cow; SPE

REFERENCE
Arenas,R.V.; Johnson,N.A. Liquid chromatographic fluorescence method for multiresidue determination of thiabendazole and 5-hydroxythiabendazole in milk, *J.AOAC Int.*, **1995**, *78*, 642–646.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin,

cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject 50 μL of a solution in RPMI-1640.

HPLC VARIABLES

Column: 250 × 4.6 Hypersil ODS
Mobile phase: MeOH:37 mM NaH_2PO_4 60:40 adjusted to pH 7.5 with triethylamine
Injection volume: 50
Detector: UV 298

CHROMATOGRAM

Retention time: 6.0

REFERENCE

Ho,N.F.H.; Sims,S.M.; Vidmar,T.J.; Day,J.S.; Barsuhn,C.L.; Thomas,E.M.; Geary,T.G.; Thompson,D.P. Theoretical perspectives on anthelmintic drug discovery: Interplay of transport kinetics, physicochemical properties, and in vitro activity of anthelmintic drugs, *J.Pharm.Sci.*, **1994**, *83*, 1052–1059.

SAMPLE

Matrix: solutions
Sample preparation: Centrifuge at 10000 rpm, dilute the supernatant with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil MOS
Mobile phase: MeCN:10 mM KH_2PO_4 60:40
Detector: UV 254

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

SAMPLE
Matrix: tissue
Sample preparation: Wash 22 g bulk 40 μm 18% load end-capped C18 material (Analytichem) in a syringe barrel with 100 mL hexane, with 100 mL dichloromethane, and with 100 mL MeOH and dry under vacuum aspiration. Gently blend 2 g C18 material, 0.5 g liver, and 10 μL 40 μg/mL mebendazole in DMF in a glass pestle for 1 min until homogeneous in appearance. Place in a 10 mL syringe barrel plugged with filter paper (Whatman No. 1), cover with filter paper, compress to 4.5 mL, place a 100 μL pipette tip on the barrel to restrict flow, wash with 8 mL hexane, elute with 8 mL MeCN. Pass the eluate through 0.5 g activated alumina (EM Science Type F-20 80-200 mesh) between filter paper in a 10 mL syringe barrel (wash column with 4 mL MeCN just before use). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH and 400 μL 17 mM phosphoric acid, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micro Pak ODS (Varian)
Mobile phase: MeCN:17 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 5
Internal standard: mebendazole (9)
Limit of detection: 100 ng/g

OTHER SUBSTANCES
Extracted: albendazole, oxfendazole, fenbendazole

KEY WORDS
matrix solid-phase dispersion; liver

REFERENCE
Long,A.R.; Malbrough,M.S.; Hsieh,L.C.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of five benzimidazole anthelmintics in fortified beef liver, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 860–863.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 2.8 mL 500 mg 40 μm 60 Å Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Minced tissue + 5 mL 1 M sodium carbonate + 150 mL ethyl acetate + 1 mL 10 mg/mL BHT in ethyl acetate, blend (Waring) at high speed for 5 min, add 80 g anhydrous sodium sulfate, blend at low speed for 1 min. Decant the organic layer and filter it (No. 41 paper), add 150 mL acetone to material remaining in blender, blend at low speed for 2-3 min, filter, wash solid with 10 mL EtOH. Combine all the filtrates and evaporate them to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid, combine rinses, shake vigorously for 2 min, allow to separate for 10 min, extract the hexane layer twice more with 10 mL portions of 1 M phosphoric acid. Combine all the aqueous layers and wash them with 10 mL hexane, adjust the pH of the aqueous layer to 8.5 ± 1.0 by slowly adding about 9 mL 10 M KOH while using an ice bath. Extract twice with 50 mL ethyl acetate (2 min shaking),

pass ethyl acetate layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the ethyl acetate layers, add 200 μL 10 mg/mL BHT in ethyl acetate, evaporate to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with three 3 mL portions of dichloromethane, add rinses to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex, filter (0.2 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Brownlee RP-18 Spheri-10 MPLC
Column: 250 × 4.6 5 μm C18 (Alltech)
Mobile phase: MeOH:buffer 53:47 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 298

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: oxfendazole, mebendazole, metabolites
Simultaneous: chloramphenicol
Noninterfering: amprolium, chlortetracycline, erythromycin, levamisole, morantel, oxytetracycline, phenothiazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline

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KEY WORDS
cow; liver; SPE

REFERENCE
LeVan,L.W.; Barnes,C.J. Liquid chromatographic method for multiresidue determination of benzimidazoles in beef liver and muscle: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 487–493.

SAMPLE
Matrix: vegetables
Sample preparation: Condition a 10 mL 500 mg Bond Elut LRC, PRS (propylsulfonic acid) SPE cartridge with 10 mL conditioning solution, 3 mL MeOH, and 5 mL ethyl acetate. Homogenize with an equal amount of water. 10 g Potato homogenate or 20 g sweet potato homogenate + 20 mL ethyl acetate, shake on a reciprocating shaker for 10 min, centrifuge at 2500-3000 rpm for 15 min, repeat the extraction, add the ethyl acetate layers to the SPE cartridge, wash with 5 mL ethyl acetate, dry the SPE cartridge under vacuum, elute with 9.8 (potato) or 4.8 (sweet potato) mL elution solution, make up to 5 (sweet potato) or 10 (potato) mL with elution solution, dilute with mobile phase if necessary, inject a 50 μL aliquot. (Prepare conditioning solution by mixing 40 mL water and 2 mL 85% phosphoric acid, make up to 200 mL with MeOH. Prepare elution solution by dissolving 2.8 g KH_2PO_4 in 100 mL water, add 60 mL MeCN, make up to 200 mL water.)

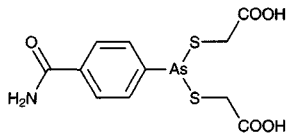
HPLC VARIABLES
Column: 125 × 4.6 5 μm PartiSphere SCX (benzenesulfonic acid) (Whatman)
Mobile phase: MeCN:buffer 25:75, adjusted to pH 3.4 with 85% phosphoric acid. (Prepare mobile phase by dissolving 13.6 g KH_2PO_4 in 1 L water, add 500 mL MeCN, make up to 2 L with water, adjust pH to 3.4 with 85% phosphoric acid.)
Column temperature: 25
Flow rate: 1
Injection volume: 50
Detector: F ex 305 em 380

CHROMATOGRAM
Retention time: 5
Limit of detection: 2.5 ppb
Limit of quantitation: 5 ppb

KEY WORDS
potato; sweet potato; SPE

REFERENCE
Arenas,R.V.; Rahman,H.; Johnson,N.A. Determination of thiabendazole residues in white and sweet potatoes by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1995**, *78*, 1455–1458.

Thiacetarsamide



Molecular formula: $C_{11}H_{12}AsNO_5S_2$
Molecular weight: 377.27
CAS Registry No.: 531-72-6, 7681-85-8 (di Na salt)
Merck Index: 831

SAMPLE
Matrix: solutions

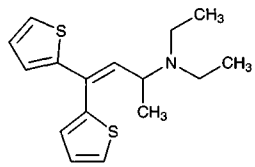
HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax C-8
Mobile phase: MeOH:0.25 mM pH 7 sodium phosphate buffer containing 0.125 mM EDTA
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 1 (thiacetarsamide), 4.5 (p-arsenobenzamide, the major degradation product)

KEY WORDS
protect from light

REFERENCE
Leadbetter,M.G.; Allen,E.H. Liquid chromatographic determination of the composition of thiacetarsamide solution, *J.Liq.Chromatogr.*, **1986**, *9*, 1075–1094.

Thiambutene



Molecular formula: $C_{16}H_{21}NS_2$
Molecular weight: 291.48
CAS Registry No.: 96-14-6
Merck Index: 9429

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

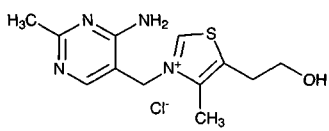
HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Thiamine



Molecular formula: $C_{12}H_{17}ClN_4OS$
Molecular weight: 300.81
CAS Registry No.: 59-43-8, 67-03-8 (HCl), 532-43-4 (mononitrate)
Merck Index: 9430

SAMPLE
Matrix: blood
Sample preparation: 1 mL Hemolysate + 30 µL 4 µM IS in 100mM HCl, shake thoroughly. Slowly add 2 mL MeOH, mix, let stand for 30 min. Centrifuge at 2000 g for 10 min. Add 50 µL freshly prepared 30.4 mM potassium ferricyanide and 50 µL 0.8 mM NaOH to 1 mL supernatant. Filter (0.45 µm) and inject a 50 µL aliquot.

CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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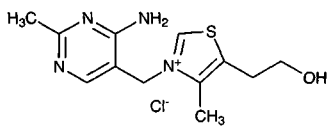
Thiamine



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Merck Index: 9430

SAMPLE
Matrix: blood
Sample preparation: 1 mL Hemolysate + 30 μL 4 μM IS in 100mM HCl, shake thoroughly. Slowly add 2 mL MeOH, mix, let stand for 30 min. Centrifuge at 2000 g for 10 min. Add 50 μL freshly prepared 30.4 mM potassium ferricyanide and 50 μL 0.8 mM NaOH to 1 mL supernatant. Filter (0.45 μm) and inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.0 Spherisorb NH2
Column: 125 × 4.0 5 μm Spherisorb NH2
Mobile phase: MeOH:100 mM pH 7.5 potassium phosphate buffer 45:55
Flow rate: 1
Injection volume: 50
Detector: F ex 375 em 430

CHROMATOGRAM
Retention time: 4.0
Internal standard: acetylaneurine (3.0)
Limit of detection: 2 nM

KEY WORDS
erythrocytes

REFERENCE
Lynch,P.L.M.; Trimble,E.R.; Young,I.S. High-performance liquid chromatographic determination of thiamine diphosphate in erythrocytes using internal standard methodology, *J.Chromatogr.B*, **1997**, *701*, 120–123.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma, whole blood, or erythrocytes + 200 μL 100 mg/mL trichloroacetic acid, vortex vigorously, centrifuge at 35000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 μBondapak C18
Mobile phase: MeCN:buffer 3.8:96.2 (Mobile phase was 200 mM NaH_2PO_4 in 3 g/L MeCN in water.)
Flow rate: 1
Injection volume: 100
Detector: F ex 375 em 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and flowed to the detector. (Reagent was 100 μg/mL potassium ferricyanide in 150 g/L NaOH.)

CHROMATOGRAM
Retention time: 8.0 (thiamine), 3.1 (thiamine triphosphate), 3.8 (thiamine pyrophosphate), 5.0 (thiamine monophosphate)
Limit of detection: 30 fmole

KEY WORDS
post-column reaction; pharmacokinetics; plasma; whole blood; erythrocytes

REFERENCE
Kimura,M.; Itokawa,Y. Determination of thiamin and thiamin phosphate esters in blood by liquid chromatography with post-column derivatization, *Clin.Chem.*, **1983**, *29*, 2073–2075.

SAMPLE
Matrix: blood
Sample preparation: Hemolyze whole blood by freezing at -20° for 20 min, thaw, homogenize. Add a 200 μL aliquot of hemolyzed blood or serum to 200 μL chilled 10% perchloric acid, let stand below 4° for 15 min, centrifuge at 10000 g for 1 min. Remove a 200 μL aliquot of the supernatant and add it to 1.8 M sodium acetate containing 600 mM NaOH, mix, filter (Costar filter unit) while centrifuging for 30 s, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: present but not specified
Column: 110 × 4.7 Partisphere 5 C18 (Whatman)
Mobile phase: Gradient. A was 15 mM citric acid adjusted to pH 4.2 with 50% ammonium hydroxide, prepare fresh each day. B was 100 mM formic acid containing 4% diethylamine, pH

3.2. A:B from 90:10 to 50:50 over 2.5 min, to 5:95 over 0.5 min, maintain at 5:95 over 3.5 min, to 95:5 over 0.5 min, maintain at 95:5 for 3 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 435 following post-column reaction. The column effluent mixed with the reagent pumped at 0.2 mL/min and the mixture flowed through a 90 cm × 0.5 mm ID PTFE coil to the detector. (Prepare reagent by dissolving 100 mg potassium ferricyanide in 120 mL 3 M NaOH.)

CHROMATOGRAM
Retention time: 7.6
Limit of quantitation: 2 nM

OTHER SUBSTANCES
Extracted: thiamine monophosphate, thiamine pyrophosphate, thiamine triphosphate

KEY WORDS
post-column reaction; whole blood; serum

REFERENCE
Lee,B.L.; Ong,H.Y.; Ong,C.N. Determination of thiamine and its phosphate esters by gradient-elution high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *567*, 71–80.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 150 μL 3 M perchloric acid, vortex, centrifuge at >1500 g. Remove 500 μL of the supernatant and add it to 300 μL 1 M pH 4.6 acetate buffer, add 100 μL 10 mg/mL acidic phosphatase (2 U/mg, grade II, Boehringer Mannheim) in water, heat at 40° for 16 h, add 150 μL 3 M perchloric acid, vortex, centrifuge at >1500 g, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 Nucleosil 120 5 C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 10 mM perchloric acid containing 10 mM octanesulfonic acid.)
Flow rate: 2
Injection volume: 20
Detector: F ex 365 em 435 following post-column reaction. The column effluent mixed with 0.8 g/L potassium ferricyanide in 3 M NaOH pumped at 1 mL/min, the mixture flowed through a 10 m × 0.3 mm i.d. PTFE coil at 30° to the detector.

CHROMATOGRAM
Retention time: 2.3
Limit of detection: 2 ng/mL

KEY WORDS
post-column reaction; plasma; pharmacokinetics

REFERENCE
Mascher,H.; Kikuta,C. High-performance liquid chromatographic determination of total thiamine in human plasma for oral bioavailability studies, *J.Pharm.Sci.*, **1993**, *82*, 56–59.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 2.597

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: feed
Sample preparation: 1 g Ground feed + 20 mL 100 mM HCl, shake vigorously, heat on a boiling water bath for 30 min (shake every 5 min), cool in an ice bath for 5 min, centrifuge at 1000 rpm for 10 min. Remove a 5 mL aliquot and make it up to 50 mL with buffer, centrifuge at 1000 rpm for 5 min, inject a 10 μL aliquot. (Buffer was water adjusted to pH 4.0 with acetic acid.)

HPLC VARIABLES
Column: 250 × 4.6 SynChropack SCD-100 (SynChrome Inc.)
Mobile phase: MeOH:water 40:60 containing 50 mM sodium pentanesulfonate, pH adjusted to 4.0 with acetic acid
Flow rate: 1.5
Injection volume: 10
Detector: F ex 370 em 430 following post-column derivatization. The column effluent was mixed with 200 mM KOH and 0.01% potassium ferricyanide, each pumped at 0.5 mL/min. The mixture flowed in the dark through a 3 m × 0.8 mm i.d. knotted coil of PTFE tubing to the detector.

CHROMATOGRAM
Retention time: 5
Limit of detection: 5 pg

KEY WORDS
post-column reaction; derivatization

REFERENCE
Gehring,T.A.; Cooper,W.M.; Holder,C.L.; Thompson,H.C.,Jr. Liquid chromatographic determination of thiamine in rodent feed by postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1995**, *78*, 307–309.

SAMPLE
Matrix: food
Sample preparation: Condition a CBA (methylcarboxylate in acid form) SPE cartridge with 1 mL MeOH and 1 mL 10 mM pH 4.0 phosphate buffer. Add a sample of finely-ground food to 20 mL 100 mM HCl and heat at 100° for 30 min. Cool and adjust pH to 4.4-4.5 with sodium acetate. Add a 6 mg/mL solution of takadiastase (Fluka). Heat at 47° for 3 h. Filter through a

cellulose acetate filter and dilute with water to 50 mL. Add a 2 mL aliquot to the SPE cartridge. Wash twice with 500 μL portions of pH 4.0 phosphate buffer. Elute with three 200 μL portions of 100 mM barium chloride. Inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100RP-18
Mobile phase: MeOH:10 mM pH 2.8 phosphate buffer:triethylamine 25:85:0.1 containing 5 mM hexanesulfonic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.25
Limit of detection: 408 ng/g

KEY WORDS
baby food; cereal; dietetic cookies; SPE

REFERENCE
Blanco,D.; Llaneza,M.B.; Gutierrez,M.D. A paired ion liquid chromatographic method for thiamine determination in selected foods, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2155–2164.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 48
Limit of quantitation: ≤100 ng/mL

OTHER SUBSTANCES
Extracted: riboflavin, pyridoxine, vitamin B12, folic acid, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albal-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 75 mg powdered tablets with 25 mL mobile phase for 15 min, filter (paper), inject a 135 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Scharlau Science, Spain)
Mobile phase: MeOH:20 mM KH_2PO_4 30:70 adjusted to pH 4.0 with orthophosphoric acid

Flow rate: 1.5
Injection volume: 135
Detector: UV 246

CHROMATOGRAM
Retention time: 2.3
Limit of quantitation: 1.9 μg/mL

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, salicylic acid

KEY WORDS
tablets

REFERENCE
Gámiz-Gracia,L.; Luque de Castro,M.D. An HPLC method for the determination of vitamin B1, caffeine, acetylsalicylic acid, and the impurities of salicylic acid in a pharmaceutical preparation, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2123–2133.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: pantothenic acid, riboflavin, niacinamide, pyridoxine

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE
Matrix: formulations
Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1

L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES
Column: 100 × 8 Radial Pak A C18 (Waters)
Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)
Flow rate: 2
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: niacinamide, riboflavin, pyridoxine, ascorbic acid (UV 254)

KEY WORDS
multi-vitamin; protect from light; tablets; capsules

REFERENCE
Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 31 (step gradient), 24 (curve gradient)

OTHER SUBSTANCES
Simultaneous: folic acid (UV 280), niacin, niacinamide, pyridoxamine (UV 280), riboflavin, pyridoxine (UV 280), ascorbic acid (UV 280)

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, niacinamide, pyridoxine, riboflavin, ascorbic acid

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, pyridoxine, riboflavin, saccharin, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1

Injection volume: 5
Detector: UV 272

CHROMATOGRAM
Retention time: 9.90
Internal standard: theobromine (8)
Limit of detection: 0.430 ng

OTHER SUBSTANCES
Simultaneous: folic acid, niacin, niacinamide, riboflavin, pyridoxine (UV 290)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: rice
Sample preparation: Heat ground rice with at least 10 volumes of 100 mM HCl at 95-100° for 30 min with frequent mixing, cool, dilute to a thiamine concentration of 200 ng/mL with 100 mM HCl, adjust the pH of a 65 mL aliquot to 4.0-4.5 with about 5 mL 2 M sodium acetate, add 5 mL 10% takadiastase in water, heat at 45-50° for 3 h, adjust pH to 3.5, make up to 100 mL with water, filter (paper) (AOAC Official Methods of Analysis, 1990, 1049), centrifuge at 3500 rpm for 20 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 5 C18
Mobile phase: 10 mM NaH_2PO_4 containing 150 mM sodium perchlorate, adjusted to pH 2.2 with perchloric acid
Column temperature: 55
Flow rate: 0.6
Injection volume: 25
Detector: F ex 375 em 435 following post-column reaction. The column effluent mixed with 0.1% potassium hexacyanoferrate(III) in 12% NaOH pumped at 0.6 mL/min and this mixture flowed through a 30 cm × 0.8 mm ID stainless steel coil at 55° to the detector.

CHROMATOGRAM
Retention time: 8.5

KEY WORDS
post-column reaction

REFERENCE
Ohta,H.; Baba,T.; Suzuki,Y.; Okada,E. High-performance liquid chromatographic analysis of thiamine in rice flour with fluorimetric post-column derivatization, *J.Chromatogr.*, **1984**, *284*, 281–284.

SAMPLE
Matrix: rice
Sample preparation: Grind rice to pass 30-mesh screen. Stir 3 g ground rice and 50 mL MeOH: 100 mM HCl 40:60 with a glass rod until homogeneous, reflux for 30 min, vortex for 1 min, sonicate for 20 min, centrifuge at 3000 g for 20 min, filter (0.45 μm) the supernatant, inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax TMS
Mobile phase: 10 mM NaH_2PO_4 containing 500 mM sodium perchlorate adjusted to pH 2.5 with 3 M perchloric acid
Column temperature: 55
Flow rate: 0.4

Injection volume: 5
Detector: F ex 375 em 435 following post-column reaction. The column effluent mixed with reagent pumped at 0.4 mL/min, the mixture flowed through a 300 × 0.8 stainless steel mixing coil to the detector. (Reagent was 0.1% potassium hexacyanoferrate(III) in 15% NaOH.)

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 1.5 μg/g

KEY WORDS
post-column reaction

REFERENCE
Ohta,H.; Maeda,M.; Nogata,Y.; Yoza,K.-I.; Takeda,Y.; Osajima,Y. A simple determination of thiamine in rice (*Oryza sativa* L.) by high-performance liquid chromatography with post-column derivatization, *J.Liq.Chromatogr.*, **1993**, *16*, 2617–2629.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10:90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, pyridoxamine, riboflavin, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: niacin, pantothenic acid, pyridoxine, riboflavin, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 780.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: niacin, pyridoxine, riboflavin, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, predni-

solone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (motor-driven glass homogenizer) 100 mg tissue with 300 μL 5% trichloroacetic acid at 4°, centrifuge at 4° at 5000 g for 1 h. Wash the supernatant with 3 volumes of water-saturated diethyl ether for 1 h. Remove an 80 μL aliquot of the aqueous phase and add it to 50 μL reagent, mix for 5-10 s, inject an aliquot 1 min after the addition of the reagent. (Prepare reagent by mixing 50 μL 10 mg/mL potassium ferricyanide with 2.5 mL 15% NaOH, store in the dark, discard after 1 day.)

HPLC VARIABLES

Guard column: 4.2 × 3.2 30-44 μm 201RP (Vydac)
Column: 150 or 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. MeOH:25 mM pH 8.4 phosphate buffer 10:90 for 1 min, to 100:0 over 3 min, maintain at 100:0 for 2 min, return to initial conditions over 2 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 390 em 475

CHROMATOGRAM

Retention time: 4.2 (thiamine triphosphate), 4.5 (thiamine pyrophosphate), 5 (thiamine monophosphate), 6 (thiamine)
Limit of detection: 0.05 pmole

KEY WORDS

derivatization; rat; nerve; heart

REFERENCE

Bontemps,J.; Philippe,P.; Bettendorff,L.; Lombet,J.; Dandrifosse,G.; Schoffeniels,E.; Crommen,J. Determination of thiamine and thiamine phosphates in excitable tissues as thiochrome derivatives by reversed-phase high-performance liquid chromatography on octadecyl silica, *J.Chromatogr.*, **1984**, *307*, 283–294.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Nucleosil C18 SPE cartridge with 2 mL MeOH, 2 mL MeOH containing 5 mM sodium heptanesulfonate, and two 2 mL portions of water. Suspend 5 g homogenized tissue with 35 mL 10 mM HCl, autoclave at 121° for 30 min, add 2 mL 25 mg/mL taka-distase (Fluka) in 2.5 M sodium acetate, add 2 mL 10 (muscle) or 20 (liver) mg/mL clara-distase (Fluka) in water, add 2 mL 50 mg/mL papain (Merck) in water, adjust pH to 4.5, heat at 37° for 16-18 h, filter (paper), adjust pH to 6.5, filter again, make up to 50 mL with water, add 4 mL to the SPE cartridge, wash with 2 mL MeOH:water 20:80 containing 5 mM sodium heptanesulfonate, elute with 2 mL MeOH:water 50:50 containing 5 mM sodium heptanesulfonate, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 10 μm Nucleosil C18
Column: 150 × 4.6 3 μm Nucleosil C18

Mobile phase: MeCN:10 mM pH 3.0 KH_2PO_4 16:84 (muscle) or 15:85 (liver) containing 5 mM sodium heptane sulfonate (Wash with MeCN:water 20:80 at the end of the day, store column in MeCN.)
Column temperature: 45
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5 (muscle), 8 (liver)
Limit of detection: 500 ng/g

OTHER SUBSTANCES
Extracted: riboflavin

KEY WORDS
pig; muscle; liver; protect from light; SPE

REFERENCE
Barna,I.; Dworschák,E. Determination of thiamine (vitamin B1) and riboflavin (vitamin B2) in meat and liver by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *668*, 359–363.

SAMPLE
Matrix: yeast
Sample preparation: Condition CM-cellulose (44-105 μm CM-Cellulofine C-200, Biochemical Industry, Tokyo) by washing with 200 mM HCl, 200 mM NaOH, water, and 200 mM HCl, rinse with water until the rinses are neutral. Add a 2.5 mL aliquot to a 170 × 10 column, wash with 300 mM phosphoric acid, wash with water until the eluate is neutral. Heat 1 g dried yeast, 1 mL 10% HCl, and 80 mL water with frequent shaking at 80-85° for 30 min, cool, make up to 100 mL with water, centrifuge for 10 min. Remove a 4 mL aliquot of the supernatant and add it to 5 mL 200 mM pH 4.5 acetic acid/sodium acetate buffer and 1 mL 30 mg/mL Taka-diastase supernatant (Sankyo) in 5 mM HCl, heat at 45-50° for 3 h, add 2 mL of this mixture to the column at 0.5 mL/min, wash with two 10 mL portions of water at 1 mL/min, elute with two 2.5 mL portions of 300 mM phosphoric acid at 0.5 mL/min. Add 1 mL 1 μg/mL IS and 10 mg sodium 1-octanesulfonate to the eluate, mix, inject a 200 μL aliquot.

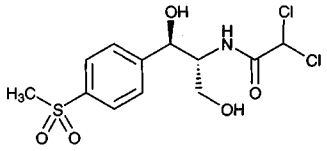
HPLC VARIABLES
Column: 150 × 4.6 Capcell-pak C18 (Shiseido)
Mobile phase: MeCN:buffer 20:80 (Buffer was 20 mM pH 3.5 phosphate buffer containing 0.2% sodium 1-octanesulfonate.)
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: phenacetin (10)
Limit of quantitation: 250 ng/mL

KEY WORDS
SPE

REFERENCE
Yamanaka,K.; Matsuoka,M.; Banno,K. Determination of thiamine in dried yeast by high-performance liquid chromatography using a clean-up column of CM-cellulose, *J.Chromatogr.A*, **1996**, *726*, 237–240.

Thiamphenicol



Molecular formula: $C_{12}H_{15}Cl2NO_5S$
Molecular weight: 356.23
CAS Registry No.: 15318-45-3
Merck Index: 9436
Lednicer No.: 2 45

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 7

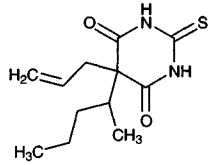
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Thiamylal



Molecular formula: $C_{12}H_{18}N_2O_2S$
Molecular weight: 254.35
CAS Registry No.: 77-27-0, 337-47-3 (Na salt)
Merck Index: 9437
Lednicer No.: 1 274

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Homogenize tissue with four volumes 100 mM pH 5 phthalate buffer. 2 mL Blood, bile, urine, stomach contents, or tissue homogenate + 2 mL 100 mM pH 5 phthalate buffer + 100 μL 1 mg/mL phenolphthalein in MeOH, vortex for 10 s, add to a Clin Elut SPE

cartridge (Analytichem), let stand for 10 min, elute with three 5 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of air at 60°, reconstitute with 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS (Altex)
Mobile phase: MeOH:water 5:1
Flow rate: 2
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Internal standard: phenolphthalein

KEY WORDS
SPE; liver; kidney

REFERENCE
Costantino,A.G.; Caplan,Y.H.; Levine,B.S.; Dixon,A.M.; Smialek,J.E. Thiamylal: review of the literature and report of a suicide, *J.Forensic Sci.*, **1990**, *35*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL MeCN, vortex for 10 s, let stand for 10 min, vortex for 10 s, centrifuge at 12000 g for 2 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeCN:water 55:45
Flow rate: 1.2
Injection volume: 20
Detector: UV 288

CHROMATOGRAM
Retention time: 4.9
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: thiopental
Noninterfering: acetaminophen, allopurinol, amikacin, amobarbital, amphotericin B, ampicillin, aspirin, barbital, caffeine, carbenicillin, chloramphenicol, chlorpromazine, cimetidine, cisplatin, cyclophosphamide, cyclosporin A, cytarabine, dactinomycin, doxorubicin, droperidol, ethosuximide, 5-fluorocytosine, 5-fluorouracil, furosemide, gentamicin, hexobarbital, ketamine, ketoconazole, 6-mercaptopurine, metharbital, methotrexate, miconazole, mizoribine, pentobarbital, phenobarbital, procainamide, secobarbital, tegafur, vancomycin

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Hosotsubo,H.; Takeda,K.; Hosotsubo,K.; Yoshiya,I. Measurement of thiamylal in human plasma using reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *487*, 204–209.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 100 μL MeOH + 1 mL 70 mM pH 6.4 phosphate buffer + 5 mL n-pentane, shake vigorously for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, vortex for 1 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: Nucleosil 5 C8
Mobile phase: MeCN:buffer 35:65 (Buffer was 0.2 mM phosphoric acid containing 0.175 mM KH_2PO_4.)
Flow rate: 1.2
Injection volume: 5
Detector: UV 290

CHROMATOGRAM
Retention time: 12
Internal standard: thiamylal

OTHER SUBSTANCES
Extracted: thiopental

KEY WORDS
serum; plasma; cow; human; comparison with capillary electrophoresis; thiamylal is IS

REFERENCE
Meier,P.; Thormann,W. Determination of thiopental in human serum and plasma by high-performance capillary electrophoresis-micellar electrokinetic chromatography, *J.Chromatogr.*, **1991**, *559*, 505–513.

SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of 1 M pH 5.0 phosphate buffer, add IS, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3500 rpm for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:10 mM pH 5.0 sodium phosphate buffer 60:40
Flow rate: 1
Injection volume: 10-20
Detector: UV

CHROMATOGRAM
Internal standard: 5-(p-methylphenyl)-5-phenylhydantoin
Limit of quantitation: 1 μg/mL

KEY WORDS
serum; rat; pharmacokinetics

REFERENCE
Nakashima,E.; Matsushita,R.; Ohshima,T.; Tsuji,A.; Ichimura,F. Quantitative relationship between structure and peritoneal membrane transport based on physiological pharmacokinetic concepts for acidic drugs, *Drug Metab.Dispos.*, **1995**, *23*, 1220–1224.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut C18 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 10 mM KH_2PO_4. 100 μL Serum + 50 μL 25 μg/mL n-propyl p-hydroxybenzoate in water + 500 μL 10 mM KH_2PO_4, add to the SPE cartridge, wash with two 1 mL portions of water, elute with 300 μL MeOH. Evaporate the eluate, reconstitute in 100 μL EtOH:water 50:50, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 TSK-gel ODS 80TM
Mobile phase: EtOH:10 mM KH_2PO_4 15:85 containing 17 mM β-cyclodextrin (Suspend 17 mmoles β-cyclodextrin in 150 mL EtOH, make up to 1 L with 10 mM KH_2PO_4.)
Column temperature: 25

Flow rate: 0.9
Injection volume: 50
Detector: UV 288

CHROMATOGRAM
Retention time: 37 (S(-)), 39 (R(+))
Internal standard: n-propyl p-hydroxybenzoate (25)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: muscle relaxants, benzodiazepines

KEY WORDS
serum; chiral; SPE

REFERENCE
Sueyasu,M.; Ikeda,T.; Otsubo,K.; Taniyama,T.; Aoyama,T.; Oishi,R. Enantioselective determination of thiamylal in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 133–137.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 5 μm C-18 (Perkin-Elmer)
Mobile phase: MeOH:THF:buffer 50:7:43 (Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)
Flow rate: 2
Injection volume: 6
Detector: UV 240

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetazolamide, amobarbital, aspirin, barbital, butabarbital, cefazolin, chlorothiazide, chlorzoxazone, dicloxacillin, ethosuximide, furosemide, hydrochlorothiazide, ibuprofen, oxacillin, pentobarbital, phenobarbital, phenylbutazone, phenytoin, salicylic acid, secobarbital, sulfamethoxazole, theophylline, thiopental, ascorbic acid
Noninterfering: ampicillin, penicillin G, valproic acid

REFERENCE
Kelner,M.; Bailey,D.N. Reversed-phase liquid-chromatographic simultaneous analysis for thiopental and pentobarbital in serum, *Clin.Chem.*, **1983**, *29*, 1097–1100.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 25:75, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: MeCN:4 mM potassium phosphate buffer 50:50, pH 4.0
Flow rate: 1.2
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 6.9

OTHER SUBSTANCES
Simultaneous: amobarbital, heptabarbital, hexobarbital, methohexital, pentobarbital, phenobarbital, secobarbital, thiopental

Flow rate: 0.9
Injection volume: 50
Detector: UV 288

CHROMATOGRAM
Retention time: 37 (S(-)), 39 (R(+))
Internal standard: n-propyl p-hydroxybenzoate (25)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: muscle relaxants, benzodiazepines

KEY WORDS
serum; chiral; SPE

REFERENCE
Sueyasu,M.; Ikeda,T.; Otsubo,K.; Taniyama,T.; Aoyama,T.; Oishi,R. Enantioselective determination of thiamylal in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 133–137.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 5 μm C-18 (Perkin-Elmer)
Mobile phase: MeOH:THF:buffer 50:7:43 (Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)
Flow rate: 2
Injection volume: 6
Detector: UV 240

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetazolamide, amobarbital, aspirin, barbital, butabarbital, cefazolin, chlorothiazide, chlorzoxazone, dicloxacillin, ethosuximide, furosemide, hydrochlorothiazide, ibuprofen, oxacillin, pentobarbital, phenobarbital, phenylbutazone, phenytoin, salicylic acid, secobarbital, sulfamethoxazole, theophylline, thiopental, ascorbic acid
Noninterfering: ampicillin, penicillin G, valproic acid

REFERENCE
Kelner,M.; Bailey,D.N. Reversed-phase liquid-chromatographic simultaneous analysis for thiopental and pentobarbital in serum, *Clin.Chem.*, **1983**, *29*, 1097–1100.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 25:75, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: MeCN:4 mM potassium phosphate buffer 50:50, pH 4.0
Flow rate: 1.2
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 6.9

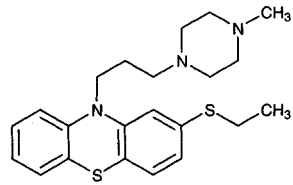
OTHER SUBSTANCES
Simultaneous: amobarbital, heptabarbital, hexobarbital, methohexital, pentobarbital, phenobarbital, secobarbital, thiopental

mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Thiethylperazine



Molecular formula: $C_{22}H_{29}N_3S_2$
Molecular weight: 399.62
CAS Registry No.: 1420-55-9, 52239-63-1 (maleate)
Merck Index: 9449

SAMPLE

Matrix: blood

Sample preparation: 3 mL Serum + 3 mL diluted Titrisol (pH 10 borate buffer, Merck) + 4 mL heptane:isoamyl alcohol 97:3, shake thoroughly for 15 s, centrifuge at 2500 g for 5 min. Remove the organic phase and add it to 1.5 mL 50 mM sulfuric acid containing 0.1% $Na_2S_2O_2$, mix for 15 s, centrifuge at 2500 g for 10 min. Remove the aqueous phase. Repeat the extraction and back extraction. Combine the aqueous phases and add them to 1.5 mL 2 M pH 9.1 glycine buffer, add 200 µL n-hexane:isoamyl alcohol 97:3, vortex for 25 s. Remove the organic phase and evaporate it to dryness in a desiccator, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: MeCN:water:acetic acid 65:35:3 containing 10 mM dodecyl hydrogen sulfate
Flow rate: 2
Detector: UV 257

CHROMATOGRAM

Retention time: 13
Internal standard: thiethylperazine

OTHER SUBSTANCES

Extracted: perphenazine

Noninterfering: alimemazine, biperidine, carbamazepine, chlorpromazine, clomipramine, diazepam, dihydroergotamine, disulfiram, dixyrazine, haloperidol, levomepromazine, nitrazepam, orphenadrine, promethazine, propiomazine, thioridazine, trimipramine, vitamins

KEY WORDS
serum; thiethylperazine is IS

REFERENCE
Larsson,M.; Forsman,A. A high-performance liquid chromatographic method for the assay of perphenazine and its dealkylated metabolite in serum after therapeutic doses, *Ther.Drug Monit.*, **1983**, *5*, 225–228.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Lichrosorb SI60
Mobile phase: MeCN:MeOH:ammonium hydroxide 250:55:13
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 3.4

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, amitriptyline, chlorimipramine, chlorpromazine, codeine, desipramine, dimethacrine, diphenhydramine, disopyramide, doxepin, hydroquinidine, maprotiline, melitracene, mesoridazine, nortriptyline, opipramol, perazine, perphenazine, procainamide, prochlorperazine, promazine, prothipendyl, protriptyline, thioperazine, thioridazine, trifluoperazine
Noninterfering: acenocoumaron, acetaminophen, acetophenetidine, aspirin, benzodiazepines, bibenzepin, butriptyline, caffeine, chlorprothixene, clopenthixol, clothiapine, dixyrazine, droperidol, fluphenazine, haloperidol, hydroxyzine, isoniazid, methotrimeprazine, metopimazine, moperone, noxiptyline, orphenadrine, pericyazine, phenprocoumon, pipothiazine, promethazine, salicylic acid, theophylline, thiopropazate, trimeprazine, trimipramine
Interfering: butaperazine, imipramine, pipamperone, quinidine, thiothixene

REFERENCE
Edelbroek,P.M.; de Haas,E.J.M.; de Wolff,F.A. Liquid-chromatographic determination of amitryptyline and its metabolites in serum, with adsorption onto glass minimized, *Clin.Chem.*, **1982**, *28*, 2143–2148.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cy-

clizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 RP-8
Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.45

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxy-

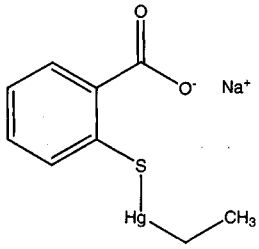
chloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Thimerosal

Molecular formula: $C_9H_9HgNaO_2S$
Molecular weight: 404.82
CAS Registry No.: 54-64-8
Merck Index: 9451



SAMPLE
Matrix: formulations
Sample preparation: Add IS to vaccine at a concentration of 40 μg/mL, centrifuge at 3400 g for 15 min, inject a 25 μL aliquot of the supernatant. (IS stock solution was prepared in mobile phase:water 1:4.)

HPLC VARIABLES
Guard column: 5 × 4 5 μm Hypersil C18
Column: 210 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:water:orthophosphoric acid 35:35:0.9, pH 2.5
Flow rate: 0.6
Injection volume: 25
Detector: UV 222

CHROMATOGRAM
Retention time: 10.1
Internal standard: salicylic acid (6.5)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
vaccine

REFERENCE
Tleugabulova,D.; Gonzalez Perez,I. Reversed-phase high-performance liquid chromatographic study of thimerosal stability in Cuban recombinant hepatitis B vaccine, *J.Chromatogr.A*, **1996**, *729*, 219–227.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 6.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: benzalkonium chloride, domiphen bromide, xylometazoline
Interfering: inorganic salts

KEY WORDS
nasal drops

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: formulations
Sample preparation: 9.5 mL Contact lens solution + 0.5 mL 3 mg/mL methylparaben, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 7 μm Nucleosil C18 pre-column
Column: 7 μm Nucleosil C18
Mobile phase: MeOH:100 mM KH_2PO_4 adjusted to pH 3.5 with phosphoric acid 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11.4
Internal standard: methyl paraben (7.8)
Limit of detection: 100 ng

OTHER SUBSTANCES
Simultaneous: chlorhexidine gluconate, thiosalicylic acid

KEY WORDS
stability-indicating; contact lens solutions

REFERENCE
Hu,O.Y.-P.; Wang,S.-Y.; Fang,Y.-J.; Chen,Y.-H.; King,M.-L. Simultaneous determination of thimerosal and chlorhexidine in solutions for soft contact lenses and its applications in stability studies, *J.Chromatogr.*, **1990**, *523*, 321–326.

SAMPLE
Matrix: formulations
Sample preparation: Inject directly or extract as follows. Condition a Sep-Pak C18 SPE cartridge with MeOH and water. 10 mL Ophthalmic solution + 100 μL concentrated phosphoric acid, add to the SPE cartridge, wash with 5 mL water, wash with 2 mL MeOH:water 20:80, elute with 2 mL MeOH, dilute the eluate to 10 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb C18
Mobile phase: MeOH:water 50:50 containing 2 mM tetraethylammonium perchlorate, adjusted to pH 4.8 with perchloric acid
Flow rate: 1
Injection volume: 20
Detector: E, Metrohm Model 461, Metrohm Model 656 flow cell, carbon paste electrode, 0.9 V

CHROMATOGRAM
Retention time: 5
Limit of detection: 90 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products, 2,2'-dithiodibenzoic acid, thiosalicylic acid

KEY WORDS
ophthalmic solutions; SPE

REFERENCE
del Pilar da Silva,M.; Procopio,J.R.; Hernández,L. Evaluation of the capability of different chromatographic systems for the monitoring of thimerosal and its degradation products by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.A*, **1993**, *653*, 267–273.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 10 μm Spherisorb 10 ODS
Mobile phase: MeOH:water:phosphoric acid 60:50:1
Flow rate: 2.6
Injection volume: 25
Detector: UV 222

CHROMATOGRAM
Retention time: 3
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products, 2,2'-dithiosalicylic acid, thiosalicylic acid

REFERENCE
Reader,M.J.; Lines,C.B. Decomposition of thimerosal in aqueous solution and its determination by high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 1406–1409.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of the ophthalmic solution directly.

HPLC VARIABLES
Column: two 250 × 4 10 μm Spherisorb ODS 10 columns in series
Mobile phase: MeOH:water:phosphoric acid 60:50:1
Flow rate: 4
Injection volume: 50

Detector: UV 222

KEY WORDS
ophthalmic solutions

REFERENCE
Reader,M.J. Influence of isotonic agents on the stability of thimerosal in ophthalmic formulations, *J.Pharm.Sci.*, **1984**, *73*, 840–841.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 210 × 4.6 5 μm Spherisorb RP-18
Mobile phase: MeOH:water:phosphoric acid 65:35:0.9
Flow rate: 0.6
Injection volume: 20
Detector: UV 222

CHROMATOGRAM
Retention time: 7.6
Limit of quantitation: 5 ppm

OTHER SUBSTANCES
Simultaneous: degradation products, dithiosalicylic acid, thiosalicylic acid

REFERENCE
Caraballo,I.; Rabasco,A.M.; Fernández-Arévalo,M. Study of thimerosal degradation mechanism, *Int.J.Pharm.*, **1993**, *89*, 213–221.

SAMPLE
Matrix: urine
Sample preparation: Add 0.05% thymol to urine, filter, dilute 1:10 with water, inject an aliquot.

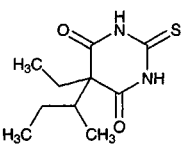
HPLC VARIABLES
Guard column: Guard-Pak C18
Column: 300 × 3.9 μBondapak C18
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Simultaneous: creatinine, thymol, uric acid

REFERENCE
Chen,Y.; Pietrzyk,R.A.; Whitson,P.A. Quantification of urinary uric acid in the presence of thymol and thimerosal by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *763*, 187–192.

Thiobutabarbital



Molecular formula: $C_{10}H_{16}N_2O_2S$, $C_{10}H_{15}N_2NaO_2S$
Molecular weight: 228.32
CAS Registry No.: 2095-57-0, 947-08-0 (sodium salt)
Merck Index: 9457

SAMPLE
Matrix: solutions

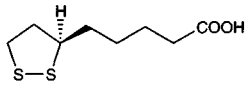
HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Column: 125 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Mobile phase: MeCN:buffer 50:50 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)
Flow rate: 2
Injection volume: 50
Detector: UV 283

CHROMATOGRAM
Retention time: 2.70

OTHER SUBSTANCES
Simultaneous: thiopental

REFERENCE
Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, *728*, 307–310.

Thioctic acid



Molecular formula: $C_8H_{14}O_2S_2$
Molecular weight: 206.33
CAS Registry No.: 62-46-4, 1200-22-2 (d-form), 1077-28-7 (dl-form), 1077-27-6 (l-form), 2319-84-8 (sodium salt)
Merck Index: 9462

SAMPLE
Matrix: solutions
Sample preparation: Mix 200 μL of a solution in MeOH:water 10:90 with 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer and 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate for 3 min, let stand for 22 min, add 150 μL 4.5 μg/mL IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES
Column: 250 × 4.5 Hypersil 5 ODS
Mobile phase: MeCN:MeOH:water 37.4:30.6:32
Column temperature: 35
Flow rate: 1.1
Injection volume: 50
Detector: F ex 300 em 460

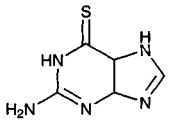
CHROMATOGRAM
Retention time: 16.5
Internal standard: n-hexanoic acid 6-methoxynaphthacyl ester (?) (Dissolve 2 mmole n-hexanoic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 500 μL triethylamine, heat to 60° for 30 min, cool, add 30 mL water, extract three times with 10 mL portions of diethyl ether. Combine the organic layers and wash them with 5% sodium bicarbonate solution, wash three times with 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from MeOH/water

to give 6-methoxynaphthacyl ester of n-hexanoic acid (mp 79-80°) (J. Pharm. Biomed. Anal. 1993, 11, 761) (19)

KEY WORDS
derivatization

REFERENCE
Gatti,R.; Bousquet,E.; Bonazzi,D.; Cavrini,V. Determination of carboxylic acid salts in pharmaceuticals by high-performance liquid chromatography after pre-column fluorogenic labelling, *Biomed.Chromatogr.*, **1996**, *10*, 19–24.

Thioguanine



Molecular formula: $C_5H_5N_5S$
Molecular weight: 167.19
CAS Registry No.: 154-42-7, 5580-03-0 (hemihydrate)
Merck Index: 9473
Lednicer No.: 2 464

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 1 mL cold 2 M perchloric acid, mix, centrifuge at 4° at 48000 g for 20 min. Remove a 1 mL aliquot of the supernatant and adjust to pH 10-12 with 150 μL 4 M KOH, let stand at 4° for 2 days. Adjust the pH of the supernatant to 2-3 with 150 μL 1 M HCl, centrifuge at 700 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 10 mM acetic acid adjusted to pH 3.5 with 5 M NaOH. Flush column daily with MeOH:water 50:50.)
Flow rate: 2
Injection volume: 100
Detector: UV 340

CHROMATOGRAM
Retention time: 4
Limit of detection: 800 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Andrews,P.A.; Egorin,M.J.; May,M.E.; Bachur,N.R. Reversed-phase high-performance liquid chromatography analysis of 6-thioguanine applicable to pharmacologic studies in humans, *J.Chromatogr.*, **1982**, *227*, 83–91.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 400 mM NaOH + 1 mL 0.3% phenylmercuric acetate in ethyl acetate + 3 mL diethyl ether, shake on a tumble mixer for 10 min, centrifuge for 5 min. Remove the organic layer and add it to 500 μL 100 mM HCl, whirlmix for 2 min, centrifuge for 5 min, discard the organic layer, evaporate traces of organic solvent under a stream of nitrogen at room temperature for 15 min, add 10 μL 3 mg/mL dithioerythritol in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 LiChrosorb 10 RP-18
Mobile phase: Isopropanol:water 3:97 containing 13.80 g/L $NaH_2PO_4.H_2O$, 200 μL/L 85% phosphoric acid, 60 mg/L dithioerythritol, and 500 mg/L sodium octanesulfonate, pH 3.6-3.7
Flow rate: 1.5
Detector: F ex 295 em 380 following post-column reaction. The column effluent mixed with 8 mM potassium chromate in 500 mM HCl pumped at 0.16 mL/min and with air flowing at 0.32 mL/min and the mixture flowed through a single mixing coil. The effluent from this coil mixed with 1.6% sodium metabisulfite pumped at 0.16 mL/min and this mixture flowed through a single mixing coil. The effluent from this coil mixed with 4 M ammonium hydroxide pumped at 0.23 mL/min and this mixture flowed through a double mixing coil to a debubbler. The liquid effluent from the debubbler flowed to the detector.

CHROMATOGRAM

Retention time: 8
Internal standard: 6-thioguanine

OTHER SUBSTANCES

Extracted: 6-mercaptopurine

KEY WORDS

post-column reaction; plasma; 6-thioguanine is IS

REFERENCE

Jonkers,R.E.; Oosterhuis,B.; ten Berge,R.J.M.; van Boxtel,C.J. Analysis of 6-mercaptopurine in human plasma with a high-performance liquid chromatographic method including post-column derivatization and fluorimetric detection, *J.Chromatogr.*, **1982**, *233*, 249–255.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 80 μL water + 10 μL 1 M dithiothreitol, vortex for 10 s, add 2 mL MeCN, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and add it to 2 mL dichloromethane, shake on a reciprocating shaker for 5 min, centrifuge at 2000 g for 5 min. Remove 750 μL from the top aqueous layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 150 μL distilled water, vortex for 1 min, inject a 15 μL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:acetic acid:water 3.5:0.2:96.3
Flow rate: 1.4
Injection volume: 15
Detector: UV 322

CHROMATOGRAM

Retention time: 6.5
Internal standard: 6-thioguanine

OTHER SUBSTANCES

Extracted: 6-mercaptopurine
Noninterfering: caffeine, cytarabine, 5-fluorouracil, prednisone, theophylline, vinblastine, vincristine

KEY WORDS

plasma; protect from light; monkey; human; 6-thioguanine is IS

REFERENCE

Narang,P.K.; Yeager,R.L.; Chatterji,D.C. Quantitation of 6-mercaptopurine in biologic fluids using high-performance liquid chromatography: a selective and novel procedure, *J.Chromatogr.*, **1982**, *230*, 373–380.

SAMPLE
Matrix: blood
Sample preparation: Dilute 10 mL blood with 15 mL phosphate buffered saline containing 3 mM ethyleneglycoltetraacetic acid. Layer 8 mL diluted blood on 2 mL Ficoll-Hypaque (Sigma), centrifuge at 9000 g for 10 min, collect lymphocyte band, wash with PBS. 200 μL Lymphocytes + 50 μL 1 M sulfuric acid, heat at 100° for 45 min, cool, centrifuge. Remove 200 μL and add to 55 μL 1 M pH 10.1 sodium bicarbonate, add 1.5 mL ethyl acetate:dichloromethane containing 100 ng/mL sulfamethoxazole, vortex, centrifuge. Remove 175 μL of the upper aqueous layer and add it to 25 μL 1 M pH 10.1 sodium bicarbonate, add 25 μL 0.5% potassium permanganate (freshly prepared), let stand for 5 min, add 5 μL 15% hydrogen peroxide, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 11:89 (Buffer was 10 mM pH 7 sodium phosphate containing 0.06% tetrabutylammonium chloride.)
Column temperature: 45
Flow rate: 1.5
Injection volume: 10
Detector: F ex 330 em 412 (370 nm cut-off filter)

CHROMATOGRAM
Retention time: 2.30
Limit of detection: 1 ng

KEY WORDS
whole blood; lymphocytes

REFERENCE
Erdmann,G.R.; Steury,J.C.; Carleton,B.C.; Stafford,R.J.; Bostrom,B.C.; Canafax,D.M. Reversed-phase high-performance liquid chromatographic approach to determine total lymphocyte concentrations of 6-thioguanine, methylmercaptopurine and methylthioguanine in humans, *J.Chromatogr.*, **1991**, *571*, 149–156.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 25 μL 1 M aluminum perchlorate in water, let stand at room temperature for 15 min, chill in ice water for 15 min, centrifuge at 15600 g for 15 min, discard the supernatant. Suspend the precipitate in 500 μL 50 mM aluminum perchlorate in water by stirring to break up the precipitate, vortex for 20 s, centrifuge at 15600 g for 15 min, discard the supernatant. Suspend the precipitate in 150 μL 400 mM perchloric acid, add 5 μL freshly prepared 200 mM aqueous sodium hydrosulfite, mix, let stand at room temperature for 30 min, chill in ice water, centrifuge at 15600 g for 15 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 45 × 2 40 μm ODS
Column: 150 × 4.3 5 μm Ultrasphere ODS
Mobile phase: Water:85% phosphoric acid 99.32:0.68 containing 154.3 mg/L dithiothreitol
Flow rate: 1
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 4.73
Internal standard: 6-thioguanine
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: 6-mercaptopurine, 6-thiouric acid

KEY WORDS
plasma; pharmacokinetics; 6-thioguanine is IS

REFERENCE

Lin,K.T.; Varin,F.; Rivard,G.E.; Leclerc,J.M. Isolation of 6-mercaptopurine in human plasma by aluminum ion complexation for high-performance liquid chromatographic analysis, *J.Chromatogr.*, **1991**, *536*, 349–355.

SAMPLE

Matrix: blood

Sample preparation: Add 1 volume ice-cold 8 M perchloric acid to 20 volumes plasma, mix, keep on ice for 10 min, centrifuge at 10 000 g for 15 min, remove the supernatant. Adjust the pH of the supernatant to 6-7 with 10 volumes ice-cold 4 M K_2KHPO_4, keep on ice for 10 min, centrifuge at 10 000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-18-DB

Mobile phase: Gradient. A was 25 mM KH_2PO_4. B was MeOH:50 mM KH_2PO_4 25:75. A:B from 98:2 to 96:4 over 5 min, to 85:15 over 3 min, to 80:20 over 2 min, to 40:60 over 10 min, maintain at 40:60 over 2 min, to 20:80 over 3 min, maintain at 20:80 for 20 min, return to initial conditions over 3 min, re-equilibrate for 12 min.

Flow rate: 1.25

Injection volume: 100

Detector: UV 342

CHROMATOGRAM

Retention time: 10

Limit of detection: 20-50 nM

OTHER SUBSTANCES

Extracted: metabolites, mercaptopurine (UV 320)

KEY WORDS

plasma

REFERENCE

Keuzenkamp-Jansen,S.W.; De Abreu,R.A.; Bökkerink,J.P.M.; Trijbels,J.M.F. Determination of extracellular and intracellular thiopurines and methylthiopurines by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *672*, 53–61.

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Blood, CSF. Collect 2 mL blood in tubes containing heparin and 120 μg dithiothreitol (DTT). Mix, centrifuge at 2000 g for 5 min, remove plasma. CSF. Collect 0.5 mL CSF in tubes containing 30 μg DTT. Cool CSF and plasma samples on ice, add freshly prepared ice-cold 50% trichloroacetic acid equal to 10% of sample volume. Urine. Collect 2 mL urine in tubes containing 120 μg DTT, filter (0.22 μm), inject an aliquot.

HPLC VARIABLES

Column: two 250 × 4.6 10μm Nucleosil 10 C18 columns in series

Mobile phase: Gradient. A was 25 mM pH 2.75 phosphoric acid. B was MeOH:water 50:50. C was 100 mM pH 6.6 KH_2PO_4. A:B:C from 100:0:0 to 98:2:0 over 5 min, to 30:3.5:66.5 over 5 min, maintain at 30:3.5:66.5 for 10 min, re-equilibrate at initial conditions for 10 min.

Column temperature: 33

Flow rate: 1.7

Injection volume: 195, 500

Detector: UV 342

CHROMATOGRAM

Retention time: 13

Limit of detection: 25 nM

OTHER SUBSTANCES

Extracted: metabolites, mercaptopurine (UV 312), 6-mercaptopurine riboside, 6-thioguanosine

KEY WORDS
plasma; goat; pharmacokinetics

REFERENCE
van Baal,J.M.; van Leeuwen,M.B.; Schouten,T.J.; De Abreu,R.A. Sensitive high-performance liquid chromatographic determination of 6-mercaptopurine, 6-thioguanine, 6-mercaptopurine riboside and 6-thioguanosine in biological fluids, *J.Chromatogr.*, **1984**, *336*, 422–428.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Filter (Amicon Model MM 302, type PM 10 Diaflo membrane) plasma while centrifuging at 8000 g for 3 min, inject a 20-100 μL aliquot of the ultrafiltrate. Urine. Inject an aliquot directly.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 5C8
Mobile phase: 50 mM pH 7.0 citrate-phosphate buffer (Buffer was 9.61 g $Na_2HPO_4.H_2O$ and 1.33 g citric acid monohydrate in 1 L water, pH 7.0.)
Flow rate: 1.4
Injection volume: 20-100
Detector: UV 343

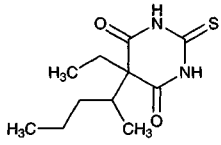
CHROMATOGRAM
Retention time: 5.7
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: 6-mercaptopurine
Simultaneous: allopurinol, azaguanine, guanine, oxipurinol, uric acid
Noninterfering: adenine, 2-amino-6-methylthiopurine, aspirin, benzbromarone, caffeine, diazepam, dihydralazine, dipyridamole, fluorouracil, hypoxanthine, methotrexate, procarbazine, propranolol, spironolactone, sulfamethoxazole, sulfinpyrazone, theophylline, thiouric acid, thioxanthine, trimethoprim, xanthine

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Breithaupt,H.; Goebel,G. Quantitative high pressure liquid chromatography of 6-thioguanine in biological fluids, *J.Chromatogr.Sci.*, **1981**, *19*, 496–499.

Thiopental



Molecular formula: $C_{11}H_{18}N_2O_2S$
Molecular weight: 242.34
CAS Registry No.: 76-75-5, 71-73-8 (Na salt)
Merck Index: 9487
Lednicer No.: 1 274

SAMPLE
Matrix: blood
Sample preparation: Precipitate 100 μL serum with 200 μL 10 μg/mL IS in MeCN, centrifuge at 12000 g for 5 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART LiChrospher 60 RP Select B

KEY WORDS
plasma; goat; pharmacokinetics

REFERENCE
van Baal,J.M.; van Leeuwen,M.B.; Schouten,T.J.; De Abreu,R.A. Sensitive high-performance liquid chromatographic determination of 6-mercaptopurine, 6-thioguanine, 6-mercaptopurine riboside and 6-thioguanosine in biological fluids, *J.Chromatogr.*, **1984**, *336*, 422–428.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Filter (Amicon Model MM 302, type PM 10 Diaflo membrane) plasma while centrifuging at 8000 g for 3 min, inject a 20-100 μL aliquot of the ultrafiltrate. Urine. Inject an aliquot directly.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 5C8
Mobile phase: 50 mM pH 7.0 citrate-phosphate buffer (Buffer was 9.61 g $Na_2HPO_4.H_2O$ and 1.33 g citric acid monohydrate in 1 L water, pH 7.0.)
Flow rate: 1.4
Injection volume: 20-100
Detector: UV 343

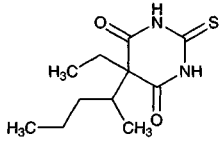
CHROMATOGRAM
Retention time: 5.7
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: 6-mercaptopurine
Simultaneous: allopurinol, azaguanine, guanine, oxipurinol, uric acid
Noninterfering: adenine, 2-amino-6-methylthiopurine, aspirin, benzbromarone, caffeine, diazepam, dihydralazine, dipyridamole, fluorouracil, hypoxanthine, methotrexate, procarbazine, propranolol, spironolactone, sulfamethoxazole, sulfinpyrazone, theophylline, thiouric acid, thioxanthine, trimethoprim, xanthine

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Breithaupt,H.; Goebel,G. Quantitative high pressure liquid chromatography of 6-thioguanine in biological fluids, *J.Chromatogr.Sci.*, **1981**, *19*, 496–499.

Thiopental



Molecular formula: $C_{11}H_{18}N_2O_2S$
Molecular weight: 242.34
CAS Registry No.: 76-75-5, 71-73-8 (Na salt)
Merck Index: 9487
Lednicer No.: 1 274

SAMPLE
Matrix: blood
Sample preparation: Precipitate 100 μL serum with 200 μL 10 μg/mL IS in MeCN, centrifuge at 12000 g for 5 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART LiChrospher 60 RP Select B

Column: 125 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Mobile phase: MeCN:buffer 50:50 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)
Flow rate: 2
Injection volume: 50
Detector: UV 283

CHROMATOGRAM
Retention time: 3.46
Internal standard: thiobutabarbital (2.70)
Limit of detection: 230 ng/mL

KEY WORDS
serum

REFERENCE
Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, *728*, 307–310.

SAMPLE
Matrix: blood
Sample preparation: Mix 250 μL plasma with IS and 400 μL MeCN, vortex, centrifuge. Inject a 20 μL aliquot of the clear supernatant.

HPLC VARIABLES
Column: C18 reverse phase
Mobile phase: MeOH:0.5% tetrabutylammonium phosphate in water 50:50
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Internal standard: methohexital
Limit of detection: 100 ng/mL

KEY WORDS
sheep; plasma; pharmacokinetics

REFERENCE
Upton,R.N.; Huang,Y.F.; Grant,C.; Gray,E.C.; Ludbrook,G.L. Myocardial pharmacokinetics of thiopental in sheep after short-term administration: Relationship to thiopental-induced reductions in myocardial contractility, *J.Pharm.Sci.*, **1996**, *85*, 863–867.

SAMPLE
Matrix: blood
Sample preparation: Buffer serum to pH 5.6 with 100 mM acetate buffer, extract with hexane. Remove the hexane and extract it with 250 mM NaOH. Neutralize the aqueous layer with phosphoric acid and inject an aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:0.1 mM pH 4.2 phosphate buffer 43:57
Flow rate: 1
Detector: UV 195

CHROMATOGRAM
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: methohexital

KEY WORDS
serum; pharmacokinetics

REFERENCE
Hudson,R.J.; Stanski,D.R.; Burch,P.G. Pharmacokinetics of methohexital and thiopental in surgical patients, *Anesthesiology*, **1983**, *59*, 215–219.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 1 mL buffer, vortex, add 10 mL n-butyl chloride containing 10 μg/mL barbital and 4 μg/mL thiamylal, extract vigorously for 3 min, centrifuge at 3000 g for 5 min. Remove the upper organic layer and add it to 100 μL 450 mM NaOH, extract vigorously for 3 min, centrifuge for 10 min or until lower aqueous phase is clear, inject a 15 μL aliquot of the lower aqueous phase. (Soak glassware in 1 M HCl overnight, rinse with water, dry. Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)

HPLC VARIABLES
Column: 125 × 4.6 5 μm C-18 (Perkin-Elmer)
Mobile phase: MeOH:THF:buffer 50:7:43 (Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)
Flow rate: 2
Injection volume: 6
Detector: UV 240

CHROMATOGRAM
Retention time: 4.1
Internal standard: barbital (0.8), thiamylal (5.2)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: pentobarbital
Simultaneous: acetaminophen, acetazolamide, amobarbital, aspirin, butabarbital, cefazolin, chlorothiazide, chlorzoxazone, dicloxacillin, ethosuximide, furosemide, hydrochlorothiazide, ibuprofen, oxacillin, phenobarbital, phenylbutazone, phenytoin, salicylic acid, secobarbital, sulfamethoxazole, theophylline, ascorbic acid
Noninterfering: ampicillin, penicillin G, valproic acid

KEY WORDS
serum

REFERENCE
Kelner,M.; Bailey,D.N. Reversed-phase liquid-chromatographic simultaneous analysis for thiopental and pentobarbital in serum, *Clin.Chem.*, **1983**, *29*, 1097–1100.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma or hemolyzed (frozen and thawed) whole blood + 500 μL 2 μg/mL hexobarbital in water + 500 μL 250 mM HCl + 40 mg NaCl + 3 mL toluene, rotate, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at 50 ± 5°, reconstitute the residue in 200 μL MeCN and 200 μL 50 mM NaH_2PO_4, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 Perisorb RP-18 (Merck)
Column: 250 × 4 7 μm LiChroCart RP-18 (Merck)
Mobile phase: MeCN:50 mM pH 4.6 NaH_2PO_4 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 195

CHROMATOGRAM
Retention time: 6.8
Internal standard: hexobarbital (4.8)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Extracted: methohexital
Simultaneous: barbital, caffeine, indomethacin, pentobarbital, phenobarbital
Noninterfering: aspirin, salicylic acid

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Bjorkman,S.; Idvall,J. A high-performance liquid chromatographic method for methohexital and thiopental in plasma or whole blood, *J.Chromatogr.*, **1984**, *307*, 481–487.

SAMPLE
Matrix: blood
Sample preparation: Whole blood, serum. 500 μL Blood or serum + 500 μL buffer + 200 μg phenolphthalein + 5 mL dichloromethane, rotate, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 300 μL MeOH, inject a 20 μL aliquot. (Prepare buffer by mixing 500 mM KH_2PO_4 and 500 mM Na_2HPO_4 to obtain a pH of 5.5.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm RP-8
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 20
Detector: UV 290

KEY WORDS
whole blood; serum

REFERENCE
Levine,B.S.; Blanke,R.V.; Valentour,J.C. Postmortem stability of barbiturates in blood and tissues, *J.Forensic Sci.*, **1984**, *29*, 131–138.

SAMPLE
Matrix: blood
Sample preparation: 50 μL 25 μg/mL 5-Ethyl-5-p-tolylbarbituric acid in MeOH added to 150 × 10 mm glass centrifuge tube and blow dry under a stream of nitrogen, add 500 μL plasma, add 5 mL dichloromethane, mix on rotary mixer for 5 min, centrifuge. Remove organic layer, evaporate to dryness under nitrogen, take up in 500 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 μBondapak C18 Corasil B
Column: 300 × 4 5 μm μBondapak C18
Mobile phase: MeOH:10 mM potassium phosphate adjusted to pH 4.40 ± 0.05 with 150 mM phosphoric acid 50:50
Flow rate: 1.7
Injection volume: 40
Detector: UV 284

CHROMATOGRAM
Retention time: 8.90
Internal standard: 5-ethyl-5-p-tolylbarbituric acid (UV 212) (4.62)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: pentobarbital (UV 212)

Simultaneous: acetaminophen, amobarbital, barbital, butalbital, butabarbital, caffeine, carbamazepine, phenacetin, phenobarbital, phenytoin, secobarbital, theobromine, theophylline, vinbarbital

KEY WORDS
plasma

REFERENCE
Houdret,N.; Lhermitte,M.; Lalau,G.; Izydorczak,J.; Roussel,P. Determination of thiopental and pentobarbital in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 437–442.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C8 SPE cartridge with 2 volumes of MeOH, 2 volumes of water, and 1 volume of 100 mM pH 5.59 Sörensen's phosphate buffer. 200 μL Plasma + 10 μL 1 mg/mL sodium secobarbital in EtOH, add to the SPE cartridge, wash with 1 volume of water, elute with 250 μL MeOH. Evaporate the eluate to dryness under vacuum, reconstitute in 50 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak C18 (Waters)
Column: 100 × 8 10 μm Radial-Pak C8 (Waters)
Mobile phase: MeOH:THF:100 mM pH 7.72 Sörensen's phosphate buffer 28:16:52 containing 5 ng/mL sodium thiopental
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8.54
Internal standard: secobarbital (6.38)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Extracted: methohexital, pentobarbital
Noninterfering: ketamine

KEY WORDS
plasma; dog; pharmacokinetics; SPE

REFERENCE
Avram,M.J.; Krejcie,T.C. Determination of sodium pentobarbital and either sodium methohexital or sodium thiopental in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1987**, *414*, 484–491.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 285

CHROMATOGRAM
Retention time: 7.87
Internal standard: 3-isobutyl-1-methylxanthine (3.15)

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline
Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

KEY WORDS
serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL MeCN, vortex for 10 s, let stand for 10 min, vortex for 10 s, centrifuge at 12000 g for 2 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeCN:water 55:45
Flow rate: 1.2
Injection volume: 20
Detector: UV 288

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Extracted: thiamylal
Noninterfering: acetaminophen, allopurinol, amikacin, amobarbital, amphotericin B, ampicillin, aspirin, barbital, caffeine, carbenicillin, chloramphenicol, chlorpromazine, cimetidine, cisplatin, cyclophosphamide, cyclosporin A, cytarabine, dactinomycin, doxorubicin, droperidol, ethosuximide, 5-fluorocytosine, 5-fluorouracil, furosemide, gentamicin, hexobarbital, ketamine, ketoconazole, 6-mercaptopurine, metharbital, methotrexate, miconazole, mizoribine, pentobarbital, phenobarbital, procainamide, secobarbital, tegafur, vancomycin

KEY WORDS
plasma

REFERENCE
Hosotsubo,H.; Takeda,K.; Hosotsubo,K.; Yoshiya,I. Measurement of thiamylal in human plasma using reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *487*, 204–209.

SAMPLE
Matrix: blood
Sample preparation: Vigorously shake equal volumes of plasma and MeCN, centrifuge at 10000 g for 3 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 110 × 4.6 PartiSphere C8 (Whatman)
Mobile phase: MeCN:120 mM pH 6.2 phosphate buffer 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted PTFE coil irradiated with a Sylvania G8-T5 lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 4.25
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: pentobarbital

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma

REFERENCE
Schmid,R.W.; Wolf,C. Simultaneous determination of thiopental and its metabolite, pentobarbital, in blood by high-performance liquid chromatography and post-column photochemical reaction, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1749–1755.

SAMPLE
Matrix: blood
Sample preparation: 200-500 μL Whole blood + 1 mL 100 mM pH 7.5 phosphate buffer, vortex for 1 min, add 7 mL n-hexane:diethyl ether 50:50, add 50 μL 100 μg/mL secobarbital in EtOH:water 75:25, shake for 15 min, centrifuge at 4° at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, vortex, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Nucleosil C18
Mobile phase: MeCN:water 32:68
Flow rate: 0.3
Injection volume: 5-20
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Internal standard: secobarbital (9)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: pentobarbital

KEY WORDS
whole blood; pharmacokinetics

REFERENCE
Celardo,A.; Bonati,M. Determination of thiopental measured in human blood by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 220–225.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 1.2 mL buffer, mix gently, add 10 mL toluene, shake for 5 min (break any emulsion with sonication), centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 μL MeOH, inject a 50 μL aliquot. (Buffer was 100 mM acetic acid:100 mM sodium acetate 76:24, pH 4.5.)

HPLC VARIABLES
Column: 300 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer:150 mM NaCl 27:27:36:10 (Buffer was 9.00 g KH_2PO_4 and 140 mg $Na_2HPO_4.7H_2O$ in 1 L water, pH 5.05.)
Flow rate: 2.5
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 4
Internal standard: thiopental

OTHER SUBSTANCES
Extracted: progabide

KEY WORDS
plasma; thiopental is IS

REFERENCE
Decourt,J.P.; Mura,P.; Papet,Y.; Piriou,A.; Reiss,D. Simultaneous determination of progabide and its acid metabolite by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 214–219.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 100 μL 20 μg/mL thiamylal in MeOH + 1 mL 70 mM pH 6.4 phosphate buffer + 5 mL n-pentane, shake vigorously for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, vortex for 1 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: Nucleosil 5 C8
Mobile phase: MeCN:buffer 35:65 (Buffer was 0.2 mM phosphoric acid containing 0.175 mM KH_2PO_4.)
Flow rate: 1.2
Injection volume: 5
Detector: UV 290

CHROMATOGRAM
Retention time: 10
Internal standard: thiamylal (12)

KEY WORDS
serum; plasma; cow; human; comparison with capillary electrophoresis

REFERENCE
Meier,P.; Thormann,W. Determination of thiopental in human serum and plasma by high-performance capillary electrophoresis-micellar electrokinetic chromatography, *J.Chromatogr.*, **1991**, *559*, 505–513.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 20 μL 500 μg/mL phenylbutazone in 2 mM NaOH + 1.1 mL ether:n-hexane 20:80 + 20 μL 3 M phosphoric acid, vortex at 1200 rpm for 1 min, centrifuge at 2000 g for 5 min, freeze in dry ice for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure (16 mbar) at 40° for 10 min, reconstitute the residue in 50 μL 400 μM NaOH, inject a 2-40 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 AGP (ChromTech)
Column: 100 × 4 AGP-CSP (ChromTech)
Mobile phase: Isopropanol:100 mM pH 6.2 phosphate buffer 4.5:95.5

Flow rate: 0.9
Injection volume: 2-40
Detector: UV 287

CHROMATOGRAM
Retention time: 7.1 (R(+)), 10.8 (S(-))
Internal standard: phenylbutazone (15.7)
Limit of quantitation: 6 ng/mL (S(-)), 5 ng/mL (R(+))

OTHER SUBSTANCES
Extracted: pentobarbital (UV 220)

KEY WORDS
sheep; plasma; chiral; pharmacokinetics

REFERENCE
Huang,J.L.; Mather,L.E.; Duke,C.C. High-performance liquid chromatographic determination of thiopentone enantiomers in sheep plasma, *J.Chromatogr.B*, **1995**, *673*, 245–250.

SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of 1 M pH 5.0 phosphate buffer, add IS, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3500 rpm for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:10 mM pH 5.0 sodium phosphate buffer 55:45
Flow rate: 1
Injection volume: 10-20
Detector: UV

CHROMATOGRAM
Internal standard: 5-(p-methylphenyl)-5-phenylhydantoin
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Also analyzed: pentobarbital

KEY WORDS
serum; rat; pharmacokinetics

REFERENCE
Nakashima,E.; Matsushita,R.; Ohshima,T.; Tsuji,A.; Ichimura,F. Quantitative relationship between structure and peritoneal membrane transport based on physiological pharmacokinetic concepts for acidic drugs, *Drug Metab.Dispos.*, **1995**, *23*, 1220–1224.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 2 mL 34 mg/mL pH 5.5 KH_2PO_4 + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 10000 g for 5 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:THF:0.68 mg/mL pH 2.6 KH_2PO_4 65:5:30
Column temperature: 30

Flow rate: 0.8
Injection volume: 50
Detector: UV 293

CHROMATOGRAM
Retention time: 6.0
Internal standard: thiopental

OTHER SUBSTANCES
Extracted: dapsone

KEY WORDS
plasma; thiopental is IS

REFERENCE
Tracqui,A.; Gutbub,A.M.; Kintz,P.; Mangin,P. A case of acute dapsone poisoning: Toxicological data and review of the literature, *J.Anal.Toxicol.*, **1995**, *19*, 229–235.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 287

CHROMATOGRAM
Retention time: 5.26
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazo-

lam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 250 μL Plasma + 50 μL 200 μg/mL ketamine in MeOH + 1 mL 10 mM pH 6.0 KH_2PO_4 buffer + 3 mL pentane, vortex for 20 s, centrifuge at 4000 g for 10 min, freeze in dry ice. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 120 μL mobile phase, vortex for 10 s, inject a 10-100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 Chromtec chiral AGP (J.T. Baker)

Column: 100 × 4 Chromtec chiral AGP (J.T. Baker)

Mobile phase: MeOH:isopropanol:20 mM phosphate buffer 1.5:5:93.5, pH 5.0 (At the end of each day wash with isopropanol:water 10:90 at 0.1 mL/min overnight.)

Flow rate: 0.9

Injection volume: 10-100

Detector: UV 280

CHROMATOGRAM

Retention time: 4.84 (R-(+)), 6.18 (S-(-))

Internal standard: ketamine (1.84)

Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; chiral; pharmacokinetics

REFERENCE

Jones,D.J.; Nguyen,K.T.; McLeish,M.J.; Crankshaw,D.P.; Morgan,D.J. Determination of (*R*)-(+)- and (*S*)-(-)-isomers of thiopentone in plasma by chiral high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 174–179.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 14.1
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize tissue with 2 volumes of water. 1 mL Homogenate + 300 μL 1 mg/mL phenolphthalein in MeOH + 1 mL buffer + 10 mL dichloromethane, rotate, centrifuge. Remove the organic layer and add it to 3 mL 100 mM NaOH, rotate, centrifuge. Remove the aqueous layer and acidify it with 1 mL 1 M HCl, extract with 10 mL dichloromethane. Remove the organic layer and evaporate it to dryness at 40°, reconstitute the residue in 300 μL MeOH, inject a 20 μL aliquot. Blood. 500 μL Whole blood + 500 μL buffer + 200 μL 1 mg/mL phenolphthalein in MeOH + 5 mL dichloromethane, rotate, centrifuge. Remove the organic layer and evaporate it to dryness at 40°, reconstitute the residue in 300 μL MeOH, inject a 20 μL aliquot. (Buffer was prepared by mixing 500 mM Na_2HPO_4 with 500 mM KH_2PO_4 to pH 5.5.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm RP-8 (Hewlett-Packard) or 260 × 4.6 10 μm Spherisorb C18
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 5.97
Internal standard: phenolphthalein (2.96)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: carbamazepine, pentobarbital (UV 210)
Noninterfering: amobarbital, butabarbital, glutethimide, meprobamate, methaqualone, methyprylon, phenobarbital, phenytoin, secobarbital

KEY WORDS
whole blood

REFERENCE
Levine,B.; Blanke,R.; Valentour,J. Liquid chromatographic analysis of thiopental in blood and tissues, *J.Anal.Toxicol.*, **1983**, 7, 207–208.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 285.5

CHROMATOGRAM
Retention time: 19.202

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute equal volume 10 mg/mL propofol and 25 mg/mL thiopental injections 1:200 with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax SB
Mobile phase: MeCN:buffer 45:55 (Buffer was 10 mM KH_2PO_4, adjusted to pH 4.0 with 10% phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 4.9

OTHER SUBSTANCES
Simultaneous: propofol

KEY WORDS
stability-indicating; injections

REFERENCE
Chernin,E.L.; Stewart,J.T.; Smiler,B. Stability of thiopental sodium and propofol in polypropylene syringes at 23 and 4°C, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1576–1579.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with saline, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Lichrosorb 10 RP 8
Mobile phase: MeOH:THF:water 50:5:50
Flow rate: 3
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11.2

OTHER SUBSTANCES
Simultaneous: diazepam, lorazepam

KEY WORDS
injections; saline

REFERENCE
Martens,H.J.; de Goede,P.N.; van Loenen,A.C. Sorption of various drugs in polyvinyl chloride, glass, and polyethylene-lined infusion containers, *Am.J.Hosp.Pharm.*, **1990**, *47*, 369–373.

SAMPLE
Matrix: solutions
Sample preparation: Add 380 μg propofol and 1.03 mg thiopental sodium to 0.9% sodium chloride, shake vigorously for 2 min, make up to 10 mL with 0.9% sodium chloride, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax SB phenyl
Mobile phase: MeCN:buffer 45:55 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 4.9
Limit of detection: 317 ng/mL

OTHER SUBSTANCES
Simultaneous: propofol

REFERENCE
King,D.T.; Stewart,J.T.; Venkateshwaran,T.G. HPLC determination of propofol-thiopental sodium and propofol-ondansetron mixtures, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2285–2294.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 232, 282

CHROMATOGRAM
Retention time: 10.4
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES
Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, triamterene, verapamil, zolpidem, zopiclone

REFERENCE
Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 4.07

OTHER SUBSTANCES
Simultaneous: aprobarbital, pentobarbital, amobarbital, butabarbital, butalbital, phenobarbital, secobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 7.04 (A), 7.33 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide,

ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Brinkman Polytron Model PT 10/35) 0.5-1.5 g tissue in 3 mL 70 mM pH 7.4 Sorensen's phosphate buffer with 10 s bursts while chilling with ice. 0.2-1 mL Plasma or tissue homogenate + 1 mL 70 mM pH 6.3 Sorensen's phosphate buffer + 50 μL 20 μg/mL thiamylal in MeOH:water 50:50 + 5 mL pentane, shake at 40 cycles/min for 10 min, centrifuge, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeCN:water 25:75, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: MeCN:4 mM potassium phosphate buffer 50:50, pH 4.0
Flow rate: 1.2
Injection volume: 100
Detector: UV 290

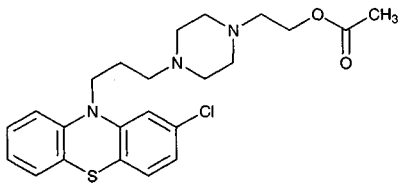
CHROMATOGRAM
Retention time: 5.5
Internal standard: thiamylal (6.5)
Limit of detection: 10 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: amobarbital, heptabarbital, hexobarbital, methohexital, pentobarbital, phenobarbital, secobarbital

KEY WORDS
rat; plasma; brain; fat; heart; intestine; kidney; liver; lung; muscle; pancreas; plasma; spleen; testes

REFERENCE
Ebling,W.F.; Mills-Williams,L.; Harapat,S.R.; Stanski,D.R. High-performance liquid chromatographic method for determining thiopental concentrations in twelve rat tissues: application to physiologic modeling of disposition of barbiturate, *J.Chromatogr.*, **1989**, *490*, 339–353.

Thiopropazate



Molecular formula: $C_{23}H_{28}ClN_3O_2S$
Molecular weight: 446.01
CAS Registry No.: 84-06-0, 146-28-1 (2.HCl), 104999-18-0 (dimaleate)
Merck Index: 9493
Lednicer No.: 1 383

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.8

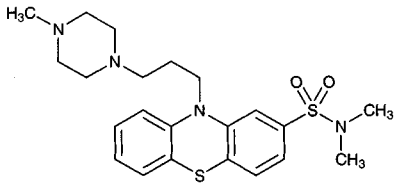
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, pro-

heptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Thioproperazine



Molecular formula: $C_{22}H_{30}N_4O_2S_2$
Molecular weight: 446.64
CAS Registry No.: 316-81-4, 2347-80-0 (dimethanesulfonate)
Merck Index: 9494

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 266

CHROMATOGRAM

Retention time: 7.47
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; almino-

profen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 265.3

CHROMATOGRAM

Retention time: 15.212

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.5

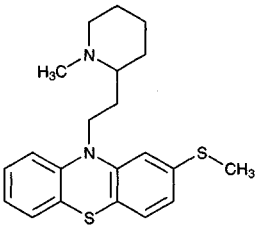
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Thioridazine



Molecular formula: $C_{21}H_{26}N_2S_2$
Molecular weight: 370.58
CAS Registry No.: 50-52-2, 130-61-0 (HCl)
Merck Index: 9497
Lednicer No.: 1 389

SAMPLE

Matrix: blood
Sample preparation: 1-5 mL Plasma + 1 mL 1 M NaOH + hexanes, extract for 30 min, centrifuge. Remove a 9 mL aliquot of the organic phase and evaporate it to dryness at 30° under a stream of nitrogen. Dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:5 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 29.5
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, fluphenazine, promethazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, chlorpromazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, metabolites

KEY WORDS

plasma

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL water + 2 mL 2 M NaOH, mix well, add 10 mL heptane:isoamyl alcohol 99:1, shake slowly on a reciprocating shaker for 15 min, centrifuge at 5-10° at 1207 g for 5 min. Remove 8.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 μL MeCN:water 60:40, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-PCN (cyano) (Supelco)
Mobile phase: MeCN:20 mM pH 4.5 KH_2PO_4 60:40
Column temperature: 40
Flow rate: 2
Injection volume: 50
Detector: UV 229 or E, IBM Model 230, Model 3892 glassy carbon electrode, 1000 mV vs saturated calomel electrode

CHROMATOGRAM

Retention time: 8.5
Internal standard: thioridazine

OTHER SUBSTANCES
Extracted: chlorprothixene

KEY WORDS
plasma; thioridazine is IS

REFERENCE
Brooks,M.A.; DiDonato,G.; Blumenthal,H.P. Determination of chlorprothixene and its sulfoxide metabolite in plasma by high-performance liquid chromatography with ultraviolet and amperometric detection, *J.Chromatogr.*, **1985**, *337*, 351–362.

SAMPLE
Matrix: blood
Sample preparation: Condition a Varian AASP C18 SPE cartridge with 1 mL MeOH and 1 mL water. Mix 500 μL serum with 500 μL 200 mM phosphoric acid (pH was ca. 2.3), let stand for 5 min, add 400 μL to the SPE cartridge, wash with 1 mL water, wash with 1 mL MeCN:water 1:1, elute cartridge with mobile phase for 30 s directly onto the analytical column.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS-2 Superpac
Mobile phase: MeCN:MeOH:buffer 25:50:25, pH was 4.1. (Buffer was 4.5 mL 85% orthophosphoric acid and 4.5 mL triethylamine in 1 L water.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 10.95
Limit of detection: about 0.2 μM

OTHER SUBSTANCES
Simultaneous: metabolites, clonazepam, nitrazepam, flunitrazepam, lorazepam, oxazepam, alprazolam, haloperidol, diazepam, zuclopenthixol, protriptyline, nortriptyline, maprotiline, promethazine, imipramine, amitriptyline, levomepromazine, trimipramine, chlorpromazine, clomipramine, perphenazine, fluphenazine, prochlorperazine
Noninterfering: carbamazepine, phenytoin

KEY WORDS
serum; SPE

REFERENCE
Svensson,C.; Nyberg,G.; Soomägi,M.; Mårtensson,E. Determination of the serum concentrations of thioridazine and its main metabolites using a solid-phase extraction technique and high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 229–236.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 25 μL 25 μg/mL promethazine in MeOH + 100 μL 2 M NaOH + 4 mL hexane:ethyl acetate 1:1, rotate at 60 rpm for 5 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness at 40° under a stream of air, reconstitute with 250 μL mobile phase A, vortex for 20 s, inject a 120 μL aliquot onto column A with mobile phase A. Collect the effluent corresponding to the thioridazine peak, add 25 μL 25 μg/mL thiothixene in MeOH, vortex for 20 s, evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 250 μL mobile phase B, inject a 120 μL aliquot onto column B with mobile phase B.

HPLC VARIABLES
Column: A 15 × 3.2 Alltech RP-18 guard column + 250 × 4.6 5 μm Supelcosil LC18-DB; B 250 × 4.6 5 μm Spherisorb Chiral 1 (phenylmethylurea)
Mobile phase: A MeOH:MeCN:water:1 M ammonium hydroxide 500:350:150:3, adjusted to pH 6.7-8.0 with 6 M HCl; B Hexane:dichloromethane:MeOH:1 M ammonium acetate in MeOH 450:450:100:0.075

Flow rate: A 1; B 1
Injection volume: 120
Detector: A UV 263; B UV 263

CHROMATOGRAM
Retention time: 7.4 (A), 9 (B) (+), 10 (-)
Internal standard: A promethazine (5.8); B thiothixene (4)
Limit of detection: 12.5 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: desipramine, nortriptyline, amitriptyline, imipramine, clozapine, maprotiline, chlordiazepoxide, thiothixene, mesoridazine, sulforidazine, fluoxetine
Noninterfering: flurazepam, methylphenidate, ephedrine
Interfering: chlorpromazine

KEY WORDS
serum; chiral

REFERENCE
Jortani,S.A.; Poklis,A. Determination of thioridazine enantiomers in human serum by sequential achiral and chiral high-performance liquid chromaqtography, *J.Anal.Toxicol.*, **1993**, *17*, 374–377.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 ng pipothiazine + 400 μL 2 M NaOH + 4 mL diethyl ether:hexane 75:25, shake for 20 min, centrifuge at 2800 g for 5 min. Remove the organic layer and add it to 1.2 mL 100 mM HCl, shake for 15 min, centrifuge. Remove the aqueous layer and add it to 200 μL 2 M NaOH, extract twice with 2 mL portions of diethyl ether:hexane 75:25. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 120 μL isooctane:dichloromethane:MeOH 83:10:7, inject a 100 μL aliquot on to an 8 mm dia column of 5 μm silica (Waters Z-module radial compression) protected by a guard column of the same material, and elute with isooctane:dichloromethane:MeOH 83:10:7 at 2.2 mL/min (detector F ex 262 em 458). Collect the fraction at 2.3 min containing thioridazine and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute with 125 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Cyclobond I 2000 ac β-acetylated cyclodextrin (Astec, Whippany)
Mobile phase: MeCN:buffer 16:84 (Buffer was 1% triethylamine adjusted to pH 3.0 with orthophosphoric acid. Wash column with MeCN:water 90:10 at the end of each day.)
Flow rate: 1.2
Injection volume: 100
Detector: F ex 262 em 458

CHROMATOGRAM
Retention time: 17.9 (R), 19.7 (S)
Internal standard: pipothiazine (9.4 min on achiral system)
Limit of quantitation: 60 ng/mL (chiral), 15 ng/mL (achiral)

KEY WORDS
plasma; chiral; normal phase; achiral

REFERENCE
Eap,C.B.; Koeb,L.; Powell,K.; Baumann,P. Determination of the enantiomers of thioridazine, thioridazine 2-sulfone, and of the isomeric pairs of thioridazine 2-sulfoxide and thioridazine 5-sulfoxide in human plasma, *J.Chromatogr.B*, **1995**, *669*, 271–279.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 264

CHROMATOGRAM

Retention time: 13.43
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 11.33
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 77.76
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thiothixene, tranylcypromine, trazodone, trihexiphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 262.9

CHROMATOGRAM

Retention time: 17.168

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Crush tablet or capsule, to 2 mg amitriptyline add 20 mL MeOH, shake 30 min, centrifuge at 2000 rpm for 5 min, to 5 mL supernatant add 4 mL 1.25 mg/mL norephedrine.HCl in MeOH, dilute to 10 mL with MeOH.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Zorbax CN

Mobile phase: MeCN:MeOH:25 mM pH 4.8 sodium acetate-acetic acid buffer 35:45:20
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7
Internal standard: norephedrine (2.7)

OTHER SUBSTANCES
Also analyzed: chlorpromazine, amitriptyline, imipramine, trifluoperazine

KEY WORDS
tablets; capsules

REFERENCE
Lovering,E.G.; Beaulieu,N.; Lawrence,R.C.; Sears,R.W. Liquid chromatographic method for identity, assay, and content uniformity of five tricyclic drugs, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 168–171.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane: butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak phenyl
Mobile phase: MeCN:buffer 55:45 (Buffer was 10 mM pH 3.0 KH_2PO_4.)
Flow rate: 1.5
Injection volume: 30
Detector: UV 265

CHROMATOGRAM
Internal standard: protriptyline (UV 214) (4)

OTHER SUBSTANCES
Extracted: chlorpromazine (UV 255)

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 2.5 mL MTBE:hexane 75:25, vortex for 20 s, centrifuge at 2000 g for 5 min, remove and retain the organic layer. Add 750 μL 1 M sodium carbonate , 200 μL 2 M NaOH, and 2.5 mL MTBE:hexane 75:25 to the aqueous layer, vortex for 20 s, centrifuge at 2000 g for 5 min. Repeat the extraction with 2.5 mL dichloromethane. Combine all the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 5 μm silica RCM 8 × 10 radial compression (Waters)
Mobile phase: Isooctane:MeOH:dichloromethane 80:10:10 containing 0.036% methylamine
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; liver; normal phase

REFERENCE
Blake,B.L.; Rose,R.L.; Mailman,R.B.; Levi,P.E.; Hodgson,E. Metabolism of thioridazine by microsomal monooxygenases: relative roles of P450 and flavin-containing monooxygenase, *Xenobiotica*, **1995**, *25*, 377–393.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: mesoridazine, promethazine, acetophenazine, chlorpromazine, prochlorperazine, butaperazine, thiethylperazine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 16.2

OTHER SUBSTANCES
Simultaneous: mesoridazine, promazine, thiothixene, chlorpromazine, trifluoperazine
Also analyzed: amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl

Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM
Retention time: 5.38

OTHER SUBSTANCES
Simultaneous: benactyzine, buclizine, hydroxyzine, perphenazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine,

pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3022 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 58:35:7 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 264

KEY WORDS

chiral; α = 1.10 for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 RP-8
Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.82

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide,

ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 25 ng/mL solution in pH 4.0 acetate/citrate buffer.

HPLC VARIABLES
Column: 150 × 0.32 3 μm Hypersil C18
Mobile phase: MeCN:pH 4.0 acetate/citrate buffer 45:55
Injection volume: 20
Detector: UV

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Simultaneous: chlorpromazine, methotrimeprazine (levomepromazine)

KEY WORDS
microcolumn

REFERENCE
Streel,B.; Ceccato,A.; Chiap,P.; Hubert,P.; Crommen,J. Injection-generated solvent and pH gradients for sample enrichment on injection of large volumes in microcolumn liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 254–256.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)

Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 22.39

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: Perform all procedures in subdued light. 10 mL Urine + 10 μL 100 μg/mL IS, lyophilize, extract residue with 3 mL MeOH by shaking for 15 min, repeat extraction twice, combine extracts and evaporate them to dryness under vacuum below 45°, dissolve residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane, wash the combined organic layers twice with 2 mL phosphate buffer, twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute with 100 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb cyano
Mobile phase: 2,2,4-Trimethylpentane:dichloromethane:MeOH:diethylamine 82:10:8:0.1
Flow rate: 1.1
Injection volume: 10
Detector: UV 254

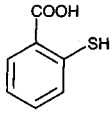
CHROMATOGRAM
Retention time: 2.9
Internal standard: mesoridazine lactam (18)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
human; rat; dog; normal phase

REFERENCE
Lin,G.; Hawes,E.M.; McKay,G.; Korchinski,E.D.; Midha,K.K. Metabolism of piperidine-type phenothiazine antipsychotic agents. IV. Thioridazine in dog, man and rat, *Xenobiotica*, **1993**, *23*, 1059–1074.

Thiosalicylic acid



Molecular formula: $C_7H_6O_2S$
Molecular weight: 154.19
CAS Registry No.: 147-93-3
Merck Index: 9498

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 50 μL 500 ng/mL mefenamic acid or indomethacin + 1 mL 100 mM HCl + 10 mL dichloromethane, rotate for 10 min, centrifuge at 1500 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°. Redissolve the residue in mobile phase, inject a 20 μL aliquot. Urine. 50 μL Urine + 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 75 × 4.6 3 μm Supelcosil LC-8
Mobile phase: MeCN:50 mM phosphoric acid 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM

Retention time: 2.2
Internal standard: mefenamic acid (8) or indomethacin (5)
Limit of detection: 50-250 ng/mL

OTHER SUBSTANCES

Simultaneous: naproxen, flunixin, ethacrynic acid, phenylbutazone

KEY WORDS

plasma

REFERENCE

Singh,A.K.; Jang,Y.; Mishra,U.; Granley,K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicyclic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *568*, 351–361.

SAMPLE

Matrix: formulations
Sample preparation: 9.5 mL Contact lens solution + 0.5 mL 3 mg/mL methylparaben, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 7 μm Nucleosil C18 pre-column
Column: 7 μm Nucleosil C18
Mobile phase: MeOH:100 mM KH_2PO_4 adjusted to pH 3.5 with phosphoric acid 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 10.9
Internal standard: methyl paraben (7.8)

OTHER SUBSTANCES

Simultaneous: chlorhexidine gluconate, thimerosal

Interfering: 2,2-dithisalicylic acid

KEY WORDS
contact lens solutions

REFERENCE
Hu,O.Y.-P.; Wang,S.-Y.; Fang,Y.-J.; Chen,Y.-H.; King,M.-L. Simultaneous determination of thimerosal and chlorhexidine in solutions for soft contact lenses and its applications in stability studies, *J.Chromatogr.*, **1990**, *523*, 321–326.

SAMPLE
Matrix: formulations
Sample preparation: Inject directly or extract as follows. Condition a Sep-Pak C18 SPE cartridge with MeOH and water. 10 mL Ophthalmic solution + 100 µL concentrated phosphoric acid, add to the SPE cartridge, wash with 5 mL water, wash with 2 mL MeOH:water 20:80, elute with 2 mL MeOH, dilute the eluate to 10 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4 5 µm Spherisorb C18
Mobile phase: MeOH:water 50:50 containing 2 mM tetraethylammonium perchlorate, adjusted to pH 4.8 with perchloric acid
Flow rate: 1
Injection volume: 20
Detector: E, Metrohm Model 461, Metrohm Model 656 flow cell, carbon paste electrode, 0.9 V

CHROMATOGRAM
Retention time: 3
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Simultaneous: 2,2'-dithiodibenzoic acid, thimerosal

KEY WORDS
ophthalmic solutions; SPE

REFERENCE
del Pilar da Silva,M.; Procopio,J.R.; Hernández,L. Evaluation of the capability of different chromatographic systems for the monitoring of thimerosal and its degradation products by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.A*, **1993**, *653*, 267–273.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 10 µm Spherisorb 10 ODS
Mobile phase: MeOH:water:phosphoric acid 60:50:1
Flow rate: 2.6
Injection volume: 25
Detector: UV 222

CHROMATOGRAM
Retention time: 3
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: 2,2'-dithiosalicylic acid, thimerosal

REFERENCE
Reader,M.J.; Lines,C.B. Decomposition of thimerosal in aqueous solution and its determination by high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 1406–1409.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 210 × 4.6 5 μm Spherisorb RP-18
Mobile phase: MeOH:water:phosphoric acid 65:35:0.9
Flow rate: 0.6
Injection volume: 20
Detector: UV 222

CHROMATOGRAM
Retention time: 5.8
Limit of quantitation: 0.15 ppm

OTHER SUBSTANCES
Simultaneous: degradation products, dithiosalicylic acid, thimerosal

REFERENCE
Caraballo,I.; Rabasco,A.M.; Fernández-Arévalo,M. Study of thimerosal degradation mechanism, *Int.J.Pharm.*, **1993**, *89*, 213–221.

SAMPLE
Matrix: solutions

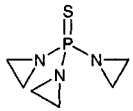
HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic

acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Thiotepa



Molecular formula: $C_6H_{12}N_3PS$
Molecular weight: 189.22
CAS Registry No.: 52-24-4
Merck Index: 9805

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 2.2 mL water, add 3 mL to an Extrelut 3 SPE cartridge, let stand for 15 min, elute with chloroform. Collect the first 8 mL of effluent, evaporate to dryness under a stream of nitrogen at 20°, reconstitute the residue in 500 µL 1-propanol. Remove a 100 µL aliquot and add 10 µL reagent, heat at 80° for 30 min, cool in an ice bath, add 400 µL taurine solution, add 400 µL OPA solution, let stand for 10 min, inject a 20 µL aliquot. (Reagent was prepared by mixing equal volumes of 80 mM sodium sulfide solution and 100 mM disodium EDTA, prepare fresh daily. Taurine solution was 0.2 mM taurine in 100 mM pH 8.0 phosphate buffer. OPA solution was 0.3 mM o-phthalaldehyde in 100 mM pH 8.0 phosphate buffer.)

HPLC VARIABLES
Column: 250 × 4 5 µm LiChrosorb RP-18
Mobile phase: MeCN:100 mM pH 5.7 phosphate buffer 28:72
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 440

CHROMATOGRAM
Retention time: 17.0
Limit of detection: 10 mg/mL

OTHER SUBSTANCES
Extracted: metabolites, TEPA

KEY WORDS
rabbit; plasma; SPE; derivatization; pharmacokinetics

REFERENCE
Sano,A.; Matsutani,S.; Takitani,S. High-performance liquid chromatography of the anti-tumour agent triethylenethiophosphoramide and its metabolite triethylenephosphoramide with sodium sulphide, taurine and o-phthalaldehyde as pre-column fluorescent derivatization reagents, *J.Chromatogr.*, **1988**, *458*, 295–301.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 10 mL MeOH and 10 mL water. Add 1 mL plasma to the SPE cartridge, wash with 10 mL water, wash with 1 mL MeCN: water 20:80, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 500 μL 20% MeCN, filter (0.22 μm), inject a 100 μL aliquot. (Use preservative-free heparin when collecting blood.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS II
Mobile phase: MeCN:water 20:80
Flow rate: 1
Injection volume: 100
Detector: UV 200

CHROMATOGRAM
Retention time: 11.4
Limit of quantitation: 25 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Tinsley,P.W.; O'Dwyer,P.J.; LaCreta,F.P. High-performance liquid chromatographic analysis of N,N',N"-triethylenethiophosphoramide in human plasma, *J.Chromatogr.*, **1989**, *495*, 318–323.

SAMPLE
Matrix: formulations
Sample preparation: Dilute sample with water.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Spherisorb CN5
Mobile phase: MeOH:water 18:82
Flow rate: 1.2
Injection volume: 10
Detector: UV 216

CHROMATOGRAM
Retention time: 6.2

KEY WORDS
injections; stability-indicating

REFERENCE
Xu,Q.A.; Trissel,L.A.; Zhang,Y.; Martinez,J.F.; Gilbert,D.L. Stability of thiotepa (lyophilized) in 5% dextrose injection at 4 and 23°C, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2728–2730.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute thiotepa injection with water to a thiotepa concentration of 10 mg/mL. Filter through a 0.22 μm filter. Dilute with 5% dextrose injection to a thiotepa concentration of 500 μg/mL or 5 mg/mL. Dilute a 5 mg/mL solution 10-fold with water. Inject a 10 μL aliquot. Inject a 10 μL aliquot of the 500 μL/mL solution directly.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Spherisorb CN5 (Alltech)

Mobile phase: MeOH:water 18:82
Flow rate: 1.2
Injection volume: 10
Detector: UV 216

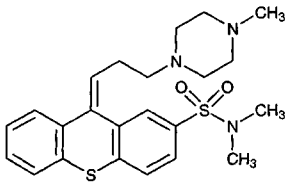
CHROMATOGRAM
Retention time: 6.2

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Xu,Q.A.; Trissel,L.A.; Zhang,Y.; Martinez,J.F.; Gilbert,D.L. Stability of thiotepa (lyophilized) in 5% dextrose injection at 4 and 23°C, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2728–2730.

Thiothixene



Molecular formula: $C_{23}H_{29}N_3O_2S_2$
Molecular weight: 443.63
CAS Registry No.: 5591-45-7, 22189-31-7 (HCl dihydrate), 49746-09-0 (Z HCl dihydrate), 58513- 59-0 (HCl), 49746-04-5 (Z HCl)
Merck Index: 9503
Lednicer No.: 1 400

SAMPLE
Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES
Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.0
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promethazine, thioridazine, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine
Interfering: promazine, imipramine

KEY WORDS
plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane: isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: k' 3.39
Internal standard: loxapine (k' 7.18)

OTHER SUBSTANCES

Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, diazepam, doxepin, fluphenazine, haloperidol, imipramine, oxazepam, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine
Interfering: haloperidol, nortriptyline

KEY WORDS

plasma

REFERENCE

Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 10 μL 10 μg/mL trans-thiothixene in water + 1 mL 2 M pH 9.8 sodium carbonate + 5 mL hexane:isoamyl alcohol 98.5:1.5, vortex twice for 15 s each time, centrifuge at 700 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, vortex for 15 s, centrifuge for 5 min, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb cyanopropyl
Mobile phase: MeCN:MeOH:10 mM pH 7.0 KH_2PO_4 48:12:40
Flow rate: 2
Injection volume: 20
Detector: UV 229

CHROMATOGRAM

Retention time: 3
Internal standard: trans-thiothixene (4)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites amitriptyline, amoxapine, benztropine, chlordiazepoxide, chlorohaloperidol, chlorpromazine, clozapine, desipramine, desmethyldiazepam, desmethyldoxepin, diazepam, doxepin, fluphenazine, flurazepam, haloperidol, imipramine, loxapine, maprotiline, mesoridazine, nortriptyline, protriptyline, thioproperazine, thioridazine, trazodone, trimipramine

KEY WORDS

plasma

REFERENCE

Narasimhachari,N.; Dorey,R.C.; Landa,B.L.; Friedel,R.O. Improved high-performance liquid chromatographic method for the quantitation of cis-thiothixene in plasma samples using trans-thiothixene as internal standard, *J.Chromatogr.*, **1984**, *311*, 424–429.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 100 ng/mL trifluoperazine dihydrochloride in 50 mM HCl + 200 μL concentrated ammonium hydroxide + 7 mL n-pentane:isopropanol 95:5, shake horizontally for 30 min, centrifuge at 2000 g. Remove the top organic layer and add it to 2 mL 100 mM perchloric acid, agitate for 10 min, centrifuge. Remove the aqueous layer and add it to 200 μL concentrated ammonium hydroxide, add 6 mL n-pentane:isopropanol 95:5, agitate for 30 min, centrifuge. Remove the top organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μL MeCN, inject a 20-40 μL aliquot. (Clean glassware scrupulously by soaking overnight in 50 mL/L Contrad (Curtin Matheson), rinse several times with water, and air dry.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano

Mobile phase: MeCN:10 mM pH 2.5 KH_2PO_4 60:40

Flow rate: 2.5

Injection volume: 20-40

Detector: E, Environmental Science Associates Coulochem Model 5100A, Model 5100 guard cell +0.85 V (between pump and injector), Model 5010 analytical cell +0.8 V, preanalytical cell +0.3 V

CHROMATOGRAM

Retention time: 6.4

Internal standard: trifluoperazine dihydrochloride (8.2)

Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, chlorpromazine, desipramine, doxepin, haloperidol, imipramine, loxapine, mesoridazine, nortriptyline, pheniramine, phenylephrine, prochlorperazine, promazine, promethazine, trazodone, trimeprazine, tripelennamine

Noninterfering: diazepam, diphenhydramine, ethopropazine, fluoxetine, nordiazepam, oxazepam, phenylpropanolamine, pseudoephedrine

Interfering: fluphenazine, perphenazine, thioridazine, triflupromazine

KEY WORDS

plasma

REFERENCE

Hariharan,M.; VanNoord,T.; Kindt,E.K.; Tandon,R. A simple, sensitive liquid chromatographic assay of cis-thiothixene in plasma with coulometric detection, *Ther.Drug Monit.*, **1991**, *13*, 79–85.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Whole blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge,

wash with water, wash with 1 mM pH 3.3 acetic acid, dry under suction, wash with 2 mL acetone:chloroform 50:50, elute with 3 mL freshly prepared ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm Asahipak ODP-50
Mobile phase: MeCN:50 mM ammonium acetate 85:15
Flow rate: 0.6
Injection volume: 10
Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, Finnigan MAT TSP-2 interface, collision gas argon 2.5 mTorr, collision offset -15 V, repeller 70 V, vaporizer 130-5°, source 200°, filament off, multiplier 1500 V, dynode power 15 kV, scantime 1.20 s, MSMSC factor 0, monitor 444–335. (The effluent from the column was mixed with 50 mM ammonium acetate pumped at 0.6 mL/min. The mixture flowed to the detector.)

CHROMATOGRAM

Retention time: 2.50
Limit of detection: 5 ng

OTHER SUBSTANCES

Extracted: chlorprothixene, flupenthixol, zuclopenthixol

KEY WORDS

whole blood; SPE

REFERENCE

Verweij,A.M.A.; Hordijk,M.L.; Lipman,P.J.L. Quantitative liquid chromatography, thermospray/tandem mass spectrometric (LC/TSP/MS/MS) analysis of some tranquilizers of the thioxanthene group in whole-blood, *J.Liq.Chromatogr.*, **1994**, *17*, 4009–4110.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 17.62
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Capsules. Shake the contents of a capsule with mobile phase for 30 min, make up to 100 mL with mobile phase, filter, dilute as necessary with mobile phase, inject an aliquot. Bulk. Prepare a 30-35 μg/mL aliquot in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm SiAl (ES Industries)
Mobile phase: MeCN:water:buffer 60:20:20 (Buffer was 3.15 g $LiOH.H_2O$ in 950 mL water, adjusted to pH 5.5 ± 0.05 with phosphoric acid, and made up to 1 L with water.) (A column of 18 μm silica (Supelco 5-8411) was placed between the pump and the injection valve.)
Flow rate: 3
Injection volume: 10
Detector: UV 225

CHROMATOGRAM
Retention time: 1.7

OTHER SUBSTANCES
Simultaneous: E-thiothixene, impurities

KEY WORDS
capsules; stability-indicating

REFERENCE
Severin,G. Comprehensive high-performance liquid chromatographic methodology for the determination of thiothixene in bulk drug, finished product, and dissolution testing samples, *J.Pharm.Sci.*, **1987**, *76*, 231–234.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Lichrosorb SI60
Mobile phase: MeCN:MeOH:ammonium hydroxide 250:55:13
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 3.5
Internal standard: perazine (5)

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, butaperazine, chlorimipramine, chlorpromazine, codeine, desipramine, dimethacrine, diphenhydramine, disopyramide, doxepin, hydroquinidine, maprotiline, melitracene, mesoridazine, nortriptyline, opipramol, perphenazine, procainamide, prochlorperazine, promazine, prothipendyl, protriptyline, quinidine, thioperazine, thioridazine, trifluoperazine

Noninterfering: acenocoumaron, acetaminophen, acetophenetidine, aspirin, benzodiazepines, bibenzepin, butriptyline, caffeine, chlorprothixene, clopenthixol, clothiapine, dixyrazine, droperidol, fluphenazine, haloperidol, hydroxyzine, isoniazid, methotrimeprazine, metopimazine, moperone, noxiptyline, orphenadrine, pericyazine, phenprocoumon, pipothiazine, promethazine, salicylic acid, theophylline, thiopropazate, trimeprazine, trimipramine
Interfering: imipramine, pipamperone, thiethylperazine, amitriptyline

KEY WORDS
maprotiline prevents adsorption on glass

REFERENCE
Edelbroek,P.M.; de Haas,E.J.M.; de Wolff,F.A. Liquid-chromatographic determination of amitryptyline and its metabolites in serum, with adsorption onto glass minimized, *Clin.Chem.*, **1982**, *28*, 2143–2148.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine,

thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Make up a solution in mobile phase, inject a 120 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Alltech RP-18 guard column
Column: 250 × 4.6 5 μm Supelcosil LC18-DB
Mobile phase: MeOH:MeCN:water:1 M ammonium hydroxide 50:35:15:0.3, adjusted to pH 6.7-8.0 with 6 M HCl
Flow rate: 1
Injection volume: 120
Detector: UV 263

CHROMATOGRAM

Retention time: 5.6

OTHER SUBSTANCES

Simultaneous: chlorpromazine, amitriptyline, clozapine, chlordiazepoxide, thioridazine, mesoridazine, sulforidazine, fluoxetine
Noninterfering: flurazepam, methylphenidate, ephedrine
Interfering: desipramine, nortriptyline, imipramine, maprotiline

REFERENCE

Jortani,S.A.; Poklis,A. Determination of thioridazine enantiomers in human serum by sequential achiral and chiral high-performance liquid chromaqtography, *J.Anal.Toxicol.*, **1993**, *17*, 374–377.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM

Retention time: 14

OTHER SUBSTANCES

Simultaneous: mesoridazine, promazine, chlorpromazine, trifluoperazine, thioridazine
Also analyzed: amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 8.3

OTHER SUBSTANCES
Simultaneous: trifluprομazine, carphenazine, methotrimeprazine, promazine, perphenazine, chlorprothixene, deserpidine, reserpine
Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam,

mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 16.49 (A), 6.57 (B)

OTHER SUBSTANCES

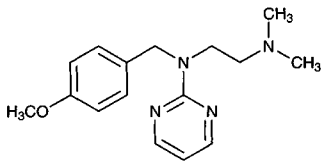
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, phen-

iramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Thonzylamine



Molecular formula: $C_{16}H_{22}N_4O$
Molecular weight: 286.38
CAS Registry No.: 63-56-9
Merck Index: 9513
Lednicer No.: 1 52

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.8

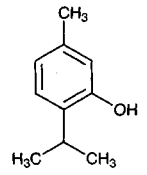
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamyl-

amine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Thymol



Molecular formula: $C_{10}H_{14}O$
Molecular weight: 150.22
CAS Registry No.: 89-83-8, 528-79-0 (acetate)
Merck Index: 9540

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 272

OTHER SUBSTANCES

Also analyzed: amitriptyline, chlorpromazine, promazine, clomipramine, promethazine

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

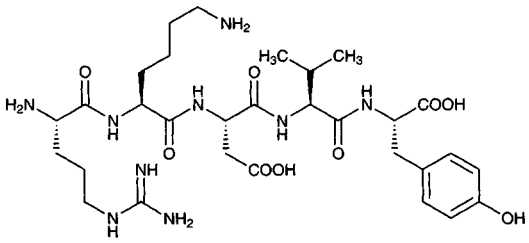
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Thymopentin

Molecular formula: $C_{30}H_{49}N_9O_9$
Molecular weight: 679.77
CAS Registry No.: 69558-55-0
Merck Index: 9544



SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge lymphocyte suspension for 10 s, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm C18 (Waters)
Mobile phase: MeCN:80 mM triethylammonium phosphate 4:96, pH 4.0 (Prepare buffer by adjusting 80 mM phosphoric acid to pH 4.0 with triethylamine.)
Flow rate: 1
Detector: UV 280

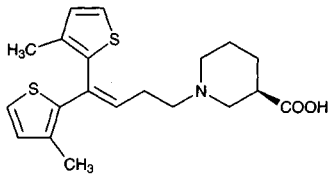
CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Amoscato,A.A.; Balasubramaniam,A.; Alexander,J.W.; Babcock,G.F. Degradation of thymopentin by human lymphocytes: evidence for aminopeptidase activity, *Biochim.Biophys.Acta*, **1988**, *955*, 164–174.

Tiagabine

Molecular formula: $C_{20}H_{25}NO_2S_2$
Molecular weight: 375.56
CAS Registry No.: 115103-54-3
Merck Index: 9557



SAMPLE
Matrix: bulk
Sample preparation: Weigh out approximately 50 mg tiagabine, add 5 to 10 drops of MeOH, make up to 25 mL with isopropanol, inject an aliquot.

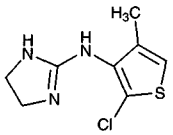
HPLC VARIABLES
Column: 250 × 4.6 Chiracel-OD (Daicel)
Mobile phase: EtOH:hexane:isopropanol:trifluoroacetic acid 6:80:14:0.5
Flow rate: 0.8
Injection volume: 10
Detector: UV 260

CHROMATOGRAM
Retention time: 10.1 (S(+)), 14.2 (R(-))

KEY WORDS
chiral

REFERENCE
Rustum,A.M.; Estrada,V. Separation and quantitation of the S-(+)-enantiomer in the bulk drug tiagabine.HCl by chiral high-performance-liquid chromatography using a Chiralcel-OD column, *J.Chromatogr.B*, **1998**, *705*, 111–117.

Tiamenidine



Molecular formula: $C_8H_{10}ClN_3S$
Molecular weight: 215.71
CAS Registry No.: 31428-61-2, 51274-83-0 (HCl)
Merck Index: 9558
Lednicer No.: 3 137

SAMPLE
Matrix: solutions

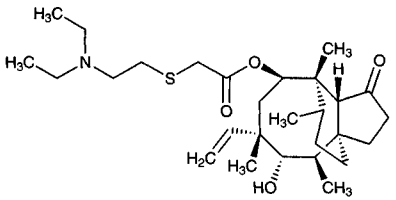
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.72

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Tiamulin



Molecular formula: $C_{28}H_{47}NO_4S$
Molecular weight: 493.75
CAS Registry No.: 55297-95-5, 55297-96-6 (fumarate)
Merck Index: 9559

SAMPLE
Matrix: feed
Sample preparation: 50 g Milled feed + 250 mL 1% sodium carbonate + 250 mL hexane:ethyl acetate 75:25, homogenize (Silverson) for 1 min, centrifuge at 2000 rpm for 5 min. Remove a 10 mL aliquot of the upper solvent layer and add it to 10 mL 0.1% tartaric acid, rotate for 1 min, centrifuge at 2000 rpm for 5 min, inject a 0.4-3 mL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil-ODS
Mobile phase: MeCN:MeOH:1% ammonium carbonate 30:60:20
Flow rate: 1.5
Injection volume: 400-3000
Detector: UV 250

CHROMATOGRAM
Retention time: 12
Limit of detection: <5 μg/g

OTHER SUBSTANCES
Noninterfering: furazolidone, monensin, sulfadimidine

REFERENCE
Howard,D.; Cowen,T. Determination of tiamulin hydrogen fumarate in animal feeds using high-performance liquid chromatography, *Analyst*, **1982**, *107*, 319–323.

SAMPLE
Matrix: feed, formulations, premix
Sample preparation: Premix. 5 g Premix + 250 mL 1% sodium carbonate, shake for 30 min, add 250 mL hexane:ethyl acetate 75:25, shake for 1 h, shake vigorously by hand for 15-20 s, let stand until layers separate (centrifuge if necessary). Remove a 10 mL aliquot of the upper organic layer and add it to 30 mL 0.1% tartaric acid, shake gently horizontally for 30 s, inject a 50 μL aliquot of the lower aqueous layer. Formulations. Weigh out 660 mg formulation, add 100 mL water, shake or sonicate for 1 h, filter (44 μm) an aliquot, inject an aliquot of the filtrate (J. AOAC Int. 1993, 76, 447). Feed. 50 g Feed + 250 mL hexane:ethyl acetate 75:25 + 250 mL 1% sodium carbonate, shake in an orbital shaker at 250 rpm for 1 h, let stand for 1 h, centrifuge the supernatant at 1500 rpm for 15 min. Remove a 75 mL aliquot of the upper organic layer and add it to 5 mL 0.1% tartaric acid, shake gently horizontally for 30 s, repeat extraction twice more, combine the aqueous layers, inject a 50 μL aliquot of the lower aqueous layer (J. AOAC Int. 1993, 76, 449).

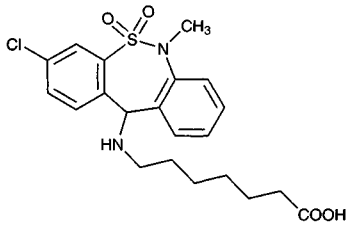
HPLC VARIABLES
Guard column: CO:PELL ODS
Column: 250 × 4.6 5 μm Hypersil ODS (premix, feed) or 300 × 3.9 10 μm C18 (formulations)
Mobile phase: MeCN:MeOH:1% ammonium carbonate 30:60:25 (Pass mobile phase through a column of 200-425 mesh Adsorbosil silica (Alltech) before injector. Periodically wash column with 60 mL MeOH:1% acetic acid and 30 mL MeOH, then re-equilibrate with mobile phase.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7

KEY WORDS
powders

REFERENCE
Markus,J.R.; Sherma,J. Method. I. Liquid chromatographic determination of tiamulin hydrogen fumarate in feed premixes, *J.AOAC Int.*, **1993**, *76*, 444–446.

Tianeptine



Molecular formula: $C_{21}H_{25}ClN_2O_4S$
Molecular weight: 436.96
CAS Registry No.: 66981-73-5
Merck Index: 9560

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 269

CHROMATOGRAM

Retention time: 5.40

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidra-

mine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Plasma, urine. Centrifuge urine at 900 g. 2 mL Plasma or centrifuged urine + 1 mL 50 (plasma) or 500 (urine) mM pH 7.0 phosphate buffer + 100 μL 10 μg/mL IS in water + 10 mL heptane:octanol 98:2 containing 5 g/L tetraheptylammonium bromide, shake for 10 min, centrifuge at 900 g for 10 min. Remove an 8 mL aliquot of the upper aqueous phase and add it to 200 μL MeOH:170 mM acetic acid 10:90, shake on a rotary agitator at 10 rpm for 5 min, centrifuge at 900 g for 5 min, inject a 50 μL aliquot of the aqueous phase. Brain. Homogenize (Ultra Turrax) 1 g brain tissue and 2 mL 50 mM pH 7.0 phosphate buffer for 30 s, vortex for 15 s, centrifuge. 2 mL Supernatant + 1 mL 50 mM pH 7.0 phosphate buffer + 100 μL 10 μg/mL IS in water + 10 mL heptane:octanol 98:2 containing 5 g/L tetraheptylammonium bromide, shake for 10 min, centrifuge at 900 g for 10 min. Remove an 8 mL aliquot of the upper aqueous phase and add it to 200 μL MeOH:170 mM acetic acid 10:90, shake on a rotary agitator at 10 rpm for 5 min, centrifuge at 900 g for 5 min, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil ODS

Mobile phase: MeCN:buffer 45:55 (Buffer was 2.7 g/L sodium pentanesulfonate adjusted to pH 3.0 with phosphoric acid.)

Flow rate: 1.3

Injection volume: 50

Detector: UV 220

CHROMATOGRAM

Retention time: 4.5

Internal standard: (dihydro-10,11-dibenzo[a,d]cycloheptenyl-5-amino)-7-octanoic acid (Servier Labs) (6.0)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: bromazepam, clobazam, clonazepam, clorazepate, desmethyldiazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, triazolam

Noninterfering: clomipramine, desipramine, desmethylclomipramine, imipramine, medazepam, meprobamate, nortriptyline, protriptyline, tetrazepam, trimipramine

Interfering: amineptine, diazepam

KEY WORDS

plasma; human; rat; brain; pharmacokinetics

REFERENCE

Nicot,G.; Lachatre,G.; Gonnet,C.; Mallon,J.; Mocaer,E. Ion-pair extraction and high-performance liquid chromatographic determination of tianeptine and its metabolites in human plasma, urine and tissues, *J.Chromatogr.*, **1986**, *381*, 115–126.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the

organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 14.877

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

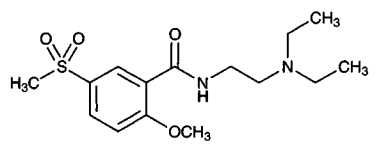
SAMPLE
Matrix: urine
Sample preparation: Inject directly.

HPLC VARIABLES
Column: 125 × 4.8 10 μm Lichrosorb RP 18
Mobile phase: Gradient. MeCN:10 mM pH 5.5 phosphate buffer from 0:100 to 100:0 over 105 min.
Flow rate: 1
Injection volume: 72
Detector: radioactivity

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Grislain,L.; Gele,P.; Bertrand,M.; Luijten,W.; Bromet,N.; Salvadori,C.; Kamoun,A. The metabolic pathways of tianeptine, a new antidepressant, in healthy volunteers, *Drug Metab.Dispos.*, **1990**, *18*, 804–808.

Tiapride



Molecular formula: $C_{15}H_{24}N_2O_4S$
Molecular weight: 328.43
CAS Registry No.: 51012-32-9, 51012-33-0 (HCl)
Merck Index: 9561

SAMPLE
Matrix: blood

Sample preparation: Mix 1 mL plasma with 4 mL 500 mM NaOH and 2 g NaCl. Add 10 mL MTBE, shake for 10 min and centrifuge at 850 g for 10 min. Remove the solvent layer, mix with 2.5 mL 100 mM HCl, shake for 10 min and centrifuge at 850 g for 10 min. Remove the aqueous layer, add it to 1 mL 500 mM NaOH, 1.5 g NaCl and 2 mL MTBE, shake for 10 min and centrifuge. Remove the solvent layer and evaporate to dryness under a stream of nitrogen. Reconstitute the residue in 50 μL MeCN and inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 I.D. 5 μm Hypersil
Mobile phase: MeCN:100 mM ammonium acetate 94:6
Column temperature: 40
Flow rate: 0.4
Injection volume: 10
Detector: UV 240; MS, Hewlett-Packard Model 59980A, particle beam nebulizer helium 35 psi, solvation chamber 60°, Model 5989A, NICI mode, reagent gas methane at 1 torr, source 250°, negative ion chemical ionization mode, reagent gas methane at 1 torr, source 250 .deg., m/z 313.

CHROMATOGRAM
Retention time: 11.0
Internal standard: tiapride

OTHER SUBSTANCES
Extracted: sultopride

KEY WORDS
plasma; tiapride is IS

REFERENCE
Jitsufuchi,N.; Kudo,K.; Tokunaga,H.; Imamura,T. Selective determination of sultopride in human plasma using high-performance liquid chromatography with ultraviolet detection and particle beam mass spectrometry, *J.Chromatogr.B*, **1997**, *690*, 153–159.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

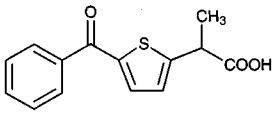
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 5.468

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tiaprofenic acid



Molecular formula: $C_{14}H_{12}O_3S$
Molecular weight: 260.31
CAS Registry No.: 33005-95-7
Merck Index: 9562

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 307

CHROMATOGRAM
Retention time: 4.34
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclam-

ide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 50 μL 100 μg/mL ketorolac + 100 μL 600 mM sulfuric acid + 3 mL isooctane:isopropanol 95:5, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL 2 mg/mL 4-(dimethylamino)pyridine in MeCN, add 100 μL 60 mM trichloroethyl chloroformate in MeCN, add 1 M L-leucinamide in MeCN, let stand for 2 min, add 500 μL 250 mM HCl, extract with chloroform. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10-100 μL aliquot. (A 7% conversion of S to R is observed during the derivatization procedure. No racemization is observed using a direct procedure with a chiral column.)

HPLC VARIABLES

Column: 100 × 4.6 5 μm Partisil 5 ODS-2
Mobile phase: MeCN:60 mM KH_2PO_4:triethylamine 30:70:0.02
Flow rate: 1
Injection volume: 10-100
Detector: UV 310

CHROMATOGRAM

Retention time: 17.2 (R), 19.5 (S)
Internal standard: ketorolac (10.7 (R), 19.5 (S))
Limit of quantitation: 50 ng/mL

KEY WORDS

derivatization; chiral; plasma; comparison with a method involving a chiral column; pharmacokinetics

REFERENCE

Vakily,M.; Jamali,F. Pharmacokinetics of tiaprofenic acid in humans: Lack of stereoselectivity in plasma using both direct and precolumn derivatization methods, *J.Pharm.Sci.*, **1996**, *85*, 638–642.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 2 mL Plasma + 50 μL 50 μg/mL IS in MeOH + 250 μL 1 M HCl + 5 mL diethyl ether, mix, centrifuge at 1100 g for 10 min. Remove the organic phase, repeat the extraction, combine the organic extracts, evaporate under nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, vortex, inject a 20 μL aliquot. Urine. 1 mL Urine + 250 μL 1 M HCl + 50 μL IS + 5 mL diethyl ether, rotate for 15 min. Remove the organic layer, add 1 mL 1% sodium hydrogen carbonate, vortex for 1 min. Remove the organic layer and evaporate it under nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 3 5 μm Nucleosil
Mobile phase: MeCN:water:acetic acid 40:59.4:0.6

Column temperature: 35
Flow rate: 0.8
Injection volume: 20
Detector: UV 310

CHROMATOGRAM
Retention time: 2.7
Internal standard: feprazone (8.3, UV 245))
Limit of quantitation: 100 ng/mL (plasma), 500 ng/mL (urine)

OTHER SUBSTANCES
Noninterfering: alclofenac, diclofenac, fenoprofen, flunixin, flurbiprofen, ibuprofen, indomethacin, naproxen, oxyphenbutazone, phenylbytazone, piroxicam

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Delbeke,F.T.; Baert,K.; De Backer,P. Disposition of human drug preparations in the horse. VI. Tiaprofenic acid, *J.Chromatogr.B*, **1997**, *704*, 207–214.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

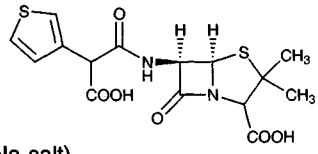
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 17.653

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ticarcillin



Molecular formula: $C_{15}H_{16}N_2O_6S_2$
Molecular weight: 384.43
CAS Registry No.: 34787-01-4, 4697-14-7 (di Na salt), 74682-62-5 (Na salt)
Merck Index: 9568
Lednicer No.: 2 437

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10

HPLC VARIABLES

Column: 75 × 4.6 3 μm octadecylsilane
Mobile phase: 36:64 MeCN:10 mM citrate buffer adjusted to pH 2 with HCl
Flow rate: 1
Injection volume: 5
Detector: UV 214

CHROMATOGRAM

Retention time: 1.6
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, piperacillin

KEY WORDS

serum

REFERENCE

Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE

Matrix: blood
Sample preparation: 350 μL Serum + 150 μL 150 μg/mL temocillin in water + 250 μL 400 mM HCl + 3.5 mL chloroform:n-amyl alcohol (3:1), mix for 5 min, centrifuge for 5 min. Remove the organic layer and add it to 350 μL 10 mM pH 7.0 phosphate buffer, mix for 5 min, centrifuge for 5 min, inject a 20 μL aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 15:85 (Buffer was 100 mM ammonium acetate adjusted to pH 4.0 with glacial acetic acid.)
Flow rate: 1.8
Injection volume: 20
Detector: UV 242

CHROMATOGRAM

Retention time: 6.8
Internal standard: temocillin (5.4)

OTHER SUBSTANCES
Simultaneous: cefoxitin
Noninterfering: acetaminophen, acetazolamide, allopurinol, amikacin, ampicillin, azlocillin, caffeine, cefamandole, cefoperazone, cefotaxime, cefsulodin, ceftazidime, ceftizoxime, chloramphenicol, chlorpromazine, clindamycin, dicloxacillin, 5-fluorocytosine, flurazepam, gentamicin, methicillin, metronidazole, mezlocillin, moxalactam, nafcillin, penicillin, phenobarbital, piperacillin, procainamide, rifampin, sulfamethoxazole, theophylline, thienamycin, tobramycin, trimethoprim, vancomycin
Interfering: cefuroxime, cephalothin

KEY WORDS
serum

REFERENCE
Shull,V.H.; Dick,J.D. Determination of ticarcillin levels in serum by high-pressure liquid chromatography, *Antimicrob.Agents Chemother.*, **1985**, *28*, 597–600.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 200 μL Serum + 200 μL 10 M urea, mix, filter (Amicon MPS-1 micropartition system with Amicon YMT membranes) while centrifuging at 1500 g for 10 min. Add 200 μL of the ultrafiltrate to 200 μL reagent and heat at 60° for 10 min, cool to room temperature, inject a 30-90 μL aliquot. Urine. Dilute urine 10-fold with water, filter (0.45 μm acrylate copolymer). Add 200 μL of the filtrate to 200 μL reagent and heat at 60° for 10 min, cool to room temperature, inject a 30-60 μL aliquot. (Prepare reagent by dissolving 13.81 g 1,2,4-triazole in 60 mL water, add 10 mL 2.7 mg/mL mercury(II) chloride in water, adjust pH to 9.0 ± 0.05 with saturated NaOH, make up to 100 mL with water.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Develosil ODS-5 (Nomura Chemicals)
Mobile phase: MeCN:buffer containing 5 mM tetrabutylammonium bromide and 5 mM sodium thiosulfate 1:1.8 (Prepare the buffer by dissolving 14.32 g $Na_2HPO_4.12H_2O$ and 6.240 g $NaH_2PO_4.2H_2O$ in 1 L water then diluting 100-fold.)
Column temperature: 40
Flow rate: 1
Injection volume: 30-90
Detector: UV 328

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 1 μg/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES
Interfering: carbenicillin

KEY WORDS
serum; derivatization; ultrafiltrate; pharmacokinetics

REFERENCE
Haginaka,J.; Wakai,J. High-performance liquid chromatographic assay of carbenicillin, ticarcillin and sulbenicillin in serum and urine using pre-column reaction with 1,2,4-triazole and mercury(II) chloride, *Analyst*, **1985**, *110*, 1185–1188.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 500 μL 2 μg/mL cefoperazone in MeCN, vortex for 20 s, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 10-15 μL aliquot. Urine. Inject a 102-20 μL aliquot directly onto the column.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere C18

Mobile phase: MeCN:water:orthophosphoric acid:10% tetramethylammonium chloride 30:69.6:0.1:0.3
Flow rate: 1
Injection volume: 10-20
Detector: UV 205

CHROMATOGRAM
Retention time: 8.8
Internal standard: cefoperazone (6.8)
Limit of detection: 1000 ng/mL (urine), 500 ng/mL (plasma)

OTHER SUBSTANCES
Noninterfering: xanthines, aspirin, acetaminophen, cephalosporins, penicillins

KEY WORDS
plasma; pharmacokinetics

REFERENCE
La Follette,G.; Jayewardene,A.L.; Seneviratne,A.K.; Lin,E.T.; Gambertoglio,J.G. Determination of ticarcillin in human plasma by reversed-phase LC, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 159–164.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 300 μg/mL solution in 100 mM pH 7.0 phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 15.6 g $NaH_2PO_4.2H_2O$ in 900 mL water, adjust pH to 7.0 with NaOH, make up to 1 L with water.)
Flow rate: 2
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 10.5 (R), 12.0 (S)

OTHER SUBSTANCES
Simultaneous: isomers, temocillin

REFERENCE
Bird,A.E.; Charsley,C.H.; Jennings,K.R.; Marshall,A.C. High-performance liquid chromatographic assay of temocillin and epimerisation of its diastereoisomers, *Analyst*, **1984**, *109*, 1209–1212.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water (if necessary), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: 10 mM ammonium acetate
Flow rate: 1.6
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 4.5, 5 (two isomers)

OTHER SUBSTANCES
Interfering: carbenicillin

KEY WORDS
saline; 5% dextrose; stability-indicating

REFERENCE
Das Gupta,V.; Stewart,K.R. Quantitation of carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium by high-pressure liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 1264–1267.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 195

CHROMATOGRAM
Retention time: 3.07

OTHER SUBSTANCES
Simultaneous: clavulanic acid, granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 1 mL 1 M oxalic acid, heat at 60° for 10 min, centrifuge for 10 min, remove the supernatant and add it to 20 mL water and 400 μL tributylamine, shake well, add to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

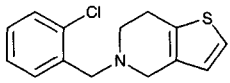
HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

OTHER SUBSTANCES
Extracted: amoxicillin, cefadroxil
Simultaneous: ampicillin, ceftiofur, cephapirin, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G

KEY WORDS
SPE

REFERENCE
Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

Ticlopidine



Molecular formula: $C_{14}H_{14}ClNS$
Molecular weight: 263.79
CAS Registry No.: 55142-85-3, 53885-35-1 (HCl)
Merck Index: 9569
Lednicer No.: 3 228

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge (Varian) with 1 mL MeOH and 1 mL 10 mM NaOH. Add 50 μL 10 μg/mL IS in MeOH and 1 mL 1 M NaOH to 1 mL plasma, vortex, add to SPE cartridge, wash with 4 mL MeOH:10 mM NaOH 50:50, dry by sucking air through the cartridge, elute with 500 μL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, dissolve the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4 BST Rutin 10 C18 (Bio-Separation Technologies, Hungary)
Column: 250 × 4 BST Rutin 10 C18 BD (BST Rutin C18 is equivalent to Hypersil BDS C18)
Mobile phase: MeCN:MeOH:10 mM pH 4 NaH_2PO_4 buffer 40:40:20
Flow rate: 1
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 11
Internal standard: 5-(2,4-o-dichlorobenzyl)(-4,5,6,7-tetrahydrotieno-[3,2-c] pyridine (18)
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Rona,K.; Ary,K.; Gachalyi,B.; Klebovich,I. Liquid chromatographic method for the determination of ticlopidine in human plasma, *J.Chromatogr.B*, **1997**, *693*, 393–398.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 250 μL water + 0.5 μg IS, vortex for 10 s, add to an Extrelut-1 SPE cartridge, let stand for 10 min, elute with 6.5 mL hexane. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL MeCN, inject a 50 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:10 mM pH 7.8 phosphate 30:70
Flow rate: 1.3
Injection volume: 50
Detector: UV 235

CHROMATOGRAM
Retention time: 7.7

Internal standard: 5-[2,4-(o-dichlorobenzyl)]-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (PCR 2735) (11.2)
Limit of detection: 5 ng/mL
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma; baboon; pharmacokinetics; SPE

REFERENCE
Arnoux,P.; Sales,Y.; Mandray,M.; Lechat,P.; Berger,Y.; Cano,J.-P. Quantitative high-performance liquid chromatographic, gas chromatographic, and gas chromatographic-mass spectrometric analysis of ticlopidine in baboon plasma after solid-phase extraction, *J.Pharm.Sci.*, **1991**, *80*, 1092–1095.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 20 μg/mL imipramine hydrochloride in MeOH + 1 mL 500 mM pH 9 phosphate buffer, vortex briefly, add 7 mL n-heptane:isoamyl alcohol 98.5:1.5, shake on a rotating shaker at 32 rpm for 15 min, centrifuge at 1500 g for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex for 10 s, centrifuge at 1500 g for 3 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-8-DB (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 20:25:55 (Buffer was 50 mM pH 3.0 KH_2PO_4 containing 0.2% triethylamine.)
Flow rate: 1
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 7.6
Internal standard: imipramine hydrochloride (11.6)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Dal Bo,L.; Verga,F.; Marzo,A.; Ambrosoli,L.; Poli,A. Determination of ticlopidine in human plasma by high-performance liquid chromatography and ultraviolet absorbance detection, *J.Chromatogr.B*, **1995**, *665*, 404–409.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 234

CHROMATOGRAM
Retention time: 4.91
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

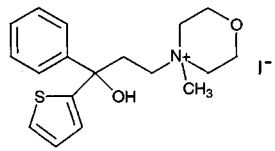
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.813

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tiemonium iodide



Molecular formula: $C_{18}H_{24}INO_2S$
Molecular weight: 445.36
CAS Registry No.: 144-12-7
Merck Index: 9571

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

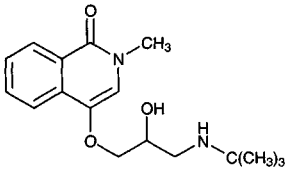
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.795

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tilisolol



Molecular formula: $C_{17}H_{24}N_2O_3$
Molecular weight: 304.39
CAS Registry No.: 85136-71-6
Merck Index: 9579

SAMPLE

Matrix: aqueous humor, blood, vitreous body
Sample preparation: Aqueous humor, vitreous body. Mix 200 µL aqueous humor or vitreous body with 20 µL 1 M HCl and 300 µL 100 µg/mL o-ethoxybenzamide in MeOH. Centrifuge at 12000 g for 15 min and inject a 50 µL aliquot of the supernatant. Plasma. Mix 700 µL plasma with 300 µL 2 M perchloric acid and centrifuge at 12000 g for 15 min. Shake 800 µL supernatant with 200 µL 5 M NaOH and 6 mL chloroform for 15 min, centrifuge at 650 g for 15 min. Remove a 5 mL aliquot of the organic layer and mix with 100 µL 100 µg/mL o-ethoxybenzamide in MeOH, evaporate under reduced pressure. Dissolve residue in 250 µL MeOH: phosphate-buffered saline 20:80, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5 C18-P
Mobile phase: MeOH:50 mM NaH_2PO_4 37:63
Flow rate: 1
Injection volume: 50
Detector: F ex 315 em 420

CHROMATOGRAM

Internal standard: o-ethoxybenzamide

KEY WORDS

rabbit; plasma

REFERENCE

Sasaki,H.; Ichikawa,M.; Kawakami,S.; Yamamura,K.; Nishida,K.; Nakamura,J. In situ ocular absorption of tilisolol through ocular membranes in albino rabbits, *J.Pharm.Sci.*, **1996**, *85*, 940–943.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M perchloric acid, shake for a few min, centrifuge at 1600 g for 10 min. Remove a 1.5 mL aliquot of the supernatant and add it to 500 µL 6 M NaOH and 5 mL chloroform, vortex for 1 min, centrifuge at 700 g for 5 min. Remove 4 mL of the chloroform extract and add it to 1 mL 400 ng/mL reserpine in chloroform, evaporate to dryness under reduced pressure, reconstitute with 200 µL MeOH, inject a 40 µL aliquot.

HPLC VARIABLES

Column: 400 × 3 5 µm Nucleosil C18
Mobile phase: MeCN:MeOH:acetic acid:water 30:10:0.4:15
Flow rate: 1.2
Injection volume: 40
Detector: F ex 313 em 420

CHROMATOGRAM

Retention time: 8.4
Internal standard: reserpine (14.7)
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; dog; human; pharmacokinetics

REFERENCE

Yonezawa,K.; Sato,K.; Kobayashi,A. High-performance liquid chromatography of a new β-blocker, 4-[3-(tert.-butylamino)-2-hydroxypropoxy]-N-methylisocarbostyril hydrochloride, in plasma using fluorometric detection, *J.Chromatogr.*, **1985**, *339*, 219–222.

SAMPLE

Matrix: perfusate
Sample preparation: 50 μL Perfusate + 50 μL pH 7.4 phosphate-buffered saline or 100 mM HCl + 100 μL 300 μg/mL o-ethoxybenzamide in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 37:63
Flow rate: 1
Injection volume: 50
Detector: F ex 315 em 420

CHROMATOGRAM

Internal standard: o-ethoxybenzamide

KEY WORDS

rabbit

REFERENCE

Sasaki,H.; Igarashi,Y.; Nagano,T.; Nishida,K.; Nakamura,J. Different effects of absorption promoters on corneal and conjunctival penetration of ophthalmic β-blockers, *Pharm.Res.*, **1995**, *12*, 1146–1150.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL Solution + 50 μL pH 7.4 PBS + 100 μL 300 μg/mL o-ethoxybenzamide in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 37:63
Flow rate: 1
Injection volume: 50
Detector: F ex 315 em 420

CHROMATOGRAM

Internal standard: o-ethoxybenzamide

KEY WORDS

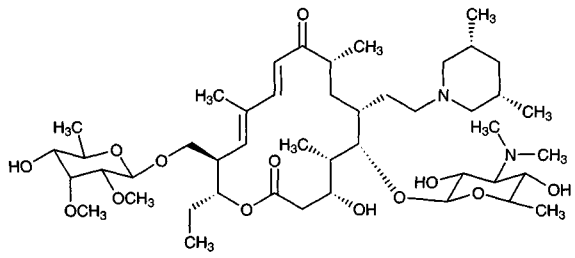
buffer; Earle's balanced salt solution

REFERENCE

Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

Tilmicosin

Molecular formula: $C_{46}H_{80}N_2O_{13}$
Molecular weight: 869.15
CAS Registry No.: 108050-54-0
Merck Index: 9580



cis-form

SAMPLE

Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm C18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
Flow rate: 0.5
Injection volume: 200
Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 677.4-869.6 in NCI and 869.6-679.4 in PCI

CHROMATOGRAM

Retention time: 5.7
Limit of detection: 50 μg/kg

OTHER SUBSTANCES

Extracted: erythromycin, josamycin, spiramycin, tylosin

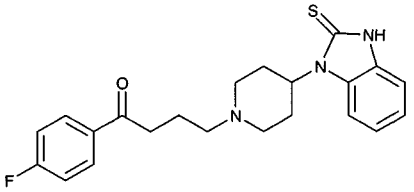
KEY WORDS

muscle; cow; SPE

REFERENCE

Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

Timiperone



Molecular formula: $C_{22}H_{24}FN_3OS$
Molecular weight: 397.52
CAS Registry No.: 57648-21-2
Merck Index: 9584

SAMPLE
Matrix: blood
Sample preparation: 500 µL Plasma + 100 µL 250 ng/mL IS in 100 mM pH 3.5 phosphate buffer + 500 µL 500 mM pH 8.5 phosphate buffer, extract with 2.5 mL heptane:isoamyl alcohol 98:2. Shake for 5 min, centrifuge at 1700 g for 10 min. Mix 2.0 mL organic layer with 100 µL 3 M acetic acid, shake for 20 min, centrifuge at 1700 g for 10 min. Aspirate organic layer, inject a 20 µL aliquot of the aqueous layer.

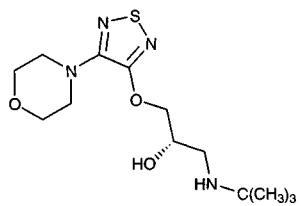
HPLC VARIABLES
Column: 150 × 4.6 5 µm EicomPak MA-5ODS (Eicom, Japan)
Mobile phase: MeCN:MeOH:buffer 20:15:65 containing 500 µg/L (?) disodium EDTA (Buffer was 100 mM KH_2PO_4 adjusted to pH 3.5 with 100 mM phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: E, Eicom ECD-100, glassy carbon electrode +1 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 11.3
Internal standard: spiperone (14.9)
Limit of quantitation: 500 pg/mL

KEY WORDS
plasma; rat

REFERENCE
Takayasu,T.; Kakubari,I.; Fukamachi,A.; Mafune,E.; Takasugi,N.; Takayama,K.; Nagai,T. Determination of timiperone in rat plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1996**, *679*, 161–165.

Timolol



Molecular formula: $C_{13}H_{24}N_4O_3S$
Molecular weight: 316.42
CAS Registry No.: 26839-75-8, 91524-16-2 (hemihydrate), 26921-17-5 maleate
Merck Index: 9585
Lednicer No.: 2 272

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 3 mL 500 mg Bakerbond C18 SPE cartridge with 2.5 mL MeOH and 2.5 mL water. Urine. 200 µL Urine + 800 µL 100 mM pH 7.4 phosphate buffer + 100 µL 50 mM HCl + 100 µL 20 ng/mL IS in 50 mM HCl, mix. Add to the SPE cartridge. Wash with 2.5 mL 100 mM pH 10.6 K_2HPO_4, then with 2.5 mL water. Elute with 2.5 mL MeOH, evaporate the eluate to dryness at 50°. Reconstitute in 100 µL MeCN:MeOH:0.1% TFA 27:7:66 sonicate for 20 min, inject an aliquot. Plasma. 1 mL Plasma + 100 µL 50 mM HCl + 100 µL 20 ng/mL IS in 50 mM HCl + 2 mL 100 mM pH 10.6 K_2HPO_4, mix. Add to the SPE cartridge. Wash with 2.5 mL 100 mM pH 10.6 K_2HPO_4 and with 2.5 mL water. Elute with 2.5 mL MeOH,

evaporate the eluate to dryness under reduced pressure at 50°. Add 20 μL 1% pyridine in THF to the residue, vortex, add 40 μL 20% phosgene in toluene, heat at 85° for 2 h. Remove the excess reagent under nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:0.1% TFA in water 36:6:58, sonicate for 20 min. Inject an aliquot. (Phosgene reacts with the hydroxy and secondary amino groups of timolol to form the oxazolidinone derivative.)

HPLC VARIABLES
Guard column: 20 Supelco Pelliguard LC-CN
Column: 50 × 4.6 3 μm Spherisorb CN
Mobile phase: MeCN:MeOH:0.1% TFA in water 45:5:50
Flow rate: 0.5
Injection volume: 10-50
Detector: MS, Sciex API IIIplus triple quadrupole, nebulizer 500°, nebulizing gas air 80 psi, curtain gas nitrogen, collision gas argon 50 eV, multiplier 3500 V, positive mode ionization

CHROMATOGRAM
Retention time: 5.53
Internal standard: N-isopropyl timolol (4.88)
Limit of quantitation: 200 pg/mL (plasma), 50 ng/mL (urine)

KEY WORDS
plasma; urine; dog; SPE; pharmacokinetics; derivatization

REFERENCE
Gilbert,J.D.; Olah,T.V.; Morris,M.J.; Bortnick,A.; Brunner,J. The use of stable isotope labeling and liquid chromatography-tandem mass spectrometry techniques to simultaneously determine the oral and ophthalmic bioavailability of timolol in dogs, *J.Chromatogr.Sci.*, **1998**, *36*, 163–168.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 296.1

CHROMATOGRAM
Retention time: 10.295

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate: benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane: acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25

mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylamino-sulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 35:65:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM
Retention time: 28.8, 29.9 (enantiomers)
Limit of detection: 0.00595-0.00642 fmole

OTHER SUBSTANCES
Also analyzed: atenolol, carteolol

KEY WORDS
derivatization; chiral

REFERENCE
Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

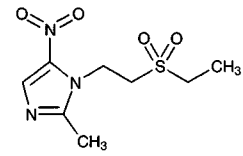
SAMPLE
Matrix: urine
Sample preparation: Mix 4 mL urine with 500μL 5 M NaOH, 4 mL diethyl ether and 1 g sodium sulfate. Shake the mixture mechanically for 15 min, centrifuge at 734 g for 5 min, separate, evaporate the diethyl layer to dryness at 60° under a gentle stream of nitrogen. Dissolve the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 30:70 containing 5 mM pH 6.5 KH_2PO_4/K_2HPO_4
Column temperature: 30
Flow rate: 1.3
Injection volume: 20
Detector: E, PAR Model 400, glassy carbon electrode + 1 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.47
Limit of quantitation: 10 ng/mL

REFERENCE
Maguregui,M.I.; Alonso,R.M.; Jiménez,R.M. A rapid quantitative analysis of the β-blocker timolol in human urine by HPLC-electrochemical detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1643–1652.

Tinidazole



Molecular formula: $C_8H_{13}N_3O_4S$
Molecular weight: 247.28
CAS Registry No.: 19387-91-8
Merck Index: 9588

SAMPLE

Matrix: blood
Sample preparation: Add 50 μL 1M NaOH to 1 mL plasma, add 7 mL dichloromethane, mix on a rotary agitator for 20 min and centrifuge at 1636 g for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute the dry residue by vortex agitation with 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm Kromasil C8 (Bischoff Chromatography, Germany)
Mobile phase: MeCN:MeOH:68 mM pH 3 phosphate buffer 15:20:65
Flow rate: 0.7
Injection volume: 50
Detector: UV 360

CHROMATOGRAM

Retention time: 6.10
Internal standard: tinidazole

KEY WORDS

human; rat; plasma; tinidazole is internal standard

REFERENCE

Enanga,B.; Labat,C.; Boudra,H.; Chauvière,G.; Keita,M.; Bouteille,B.; Dumas,M.; Houin,G. Simple high-performance liquid chromatographic method to analyse megazol in human and rat plasma, *J.Chromatogr.B*, **1997**, *696*, 261–266.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 317.6

CHROMATOGRAM

Retention time: 10.563

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: gastric juice
Sample preparation: Mix 500 μL gastric juice with 50 μL 50% perchloric acid (w/v), vortex briefly, add 1.5 g solid anhydrous potassium carbonate to neutral pH. Add 300 μL MeCN, centrifuge at 11600 g for 6 min., remove a 180 μL aliquot of the supernatant, evaporate under a stream of nitrogen at 50°, reconstitute the residue with 500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 10:90 (Buffer was 50 mM KH_2PO_4 containing 0.1% triethylamine, adjusted to pH 7.0 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 317

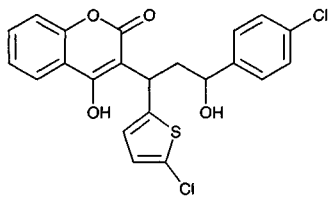
CHROMATOGRAM
Retention time: 13.9
Internal standard: tinidazole

OTHER SUBSTANCES
Extracted: metronidazole

KEY WORDS
tinidazole is IS

REFERENCE
Jessa,M.J.; Barrett,D.A.; Shaw,P.N.; Spiller,R.C. Rapid and selective high-performance liquid chromatographic method for the determination of metronidazole and its active metabolite in human plasma, saliva and gastric juice, *J.Chromatogr.B*, **1996**, *677*, 374–379.

Tioclomarol



Molecular formula: $C_{22}H_{16}Cl2O_4S$
Molecular weight: 447.34
CAS Registry No.: 22619-35-8
Merck Index: 9594

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 312

CHROMATOGRAM

Retention time: 9.41
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

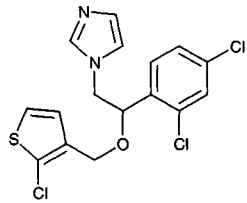
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 22.525

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tioconazole



Molecular formula: $C_{16}H_{13}Cl_3N_3OS$
Molecular weight: 387.72
CAS Registry No.: 65899-73-2
Merck Index: 9595

SAMPLE
Matrix: bulk
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil phenyl
Mobile phase: MeCN:MeOH:buffer 17.4:40.6:42 (Buffer was 50 mM pH 4 triethylamine phosphate containing 25 mM 1-octanesulfonic acid.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Wright,A.G.; Berridge,J.C.; Fell,A.F. Development and optimisation of a high-performance liquid chromatographic assay for tioconazole and its potential impurities. Part II. Selection of detection conditions for potential impurities, *Analyst*, **1989**, *114*, 53–56.

SAMPLE
Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 30 mg, add 100 mL MeOH, sonicate for 5 min, filter. Add a 2 mL aliquot of filtrate to 5 mL of 100 μg/mL ketoconazole in MeOH, make up to 25 mL with MeOH, inject 20 μL aliquot. Cream. Condition a 500 mg Bond-Elut diol cartridge with 6 mL dichloromethane. Weigh out cream equivalent to about 5 mg of drug, add 30 mL dichloromethane, sonicate for 3 min, make up to 100 mL with dichloromethane, filter. Add a 2 mL aliquot to the cartridge, wash with 2 mL dichloromethane:methanol 4:1, wash with 2 mL dichloromethane, elute with 3 mL MeOH:buffer 85:15. Add eluate to 0.5 mL 100 μg/mL ketoconazole in MeOH, make up to 5 mL with MeOH, inject 20 μL aliquot. (Buffer was 50 mM triethylamine adjusted to pH 7.0 with phosphoric acid.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: THF:buffer 30:70 (Buffer was 50 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 230 [Enhanced sensitivity with photoreactor (Beam Boost model C6808 with 10 m × 0.3 mm reaction coil) followed by UV detection at 270 nm.]

CHROMATOGRAM

Retention time: 12
Internal standard: ketoconazole (7)

OTHER SUBSTANCES

Simultaneous: clotrimazole, ketoconazole, bifonazole, econazole, isoconazole, miconazole, fenticonazole

KEY WORDS

tablets; creams; post-column photochemical derivatization

REFERENCE

Di Pietra,A.M.; Cavrini,V.; Andrisano,V.; Gatti,R. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 873–879.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100 μM solution.

HPLC VARIABLES

Column: 250 × 4.6 Cyclobond 1 (β-cyclodextrin) (Astec)
Mobile phase: MeCN:buffer 10:90 (Buffer was 3.5% triethylamine adjusted to pH 4.0 with glacial acetic acid.)
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 10.40 (-), 11.17 (+)

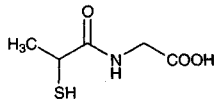
KEY WORDS

chiral; comparison with capillary electrophoresis

REFERENCE

Ferguson,P.D.; Goodall,D.M.; Loran,J.S. Systematic approach to the treatment of enantiomeric separations in capillary electrophoresis and liquid chromatography. III. A binding constant-retention factor relationship and effects of acetonitrile on the chiral separation of tioconazole, *J.Chromatogr.A*, **1996**, *745*, 25–35.

Tiopronin



Molecular formula: $C_5H_9NO_3S$
Molecular weight: 163.20
CAS Registry No.: 1953-02-2
Merck Index: 9597

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 200 mM pH 8.0 phosphate buffer + 200 μL chloroform:tributylphosphine 90:10, vortex for 15 s, heat at 50° for 30 min, cool in an ice bath, add 2 mL EtOH, vortex for 15 s, centrifuge at 5° at 1800 g for 10 min, inject a 50 μL aliquot of the supernatant within 15 min.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18e
Column: 125 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: MeCN:10 mM pH 7.0 phosphate buffer 25:75 containing 5 mM cetrimonium bromide
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: F ex 260 em 370 (long-pass filter) following post-column reaction. The column effluent mixed with MeCN:water 30:70 containing 2% triethylamine and 1% Brij-35 pumped at 0.35 mL/min and 50 μM pyrenemaleimide in MeCN pumped at 0.35 mL/min and flowed through a 3 m × 0.5 mm i.d. PTFE knitted open tubular reactor to the detector.

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; post-column reaction

REFERENCE

Leroy,P.; Nicolas,A.; Gavriloff,C.; Matt,M.; Netter,P.; Bannwarth,B.; Hercelin,B.; Mazza,M. Determination of 2-mercaptopropionylglycine and its metabolite, 2-mercaptopropionic acid, in plasma by ion-pair reversed-phase high-performance liquid chromatography with post-column derivatization, *J.Chromatogr.*, **1991**, *564*, 258–265.

SAMPLE

Matrix: solutions
Sample preparation: Mix 10 μL 12 mM reagent in MeCN with 20 μL of a 30 μM solution of tiopronin in 2 mM Na_2EDTA containing pyridine (final concentration 1%), heat at 50° for 60 min, inject a 10 μL aliquot. (Reagent was 4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole [R-(-)-DBD-PyNCS], available from Tokyo Kasei (TCI America, Portland, OR). Synthesis is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a

solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, was h, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 60F254 TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). DBD-F can also be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65?, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-Aminopyrrolidine is also available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm ULTRON VX-ODS (Shinwa Chemicals, Japan)

Mobile phase: MeCN:water containing 0.1% TFA 30:70 (A) or Gradient. C was MeCN containing 1% (v/v) TFA. D was water containing 1% TFA. C:D from 15:85 to 30:70 over 20min, to 40:60 over 30 min, maintain at 40:60 for 15 min (B)

Flow rate: 1

Injection volume: 10

Detector: F ex 455 em 568 (A), MS, Finnigan-MAT LCQ, ESI capillary temperature 275°, capillary voltage 3V, source voltage 4.8 kV, source current 100 μA, collision gas helium, positive ion mode, m/z 200-900 (B)

CHROMATOGRAM
Retention time: ca. 40 (R), 41 (S) (B)

OTHER SUBSTANCES
Simultaneous: hydrolysis product, reagent, D-cysteine, L- cysteine, (+)-2-mercaptopropionic acid, (-)-2-mercaptopropionic acid, N-(2-mercapto-2-methylpropionyl)-D-cysteine, N-(2-mercapto-2-methylpropionyl)-L-cysteine (B)
Also analyzed: N-acetyl-L-cysteine, N-acetyl-D/L-penicillamine, captopril, cysteamine, glutathione, D/L-homocysteine, 2-mercaptopropionic acid ethyl ester, D/L-penicillamine

KEY WORDS
derivatization; chiral

REFERENCE
Jin,D.; Toyo'oka,T. Indirect resolution of thiol enantiomers by high-performance liquid chromatography with a fluorescent chiral tagging reagent, *Analyst*, **1998**, *123*, 1271–1277.

SAMPLE
Matrix: solutions
Sample preparation: Add 1.05-3 equivalents 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate to 10 mL of a 100 μM solution of the thiol in MeCN:water 50:50 containing 1-3 equivalents triethylamine, vortex briefly, let stand at room temperature for 30 min, dilute with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel ODS-80TM (Tosoh)
Mobile phase: MeCN:10 mM pH 2.8 potassium phosphate buffer 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 2.12 (S), 2.63 (R)

OTHER SUBSTANCES
Simultaneous: alanine, mercaptopropionic acid

KEY WORDS
derivatization; chiral

REFERENCE
Ito,S.; Ota,A.; Yamamoto,K.; Kawashima,Y. Resolution of the enantiomers of thiol compounds by reversed-phase liquid chromatography using chiral derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, *J.Chromatogr.*, **1992**, *626*, 187–196.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 100 μL aliquot of a 0.1-1 mM solution in 10 mM HCl with 500 μL 100 mM pH 9.5 borate buffer, 100 μL 2.5 mM L-phenylalanine in 10 mM HCl, and 200 μL 5 mM o-phthalaldehyde in EtOH, let stand for 10 min, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18 end-capped
Mobile phase: MeOH:50 mM pH 6.5 phosphate buffer 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 335

CHROMATOGRAM
Retention time: k' 1.9 (α = 2.01, R_s = 5.18)

KEY WORDS
derivatization; chiral; comparison with capillary electrophoresis; comparison with other derivatizing reagents

REFERENCE
Leroy,P.; Bellucci,L.; Nicolas,A. Chiral derivatization for separation of racemic amino and thiol drugs by liquid chromatography and capillary electrophoresis, *Chirality*, **1995**, 7, 235–242.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of a 30 μM solution in 2 mM disodium EDTA containing 1.5% pyridine with 10 μL 12 mM R-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in MeCN, let stand for 40 min, inject a 10 μL aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole, (R)-(-)-NBD-PyNCS, is as follows. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL water, extract 4 times with 80 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as dark red crystals (mp 178-181°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as red crystals (mp 165-170°) (Analyst 1995, 120, 385).)

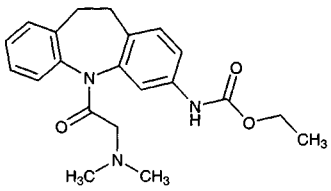
HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultron VX-ODS (Shinwa, Kyoto)
Mobile phase: MeCN:water:trifluoroacetic acid 30:70:0.1
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 455 em 568

CHROMATOGRAM
Retention time: 21.5 (R), 23 (S)
Limit of detection: 0.5 pmole

KEY WORDS
derivatization; chiral

REFERENCE
Jin,D.; Takehana,K.; Toyo'oka,T. Chiral separation of racemic thiols based on diastereomer formation with a fluorescent chiral tagging reagent by reversed-phase liquid chromatography, *Anal.Sci.*, **1997**, *13*, 113–115.

Tiracizine



Molecular formula: $C_{21}H_{25}N_3O_3$
Molecular weight: 367.45
CAS Registry No.: 83275-56-3

SAMPLE

Matrix: blood
Sample preparation: Add 1.5 mL MeCN to 500 μL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 μL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 μm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 μm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
Mobile phase: MeCN:50 mM pH 4 K_3PO_4 buffer 27:73
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 242, UV 230

CHROMATOGRAM

Retention time: 8.1

OTHER SUBSTANCES

Extracted: celiprolol, metoprolol, talinolol, oxprenolol, metabolites

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. 500 μL Serum + 500 ng talinolol + 1.5 mL MeCN, mix, centrifuge at 9000 g for 5 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 250 μL water, inject a 200 μL aliquot on to column A and elute to waste with mobile phase A, after 4 min backflush the contents of column A on to column B with mobile phase B, elute with column B, monitor the effluent from column B. Urine. Inject a 50 μL aliquot of urine on to column A and elute to waste with mobile phase A, after 4 min backflush the contents of column A on to column B with mobile phase B, elute with column B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 30-40 μm Perisorb A (Merck); B 125 × 4 LiChroCART RP18 (Merck)
Mobile phase: A water; B MeCN:50 mM pH 4 phosphate buffer 27:73
Flow rate: A 1.4; B 1
Injection volume: 200
Detector: UV 230

CHROMATOGRAM

Internal standard: talinolol
Limit of quantitation: 200 ng/mL (urine), 10 ng/mL (serum)

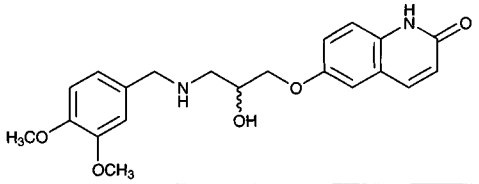
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics; column-switching

REFERENCE
Berndt,A.; Oertel,R.; Terhaag,B.; Richter,K.; Gramatté,T. Pharmacokinetics of the antiarrhythmic agent tiracizine: Steady state kinetics in comparison with single-dose kinetics, *Biopharm.Drug Dispos.*, **1995**, *16*, 427–441.

Toborinone

Molecular formula: $C_{21}H_{24}N_2O_5$
Molecular weight: 384.43
CAS Registry No.: 143343-83-3



SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Plasma, tissue. Mix plasma or homogenized tissue samples with MeOH: MeCN 50:50, sonicate, centrifuge at 1800 g for 5 min. Remove the supernatant, re-extract the residue 4 times. Combine the supernatants and lyophilize. Reconstitute the residue in mobile phase (A:B 50:50), inject an aliquot. Feces. Mix fecal homogenate with MeOH/water, extract 6 times, combine the supernatants and lyophilize. Reconstitute the residue in mobile phase(A:B 50:50), inject an aliquot. Urine. Inject urine directly or lyophilize and reconstitute in mobile phase (A:B 50:50), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Develosil ODS-5(Nomura, Japan)
Mobile phase: Gradient. A:B from 100:0 to 90:10 over 20 min, maintain at 90:10 for 10 min, to 70:30 over 10 min, to 50:50 over 5 min and back to 100:0 over 0.1 min. A was MeCN:MeOH: water:acetic acid 5:5:90:0.5. B was MeCN:MeOH:water:acetic acid 25:25:50:0.5.
Column temperature: 30
Flow rate: 1
Detector: F ex 355 em 405

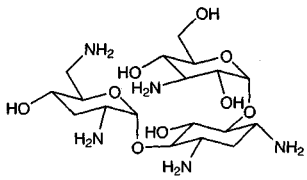
CHROMATOGRAM
Retention time: 40

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; dog; plasma; feces; liver; details of analytical and preparative HPLC

REFERENCE
Kitani,M.; Miyamoto,G.; Nagasawa,M.; Yamada,T.; Matsubara,J.; Uchida,M.; Odomi,M. Biotransformation of the novel inotropic agent toborinone (OPC-18790) in rats and dogs. Evidence for the formation of novel glutathione and two cysteine conjugates, *Drug Metab.Dispos.*, **1997**, *25*, 663–674.

Tobramycin



Molecular formula: $C_{18}H_{37}N_5O_9$
Molecular weight: 467.52
CAS Registry No.: 32986-56-4, 79645-27-5 (sulfate)
Merck Index: 9628

SAMPLE

Matrix: blood
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of water. 50 μL Serum + 25 μL 2 M pH 10.3 Tris buffer + 100 μL 10 μg/mL sisomicin in MeCN, vortex, centrifuge at 15000 g for 1 min. Remove the supernatant and add it to 30 μL 250 mg/mL 2,4,6-trinitrobenzenesulfonic acid in MeCN, vortex, heat at 70° for 30 min. Add 700 μL wash solution then 200 μL sample to the SPE cartridge, wash with 3 column volumes of wash solution, elute with 300 μL MeCN, inject a 50 μL aliquot of the eluate. (Prepare wash solution by adding 10 mL 1 M K_2HPO_4 to 90 mL water, add 100 mL MeOH, adjust pH to 8.5 with phosphoric acid.)

HPLC VARIABLES

Column: 250 × 4.6 Ultrasphere octyl
Mobile phase: MeCN:buffer 70:30 (Buffer was 6.8 g/L KH_2PO_4 adjusted to pH 3.5 with phosphoric acid.)
Column temperature: 50
Flow rate: 3
Injection volume: 50
Detector: UV 340

CHROMATOGRAM

Retention time: 3
Internal standard: sisomicin (4)
Limit of detection: 1200 ng/mL

OTHER SUBSTANCES

Simultaneous: gentamicin
Noninterfering: acetaminophen, acetazolamide, N-acetylprocainamide, amikacin, amobarbital, ampicillin, amitriptyline, caffeine, cefamandole, cefoxime, cefoxitin, cephalothin, clindamycin, chloramphenicol, chlordiazepoxide, diazepam, erythromycin, ethosuximide, glutethimide, imipramine, kanamycin, methaqualone, moxalactam, nafcillin, nitrofurantoin, penicillin G, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, secobarbital, tetracycline, theophylline, vancomycin

KEY WORDS

serum; derivatization; SPE

REFERENCE

Kabra,P.M.; Bhatnagar,P.K.; Nelson,M.A.; Wall,J.H.; Marton,L.J. Liquid-chromatographic determination of tobramycin in serum with spectrophotometric detection, *Clin.Chem.*, **1983**, *29*, 672–674.

SAMPLE

Matrix: blood
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 1 mL MeCN and 1 mL MeCN:20 mM pH 8 phosphate buffer 10:90. 100 μL Serum + 400 μL water + 500 μL 10% sulfosalicylic acid, vortex, centrifuge. Remove 60 μL supernatant and add it to 240 μL MeCN and 300 μL o-phthalaldehyde reagent, add to SPE cartridge, rinse in with 300 μL rinse + 100 μL MeCN:20 mM pH 8 phosphate buffer 10:90, wash with 500 μL MeCN:20 mM pH 8 phosphate buffer 10:90, elute with 440 μL MeCN, add 40 μL water to eluate, vortex, inject a 25 μL aliquot. (o-Phthalaldehyde reagent was 200 mg o-phthalaldehyde in 2 mL MeOH + 400 μL 2-mercaptoethanol. Mix with 1 g boric acid in 38 mL water adjusted to pH 10.4 with 450 g/L KOH, store under nitrogen at 4°.)

HPLC VARIABLES
Column: 150 × 4 MicroPak SP C8 (Varian)
Mobile phase: MeCN:20 mM pH 6.5 phosphate buffer 48:52
Flow rate: 2
Injection volume: 25
Detector: F ex 340 em 450

CHROMATOGRAM
Retention time: 8.4
Limit of detection: 10 pmol

KEY WORDS
serum; SPE; derivatization

REFERENCE
Lai,F.; Sheehan,T. Enhancement of detection sensitivity and cleanup selectivity for tobramycin through pre-column derivatization, *J.Chromatogr.*, **1992**, *609*, 173–179.

SAMPLE
Matrix: blood, dialysate, urine
Sample preparation: Plasma. Condition a 3 mL Baker cyanopropylsilane CN SPE cartridge with 2 mL MeOH, 2 mL water, and 2 mL buffer. 1 mL Plasma + 100 μL 100 μg/mL dibekacin in water, vortex for 15 s, add 1 mL buffer, vortex for 15 s, centrifuge at 3100 g at 4° for 7 min, add to SPE cartridge, wash with 500 μL water, wash with 250 μL mobile phase, elute to dryness. Elute with 250 μL mobile phase, inject an aliquot of the eluate. Urine, dialysate. Dilute 1:100 with water, add 100 μL 100 μg/mL dibekacin per 1 mL of sample, mix well, inject a 100 μL aliquot. (Buffer was 0.94 g sodium hexanesulfonate in 300 mL water, add 500 μL glacial acetic acid, dilute to 500 mL with water.)

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Hypersil C18
Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 3.76 g sodium hexanesulfonate + 28.4 g sodium sulfate in 2 L water, acidify to pH 3.4 with 2 mL glacial acetic acid.)
Column temperature: 25
Flow rate: 1.1
Injection volume: 100
Detector: F ex 338 em 418 (bandpass filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.05 mm i.d. knitted PTFE reaction coil at 25° to the detector (Derivatizing reagent was 0.4 g o-phthalaldehyde in 3 mL MeOH added to 390 mL buffer, add 2 mL β-mercaptoethanol, make up to 500 mL with water, store at 4°. Buffer was 1 M pH 10.4 borate from equal volumes of 1 M KOH and boric acid.)

CHROMATOGRAM
Retention time: 13
Internal standard: dibekacin (17)

OTHER SUBSTANCES
Simultaneous: isepamicin, kanamycin, gentamicin, netilmicin

KEY WORDS
post-column reaction; SPE; plasma

REFERENCE
Maloney,J.A.; Awni,W.M. High-performance liquid chromatographic determination of isepamicin in plasma, urine and dialysate, *J.Chromatogr.*, **1990**, *526*, 487–496.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.393

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dilute 500 μL injection to 25 mL with Sorensen's phosphate buffer, dilute a 1 mL aliquot to 100 mL with Sorensen's phosphate buffer. Dissolve tobramycin-containing polymethylmethacrylate beads in 2 mL chloroform, extract 3 times with 5 mL aliquots of 50 mM KH_2PO_4, combine and dilute the extracts. Dilute bulk samples with Sorensen's phosphate buffer. 50 μL Solution + 25 μL 242 mg/mL pH 10.4 Tris buffer + 100 μL 6 μg/mL kanamycin in MeCN:water 50:50 + 30 μL 250 mg/mL 2,4,6-trinitrobenzenesulfonic acid in MeCN:water 80:20, vortex for 10 s, heat at 70° for 15 min, add 2 mL chloroform, shake horizontally at 180 cycles/min for 5 min, centrifuge at 750 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN, vortex, inject a 20 μL aliquot. (Sorensen's phosphate buffer was 197 mL 9.08 g/L KH_2PO_4 and 1803 mL 11.88 g/L $Na_2HPO_4.2H_2O$, pH 7.4.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:50 mM KH_2PO_4 62:38, pH adjusted to 3.5 with phosphoric acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 7.7
Internal standard: kanamycin (6.4)
Limit of detection: 160 ng/mL
Limit of quantitation: 780 ng/mL

KEY WORDS
injections; beads; derivatization

REFERENCE
Dash,A.K.; Suryanarayanan,R. A liquid-chromatographic method for the determination of tobramycin, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 237–245.

SAMPLE
Matrix: fermentation solutions
Sample preparation: 5 mL Fermentation broth + 5 mL saturated aqueous solution of Tris + 20 mL MeCN, centrifuge at 3000 rpm for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 3 mL 150 mM 2,4-dinitrofluorobenzene in MeOH, heat at 100° under a reflux condenser for 45 min, make up to 4 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:water:acetic acid 55:45:0.15
Flow rate: 1.2
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 14.86

OTHER SUBSTANCES
Extracted: apramycin, kanamycin B

KEY WORDS
derivatization

REFERENCE
Harangi,J.; Deák,M.; Nánási,P.; Bacsa,G. Determination of the major factors of fermentation of the nebramycin complex by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1984**, *7*, 83–93.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 3 mg/mL ophthalmic suspension in water with 10 mM sulfuric acid to a tobramycin concentration of 240 μg/mL. Mix 4 mL diluted suspension with 10 mL 10 mg/mL 2,4-dinitrofluorobenzene in EtOH and 10 mL 15 mg/mL tris(hydroxymethyl)aminomethane in water:dimethylsulfoxide 20:80. Heat at 70 ± 2°. for 20 min, allow to cool slightly for 2 min and add 24 mL MeCN. Allow to cool to room temperature, make up to 50 mL with MeCN, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:buffer 55:45 (Prepare mobile phase as follows. Dissolve 2.0 g tris (hydroxymethyl)aminomethane in 960 mL water, add 20 mL 0.5 M sulfuric acid and 1200 mL MeCN.)
Flow rate: 1.5
Injection volume: 30
Detector: UV 365

CHROMATOGRAM
Retention time: 9.0
Limit of quantitation: 0.1%

OTHER SUBSTANCES
Simultaneous: degradation products, kanamycin, neamine, nebramine

KEY WORDS
derivatization; ophthalmic suspension; stability-indicating

REFERENCE

Russ,H.; McCleary,D.; Katimy,R.; Montana,J.L.; Miller,R.B.; Krishnamoorthy,R.; Davis,C.W. Development and validation of a stability-indicating HPLC method for the determination of tobramycin and its related substances in an ophthalmic suspension, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2165–2181.

SAMPLE

Matrix: formulations
Sample preparation: Dilute 150 μL sample to 5 mL, inject an aliquot.

HPLC VARIABLES

Guard column: C18 precolumn filter
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeCN:200 mM KH_2PO_4 30:70 adjusted to pH 6.5
Flow rate: 1
Detector: UV 210

CHROMATOGRAM

Retention time: 6.78

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

stability-indicating; ophthalmic solutions

REFERENCE

McBride,H.A.; Martinez,D.R.; Trang,J.M.; Lander,R.D.; Helms,H.A. Stability of gentamicin sulfate and tobramycin sulfate in extemporaneously prepared ophthalmic solutions at 8 degrees C, *Am.J.Hosp.Pharm.*, **1991**, *48*, 507–509.

SAMPLE

Matrix: formulations, tissue
Sample preparation: Homogenize (Polytron) kidney or lung tissue with 2.5 volumes cold sterile PBS for 30 s. 100 μL Tissue homogenate or liposome encapsulations + 1 mL MeOH, vortex for 1 min, heat at 65° for 30 min, add 900 μL PBS, vortex for 1 min, centrifuge at 4° at 5000 g for 20 min. Remove a 170 μL aliquot of the supernatant and add it to 90 μL 180 mg/mL 1-fluoro-2,4-dinitrofluorobenzene in MeOH, add 60 μL 100 mM pH 9.3 borate buffer, add 670 μL MeOH, vortex, heat at 85° for 30 min, cool to room temperature, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 4 μm Zorbax SB-C18
Mobile phase: MeCN:MeOH:10 mM potassium phosphate buffer 65:10:25, pH 3.5
Flow rate: 1.3
Injection volume: 10
Detector: UV 350

CHROMATOGRAM

Limit of detection: 200 ng/mL (PBS), 300 ng/mg (lung), 500 ng/mg (kidney)

KEY WORDS

rat; derivatization; lung; kidney; liposome encapsulations

REFERENCE

Beaulac,C.; Clement-Major,S.; Hawari,J.; Lagace,J. Eradication of mucoid Pseudomonas aeruginosa with fluid liposome-encapsulated tobramycin in an animal model of chronic pulmonary infection, *Antimicrob.Agents Chemother.*, **1996**, *40*, 665–669.

SAMPLE

Matrix: reaction mixtures

Sample preparation: 50 μL Buffered reaction mixture + 50 μL isopropanol + 50 μL reagent, heat at 60° for 10 min, centrifuge at 1000 g for 2 min, immediately inject a 50 μL aliquot of the supernatant. (Reagent was 80 mM o-phthalaldehyde and 250 mM thioglycolic acid in 1 M boric acid, pH adjusted to 10.4 with 40% KOH.)

HPLC VARIABLES
Column: 100 × 5 Hypersil ODS
Mobile phase: A was MeOH:water:acetic acid 50:45:5 containing 5 g/L heptanesulfonic acid. B was MeOH:water:acetic acid 75:20:5 containing 5 g/L heptanesulfonic acid. A:B 55:45.
Flow rate: 2
Injection volume: 50
Detector: UV 330

CHROMATOGRAM
Retention time: 18

KEY WORDS
derivatizaton

REFERENCE
Lovering,A.M.; White,L.O.; Reeves,D.S. Identification of aminoglycoside-acetylating enzymes by high-pressure liquid chromatographic determination of their reaction products, *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

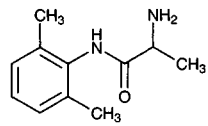
HPLC VARIABLES
Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cefamandole, cefazolin, cefotaxime, cefoxitin, cephalexin, cephalothin, cephapirin, chloramphenicol, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, vancomycin

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

Tocainide



Molecular formula: $C_{11}H_{16}N_2O$
Molecular weight: 192.26
CAS Registry No.: 41708-72-9
Merck Index: 9629
Lednicer No.: 3 55

SAMPLE
Matrix: blood
Sample preparation: 1.0 mL Serum + 500 μL saturated borate buffer + 3.0 mL dichloromethane, vortex, centrifuge. Evaporate the organic layer to dryness under a stream of nitrogen at 40°. Reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: silica (Whatman)
Column: 250 × 4.6 Supelcosil LC-8-DB
Mobile phase: MeCN:20 mM phosphoric acid containing 200 μL/L triethylamine 10:90
Flow rate: 1.7
Detector: UV 263

CHROMATOGRAM
Retention time: 9.7
Internal standard: tocainide

OTHER SUBSTANCES
Extracted: lidocaine
Noninterfering: acetaminophen, N-acetylprocainamide, amitriptyline, bupivicaine, caffeine, carbamazepine, chloramphenicol, cyclosporin A, desipramine, diazepam, disopyramide, doxepin, ethosuximide, flecainide, fluoxetine, ibuprofen, imipramine, naproxen, norchlordiazepoxide, nordiazepam, nordoxepine, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, valproic acid

KEY WORDS
serum; tocainide is IS

REFERENCE
O'Neal,C.L.; Poklis,A. Sensitive HPLC for simultaneous quantification of lidocaine and its metabolites monoethylglycinexylidide and glycinexylidide in serum, *Clin.Chem.*, **1996**, *42*, 330–331.

SAMPLE
Matrix: blood
Sample preparation: 0.05-2 mL Plasma or whole blood + 50 μL 13.5 μg/mL IS in 50 mM HCl + 200 μL 2 M NaOH + 3 mL ethyl acetate, mix with gentle tilting for 5 min, centrifuge at 2500 rpm for 10 min. Remove 2 mL of the organic layer and add it to 50 μL 50 mM sulfuric acid, vortex for 1 min, centrifuge for 5 min, discard the organic phase. Wash the aqueous phase with 1 mL hexane, freeze in dry ice/acetone, discard the organic layer. Thaw the aqueous layer and add 50 μL 1 M sodium bicarbonate and 200 μL reagent, heat at 40° for 30 min, add 100 μL 500 mM NaOH, evaporate under reduced pressure at 40° for 3 min, add 1 mL buffer and 200 μL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), mix for 2 min, centrifuge for 5 min, inject a 2-5 μL aliquot of the organic layer. (Reagent was 1 mg/mL dansyl chloride in acetone, store in the dark, prepare fresh each week. Buffer was a half-saturated solution of disodium citrate in water, adjusted to pH 6.0 with 85% phosphoric acid.)

HPLC VARIABLES
Column: 300 × 4 μBondapak NH2
Mobile phase: Hexane:dichloromethane:MeOH 50:50:1
Flow rate: 5
Injection volume: 2-5
Detector: F ex 360 (Corning 7-51 filter) em 490 (Wratten 8 filter)

CHROMATOGRAM
Retention time: 2.4
Internal standard: 2-amino-N-(2,6-dimethylphenyl)butanamide hydrochloride (Astra Pharmaceuticals, Worcester, MA) (1.9)
Limit of quantitation: 100 ng/mL

KEY WORDS
derivatization; plasma; whole blood; pharmacokinetics

REFERENCE
Meffin,P.J.; Harapat,S.R.; Harrison,D.C. High-pressure liquid chromatographic analysis of drugs in biological fluids II: Determination of an antiarrhythmic drug, tocainide, as its dansyl derivative using a fluorescence detector, *J.Pharm.Sci.*, **1977**, *66*, 583–586.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL pH 11 sodium borate buffer + 2.5 mL diisopropyl ether:EtOH 100:1.5 (Caution! Diisopropyl ether readily forms explosive peroxides!), shake for 30 min, centrifuge, repeat the extraction. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 100 μL toluene, evaporate to dryness, add 100 μL 400 μg/mL S-naproxen chloride in anhydrous dichloromethane, heat at 50° for 1 h, evaporate to dryness, reconstitute with mobile phase, inject an aliquot (cf Arch. Pharm. (Weinheim) 1990, 323, 465). (Synthesis of S-naproxen chloride is as follows. Protect all compounds from light. Dissolve 500 mg naproxen in 50 mL dry toluene, slowly add 5 mL thionyl chloride (freshly distilled from linseed oil), reflux for 1 h, evaporate to dryness under reduced pressure, dry over KOH under vacuum overnight to obtain S-naproxen chloride (mp 96°).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:20:20
Detector: F ex 313 em 365

CHROMATOGRAM

Retention time: k' 4.0 (R), k' 5.2 (S)

KEY WORDS

derivatization; protect from light; chiral; plasma; normal phase

REFERENCE

Spahn,H. S-(+)-Naproxen chloride as acylating agent for separating the enantiomers of chiral amines and alcohols, *Arch.Pharm.(Weinheim)*, **1988**, *321*, 847–850.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 25 μL 10 μg/mL acebutolol in water + 200 μL 1 M NaOH + 5 mL chloroform, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL 0.05% S-(+)-1-(1-naphthyl)ethylisocyanate in chloroform, vortex for 30 s, evaporate to dryness under reduced pressure, reconstitute with 200 μL chloroform, inject a 50-175 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 5 silica
Mobile phase: Hexane:chloroform:MeOH 60:38:2
Flow rate: 2
Injection volume: 50-175
Detector: F ex 220 em 345

CHROMATOGRAM

Retention time: 3.5 (S), 4.5 (R) (tentative assignment)
Internal standard: (±)-acebutolol (14.9 (R), 16.5 (S))
Limit of detection: 25 ng/mL
Limit of quantitation: 250 ng/mL

KEY WORDS

plasma; derivatization; chiral; pharmacokinetics

REFERENCE

Carr,R.A.; Foster,R.T.; Freitag,D.; Pasutto,F.M. Stereospecific high-performance liquid chromatographic determination of tocainide, *J.Chromatogr.*, **1991**, *566*, 155–162.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum or plasma + 100 μL 500 mM sodium carbonate + 100 μL 15 μg/mL N-propionylprocainamide in water, vortex for 5 s, add 0.5 (procainamide) or 1 (tocainide) mL dichloromethane, vortex for 30 (procainamide) or 60 (tocainide) s, centrifuge at

9500 g for 1 min. Remove the lower organic layer and add it to 200 μL 10 mM HCl, vortex for 15 s, centrifuge, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 100 × 5 NovaPak cyano HP radial compression
Mobile phase: MeCN:buffer 10:90, final pH adjusted to 6.0 (Buffer was 5 mM acetate buffer containing 0.05% triethylamine.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3.1
Internal standard: N-propionylprocainamide (6.1)
Limit of detection: 2 μg/mL

OTHER SUBSTANCES
Extracted: N-acetylprocainamide, procainamide
Simultaneous: disopyramide, lidocaine, mexiletine, quinidine
Noninterfering: carbamazepine, desmethyldoxepin, digoxin, doxepin, ethosuximide, lithium, phenobarbital, phenytoin, primidone, propranolol, theophylline, valproic acid

KEY WORDS
serum; plasma

REFERENCE
vasBinder,E.; Annesley,T. Liquid chromatographic analysis of mexiletine in serum, with alternate application to tocainide, procainamide, and N-acetylprocainamide, *Biomed.Chromatogr.*, **1991**, *5*, 19–22.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 20 mM pH 10 potassium phosphate buffer. 1 mL Plasma + 25 μL 1.2 mg/mL benzylamine hydrochloride in water + 100 mg solid sodium carbonate, vortex, add to the SPE cartridge, wash with 1 mL 20 mM pH 10 potassium phosphate buffer, elute with 500 μL MeOH. Add 20 μL 1 M (-)-menthyl chloroformate in MeCN to the eluate, shake briefly, let stand for 3 min, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeOH:200 mM pH 5.0 potassium phosphate buffer:water 75:2.5:22.5
Flow rate: 1
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 4.70 (R), 5.07 (S)
Internal standard: benzylamine (6.22)
Limit of quantitation: 1 μg/mL

KEY WORDS
chiral; derivatization; SPE; plasma; rabbit; pharmacokinetics

REFERENCE
Christensen,E.B.; Hansen,S.H.; Rasmussen,S.N. Assay of tocainide enantiomers in plasma by solid-phase extraction and indirect chiral high-performance liquid chromatography after derivatization with (-)-menthyl chloroformate, *J.Chromatogr.B*, **1995**, *670*, 243–249.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.9

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: 50 μL 5 mg/mL Tocainide in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer was 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 16.3 mg N-acetyl-L-cysteine in 1 mL MeOH, protect from light, keep on ice.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:MeCN:buffer 40:2:60 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 425 or UV 254

CHROMATOGRAM
Retention time: 20.32 (S-(+)), 22.47 (R-(-))

KEY WORDS
derivatization; protect from light; chiral

REFERENCE
Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 6.28 (A), 3.60 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupro-

mazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of a 1 mM solution in MeOH or water with 50 μL pH 8 borate buffer and 50 μL 18 mM 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate in acetone, vortex, let stand at room temperature for 30 min, add 100 μL 10 mM trans-4-hydroxy-L-proline in water, mix, let stand for 2 min, add 2 mL dichloromethane, vortex for 30 s. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL mobile phase, inject an aliquot. Prepare 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate as follows. Stir 1.5 mmoles lithium aluminum hydride in THF, slowly add 2 mmoles (S)-naproxen in 20 mL anhydrous THF, reflux for 1 h, evaporate most of the solvent, cautiously add water with stirring, acidify with 6 N HCl, extract three times with diethyl ether. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness, chromatograph on silica gel with dichloromethane:MeOH 100:2 (flash chromatography), evaporate eluate to dryness, dry under vacuum over KOH to give 2-(6-methoxy-2-naphthyl)propanol as a white solid (mp 92-3°). Stir 0.5 mmoles 2-(6-methoxy-2-naphthyl)propanol and 0.5 mmoles triethylamine in 10 mL dry toluene at 0°, add 1 mL 20% phosgene in toluene (Caution! Phosgene is highly toxic, perform reaction in a chemical fume hood!) (Fluka), stir for 4 h, filter, evaporate to dryness under reduced pressure, dry under vacuum to give 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate (mp 60°). Store under vacuum over phosphorus pentoxide at room temperature.)

HPLC VARIABLES
Column: 250 × 4 5 μm Zorbax-SIL
Mobile phase: n-Hexane:isopropanol 100:1.5
Flow rate: 1.5
Injection volume: 100
Detector: UV 230, F ex 270 em 365

CHROMATOGRAM
Retention time: k' 15.7 (S-(+)), k' 16.9 (R-(-))

OTHER SUBSTANCES
Simultaneous: flecainide, metoprolol, propafenone

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

SAMPLE
Matrix: solutions
Sample preparation: Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmoles sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene

or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

HPLC VARIABLES
Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: MeOH:water 70:30
Flow rate: 0.8
Injection volume: 100
Detector: UV 230, F ex 270 em 350

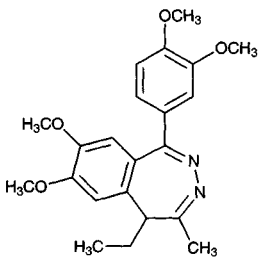
CHROMATOGRAM
Retention time: k' 10.1 (S-(+)), 11.1 (R-(-))

KEY WORDS
derivatization; chiral; F not much more sensitive than UV; α = 1.10

REFERENCE
Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

Tofisopam

Molecular formula: $C_{22}H_{26}N_2O_4$
Molecular weight: 382.46
CAS Registry No.: 22345-47-7



SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 238

CHROMATOGRAM

Retention time: 4.04
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: 4.0 mL Blood, plasma, or urine + 1.5 mL pH 8.0 dibasic ammonium phosphate + 4.5 mL dichloromethane, gently shake horizontally for 10 min, centrifuge at 3500 g for 10 min. Transfer the lower organic layer to 5 mL tube and evaporate under reduced pressure at 45° to 1.0 mL. Transfer into 1.5 mL Eppendorff-type plastic microtube and evaporate to dryness. Add 30 μL mobile phase, vortex for 10 s, centrifuge at 10 000 g for 5 min, inject a 0.6 μL aliquot of the supernatant. (Equilibrate the column at least 3 h before analyzing. At the end of each chromatographic session clean column with MeCN:water 50:50 at 0.05 mL/min for 3 h.)

HPLC VARIABLES

Guard column: 1.0 × 0.8 5 μm C18 MGU-80 (LC Packing, Switzerland)
Column: 250 × 1.0 5 μm C18 Microbore (Alltech, USA)
Mobile phase: MeCN:2 mM pH 3.0 ammonium formate 75:25
Flow rate: 0.05

Injection volume: 0.6
Detector: MS, Perkin-Elmer Sciex API-100 double-quadrupole, OR + 50 V, Q0–10 V, IQ1 (lens)–12 V, ST (lens)–15 V, Q1–13 V, EM + 2200 V, TIC m/z 100-500 or 380-405, SIM m/z 383 pm 0.5

CHROMATOGRAM
Retention time: 4.53
Internal standard: tofisopam

OTHER SUBSTANCES
Extracted: colchicine

KEY WORDS
plasma; tofisopam is IS; microbore; use PEEK tubing and injection loop

REFERENCE
Tracqui,A.; Kintz,P.; Ludes,B.; Rouge,C.; Douibi,H.; Mangin,P. High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids, *J.Chromatogr.B*, **1996**, *675*, 235–242.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

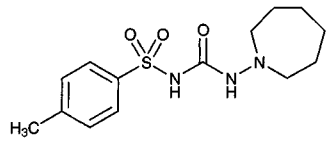
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 18.508

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tolazamide



Molecular formula: $C_{14}H_{21}N_3O_3S$
Molecular weight: 311.41
CAS Registry No.: 1156-19-0
Merck Index: 9644
Lednicer No.: 1 241

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 500 μL 5-(p-methylphenyl)-5-phenylhydantoin in chloroform + 500 μL pH 4.5 sodium acetate buffer + 5 mL dichloromethane, shake horizontally for 5 min, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 80 μL MeOH, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb C-18
Mobile phase: MeOH:pH 5.6 acetate buffer 52.3:47.7
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5
Internal standard: 5-(p-methylphenyl)-5-phenylhydantoin (7.5)
Limit of quantitation: 1000 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

Welling,P.G.; Patel,R.B.; Patel,U.R.; Gillespie,W.R.; Craig,W.A.; Albert,K.S. Bioavailability of tolazamide from tablets: comparison of in vitro and in vivo results, *J.Pharm.Sci.*, **1982**, *71*, 1259–1263.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 2 mL water + 200 μL 1 (?) M HCl + 200 μL 2.5 μg/mL glibornuride in MeOH + 7 mL diethyl ether, mix, centrifuge at 2000 rpm for 5 min. Remove 6.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 2 mg/mL dinitrofluorobenzene in butyl acetate, heat at 120° for 1 h, cool, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 120 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS 2
Mobile phase: MeCN:0.4% aqueous phosphoric acid 75:25
Column temperature: 40
Flow rate: 1.2
Injection volume: 120
Detector: UV 360

CHROMATOGRAM

Retention time: 7.3
Internal standard: glibornuride (5.8)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide, glipizide, glyburide (glibenclamide), tolbutamide

KEY WORDS
serum; derivatization

REFERENCE
Starkey,B.J.; Mould,G.P.; Teale,J.D. The determination of sulphonylurea drugs by HPLC and its clinical application, *J.Liq.Chromatogr.*, **1989**, *12*, 1889–1896.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 2 M HCl + 2 mL diethyl ether, vortex for 30 s, centrifuge at 1500 g for 5 min, freeze in dry ice for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject a 25-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18 (Alltech)
Mobile phase: MeCN:10 mM orthophosphoric acid 50:50
Flow rate: 1
Injection volume: 25-50
Detector: UV 230

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Extracted: chlorpropamide, gliclazide, glyburide (glibenclamide), glipizide, tolbutamide
Simultaneous: sulfamethoxazole, N-acetylsulfamethoxazole
Noninterfering: trimethoprim

KEY WORDS
plasma

REFERENCE
Shenfield,G.M.; Boutagy,J.S.; Webb,C. A screening test for detecting sulfonylureas in plasma, *Ther.Drug Monit.*, **1990**, *12*, 393–397.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, dil-

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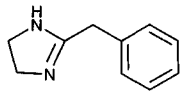
REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Tolazoline



Molecular formula: $C_{10}H_{12}N_2$
Molecular weight: 160.22
CAS Registry No.: 59-98-3, 59-97-2 (HCl)
Merck Index: 9645

SAMPLE

Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 2.5 μg/mL clonidine in 10 mM HCl + 50 μL 3 M NaOH + 1.5 mL n-butyl chloride:isopropanol 95:5, vortex 60 s, centrifuge at 2500 rpm (20 cm) for 2 min. Freeze in dry ice/isopropanol for 20 s and remove organic phase. Add 100 μL 50 mM phosphoric acid to organic phase, vortex 60 s, centrifuge at 2500 rpm (20 cm) for 2 min, remove organic layer, inject 25 μL aliquots of aqueous layer.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-PCN
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KOH/H_3PO_4, pH 3.0.)
Column temperature: 35
Flow rate: 2
Injection volume: 25
Detector: UV 214

CHROMATOGRAM
Retention time: 3.7
Internal standard: clonidine
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: amikacin, ampicillin, dopamine, furosemide, gentamicin, heparin, morphine, pancuronium

KEY WORDS
serum

REFERENCE
Todesco,L.M.; Thoma,J.J.; Barth,R.D.; Myers,N.J.; White,R.; Ward,R.M. Quantitative determination of tolazoline in human serum by high performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 78–84.

SAMPLE
Matrix: blood, urine
Sample preparation: 50-100 μL Serum or urine + 30 μL 1 (serum) or 100 (urine) μg/mL naphazoline + 250 μL buffer + 5 mL dichloromethane, shake mechanically for 20 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 (serum) or 500 (urine) μL 20 mM pH 3.75 KH_2PO_4, vortex for 15 s, inject a 10 (urine) or 90 (serum) μL aliquot. (Buffer was 100 mM potassium hydrogen carbonate and 100 mM potassium carbonate, pH 10.0.)

HPLC VARIABLES
Guard column: 50 × 2 Co:Pell ODS
Column: Resolve C18 (Waters)
Mobile phase: MeCN:buffer 40:60 (Buffer was 20 mM KH_2PO_4 adjusted to pH 3.75 with 85% phosphoric acid.)
Flow rate: 1.2
Injection volume: 10-90
Detector: UV 210

CHROMATOGRAM
Retention time: 7
Internal standard: naphazoline (10)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, carbamazepine, chloramphenicol, desipramine, digoxin, disopyramide, dopamine, ethosuximide, gentamicin, imipramine, lidocaine, methotrexate, phenobarbital, phenytoin, primidone, procainamide, propranolol, quinidine, salicylic acid, theophylline, valproic acid

KEY WORDS
serum

REFERENCE
Cwik,M.J.; Chiu,G.P.; Fischer,J.H.; Chow-Tung,E.; Currie,B.L. Quantitative determination of tolazoline in serum and urine, *J.Chromatogr.*, **1985**, *338*, 123–130.

SAMPLE
Matrix: formulations
Sample preparation: Mix 5 mL nasal solution and 10 mL 500 μg/mL tolazoline hydrochloride in MeOH:water 40:60, make up to 50 mL with MeOH:water 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 RSIL C18 (RSL, Eke, Belgium)
Mobile phase: MeOH:water 40:60 containing 20 mM sodium 1-octanesulfonate and 10 mM N,N-dimethyloctylamine, pH adjusted to 3.0 with orthophosphoric acid

Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 2.5
Internal standard: tolazoline

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline, coumazoline, lidocaine, naphazoline, oxymetazoline, prednisolone, sulfadimidine, sulfanilamide, sulfathiazole, tenaphtoxaline, tetrahydrozoline, tramazoline, xylometazoline

KEY WORDS
nasal solutions; stability-indicating; tolazoline is IS

REFERENCE
De Schutter,J.A.; Van den Bossche,W.; De Moerloose,P. Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography, *J.Chromatogr.*, **1987**, *391*, 303–308.

SAMPLE
Matrix: solutions

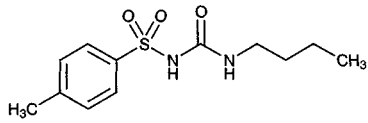
HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic

acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Tolbutamide



Molecular formula: $C_{12}H_{18}N_2O_3S$
Molecular weight: 270.35
CAS Registry No.: 64-77-7, 473-41-6 (Na salt)
Merck Index: 9646
Lednicer No.: 1 136

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 60 ng chlorpropamide + 500 µL 1 M HCl + 8 mL chloroform, shake on a reciprocal shaker, shake for 10 min in a reciprocal shaker, centrifuge at 2000 g for 15 min. Remove 7 mL of the lower organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 µL 3 mg/mL dinitrofluorobenzene in n-butyl acetate, heat at 120° for 30 min, evaporate to dryness under a stream of nitrogen at 60°, dissolve the residue in 100 µL mobile phase, inject a 30-70 µL aliquot. (Recrystallize dinitrofluorobenzene from diethyl ether. Prepare solutions weekly, store at 4° in the dark.)

HPLC VARIABLES

Column: 125 × 4.6 5 µm C8 (Perkin-Elmer)
Mobile phase: MeCN:water 50:50 containing 0.15% phosphoric acid
Flow rate: 1.5
Injection volume: 30-70
Detector: UV 350

CHROMATOGRAM

Retention time: 4.5
Internal standard: chlorpropamide (6.2)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: glyburide
Noninterfering: acetaminophen, aspirin, diazepam, chlordiazepoxide, quinidine, phenytoin, theophylline, phenobarbital

KEY WORDS

plasma; derivatization

REFERENCE
Zecca,L.; Trivulzio,S.; Pinelli,A.; Colombo,R.; Tofanetti,O. Determination of glibenclamide, chlorpropamide and tolbutamide in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1985**, *339*, 203–209.

SAMPLE
Matrix: blood
Sample preparation: 1 mL PLasma or urine + 10 μL MeOH + 10 μL concentrated HCl + 8 mL toluene, extract for 15 min, centrifuge at 1500 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm C18 (Merck)
Mobile phase: MeOH:50 mM pH 5.0 phosphate buffer 50:50
Flow rate: 1
Injection volume: 5
Detector: UV 230

CHROMATOGRAM
Retention time: 6
Internal standard: tolbutamide

OTHER SUBSTANCES
Extracted: nimesulide
Simultaneous: acetaminophen, aspirin, doxepin, glibenclamide, salicylic acid, theophylline
Noninterfering: digoxin, flurazepam, tiadenol
Interfering: bezafibrate

KEY WORDS
plasma; tolbutamide is IS

REFERENCE
Castoldi,D.; Monzani,V.; Tofanetti,O. Simultaneous determination of nimesulide and hydroxynimesulide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *425*, 413–418.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 2 mL 2 M HCl + 1 mL 2.5 μg/mL chlorpropamide in water + 5 mL diethyl ether, extract, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL DMF:water 10:90, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 μm μBondapak C16
Column: 250 × 2.5 5 μm Apex ODS (Rayonic Scientifique)
Mobile phase: MeCN:buffer 22.5:77.5 containing 3 mL/L Pic A (tetrabutylammonium phosphate) (Buffer was 392 mL 67 mM KH_2PO_4 and 608 mL 67 mM Na_2HPO_4, pH 7.0.)
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 14.8
Internal standard: chlorpropamide (10.0)
Limit of detection: 100-200 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; serum; pharmacokinetics

REFERENCE

St-Hilaire,S.; Belanger,P.M. Simultaneous determinations of tolbutamide and its hydroxy and carboxy metabolites in serum and urine: application to pharmacokinetic studies of tolbutamide in the rat, *J.Pharm.Sci.*, **1989**, *78*, 863–866.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Serum + 2 mL water + 200 μL 1 (?) M HCl + 200 μL 2.5 μg/mL glibornuride in MeOH + 7 mL diethyl ether, mix, centrifuge at 2000 rpm for 5 min. Remove 6.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 2 mg/mL dinitrofluorobenzene in butyl acetate, heat at 120° for 1 h, cool, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 120 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS 2
Mobile phase: MeCN:0.4% aqueous phosphoric acid 75:25
Column temperature: 40
Flow rate: 1.2
Injection volume: 120
Detector: UV 360

CHROMATOGRAM

Retention time: 4.9
Internal standard: glibornuride (5.8)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: chlorpropamide, glipizide, glyburide (glibenclamide), tolazamide

KEY WORDS

serum; derivatization

REFERENCE

Starkey,B.J.; Mould,G.P.; Teale,J.D. The determination of sulphonylurea drugs by HPLC and its clinical application, *J.Liq.Chromatogr.*, **1989**, *12*, 1889–1896.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 200 μL 1 M HCl + 10 μg glyburide + 5 mL toluene, shake gently for 15 min, centrifuge at 1500 g for 3 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 25 μL 6 mg/mL dinitrofluorobenzene in n-butyl acetate (prepare fresh each week, store at 4° in the dark), heat at 120° for 30 min, evaporate to dryness, reconstitute with 50 μL mobile phase, inject a 25-50 μL aliquot. Alternatively, filter (Amicon YMT membrane, 30000 MW cutoff) 200 μL 100 mM NaOH while centrifuging at 4°, rinse filter with 500 μL water, filter 1 mL serum in the same unit while centrifuging at 4° at 2500 g for 1.5 h. Remove a 700 μL aliquot of the ultrafiltrate, add 200 μL 1 M HCl, add 10 μg glyburide, add 5 mL toluene, shake gently for 15 min, centrifuge at 1500 g for 3 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 25 μL 6 mg/mL dinitrofluorobenzene in n-butyl acetate (prepare fresh each week, store at 4° in the dark), heat at 120° for 30 min, evaporate to dryness, reconstitute with 50 μL mobile phase, inject a 25-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 7 μm LiChrosorb RP18
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 80:20
Flow rate: 1
Injection volume: 25-50
Detector: UV 360

CHROMATOGRAM

Retention time: 5

Internal standard: glyburide (7)
Limit of detection: 2 ng/mL

KEY WORDS
derivatization; serum; ultrafiltrate; pharmacokinetics

REFERENCE
Arcelloni,C.; Fermo,I.; Calderara,A.; Pacchioni,M.; Pontiroli,A.E.; Paroni,R. Glibenclamide and tolbutamide in human serum: Rapid measurement of the free fraction, *J.Liq.Chromatogr.*, **1990**, *13*, 175–189.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 2 M HCl + 2 mL diethyl ether, vortex 30 s, centrifuge at 1500 g for 5 min, freeze in dry ice for 5 min. Decant ether layer and evaporate it to dryness under a stream of nitrogen at 35-40°. Reconstitute extract in 100 μL mobile phase, vortex 30 s, inject 25-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18
Mobile phase: MeCN:10 mM orthophosphoric acid 50:50
Flow rate: 1
Injection volume: 25-50
Detector: UV 230

CHROMATOGRAM
Retention time: 7.67
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: glyburide, gliclazide, chlorpropamide, glipizide, tolazamide
Noninterfering: trimethoprim, sulfamethoxazole

KEY WORDS
plasma

REFERENCE
Shenfield,G.M.; Boutagy,J.S.; Webb,C. A screening test for detecting sulfonylureas in plasma, *Ther.Drug Monit.*, **1990**, *12*, 393–397.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL water + 500 μL 100 mM HCl + 3 mL dichloromethane, mix for 15 s, centrifuge. Remove an aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 1000 × 8 10 μm radial pak C18 (Waters)
Mobile phase: MeOH:0.2% acetic acid 60:40 adjusted to pH 6.7 with 1 M NaOH (Wash with MeCN at 1 mL/min for 20 min at the end of each day.)
Flow rate: 0.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11
Internal standard: tolbutamide

OTHER SUBSTANCES
Extracted: chlorpropamide

KEY WORDS
plasma; tolbutamide is IS

REFERENCE
Bakare,M.T.; Mustapha,A.; Abdu-Aguye,I. An improved high-performance liquid chromatographic determination of chlorpropamide in human plasma, *Chromatographia*, **1994**, *39*, 107–109.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM
Retention time: 3.95
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil;

lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Plasma. 100 μL Plasma + 300 μL MeOH, mix, centrifuge at 3000 rpm for 5 min, filter (0.50 μm) the supernatant, inject an aliquot of the filtrate. Tissue. 500 μL Tissue homogenate + 2 mL MeOH, mix for 30 min, centrifuge at 4000 rpm for 20 min, filter (0.50 μm) the supernatant, inject an aliquot of the filtrate. Urine. Filter (0.45 μm) urine, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 TSKgel ODS-80TM (Tosoh)
Mobile phase: MeOH:isopropanol:0.05% phosphoric acid 35:6:60
Column temperature: 50
Detector: UV 227

CHROMATOGRAM

Limit of quantitation: 4 μg/mL

KEY WORDS

rat; pharmacokinetics; plasma; liver; small intestine; pharmacokinetics

REFERENCE

Yamao,T.; Nakagami,H.; Furuhama,K.; Onodera,T.; Kurosaki,Y.; Nakayama,T.; Kimura,T. Pharmacokinetics of tolbutamide following intravenous and oral administrations in rats with obstructive jaundice, *Biol.Pharm.Bull.*, **1994**, *17*, 691–695.

SAMPLE

Matrix: blood, urine

Sample preparation: Caution! Benzene is a carcinogen! 500 μL Plasma or urine + 1 mL 50 mM HCl + 3 mL benzene, shake gently for 15 min, centrifuge at 3250 g for 5 min. Remove organic layer and evaporate it to dryness under a stream of air. Dissolve residue in 50 μL mobile phase, vortex, inject 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm Spherisorb ODS C18
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM

Retention time: 8.2
Internal standard: tolbutamide

OTHER SUBSTANCES

Simultaneous: glipizide

KEY WORDS

plasma; tolbutamide is IS

REFERENCE

Emilsson,H. High-performance liquid chromatographic determination of glipizide in human plasma and urine, *J.Chromatogr.*, **1987**, *421*, 319–326.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or 200 μL urine + 100 μL 50 (plasma) or 250 (urine) μg/mL chlorpropamide in water + 100 μL 1 M HCl + 4 mL diethyl ether, shake for 10 min, centrifuge at 2000 g for 2-3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeCN:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm BST C8 (BST, Budapest)
Mobile phase: MeCN:isopropanol:0.1% orthophosphoric acid 17:17:66
Flow rate: 1.2
Injection volume: 5
Detector: UV 235

CHROMATOGRAM
Retention time: 10.5
Internal standard: chlorpropamide (8.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Csillag,K.; Vereczkey,L.; Gachalyi,B. Simple high-performance liquid chromatographic method for the determination of tolbutamide and its metabolites in human plasma and urine using photodiode-array detection, *J.Chromatogr.*, **1989**, *490*, 355–363.

SAMPLE
Matrix: microsomal incubation
Sample preparation: 1 mL Microsomal incubation + 100 μL 2 M HCl, cool rapidly on ice, extract with 8 mL hexane:chloroform:isobutyl alcohol 100:25:0.5, add 100 μL 10 μg/mL tolbutamide to the aqueous layer, extract with 3 mL diethyl ether. Combine (?) the organic layers and evaporate them to dryness at 60° under a stream of nitrogen, reconstitute with 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Mobile phase: MeCN:5 mM pH 4.3 acetate buffer 25:75
Flow rate: 1.5
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 15
Internal standard: chlorpropamide (8)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Rieutord,A.; Stupans,I.; Shenfield,G.M.; Gross,A.S. Gliclazide hydroxylation by rat liver microsomes, *Xenobiotica*, **1995**, *25*, 1345–1354.

SAMPLE
Matrix: microsomal incubations

Sample preparation: 200 μL Microsomal incubation + 25 μL 3 M HCl + 5 μL 1.78 μM chlorpropamide + 1 mL diethyl ether, vortex for 2 min, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL MeOH, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm C18 (Analytichem)
Mobile phase: MeOH:10 mM $(NH_4)H_2PO_4$ 55:45, pH 5.4
Flow rate: 0.7
Injection volume: 4
Detector: UV 240

CHROMATOGRAM
Retention time: 11
Internal standard: chlorpropamide (7)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Ho,J.W.; Moody,D.E. Determination of tolbutamide hydroxylation in rat liver microsomes by high-performance liquid chromatography: effect of psychoactive drugs on in vitro activity, *Life Sci.*, **1993**, *52*, 21–28.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 2 mL Microsomal incubation + 50 μL concentrated HCl + 100 μL 50 μg/mL chlorpropamide in MeOH + 4 mL chloroform, rotate for 30 min, centrifuge at 2000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: Spherisorb ODS2 C18
Mobile phase: MeOH:0.05% orthophosphoric acid 50:50
Flow rate: 1
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 12.3
Internal standard: chlorpropamide (9.5)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Ashforth,E.I.L.; Carlile,D.J.; Chenery,R.; Houston,J.B. Prediction of *in vivo* disposition from *in vitro* systems: Clearance of phenytoin and tolbutamide using rat hepatic microsomal and hepatocyte data, *J.Pharmacol.Exp.Ther.*, **1995**, *274*, 761–766.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 200 μL 1 M orthophosphoric acid + 200 μL 2% chlorpropamide in water + 11 mL MTBE, extract for 40 min. Remove the organic layer and evaporate it to dryness under vacuum at 40°, reconstitute the residue in 25 μL MeCN:water 25:75, inject a 100 μL (sic) aliquot.

HPLC VARIABLES
Column: Novapack C18 radial compression
Mobile phase: MeCN:water 26:74
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 25.6
Internal standard: chlorpropamide (16.5)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Weaver,R.J.; Dickins,M.; Burke,M.D. Hydroxylation of the antimalarial drug 58C80 by CYP2C9 in human liver microsomes: Comparison with mephenytoin and tolbutamide hydroxylations, *Biochem.Pharmacol.*, **1995**, *49*, 997–1004.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic

acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.74 (A), 6.94 (B)

OTHER SUBSTANCES

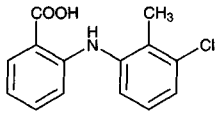
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline,

terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Tolfenamic acid



Molecular formula: $C_{14}H_{12}ClNO_2$
Molecular weight: 261.71
CAS Registry No.: 13710-19-5
Merck Index: 9650

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

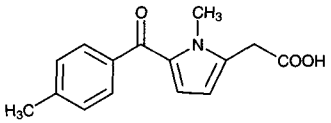
CHROMATOGRAM
Retention time: k' 3.31

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Tolmetin



Molecular formula: $C_{15}H_{15}NO_3$
Molecular weight: 257.29
CAS Registry No.: 26171-23-3, 64490-92-2 (sodium salt dihydrate), 35711-34-3 (sodium salt)
Merck Index: 9655
Lednicer No.: 2 234

SAMPLE

Matrix: blood
Sample preparation: 250 μL Plasma + 125 μL 1 M sulfuric acid + 5 mL 10 μg/mL diphenylacetic acid in dichloromethane, vortex for 10 s, centrifuge at 500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax ODS
Mobile phase: Gradient. MeOH:100 mM pH 5 acetate buffer 22:78 for 5 min, to 53:47 over 2 min, maintain at 53:47 for 8 min.
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 13.11
Internal standard: diphenylacetic acid (13.93)
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: acetaminophen, caffeine, carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone, quinidine, salicylic acid, theophylline
Noninterfering: fenoprofen, ibuprofen, indomethacin, naproxen, sulindac, valproic acid

KEY WORDS

plasma

REFERENCE

Shimek,J.L.; Rao,N.G.S.; Wahba Khalil,S.K. High performance liquid chromatographic analysis of tolmetin, indomethacin and sulindac in plasma, *J.Liq.Chromatogr.*, **1981**, *4*, 1987–2013.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 0.5 mL 1 M HCl + 10 mL dichloromethane, shake 10 min, centrifuge at 1000 g for 5 min. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute with 200 μL mobile phase, inject 10-30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 Partisil 10 ODS-3
Mobile phase: MeOH:water:phosphoric acid 700:300:1
Flow rate: 2.5
Injection volume: 10-30
Detector: UV 220

CHROMATOGRAM

Retention time: 3.2
Internal standard: tolmetin

OTHER SUBSTANCES
Simultaneous: ibuprofen, tolmetin is IS

KEY WORDS
plasma

REFERENCE
Lockwood,G.F.; Wagner,J.G. High-performance liquid chromatographic determination of ibuprofen and its major metabolites in biological fluids, *J.Chromatogr.*, **1982**, *232*, 335–343.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 90 μL 1 M HCl + 20 μL 1 mg/mL p-phenylphenol in MeCN, vortex for 10 s, add 6 mL benzene:tert-butyl alcohol 90:10 (Caution! Benzene is a carcinogen!), shake mechanically for 20 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL mobile phase, sonicate for a few s, inject an aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 30-40 μm Perisorb RP-18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:50 mM KH_2PO_4 48:52, pH adjusted to 4.7 with 8.5% phosphoric acid
Flow rate: 0.5 for 5 min then increase to 0.7 over 1 min, after another 4 min increase to 1.0 over 1 min
Injection volume: 20
Detector: UV 313

CHROMATOGRAM
Retention time: 6.5
Internal standard: p-phenylphenol (11)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: Med 5, Med 15, metabolites
Noninterfering: sulindac, naproxen, indomethacin, flufenamic acid, ibuprofen, aspirin

KEY WORDS
plasma; rat

REFERENCE
Mancinelli,A.; Bruno,G.; Cardace,G.; Morabito,E.; Marzo,A.; Arrigoni Martelli,E. High-performance liquid chromatographic evaluation of Med 15 and its metabolites Med 5 and tolmetin in rat plasma, *J.Chromatogr.*, **1991**, *553*, 81–86.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 100 mM pH 4 sodium acetate, extract twice with 5 mL diethyl ether. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL water, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:1 mM pH 3 phosphoric acid 32:68
Flow rate: 1
Injection volume: 80
Detector: UV 313

CHROMATOGRAM
Retention time: 11
Internal standard: tolmetin

OTHER SUBSTANCES
Extracted: ketorolac

KEY WORDS
plasma; pharmacokinetics; tolmetin is IS

REFERENCE
Flores-Murrieta,F.J.; Granados-Soto,V.; Castañeda-Hernández,G.; Herrera,J.E.; Hong,E. Comparative bioavailability of two oral formulations of ketorolac tromethamine: Dolac and Exodol, *Biopharm.Drug Dispos.*, **1994**, *15*, 129–136.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL pH 1.8 phosphate buffer, extract with 1-butanol/MTBE. Remove the organic layer and add it to 500 μL pH 6.1 ammonium acetate buffer, mix, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil C18
Mobile phase: MeCN:250 mM pH 5.0 ammonium acetate buffer 20:80
Flow rate: 1.8
Detector: UV 258

CHROMATOGRAM
Internal standard: tolmetin

OTHER SUBSTANCES
Extracted: ketoprofen (UV 350)

KEY WORDS
plasma; tolmetin is IS

REFERENCE
Shah,A.K.; Wei,G.; Lanman,R.C.; Bhargava,V.O.; Weir,S.J. Percutaneous absorption of ketoprofen from different anatomical sites in man, *Pharm.Res.*, **1996**, *13*, 168–172.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 322

OTHER SUBSTANCES
Also analyzed: carbamazepine, fenbufen, indomethacin, ketoprofen, α.-naphthoquinone, naproxen

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:10 mM sodium carbonate 18:82. B was MeCN:50 mM sodium carbonate 33:67. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: aspirin, ibuprofen, naproxen, fenbufen, indomethacin, carprofen, diflunisal

REFERENCE

Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, predni-

solone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.45 (A), 6.29 (B)

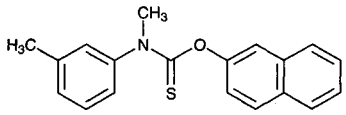
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Tolnaftate



Molecular formula: $C_{19}H_{17}NOS$
Molecular weight: 307.42
CAS Registry No.: 2398-96-1
Merck Index: 9656
Lednicer No.: 2 211

SAMPLE
Matrix: blood, tissue
Sample preparation: Skin. Homogenize 100-200 mg skin with 3 mL 1.15% KCl, add 3 mL naphthyl phenylacetate in dichloromethane (?), add 3 mL dichloromethane, agitate for 10 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 10 μL aliquot. Whole blood. 200 μL Whole blood + 200 μL water, mix, add 3 mL naphthyl phenylacetate in dichloromethane (?), add 3 mL dichloromethane, agitate for 10 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:water 80:20

KEY WORDS
whole blood; skin; mouse; pharmacokinetics

REFERENCE
Szeman,J.; Ueda,H.; Szejtli,J.; Fenyvesi,E.; Watanabe,Y.; Machida,Y.; Nagai,T. Enhanced percutaneous absorption of homogenized tolnaftate/β-cyclodextrin polymer ground mixture, *Drug Des.Deliv.*, **1987**, *1*, 325–332.

SAMPLE
Matrix: formulations
Sample preparation: Solutions. Measure out amount containing 10 mg tolnaftate, dilute to 100 mL with MeCN:water 80:20, dilute 10 fold with MeCN:water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate. Aerosol liquids. Discharge aerosol into a container, warm at 25° until propellant dissipates, dilute a 1 mL aliquot to 100 mL with MeCN: water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate. Powders. Weigh out amount containing 5 mg tolnaftate, add 50 mL MeCN:water 80:20, shake mechanically for 15 min, make up to 100 mL with MeCN:water 80:20, let stand for 15 min, dilute an aliquot 5 fold with MeCN:water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate. Aerosol powders. Discharge aerosol into a container, heat at 25° under a current of air to remove propellant, weigh out amount containing 5 mg tolnaftate, add 50 mL MeCN:water 80:20, shake mechanically for 15 min, make up to 100 mL with MeCN: water 80:20, let stand for 15 min, dilute an aliquot 5 fold with MeCN:water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate. Creams, gels. Weigh out amount containing 5 mg tolnaftate, add 50 mL MeCN:water 80:20, shake mechanically for 15 min, make up to 100 mL with MeCN:water 80:20, let stand for 15 min, dilute an aliquot 5 fold with MeCN:water 80:20, filter (0.45 μm), discard the first 1-2 mL, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 80:20 containing 500 μL/L phosphoric acid, pH 2.4
Flow rate: 1
Injection volume: 20
Detector: UV 257

CHROMATOGRAM
Retention time: 5

KEY WORDS
liquids; powders; aerosols; solutions; cream; gels

REFERENCE
Thompson,R.D.; Carlson,M. Liquid chromatographic determination of tolnaftate in commercial products, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 603–607.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μg/mL solution in MeOH, inject an aliquot.

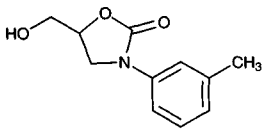
HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select-B (A) or 125 × 2 4 μm Superspher 100 RP-18 (B)
Mobile phase: MeCN:pH 2 trifluoroacetic acid 70:30 (A) or Gradient. C was MeCN:water 90:10. D was MeOH:pH 2 trifluoroacetic acid buffer 15:85. C:D from 30:70 to 80:20 over 8 min (B)
Flow rate: 1 (A) or 0.4 (B)
Detector: UV 254 (A), UV 245 (B)

CHROMATOGRAM
Retention time: 9.75 (A), 9.6 (B)

OTHER SUBSTANCES
Simultaneous: cloxyquin, chlorphenesin, naftifine, sulbentine, degradation products

REFERENCE
Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

Toloxatone



Molecular formula: $C_{11}H_{13}NO_3$
Molecular weight: 207.23
CAS Registry No.: 29218-27-7
Merck Index: 9659

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 238

CHROMATOGRAM

Retention time: 3.35
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF
Sample preparation: 50 μL Plasma or CSF + 50 μL 2 μg/mL trazodone, vortex briefly, add 20 μL 4 M NaOH, vortex briefly, add 750 μL diethyl ether, vortex for 1 min, centrifuge at 2600 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 25 × 4 Hibar LiChroCART C8 (Merck)
Column: 250 × 4 5 μm LiChrospher 100 CH-8 II C8
Mobile phase: MeCN:10 mM pH 3.0 phosphate buffer 60:40 containing 20 mM tetramethylammonium chloride
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 4.9
Internal standard: trazodone (6.0)
Limit of detection: 70 ng/mL

KEY WORDS

plasma; rabbit; pharmacokinetics

REFERENCE

Vistelle,R.; Lamiable,D.; Zinzou,M. Simple high-performance liquid chromatographic method for the measurement of toloxatone in rabbit cerebrospinal fluid and plasma, *J.Chromatogr.*, **1989**, *490*, 387–394.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM

Retention time: 14.107

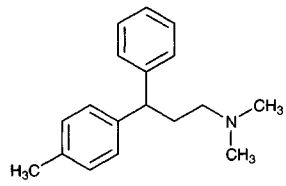
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tolpropamine



Molecular formula: $C_{18}H_{23}N$
Molecular weight: 253.39
CAS Registry No.: 5632-44-0
Merck Index: 9662

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

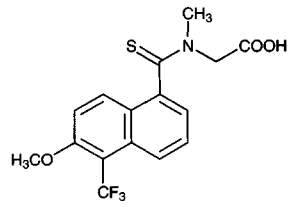
Retention time: 3.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Tolrestat



Molecular formula: $C_{16}H_{14}F_3NO_3S$
Molecular weight: 357.35
CAS Registry No.: 82964-04-3
Merck Index: 9663
Lednicer No.: 4 56

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. 1 mL Serum + 4 mL 1 M HCl + 10 mL isopropyl ether (Caution! isopropyl ether readily forms explosive peroxides!), agitate mechanically for 15 min, centrifuge at 1500 rpm for 10 min. Remove 8.5 mL of the upper organic phase and add it to 1 mL 100 mM pH 11.0 glycine buffer, agitate mechanically for 15 min, centrifuge at 1500 rpm for 10 min. Remove 800 μL of the aqueous phase and add it to 20 μL 2.5 M phosphoric acid, inject a 50-150 μL aliquot. (For increased sensitivity use 2 mL serum and 500 μL glycine buffer.) Tissue. Homogenize (all-glass, Kontes) 50-100 mg lens or nerve tissue with 3 mL isotonic saline. 2.5 mL Homogenate + 4 mL 100 mM phosphoric acid + 10 mL isopropyl ether (Caution! isopropyl ether readily forms explosive peroxides!), agitate mechanically for 30 min, centrifuge at 1500 rpm for 15 min. Remove 9.0 mL of the upper organic phase and add it to 1 mL 100 mM pH 11.0 glycine buffer, agitate mechanically for 15 min, centrifuge at 1500 rpm for 10 min. Remove 800 μL of the aqueous phase and add it to 20 μL 2.5 M phosphoric acid, inject a 50-150 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:100 mM pH 6.0 phosphate buffer 30:70
Column temperature: 50
Flow rate: 1.1
Injection volume: 50-150
Detector: UV 226

CHROMATOGRAM
Retention time: 6.4
Limit of detection: 25 ng/mL (2 mL serum), 200 ng/mL (1 mL serum), 50 ng/g (tissue)

OTHER SUBSTANCES
Extracted: rotamer
Noninterfering: acetaminophen, diazepam, glyburide, hydrochlorothiazide, indomethacin, niacin, phenobarbital, phenytoin, propoxyphene, salicylic acid, tolbutamide
Interfering: dicoumarol, phenylbutazone

KEY WORDS
rat; dog; human; serum; sciatic nerve; lens

REFERENCE
Hicks,D.R.; Kraml,M. Determination of tolrestat, a novel aldose reductase inhibitor, in serum and tissues, *Ther.Drug Monit.*, **1984**, *6*, 328–333.

SAMPLE
Matrix: bulk

Sample preparation: Prepare a 100 μg/mL solution in MeCN:buffer 35:65, inject a 5 μL aliquot. (Buffer was 10 mM phosphoric acid adjusted to pH 7.0 with 10 mM ammonium hydroxide.)

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve octadecylsilane (Waters)
Mobile phase: MeCN:THF:buffer:40% tetrabutylammonium hydroxide in water 20.5:18.5:61.5:0.3 (Buffer was 50 mM $(NH_4)H_2PO_4$ adjusted to pH 3.5 with 50 mM phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 229

CHROMATOGRAM
Retention time: 4.6
Limit of quantitation: 0.05% (of tolrestat)

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
rugged

REFERENCE
Beaulieu,N.; Lacroix,P.M.; Sears,R.W.; Lovering,E.G. Liquid chromatographic determination of tolrestat and related compounds in raw materials, *J.AOAC Int.*, **1995**, *78*, 647–650.

SAMPLE
Matrix: solutions
Sample preparation: Inject buffer solution directly.

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM pH 6.5 phosphate buffer 30:70 containing 0.8 g/L tetraethylammonium chloride (Flush column with MeOH at the end of each day.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7 (rotamer A), 3.9 (rotamer B)

OTHER SUBSTANCES
Simultaneous: rotamers

KEY WORDS
buffer

REFERENCE
Lee,H.-K.; Querijero,G. Kinetics and mechanisms of thioamide rotational isomerism: *N*-thionaphthoyl-*N*-methyl glycine derivative, *J.Pharm.Sci.*, **1985**, *74*, 273–276.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

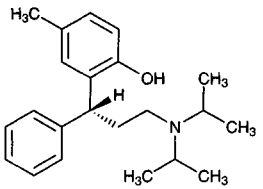
HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM KH_2PO_4:1 M tetrabutylammonium hydroxide 35:65:0.5
Flow rate: 1.5
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 8 (rotamer A), 6 (rotamer B)

OTHER SUBSTANCES
Simultaneous: degradation products, rotamers

REFERENCE
Lee,Y.J.; Lee,H.-K. Degradation kinetics of tolrestat, *J.Pharm.Sci.*, **1990**, *79*, 628–633.

Tolterodine



Molecular formula: $C_{22}H_{31}NO$
Molecular weight: 325.49
CAS Registry No.: 124937-51-5

SAMPLE
Matrix: blood
Sample preparation: Mix plasma or serum with two volumes of acetone, centrifuge. Remove the supernatant, wash the pellets with two portions of acetone:20 mM pH 4.5 ammonium acetate 50:50, evaporate the combined supernatant and pellet extract to dryness, dissolve the residue in MeOH:20 mM pH 4.5 ammonium acetate 10:90. Inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.5 Supelco PKB 100
Column: 150 × 4.5 Supelco PKB 100
Mobile phase: Gradient. A was MeOH. B was 20 mM pH 4.5 ammonium acetate. A:B from 10:90 to 20:80 in 5 min, from 20:80 to 45:55 in 30 min, from 45:55 to 100:0 in 5 min, maintain at 100:0 for 10 min
Flow rate: 1
Injection volume: 100
Detector: UV 280

CHROMATOGRAM
Retention time: 29

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
dog; mouse; serum; plasma

REFERENCE
Andersson,S.H.G.; Lindgren,A.; Postlind,H. Biotransformation of tolterodine, a new muscarinic receptor antagonist, in mice, rats, and dogs, *Drug Metab.Dispos.*, **1998**, *26*, 528–535.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Mix 250 μL or 1 mL microsomal incubation with an equal volume of acetone, centrifuge at 3200 rpm, evaporate the acetone in the supernatant under a stream of nitrogen. Inject a 100-200 μL aliquot of the remaining supernatant.

HPLC VARIABLES
Guard column: 20 mm Supelco PKB 100
Column: 150 × 4.5 Supelco PKB 100

Mobile phase: Gradient. A was MeOH. B was 20 mM pH 4.5 ammonium acetate. A:B from 10:90 to 20:80 over 5 min, to 45:55 over 30 min, to 100:0 over 5 min, maintain at 100:0 for 10 min
Flow rate: 1
Injection volume: 100-200
Detector: UV 280; Radioactivity, Beckman 171 radioisotope detector

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
pharmacokinetics; liver

REFERENCE
Postlind,H.; Danielson,Å.; Lindgren,A.; Andersson,H.,G. Tolterodine, a new muscarinic receptor antagonist, is metabolized by cytochromes P450 2D6 and 3A in human liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 289–293.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add an equal volume of acetone to the microsomal incubation, centrifuge at 3200 rpm, evaporate acetone under a stream of nitrogen. Inject a 100-200 μL aliquot of the remaining supernatant.

HPLC VARIABLES
Guard column: 20 mm long Supelco PKB 100
Column: 150 × 4.5 Supelco PKB 100
Mobile phase: Gradient. A. MeOH. B. 20 mM ammonium acetate. A:B from 10:90 to 20:80 over 5 min, to 45:55 over 30 min, to 100:0 over 5 min, maintain at 100:0 for 10 min.
Flow rate: 1
Injection volume: 100-200
Detector: UV 280

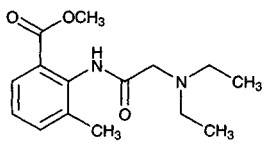
CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
liver

REFERENCE
Postlind,H.; Danielson,Å.; Lindgren,A.; Andersson,H.,G. Tolterodine, a new muscarinic receptor antagonist, is metabolized by cytochromes P450 2D6 and 3A in human liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 289–293.

Tolycaine



Molecular formula: $C_{15}H_{22}N_2O_3$
Molecular weight: 278.35
CAS Registry No.: 3686-58-6, 7210-92-6 (HCl)
Merck Index: 9679
Lednicer No.: 1 17

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

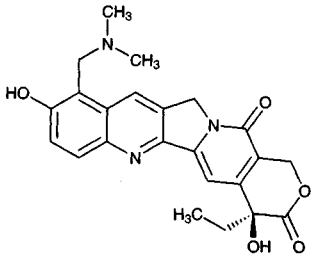
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Topotecan



Molecular formula: $C_{23}H_{23}N_3O_5$
Molecular weight: 421.45
CAS Registry No.: 123948-87-8, 119413-54-6 (HCl)
Merck Index: 9687

SAMPLE
Matrix: formulations
Sample preparation: Dilute topotecan hydrochloride injection with 5% dextrose or 0.9% NaCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5C18-AR (Nacalai Tesque, Japan)
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 15:85:0.1. B was MeCN:water:trifluoroacetic acid 40:60:0.1. A:B from 100:0 to 0:100 in 20 min, from 0:100 to 100:0 in 1 min
Flow rate: 1
Injection volume: 40
Detector: UV 228

CHROMATOGRAM
Retention time: 10.0

KEY WORDS
injections

REFERENCE
Craig,S.B.; Bhatt,U.H.; Patel,K. Stability and compatibility of topotecan hydrochloride for injection with common infusion solutions and containers, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 199–205.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute 4-mg topotecan hydrochloride injection with water to a nominal concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5C18-AR (Nacalai Tesque, Kyoto)
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 15:85:0.1. B was MeCN:water:trifluoroacetic acid 40:60:0.1. A:B 100:0 for 16 min, to 0:100 over 24 min, to 100:0 over 2 min, maintain at 100:0 for 18 min
Flow rate: 1
Injection volume: 20
Detector: UV 228

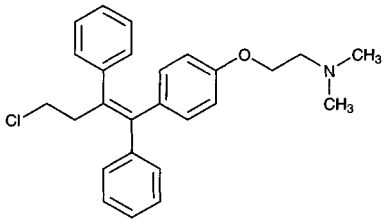
CHROMATOGRAM
Retention time: 10.7-12.0

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Patel,K.; Craig,S.B.; McBride,M.G.; Palepu,N.R. Microbial inhibitory properties and stability of topotecan hydrochloride injection, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1584–1587.

Toremifene



Molecular formula: $C_{26}H_{28}ClNO$
Molecular weight: 405.97
CAS Registry No.: 89778-26-7, 89778-27-8 (citrate)
Merck Index: 9688
Lednicer No.: 5 33

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL hexane:n-butanol 98:2, vortex for 1 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 300 μL MeOH, filter (0.2 μm). Place the filtrate in a quartz cuvette, irradiate with a mercury vapor lamp (15 W, peak wavelength 254 nm, General Electric No. G15T8) for 2 min (Caution! Protect personnel from UV radiation with aluminum foil shielding!), inject a 100 μL aliquot of the irradiated sample.

HPLC VARIABLES

Column: Ultrasphere ODS C18
Mobile phase: MeOH:water:triethylamine 92.9:7:0.1 (At the end of each day wash column with MeOH at 2 mL/min for 30 min.)
Flow rate: 2
Injection volume: 100
Detector: F ex 266

CHROMATOGRAM

Retention time: 6.03
Limit of detection: 8 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; UV irradiation; derivatization

REFERENCE

Holleran,W.M.; Gharbo,S.A.; DeGregorio,M.W. Quantitation of toremifene and its major metabolites in human plasma by high-performance liquid chromatography following fluorescent activation, *Anal.Lett.*, **1987**, *20*, 871–879.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 200 μL 2 μg/mL IS in MeCN, vortex for 10 s, centrifuge at 13000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: μBondapak C18
Column: 100 × 8 NovaPak C18 Rad-Pak
Mobile phase: MeCN:100 mM ammonium acetate:triethylamine 65:35:0.05 adjusted to pH 6.4 with acetic acid
Flow rate: 2
Injection volume: 100
Detector: UV 277

CHROMATOGRAM

Retention time: 5.4
Internal standard: (Z)-4-chloro-1,2-diphenyl-1-[4-[2-pyrroloethoxy]phenyl]-1-butene (Fc-1226a, Farmos) (6.1)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Webster,L.K.; Crinis,N.A.; Stokes,K.H.; Bishop,J.F. High-performance liquid chromatographic method for the determination of toremifene and its major human metabolites, *J.Chromatogr.*, **1991**, *565*, 482–487.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of serum to 9.5, extract 500 μL serum with diethyl ether. Remove the organic layer and evaporate it to dryness, reconstitute the residue, inject an aliquot.

HPLC VARIABLES
Column: reversed-phase C18
Mobile phase: MeOH:water 94:6 containing 18 mg/L diethylamine acetate
Flow rate: 2
Detector: F ex 267 em 377 following post-column photolysis in a PTFE coil

CHROMATOGRAM
Retention time: 7.5
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics; post-column photochemical derivatization

REFERENCE
Anttila,M.; Laakso,S.; Nyländen,P.; Sotaniemi,E.A. Pharmacokinetics of the novel antiestrogenic agent toremifene in subjects with altered liver and kidney function, *Clin.Pharmacol.Ther.*, **1995**, *57*, 628–635.

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Microsomal incubations. Add two volumes of MeOH:DMSO 80:20, centrifuge, inject an aliquot of the supernatant. Plasma. 200 μL Plasma + 400 μL MeOH:DMSO 80:20, vortex for 30 s, centrifuge at 5000 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil-ODS
Mobile phase: MeCN:250 mM pH 5.16 ammonium acetate buffer 65:35
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; liver; human

REFERENCE
Lim,C.K.; Yuan,Z.-X.; Ying,K.C.; Smith,L.L. High performance liquid chromatography of toremifene and metabolites, *J.Liq.Chromatogr.*, **1994**, *17*, 1773–1783.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 5 mL chilled chloroform to microsomal mixture, vortex, adjust aqueous phase to pH 9, extract with 5 mL chloroform. Combine the organic phases and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeOH:water 85:15, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-Select-B C8
Mobile phase: MeOH:water:triethylamine 80:20:0.01
Flow rate: 0.8
Detector: UV 238, or UV 277, or F ex 258 em 318 preceded by an on-line Knauer UV photoreactor

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Simultaneous: metabolites
Also analyzed: tamoxifen

KEY WORDS
post-column photochemical derivatization

REFERENCE
Berthou,F.; Dréano,Y. High-performance liquid chromatographic analysis of tamoxifen, toremifene and their major human metabolites, *J.Chromatogr.*, **1993**, *616*, 117–127.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 65:35 (Buffer was 100 mM ammonium acetate adjusted to pH 6.5 with glacial acetic acid.)
Flow rate: 1
Injection volume: 50
Detector: MS, VG Trio-2 quadrupole, thermospray, ion source 160°, vaporizer tip 160°, m/z 406

CHROMATOGRAM
Retention time: 11.5
Limit of detection: 10 ng/mL

REFERENCE
Martinsen,A.; Gynther,J. Liquid chromatography-thermospray mass spectrometry of toremifene and its derivatives, *J.Chromatogr.A*, **1996**, *724*, 358–363.

SAMPLE
Matrix: urine
Sample preparation: 30 mL Urine + 20 mL 100 mM pH 8.5 phosphate buffer, extract twice with 60 mL portions of n-hexane:diethyl ether 90:10. Combine the extracts and evaporate them to dryness, reconstitute with 200 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax CN
Mobile phase: MeOH:100 mM pH 8 ammonium acetate 70:30
Flow rate: 1.2
Detector: UV 237 or MS, Hitachi M-80B double-focussing MS, API source (M-8093), nebulizer 350°, vaporizer 350°, needle current 15 μA, second electrode 3 keV, drift voltage 195 V, m/z 406

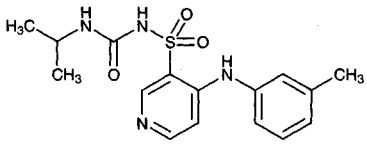
CHROMATOGRAM
Retention time: 19.5

Limit of detection: 50 ng

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Watanabe,N.; Irie,T.; Koyama,M.; Tominaga,T. Liquid chromatographic-atmospheric pressure ionization mass spectrometric analysis of toremifene metabolites in human urine, *J.Chromatogr.*, **1989**, *497*, 169–180.

Torsemide



Molecular formula: $C_{16}H_{20}N_4O_3S$
Molecular weight: 348.43
CAS Registry No.: 56211-40-6
Merck Index: 9690
Lednicer No.: 5 82

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL IS solution, mix, adjust pH to 4.5 with dilute HCl, add 4 mL ethyl acetate,, vortex for 1 min, centrifuge at 3000 rpm for 5 min, adjust pH of aqueous phase to 5, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL pH 10.2 buffer, add 4 mL ether, vortex, centrifuge. Acidify the aqueous phase to pH 4.5 and add 4 mL ethyl acetate, vortex, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 µL MeOH, inject a 2 µL aliquot. (Prepare IS solution by dissolving 30 mg IS in 10 mL 100 mM sodium bicarbonate solution, adjust pH to 7, make up to 25 mL with water.)

HPLC VARIABLES
Column: 250 × 2.6 ODS
Mobile phase: MeOH:water 30:70
Flow rate: 1
Injection volume: 2
Detector: UV 290

CHROMATOGRAM
Internal standard: 1-isopropyl-3-[[4-(4'-chloro-3'-trifluoromethylphenylamino)pyridine]-3-sulfonyl]urea (JDL 487)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lesne,M.; Clerckx-Braun,F.; Duhoux,P.; van Ypersele de Strihou,C. Pharmacokinetic study of torasemide in humans: an overview of its diuretic effect, *Int.J.Clin.Pharmacol.Ther.Toxicol.*, **1982**, *20*, 382–387.

SAMPLE
Matrix: blood
Sample preparation: Condition a C2 SPE cartridge (Analytichem) with 1 mL MeOH and 1 mL 500 mM phosphoric acid. Condition a sulfonylpropyl ion-exchange SPE cartridge (Analytichem) with 1 mL MeOH and 1 mL 75 mM HCl. Add 1 mL plasma to the C2 SPE cartridge, add 50 µL 10 µg/mL IS in water, add 500 µL 500 mM phosphoric acid, air dry for 10 s, wash with 300 µL 500 mM phosphoric acid, elute with 1 mL MeOH:water 50:50, evaporate to dryness at 60° in a vortex evaporator for 30 min, reconstitute with 1 mL 75 mM HCl, add to the sulfonylpropyl SPE cartridge, wash with 1 mL dichloromethane, air dry, elute the contents of the SPE car-

tridge on to the column with mobile phase, after 5 min remove the SPE cartridge from the circuit.

HPLC VARIABLES
Guard column: C18
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:100 mM pH 4.5 KH_2PO_4 14:86 for 8 min, 30:70 for 3.5 min, 21:79 for 8.5 min (sic), 40:60 for 2 min, 21:79 for 10 min, 14:86 for 6 min (step gradients).
Column temperature: 45
Flow rate: 1.3
Detector: UV 290

CHROMATOGRAM
Retention time: 18.2
Internal standard: 1-isopropyl-3-[[4-(3'-trifluoromethylphenylamino)pyridine]-3-sulfonyl]urea (28.0)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
March,C.; Farthing,D.; Wells,B.; Besenfelder,E.; Karnes,H.T. Solid-phase extraction and liquid chromatography of torsemide and metabolites from plasma and urine, *J.Pharm.Sci.*, **1990**, *79*, 453–457.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 100 μL IS solution, mix, adjust pH to 4.5 with pH 4 McIlvaine buffer, add 4 mL ethyl acetate, vortex for 1 min, centrifuge at 3000 rpm for 5 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL pH 11.8 buffer, wash twice with 4 mL portions of ether. Acidify the aqueous phase to pH 4.5 and extract twice with 4 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 50 μL MeOH, inject a 2 μL aliquot. (Prepare IS solution by dissolving 30 mg IS in 10 mL 100 mM sodium bicarbonate solution, adjust pH to 7, make up to 25 mL with water.)

HPLC VARIABLES
Column: 300 × 3.9 ODS
Mobile phase: MeCN:50 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1.5
Injection volume: 2
Detector: UV 290

CHROMATOGRAM
Internal standard: 1-isopropyl-3-[[4-(4'-chloro-3'-trifluoromethylphenylamino)pyridine]-3-sulfonyl]urea (JDL 487)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; dog; pharmacokinetics

REFERENCE
Ghys,A.; Denef,J.; de Suray,J.; Gerin,M.; Georges,A.; Delarge,J.; Willems,J. Pharmacological properties of the new potent diuretic torasemide in rats and dogs, *Arzneimittelforschung*, **1985**, *35(II)*, 1520–1526.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Pulverize and dissolve in MeOH, filter (0.45 μm membrane), dilute with mobile phase, inject a 20 μL aliquot. Urine. 5 mL Urine + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject a 20 μL aliquot (J. Chromatogr.A 1993, 655, 233. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 35:65 containing 5 mM KH_2PO_4/K_2HPO_4, pH 5.3
Flow rate: 1.0
Injection volume: 20
Detector: E, PAR Model 400, Ag/AgCl reference electrode, glassy carbon cell 1.3 V

CHROMATOGRAM
Retention time: 5.89
Limit of detection: 8.5 ng/mL

KEY WORDS
tablets

REFERENCE
Barroso,M.B.; Alonso,R.M.; Jiménez,R.M. Quantitative analysis of the loop diuretic torasemide in tablets and human urine by HPLC-EC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 179–186.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 10 μL 11.6 M perchloric acid, cool on ice, add 4 nmoles 4-methylumbelliferone, centrifuge at 3000 g for 5 min. Remove the supernatant and add it to 1.5 g ammonium sulfate, extract twice with dichloromethane:isopropanol 85.15. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-pak C18
Mobile phase: MeCN:10 mM pH 4.3 acetate buffer 13.5:86.5
Flow rate: 2
Injection volume: 50
Detector: UV 290

CHROMATOGRAM
Retention time: 18.9
Internal standard: 4-methylumbelliferone (7.9)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Miners,J.O.; Rees,D.L.P.; Valente,L.; Verones,M.E.; Birkett,D.J. Human hepatic cytochrome P450 2C9 catalyzes the rate-limiting pathway of torsemide metabolism, *J.Pharmacol.Exp.Ther.*, **1995**, *272*, 1076–1081.

SAMPLE
Matrix: urine

Sample preparation: Condition a C2 SPE cartridge (Analytichem) with 1 mL MeOH and 1 mL 500 mM phosphoric acid. Condition a sulfonylpropyl ion-exchange SPE cartridge (Analytichem) with 1 mL MeOH and 1 mL 75 mM HCl. Add 1 mL urine to the C2 SPE cartridge, add 700 μL 500 mM phosphoric acid, add 50 μL 10 μg/mL IS in water, wash with 1 mL 500 mM phosphoric acid, wash with 1 mL dichloromethane, elute with two 200 μL aliquots of MeOH, evaporate the eluate to dryness in a vortex evaporator, reconstitute with 1 mL 30 mM phosphoric acid, add to the sulfonylpropyl SPE cartridge, wash two 1 mL portions of water, elute with two 75 μL aliquots of MeOH:500 mM calcium chloride, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Guard column: C18
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:10 mM pH 4.5 KH_2PO_4 15:85 for 6 min, 34:66 for 2 min, 25:75 for 5 min (sic), 30:70 for 10 min, 15:85 for 7 min (step gradients).
Flow rate: 1
Injection volume: 50
Detector: UV 290

CHROMATOGRAM

Retention time: 19.0
Internal standard: 1-isopropyl-3-[[4-(3'-trifluoromethylphenylamino)pyridine]-3-sulfonyl]urea (27.1)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

SPE; pharmacokinetics

REFERENCE

March,C.; Farthing,D.; Wells,B.; Besenfelder,E.; Karnes,H.T. Solid-phase extraction and liquid chromatography of torsemide and metabolites from plasma and urine, *J.Pharm.Sci.*, **1990**, *79*, 453–457.

SAMPLE

Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN: water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

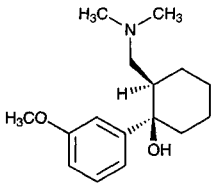
Retention time: 5.2
Internal standard: 7-propyltheophylline (4.5)

OTHER SUBSTANCES

Extracted: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, chlorthalidone, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, triamterene
Interfering: clopamide

REFERENCE
Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

Tramadol



Molecular formula: $C_{16}H_{25}NO_2$
Molecular weight: 263.38
CAS Registry No.: 27203-92-5, 22204-88-2 (HCl)
Merck Index: 9701
Lednicer No.: 2 17

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut C2 SPE cartridge with 1 mL MeOH and 1 mL pH 7.4 phosphate buffer at 6 mL/min. Centrifuge plasma at 4500 rpm for 15 min. Add 1 mL plasma to the SPE cartridge at 0.18 mL/min. Wash with 1 mL pH 7.4 phosphate buffer at 1.5 mL/min, elute with 150 μL MeOH at 1.5 mL/min and 350 μL pH 6.0 phosphate buffer containing 200 mM sodium perchlorate at 1.5 mL/min, mix the eluate, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 DIOL
Column: 250 × 4.6 10 μm Chiralcel OD-R, packed with cellulose tris-(3,5-dimethylphenylcarbamate) coated on silica
Mobile phase: MeCN:buffer 25:75 (Buffer was 50 mM phosphate buffer containing 200 mM sodium perchlorate, adjusted to pH 6.0 with NaOH solution.)
Column temperature: 30
Flow rate: 0.6
Injection volume: 200
Detector: UV 220; F ex 230 em 295

CHROMATOGRAM
Retention time: 13.3 (+), 14.9 (-)
Limit of detection: 500 pg/mL
Limit of quantitation: 1.5 ng/mL

OTHER SUBSTANCES
Extracted: active metabolite

KEY WORDS
plasma; chiral; SPE

REFERENCE
Ceccato,A.; Chiap,P.; Hubert,P.; Crommen,J. Automated determination of tramadol enantiomers in human plasma using solid-phase extraction in combination with chiral liquid chromatography, *J.Chromatogr.B*, **1997**, *698*, 161–170.

SAMPLE
Matrix: formulations
Sample preparation: Capsules. Dissolve the content of each capsule (ca. 50 mg) in 200 mL water, shake vigorously for 10 min and allow to settle down. Remove 10 mL supernatant, centrifuge at 6000 rpm for 5 min. Dilute 1 mL solution with water to a final concentration 10 μg/mL. Mix 150 μL aliquot with 150 μL 1 mg/mL metoclopramide in water. Inject a 50 μL aliquot. Intravenous ampoules. Dilute a 100 μL aliquot of the 100 mg/2 mL ampoule 100 fold with water to a final concentration 50 μg/mL. Mix 1 mL solution with 2.5 mL 1 mg/mL metoclopramide and make up to 10 mL with water. Inject a 75 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 4 5 μm Bondapak C18
Column: 150 × 3.9 5 μm Bondapak C18
Mobile phase: MeCN:10 mM pH 5.5 sodium phosphate buffer containing 5 mM triethylamine 17:83
Flow rate: 1.2
Injection volume: 25-75
Detector: UV 230

CHROMATOGRAM
Retention time: 6.2
Internal standard: metoclopramide (4.4)
Limit of detection: 75 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS
capsules; intravenous ampoules

REFERENCE
Zaghloul,I.Y.; Radwan,M.A. High performance liquid chromatographic determination of tramadol in pharmaceutical dosage forms, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 779–787.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 200 μL 2.5 μg/mL IS and 400 μL EtOH to 200 μL microsomal incubation. Mix on a whirlmix, centrifuge at 2250 g for 15 min, make the supernatant alkaline with 100 μL 25% ammonium hydroxide, add 5 mL dichloromethane, vortex for 1 min, centrifuge at 2500 g for 15 min. Discard the aqueous phase, evaporate the dichloromethane phase using nitrogen at 37°, reconstitute the residue in 200 μL EtOH:water 75:25 by agitating on a whirlmix for 30 s, inject an aliquot.

HPLC VARIABLES
Guard column: 125 × 3 Nucleosil RP 18
Column: 300 × 4 Nucleosil RP 18 (100-10)
Mobile phase: MeOH:100 mM ammonium hydrogen carbonate:25% ammonium hydroxide:triethylamine 50:49:1:0.01
Injection volume: 100
Detector: F ex 280, em 310

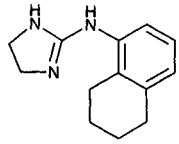
CHROMATOGRAM
Retention time: 64
Internal standard: 1-(*m*-hydroxyphenyl)-2-(N-ethyl-N-methylaminomethyl) cycloheptan -1-ol hydrochloride (35)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Paar,W.D.; Frankus,P.; Dengler,H.J. High-performance liquid chromatographic assay for the simultaneous determination of tramadol and its metabolites in microsomal fractions of human liver, *J.Chromatogr.B*, **1996**, *686*, 221–227.

Tramazoline



Molecular formula: $C_{13}H_{17}N_3$
Molecular weight: 215.30
CAS Registry No.: 1082-57-1, 3715-90-0 (H_2O)
Merck Index: 9702
Lednicer No.: 1 243

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 13.27

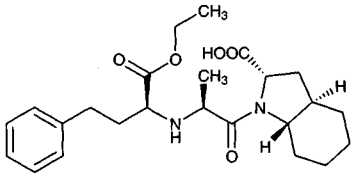
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Trandolapril



Molecular formula: $C_{24}H_{34}N_2O_5$
Molecular weight: 430.54
CAS Registry No.: 87679-37-6, 87679-71-8 (trandolaprilat)
Merck Index: 9703

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

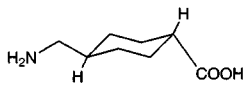
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 16.993

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tranexamic acid



Molecular formula: $C_8H_{15}NO_2$
Molecular weight: 157.21
CAS Registry No.: 1197-18-8
Merck Index: 9704
Lednicer No.: 2 9

SAMPLE
Matrix: blood
Sample preparation: 20 μL Serum + 2 μL water + 20 μL MeCN, mix, centrifuge at 10000 g for 3 min. Remove 5 μL of the supernatant and add it to 100 μL 25 mM pH 8 phosphate buffer, add 100 μL 300 μg/mL fluorescamine in acetone, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeCN:water:acetic acid:THF 30:69:0.5:0.5, containing 40 mM sodium acetate
Flow rate: 2
Injection volume: 20
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 5
Internal standard: tranexamic acid

OTHER SUBSTANCES
Extracted: 6-aminocaproic acid

KEY WORDS
serum; derivatization; tranexamic acid is IS

REFERENCE
Lacroix,C.; Levert,P.; Laine,G.; Goulle,J.P. Microdosage de deux antifibrinolytiques (acide β-aminocaproïque et acide tranexamique) par chromatographie liquide et détection fluorimétrique [Microanalysis of two antifibrinolytics (epsilon-aminocaproic acid and tranexamic acid) by liquid chromatography and fluorometry], *J.Chromatogr.*, **1984**, *309*, 183–186.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 250 μg/mL IS in water + 700 μL water, mix, add 200 μL 4 M perchloric acid, shake vigorously,let stand for 10 min, centrifuge at 3000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm cation-exchange (Brownlee)
Column: 250 × 4.6 10 μm Nucleosil SA
Mobile phase: MeOH:buffer 2:98 containing 100 μL/L caprylic acid (Buffer was 100 mM trisodium citrate adjusted to pH 4 with HCl.)
Column temperature: 26
Flow rate: 1.4
Injection volume: 100
Detector: F ex 410 em 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and the mixture flowed through a 1 m × 0.3 mm i.d. coil of tubing to the detector. (Prepare reagent by adding 800 mg o-phthalaldehyde in 10 mL MeOH to 1 L 700 mM pH 9.5 potassium borate buffer containing 2 g EDTA and 2 mL mercaptoethanol.)

CHROMATOGRAM
Retention time: 5.65
Internal standard: 4-aminomethyl bicyclo(2,2,2)octane-1-carboxylic acid (KabiVitrum, Uxbridge, UK) (6.65)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: arginine, histidine
Noninterfering: amino acids

KEY WORDS
post-column reaction; plasma

REFERENCE
Elworthy,P.M.; Tsementzis,S.A.; Westhead,D.; Hitchcock,E.R. Determination of plasma tranexamic acid using cation-exchange high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1985**, *343*, 109–117.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 5 μL 58.4 μg/mL IS, mix by swirling, add 2 mL EtOH, vortex, centrifuge at 1500 g for 10 min. Remove the supernatant and add it to 1 mL 10 mM pH 9.2 borax solution, add 13 μL phenylisothiocyanate, heat at 40° for 30 min, add 2 mL xylene, agitate, centrifuge at 1500 g for 10 min, wash twice more. Acidify the aqueous layer with 1 mL concentrated HCl, heat at 80° for 10 min, evaporate to dryness under reduced pressure, reconstitute with 1 mL 100 mM borax solution, extract twice with 2 mL portions of benzene (Caution! Benzene is a carcinogen!). Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil 5C8 (Nakarai Chemicals)
Mobile phase: EtOH:20 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10.8
Internal standard: 3-aminocyclohexanecarboxylic acid (14.1)
Limit of detection: 200 ng/mL

KEY WORDS
serum; derivatization

REFERENCE
Matsubayashi,K.; Kojima,C.; Tachizawa,H. Determination of tranexamic acid in human serum by high-performance liquid chromatography using selective pre-column derivatization with phenyl isothiocyanate, *J.Chromatogr.*, **1988**, *433*, 225–234.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out powdered tablet containing aminocaproic acid, dissolve in 100 mL water, filter (0.45 μm). Mix a 5 mL aliquot of the filtrate with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 400 μg/mL tranexamic acid in buffer, let stand in the dark at room temperature for 30 min, add 2 drops ethanolamine, mix, let stand at room temperature for 15 min, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. Injections, syrup. Weigh out amount of injection or syrup containing 250 mg aminocaproic acid, dilute with 100 mL water, dilute an aliquot 5-fold with water. Mix a 5 mL aliquot with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 400 μg/mL tranexamic acid in buffer, let stand in the dark at room temperature for 30 min, add 2 drops ethanolamine, mix, let stand at room temperature for 15 min, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. (Prepare buffer by dissolving 550 mg anhydrous sodium carbonate in 300 mL water, add 300 mL acetone, mix.)

HPLC VARIABLES
Guard column: C18 (Alltech)
Column: 150 × 4.6 5 μm Econosphere C18
Mobile phase: MeOH:water:acetic acid:triethylamine 60:38:1.5:0.5
Flow rate: 1.5
Injection volume: 20
Detector: UV 335

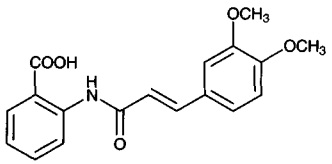
CHROMATOGRAM
Retention time: 6.5
Internal standard: tranexamic acid

OTHER SUBSTANCES
Simultaneous: aminocaproic acid

KEY WORDS
derivatization; tablets; injections; syrup; tranexamic acid is IS

REFERENCE
Lau-Cam,C.A.; Roos,R.W. Assay of aminocaproic acid in dosage forms by reversed phase high performance liquid chromatography with dansylation, *J.Liq.Chromatogr.*, **1993**, *16*, 403–419.

Tranilast



Molecular formula: $C_{18}H_{17}NO_5$
Molecular weight: 327.34
CAS Registry No.: 53902-12-8
Merck Index: 9705

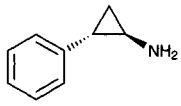
SAMPLE
Matrix: tissue
Sample preparation: Wipe the skin with liquid paraffin and EtOH. Separate the tissue by a heat separation technique, mince with scissors, homogenise, add MeOH. Centrifuge, inject an aliquot of supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Inertsil ODS-2
Mobile phase: MeOH:0.1% phosphoric acid 75:25
Flow rate: 1.0
Detector: UV 320

KEY WORDS
skin; Yucatan micropig; pig

REFERENCE
Hori,N.; Fujii,M.; Yamanouchi,S.; Miyagi,M.; Saito,N.; Matsumoto,M. In vitro release of tranilast from oily gels and penetration of the drug into Yucatan micropig skin, *Biol.Pharm.Bull.*, **1998**, *21*, 300–303.

Tranylcypromine



Molecular formula: $C_9H_{11}N$
Molecular weight: 133.19
CAS Registry No.: 155-09-9, 13492-01-8 (sulfate)
Merck Index: 9708
Lednicer No.: 1 73

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 3 mL n-hexane, shake for 20 min, centrifuge for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness using a vacuum centrifuge, reconstitute the residue in 500 μL 100 μg/mL (S)-(+)-benoxaprofen chloride in dried dichloromethane, let stand at room temperature for 30 min, inject a 10 μL aliquot. (Synthesis of benoxaprofen chloride is as follows. Dissolve 600 mg benoxaprofen in 50 mL toluene, slowly add 5 mL freshly-distilled thionyl chloride, reflux for 30 min, evaporate to dryness, recrystallize benoxaprofen chloride from dichloromethane.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax-Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:10:10
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365

CHROMATOGRAM
Retention time: 9.0 (S-(-)), 10.7 (R-(+))

OTHER SUBSTANCES
Extracted: amphetamine
Interfering: methamphetamine

KEY WORDS
plasma; derivatization; normal phase; chiral

REFERENCE
Weber,H.; Spahn,H.; Mutschler,E.; Möhrke,W. Activated α-alkyl-α-arylacetic acid enantiomers for stereoselective thin-layer chromatographic and high-performance liquid chromatographic determination of chiral amines, *J.Chromatogr.*, **1984**, *307*, 145–153.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 500 ng/mL apomorphine in 1 mM phosphoric acid + 1 mL buffer + 5 mL n-heptane:n-octanol:tetraoctylammonium bromide 89.75:10:0.25, shake

by hand for 2 min, centrifuge at 4° at 1500 g for 5 min. Remove 4 mL of the organic layer and add it to 4 mL n-octanol and 1 mL 50 mM phosphoric acid, shake by hand for 2 min, centrifuge at 4° at 1500 g for 5 min, inject a 100 μL aliquot of the aqueous layer. (Buffer was 2 M pH 8.45 ammonium chloride/ammonium hydroxide buffer containing 0.2% diphenylborate ethyleneamine and 0.5% EDTA.)

HPLC VARIABLES

Column: 150 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 20:80 (Buffer was 100 mM NaH_2PO_4 containing 0.3 g/L NaCl and 0.76 g/L sodium 1-octanesulfonate, pH adjusted to 3 with phosphoric acid.)
Flow rate: 0.5
Injection volume: 100
Detector: E, Spark-analytica model 9205, 1.0 V

CHROMATOGRAM

Retention time: 12.05
Internal standard: apomorphine (13.81)
Limit of detection: 5 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Krugers Dagneaux,P.G.L.C.; Loohuis,C.P.G.G.; Klein Elhorst,J.T.; Van der Veer,T.S. Liquid chromatographic estimation of tranylcypromine in human plasma, *Pharm.Weekbl.[Sci].*, **1992**, *14*, 46–49.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 1.43
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, mianserin, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: moclobemide, pentobarbital, metoclopramide

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL pH 11 sodium borate buffer, extract with 2.5 mL diisopropyl ether:EtOH 100:1.5 (Caution! Diisopropyl ether readily forms explosive peroxides!). Remove 2 mL of the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 200 μL 10 μg/mL S-flunoxaprofen chloride, let stand at room temperature for 1 h, add 20 μL MeOH, evaporate to dryness, reconstitute with dichloromethane, inject a 5-50 μL aliquot. Urine. 1 mL Urine + 1 mL 50 mM NaOH, extract with 2.5 mL diisopropyl ether:EtOH 100:1.5. Remove 2 mL of the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 200 μL 10 μg/mL S-flunoxaprofen chloride, let stand at room temperature for 1 h, add 20 μL MeOH, evaporate to dryness, reconstitute with dichloromethane, inject a 5-50 μL aliquot. (Prepare S-flunoxaprofen chloride as follows. Dissolve 1 mmole S-flunoxaprofen in 25 mL toluene, add a trace of DMF (J.Chromatogr. 1990, 528, 55), add 2.5 mL thionyl chloride, reflux for 30 min, remove solvent by evaporation, dry the residue under vacuum over KOH, recrystallize from dichloromethane (mp 73°).)

HPLC VARIABLES
Guard column: 4 × 4 LiChrosorb Si 60
Column: 250 × 4.6 7 μm Zorbax Sil
Mobile phase: Cyclohexane:dichloromethane:THF 70:10:10
Flow rate: 1.4
Injection volume: 5-50
Detector: F ex 305 em 355

CHROMATOGRAM
Retention time: 12 (S-(-)), 15 (R-(+))
Limit of detection: 2 ng/mL

KEY WORDS
normal phase; derivatization; chiral; plasma

REFERENCE
Spahn,H. *S*-(+)-Flunoxaprofen chloride as chiral fluorescent reagent, *J.Chromatogr.*, **1988**, *427*, 131–137.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or 500 μL urine + 1 mL 100 mM pH 11 sodium borate buffer + 20 μL 1 (plasma) or 10 (urine) μg/mL S-(+)-amphetamine in MeOH + 5 mL diethyl ether: EtOH 98.5:1.5, mix for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL reagent, vortex briefly, let stand at room temperature for 5 min, inject an aliquot. (Reagent was 10 mg o-phthaldialdehyde, 500 μL EtOH, and 40 mg N-acetyl-L-cysteine in 5 mL buffer. Buffer was 14.75 g boric acid and 160 mL 1 M NaOH made up to 1 L with water, pH 10.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:THF:buffer 60:1:50 (Buffer was 653 mL 9.07 g/L KH_2PO_4 and 347 mL 11.87 g/L $Na_2HPO_4.2H_2O$.)
Flow rate: 1.2
Detector: F ex 344 em 442

CHROMATOGRAM
Retention time: 32.5 (S-(-)), 35 (R-(+))

Internal standard: S-(+)-amphetamine (25)
Limit of detection: 2 ng/mL (urine), 0.5 ng/mL (plasma)

OTHER SUBSTANCES
Noninterfering: norephedrine, norepinephrine, norpseudoephedrine, tyramine

KEY WORDS
plasma; chiral; derivatization; pharmacokinetics

REFERENCE
Spahn-Langguth,H.; Hahn,G.; Mutschler,E.; Möhrke,W.; Langguth,P. Enantiospecific high-performance liquid chromatographic assay with fluorescence detection for the monoamine oxidase inhibitor tranylcypromine and its applicability in pharmacokinetic studies, *J.Chromatogr.*, **1992**, *584*, 229–237.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.66

OTHER SUBSTANCES
Simultaneous: levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.8

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax Sil
Mobile phase: n-Hexane:THF:isopropanol 83:12:5
Flow rate: 1
Injection volume: 20
Detector: F ex 296 em 356

CHROMATOGRAM
Retention time: 19.6 (-), 21.6 (+)

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: solutions
Sample preparation: Mix sample:50 (?) mM NaCN in 50 mM pH 9.3 borate buffer:25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was THF:50 mM pH 6.8 potassium phosphate buffer 5:95. B was MeCN:MeOH:50 mM pH 6.8 potassium phosphate buffer 55:10:35. A:B from 70:30 to 0:100 over 1 h, maintain at 0:100 for 20 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM
Retention time: 68

OTHER SUBSTANCES
Simultaneous: baclofen, amphetamine

KEY WORDS
derivatization

REFERENCE
Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE
Matrix: solutions
Sample preparation: 50 μL 5 mg/mL Tranylcypromine in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 16.3 mg N-acetyl-L-cysteine in 1 mL MeOH, protect from light, keep on ice.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:MeCN:buffer 50:2:50 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 425 or UV 254

CHROMATOGRAM
Retention time: 25.81 (first enantiomer) (α = 1.13)

KEY WORDS
derivatization; protect from light; chiral; α = 1.13

REFERENCE
Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 7.0

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate
Interfering: tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine,

chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Mix 1 mL urine with 1 mL 200 mM boric acid/KCl buffer, adjust pH to 8.5, add 5 mL n-hexane:n-octanol 90:10, invert repeatedly for 2 min, centrifuge at 1500 g for 15 min. Remove the organic layer and extract it further with 4 mL n-octanol and 1 mL 50 mM phosphoric acid, in the same manner. Centrifuge, discard the organic layer, inject a 20 µL aliquot of aqueous phase

HPLC VARIABLES

Column: 150 × 3.9 5 µm Symmetry C18
Mobile phase: MeOH:water:50 mM pH 4.55 KH_2PO_4:70:20
Column temperature: 23
Flow rate: 1
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Retention time: 6.28
Limit of detection: 5 nmol/mL
Limit of quantitation: 25 nmol/mL

REFERENCE
Aboul-Enein,H.Y.; Abou-Basha,L.I. Determination of tranylcypromine in urine and pharmaceutical formulation by HPLC using symmetry column, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 925–932.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 1 mL urine to 8.5 with 1 mL 200 mM boric acid KCl buffer, add 5 mL n-hexane:n-octanol 90:10, invert repeatedly for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and add it to 4 mL n-octanol and 1 mL 50 mM phosphoric acid, extract, centrifuge, inject a 20 μL aliquot of the aqueous layer.

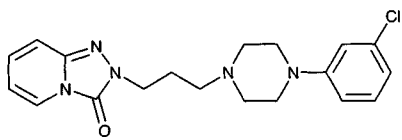
HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR (+) (Daicel)
Mobile phase: MeOH:100 mM perchloric acid 12:88
Column temperature: 10
Flow rate: 0.6
Injection volume: 20
Detector: UV 256

CHROMATOGRAM
Retention time: 12.93 (R-(+)), 16.38 (S-(-))

KEY WORDS
chiral

REFERENCE
Aboul-Enein,H.Y.; Serignese,V. Direct separation of tranylcypromine enantiomers and their profile in an atypical depressive patient, *Biomed.Chromatogr.*, **1995**, *9*, 98–101.

Trazodone



Molecular formula: $C_{19}H_{22}ClN_5O$
Molecular weight: 371.87
CAS Registry No.: 19794-93-5, 25332-39-2 (HCl)
Merck Index: 9712
Lednicer No.: 2 472

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 3.0

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, encainide, fluoxetine, flurazepam, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: diphenhydramine, doxepin, fentanyl, flecainide, haloperidol, nordoxepin

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 249

CHROMATOGRAM

Retention time: 4.98

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam;

tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: 50 µL Plasma or CSF + 20 µL 4 M NaOH, vortex briefly, add 750 µL diethyl ether, vortex for 1 min, centrifuge at 2600 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 µL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 25 × 4 Hibar LiChroCART C8 (Merck)
Column: 250 × 4 5 µm LiChrospher 100 CH-8 II C8
Mobile phase: MeCN:10 mM pH 3.0 phosphate buffer 60:40 containing 20 mM tetramethylammonium chloride
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 6.0
Internal standard: trazodone

OTHER SUBSTANCES

Extracted: toloxatone

KEY WORDS

plasma; rabbit; trazodone is IS

REFERENCE

Vistelle,R.; Lamiable,D.; Zinzou,M. Simple high-performance liquid chromatographic method for the measurement of toloxatone in rabbit cerebrospinal fluid and plasma, *J.Chromatogr.*, **1989**, *490*, 387–394.

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: Tissue homogenates were 1:2 in water. 1 mL Sample + 1 mL saturated sodium borate buffer + 100 μL 20 μg/mL methyl clonazepam in water + 5 mL n-butyl chloride, rotate at 40 rpm for 30 min, centrifuge at 2500 rcf for 5 min. Remove the organic phase and evaporate it to dryness at 70 ° under a stream of air, reconstitute the residue in 300 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 Analytichem ODS with an integral guard column
Mobile phase: MeCN:100 mM KH_2PO_4 300:700, adjust pH to 3.00 with concentrated phosphoric acid
Column temperature: 60
Flow rate: 1.5
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 1.25
Internal standard: methyl clonazepam (5.36)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: diazepam, temazepam
Also analyzed: acetaminophen, alprazolam, amitriptyline, amoxapine, carbamazepine, chlordiazepoxide, chlorpromazine, chlorprothixene, clonazepam, demoxepam, desipramine, diphenhydramine, disopyramide, doxepin, ethotoin, flurazepam, glutethimide, haloperidol, haloperidol, imipramine, lidocaine, lorazepam, loxapine, maprotiline, mesantoin, mesoridazine, methaqualone, methotrimeprazine, nordiazepam, nortriptyline, oxazepam, pentazocine, perphenazine, phenacetin, phenobarbital, phenytoin, promazine, promethazine, propranolol, protriptyline, salicylic acid, thiothixene, trifluoperazine, triflupromazine, trimipramine
Noninterfering: thioridazine, chloral hydrate, codeine, ketamine, meperidine, methamphetamine, methypyrlon, methadone

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KEY WORDS
serum; plasma; whole blood

REFERENCE
Root,I.; Ohlson,G.B. Trazodone overdose: report of two cases, *J.Anal.Toxicol.*, **1984**, *8*, 91–94.

SAMPLE
Matrix: blood, gastric contents, tissue, urine
Sample preparation: Blood, stomach contents. 1 mL Postmortem blood or stomach contents + 500 μL 1 M potassium carbonate + 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot. Urine. 1 mL Urine + 200 μL concentrated HCl, heat at 100° for 1 h, cool, adjust pH to 9.5-10 with KOH pellets and 1 M potassium carbonate, add 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot. Tissue. Add tissue to an equal volume isotonic saline, homogenize with an Ultra-Turraz mixer, remove a 2 g aliquot, add 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2.1 Chrompack pellicular reverse phase
Column: 100 × 3 5 μm Chromspher C8
Mobile phase: Gradient. MeOH:water containing 0.125% isopropylamine from 30:70 to 75:25 over 15 min
Flow rate: 0.7
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: dothiepin

REFERENCE
Lambert,W.; Van Bocxlaer,J.; Piette,M.; De Leenheer,A. A fatal case of trazodone and dothiepin poisoning: toxicological findings, *J.Anal.Toxicol.*, **1994**, *18*, 176–179.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 10 μL 20 μg/mL bupropion in MeOH, vortex briefly, add 500 μL saturated sodium borate, vortex briefly, add 5 mL MTBE, vortex briefly then mix on a reciprocating shaker for 10 min, centrifuge at 220 g for 10 min. Remove the organic phase and add it to 75 μL 10 mM phosphoric acid, vortex, centrifuge, inject a 50 μL aliquot of the aqueous layer. Tissue. Weigh whole brain and homogenize with 10 mL 340 mM perchloric acid containing 0.01 mM EDTA for 20 s (Brinkman PT 10/35). Remove a 1 mL aliquot and add 5 μL 20 μg/mL bupropion in MeOH, 500 μL 600 mM sodium carbonate, and 3 mL hexane:isoamyl alcohol 98:2 to it. Shake on a reciprocating shaker for 10 min, centrifuge at 220 g for 10 min, remove the organic layer and repeat the extraction. Combine the organic layers and add them to 75 μL 10 mM phosphoric acid, vortex, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm IBM reverse phase (trimethyl silane)
Mobile phase: MeCN:pH 3.0 phosphate buffer 27:73 containing 20 mM heptanesulfonic acid and 40 mM triethylamine
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 7.1
Internal standard: bupropion (10.6)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; rat; brain

REFERENCE
Miller,R.L.; DeVane,C.L. Analysis of trazodone and m-chlorophenylpiperazine in plasma and brain tissue by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 388–393.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)

Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 3.48
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, sulforidazine, thioridazine, thiothixene, tranylcypromine, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: nomifensine, quinine, quinidine, nordoxepin, norfluoxetine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 1.2 U/I β-glucuronidase in 200 mM pH 5.5 phosphate buffer, incubate at 37° for 2 h, add 100 μL 100 μg/mL sodium carbonate:sodium bicarbonate 1:1, extract three times with 2 mL dichloromethane:ethylene chloride:ethyl acetate 1:1:8, centrifuge at 8000 rpm for 5 min, dry organic layers over anhydrous sodium sulfate, evaporate to dryness at 35°/15 mmHg. Dissolve residue in 500 μL MeOH, vortex for 2 min, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:10 mM pH 7.5 phosphate buffer 12.5:67:20.5
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 600 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; rabbit

REFERENCE
di Tella,A.S.; Di Nunzio,C.; Ricci,P.; Parisi,G. Determination of trazodone and its metabolite, m-CPP, in serum and urine by HPLC, *J.Anal.Toxicol.*, **1986**, *10*, 233–235.

SAMPLE
Matrix: blood, urine

Sample preparation: Make 1 mL serum or urine basic (pH 12.8), extract into 5 mL diethyl ether. Remove the organic layer and evaporate it under a stream of nitrogen at room temperature, dissolve the residue in 250 μL 5 mM sulfuric acid, inject an aliquot.

HPLC VARIABLES
Column: 250 mm long 5 μm Spherisorb 5 S ODS
Mobile phase: MeCN:50 mM sulfuric acid 18:1
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 11.4
Internal standard: 2-[3-(4-m-chlorophenyl-1-piperazinyl)propyl]-5-methyl-4-phenyltriazol-3-(2H)-one (9.6)
Limit of detection: 25 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Nilsen,O.G.; Dale,O. Single dose pharmacokinetics of trazodone in healthy subjects, *Pharmacol.Toxicol.*, **1992**, *71*, 150–153.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 12.683

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Adsorbosphere C18 (PEEK column) (retention times are longer and peaks broader with stainless steel column)
Mobile phase: MeCN:20 mM pH 3.2 KH_2PO_4 23.4:76.6 containing 0.05% nonylamine
Flow rate: 1.2
Detector: UV 214

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: amitriptyline, desmethyldoxepin, desipramine, doxepin, imipramine, loxapine, maprotiline, nortriptyline

REFERENCE
Supelco Catalog, **1993,** p. 440.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

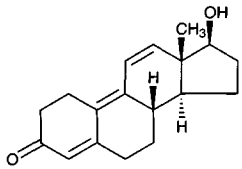
CHROMATOGRAM
Retention time: 8.37 (A), 4.58 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Trenbolone



Molecular formula: $C_{18}H_{22}O_2$
Molecular weight: 270.37
CAS Registry No.: 10161-33-8, 10161-34-9 (acetate)
Merck Index: 9716

SAMPLE

Matrix: bile, feces

Sample preparation: Condition a Bond-Elut CN SPE cartridge with 4 mL chloroform and 5 mL petroleum ether. Feces. 10 g Feces + 100 μL β-glucuronidase (type H2 Helix pomatia, Sigma) + 25 mL 200 mM pH 5.5 sodium phosphate buffer, let stand overnight at room temperature or heat at 37° for 4 h, add 60 mL MeOH, shake for 1 h, centrifuge at 4° at 2000 g for 10 min. Remove 50 mL of the supernatant and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 10 mL buffer, add 10 mL MTBE, shake vigorously for 1 min, centrifuge at 4° at 2000 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 3 mL diethyl ether, sonicate, add 3 mL petroleum ether (bp 40-60°, add to the SPE cartridge, rinse flask with 1 mL aliquots of petroleum ether:diethyl ether 50:50, add the rinses to the SPE cartridge, wash the SPE cartridge with 3 mL petroleum ether, wash with 4 mL petroleum ether:chloroform 50:50, elute with 4 mL chloroform, evaporate he eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL MeOH, sonicate, add 2 mL isotonic PBS, vortex. Apply the mixture to an immunoaffinity column (preparation details in paper), wash with 3 mL water, elute with 3 mL EtOH:water 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 100 μL MeCN, sonicate for 10 min, add 100 μL water, vortex, inject a 50 μL aliquot. Bile. 5 mL Bile + 50 μL β-glucuronidase (type H2 Helix pomatia, Sigma) + 12.5 mL 200 mM pH 7.0 sodium phosphate buffer, let stand overnight at room temperature or heat at 37° for 4 h, add to an Extrelut 20 column, let stand for 20 min, elute with two 20 mL portions of diethyl ether (let column run dry between each addition of ether), evaporate to dryness under reduced pressure at 30°, reconstitute the residue in 3 mL diethyl ether, sonicate, add 3 mL petroleum ether (bp 40-60°, add to the SPE cartridge, rinse flask with 1 mL aliquots of petroleum ether:diethyl ether 50:50, add the rinses to the SPE cartridge, wash the SPE cartridge with 3 mL petroleum ether, wash with 4 mL petroleum ether:chloroform 50:50, elute with 4 mL chloroform, evaporate he eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL MeOH, sonicate, add 2 mL isotonic PBS, vortex. Apply the mixture to an immunoaffinity column (preparation details in paper), wash with 3 mL water, elute with 3 mL EtOH:water 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 100 μL MeCN, sonicate for 10 min, add 100 μL water, vortex, inject a 50 μL aliquot. (Buffer was 100 mM pH 12.5 glycine-NaOH containing 100 mM NaCl.)

HPLC VARIABLES

Column: 125 × 4 LiChrosorb RP-18 endcapped
Mobile phase: MeCN:100 mM ammonium acetate 45:55
Flow rate: 1
Injection volume: 50
Detector: MS, Vestec Model 201A thermospray, electron beam 250 μA, electron multiplier 1600 V, source block 260°, tip heater 260°, lens assembly 135°, vaporizer probe 190°, m/z 271, SIM

CHROMATOGRAM

Retention time: 4 (17α-trenbolone)
Limit of detection: 0.5 ng/g (feces), 0.5 ng/mL (bile)

KEY WORDS

cow; SPE

REFERENCE

Hewitt,S.A.; Blanchflower,W.J.; McCaughey,W.J.; Elliott,C.T.; Kennedy,D.G. Liquid chromatography-thermospray mass spectrometric assay for trenbolone in bovine bile and faeces, *J.Chromatogr.*, **1993**, *639*, 185–191.

SAMPLE

Matrix: bile, tissue, urine

Sample preparation: Urine. 50 mL Urine + 6 g Amberlite XAD-2, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with 25 mL MeOH:ethyl acetate 50:50. Evaporate eluate to dryness at 50° and take up residue in 2 mL 250 mM pH 4.8 acetate buffer. Add 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase and incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Bile. 3 mL Bile + 2 mL 250 mM pH 4.8 acetate buffer + 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase, incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Liver, kidney. Add 80 mL 100 mM pH 9.5 Tris buffer containing 20 mg subtilopeptidase A (11.6 U/mg) to 20 g minced sample, incubate at 60° for 3.5 h, filter over glass wool, add 6 g Amberlite XAD-2 to the filtrate, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with five 10 mL portions of MeOH. Evaporate eluate to dryness and take up residue in 2 mL 250 mM pH 4.8 acetate buffer. Add 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase and incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Meat. Add 80 mL 100 mM pH 9.5 Tris buffer containing 20 mg subtilopeptidase A (11.6 U/mg) to 20 g minced sample, incubate at 60° for 3.5 h, filter over glass wool, add 6 g Amberlite XAD-2 to the filtrate, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with five 10 mL portions of MeOH. Evaporate eluate to dryness and take up residue in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Condition column A with 20 mL water then add 53 mL sample, flush column A with 10 mL water. Condition column B with 10 mL water. Elute column A onto column B with 20 mL water containing 250 μg/mL norgestrel and 5% MeCN. Switch column B into circuit with column C and elute with mobile phase. Recondition column A with MeOH:water 70:30.

HPLC VARIABLES

Column: A 10 × 10 immuno precolumn (with immunoglobulin G immobilized on cyanogen bromide-activated Sepharose 4B, prepared as J.Chromatogr. 1988, 452, 419-433); B 10 × 2 Chrompack reverse phase column; C Chromsep reverse phase guard column (Chrompack) + 100 × 3 5 μm Chromspher glass column

Mobile phase: MeCN:water 35:65

Flow rate: 0.4

Injection volume: 53000

Detector: UV 340

CHROMATOGRAM

Retention time: 7 (α), 6 (β)

OTHER SUBSTANCES

Simultaneous: nandrolone (at UV 247)

KEY WORDS

meat; liver; kidney; SPE; column-switching

REFERENCE

Haasnoot,W.; Schilt,R.; Hamers,A.R.; Huf,F.A.; Farjam,A.; Frei,R.W.; Brinkman,U.A. Determination of β-19-nortestosterone and its metabolite α-19-nortestosterone in biological samples at the sub parts per billion level by high-performance liquid chromatography with on-line immunoaffinity sample pretreatment, *J.Chromatogr.*, **1989**, *489*, 157–171.

SAMPLE

Matrix: formulations

Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 10.8 (trenbolone acetate)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate

KEY WORDS

oils; tablets; suspensions

REFERENCE

Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Add 3 volumes of MeOH to the microsomal incubation, centrifuge at 10000 g for 30 min. Evaporate the supernatant to dryness under reduced pressure, dissolve the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: RP-18 Hibar (Merck)
Mobile phase: MeCN:glacial acetic acid:water 19:1:80
Flow rate: 1
Detector: Radioactivity

CHROMATOGRAM

Retention time: 7.0

KEY WORDS

cow; liver; tritium labeled

REFERENCE

Evrard,P.; Maghuin-Rogister,G. In vitro metabolism of trenbolone: study of the formation of covalently bound residues, *Food Addit.Contam.*, **1987**, *5*, 59–65.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil ODS

Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Simultaneous: diethylstilbestrol, nandrolone, zeranol, dienestrol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE
Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 5 μL aliquot of a 10 μg/mL solution in MeOH.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:10 mM ammonium acetate buffer 45:55
Flow rate: 0.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.807

OTHER SUBSTANCES
Simultaneous: boldenone, epimethandienone, epitestosterone, fluoxymesterone, 6β-hydroxymethandienone, methandienone, norethindrone, oxymetholone (UV 280)

REFERENCE
Barrón,D.; Pascual,J.A.; Segura,J.; Barbosa,J. Prediction of LC retention of steroids using solvatochromic parameters, *Chromatographia*, **1995**, *41*, 573–580.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut silica SPE cartridge with 5 mL EtOH and 5 mL cyclohexane. Homogenize 5 g tissue with 11 mL 40 mM pH 4.1 sodium acetate buffer. Add 500 μL 17 mg/mL β-glucuronidase (type H-5) in acetate buffer, mix. Incubate at 37° overnight. Mix with 5 mL 2 M aqueous tris(hydroxymethyl)aminomethane. Fill an Extrelut cartridge with 25% of an Extrelut 20 sachet, mix the remainder of the sachet with the sample and add it to the cartridge. Rinse the sample tube with 15 mL butan-2-ol:hexane 5:95 and add the rinse to the cartridge. Elute with 100 mL butan-2-ol:hexane 5:95. Extract the eluate with two 10 mL portions of MeCN. Evaporate the combined MeCN extracts to dryness after adding two 2 mL portions of heptane. Reconstitute the residue with 2 mL cyclohexane. Add to the SPE cartridge Wash the tube with two 2 mL and two 1 mL portions of cyclohexane. Add to the SPE cartridge. Wash with 2 mL cyclohexane. Elute with 5 mL acetone:cyclohexane 25:75. Evaporate the eluate and redissolve the residue in 400 μL MeOH and 3.6 mL 85° water. Cool to room temperature and add to an immunoaffinity column (Randox Laboratories, UK). Complete the transfer with three 1 mL portions of hot water, cooled before adding to the column. Wash with 2 mL 1 mM pH 10 carbonate buffer, elute with 4 mL MeOH:water 70:30. Dilute the eluate with 12 mL water. Inject a 4 mL aliquot onto column A and then backflush the contents of column A onto column B with mobile phase. Monitor the effluent from column B.

HPLC VARIABLES
Column: A Chromspher (type R2) (Chrompack); B 200 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: MeCN:water 35:65
Flow rate: 0.5

Injection volume: 4000
Detector: UV 340

CHROMATOGRAM
Retention time: 8 (β epimer), 9 (α epimer)
Limit of detection: 100 pg/g

OTHER SUBSTANCES
Extracted: nandrolone

KEY WORDS
pig; cow; liver; corned beef; SPE; column-switching

REFERENCE
Stubbings,G.W.; Cooper,A.D.; Shepherd,M.J.; Croucher,M.; Airs,D.; Farrington,W.H.H.H.; Shearer,G. Determination of 19-nortestosterone and trenbolone in animal tissues by high-performance liquid chromatography with immunoaffinity clean-up, *Food Addit.Contam.*, **1998**, *15*, 293–301.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH: water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, add 1 mL MeOH to column, collect all eluates from column, evaporate to dryness under nitrogen at 40°, take up in 200 μL MeOH:water 50:50, add 25 μL 10 μg/mL p-chlorophenol, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: Gradient. MeCN:water from 40:60 to 65:35 in 30 min
Flow rate: 1.2
Injection volume: 50
Detector: UV 242

CHROMATOGRAM
Retention time: 24
Internal standard: p-chlorophenol (7)
Limit of detection: 1 ng/g

OTHER SUBSTANCES
Simultaneous: testosterone, progesterone

KEY WORDS
muscle; liver; chicken; ox; SPE

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21

μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES

Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM

Retention time: 9.8
Limit of detection: 100 ppb

OTHER SUBSTANCES

Extracted: dexamethasone, diethylstilbestrol, medroxyprogesterone, melengestrol acetate, triamcinolone acetonide, zeranol

KEY WORDS

cow; muscle; liver; SFE

REFERENCE

Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

SAMPLE

Matrix: urine
Sample preparation: 10 mL Urine + glucuronidase/sulfatase (Helix pomatia), incubate at 37° for 1 h, extract twice with 5 mL diethyl ether, add 225 μL water and evaporate ether under nitrogen, add 400 μL MeOH, inject a 250 μL aliquot of this mixture.

HPLC VARIABLES

Guard column: 75 × 2.1 Corasil C18
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 250
Detector: UV 240

CHROMATOGRAM

Retention time: 4.5
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES

Simultaneous: 17α-methyltestosterone, zeranol, trans-diethylstilbestrol, medroxyprogesterone, nandrolone

KEY WORDS

cow

REFERENCE
Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

SAMPLE
Matrix: urine
Sample preparation: Hydrolyze 1 mL urine with glucuronidase/sulfatase (from Helix pomatia, IBF; purified by gel filtration on Sephadex G-25M) at 37° for 2 h, cool, extract with ether. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil silica
Mobile phase: Isooctane:EtOH 97:3 for 8 min then 60:40 for 2 min to clean column
Flow rate: 2
Injection volume: 200
Detector: UV 350

CHROMATOGRAM
Retention time: 2.25 (trenbolone acetate), 6.2 (17β-trenbolone), 7.0 (17α-trenbolone)
Limit of detection: 1-2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, triendione

KEY WORDS
cow; normal phase

REFERENCE
Jansen,E.H.J.M.; Zoontjes,P.W.; van Blitterswijk,H.; Both-Miedema,R.; Stephany,R.W. Fast high-performance liquid chromatographic screening method for the presence of trenbolone and its major metabolite in urine of slaughter cattle, *J.Chromatogr.*, **1985**, *319*, 436–439.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 1 mL urine to 5,2 with 500 mM acetic acid, add 375 μL purified enzyme, heat at 37° for 1 h, cool, add to an immunosorbent column (preparation details in paper), wash with two 1 mL portions of water, wash with 5 mL water, elute with 5 mL EtOH:water 40:60. Evaporate the eluate to 800 μL under a stream of nitrogen, make up to 1 mL with water, extract with 6 mL diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 180 μL aliquot. (Enzyme was suc d'Helix pomatia (Industrie Biologique Francaise) purified by gel filtration on Pharmacia PD-10.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm LiChrosorb diol
Mobile phase: Isooctane:EtOH 95:5
Flow rate: 2
Injection volume: 180
Detector: UV 350 (with confirmation by TLC)

CHROMATOGRAM
Retention time: 6.1 (17β- and 17α-trenbolone)
Limit of detection: 2 ng/mL

KEY WORDS
cow

REFERENCE
van Ginkel,L.A.; van Blitterswijk,H.; Zoontjes,P.W.; van den Bosch,D.; Stephany,R.W. Assay for trenbolone and its metabolite 17 α-trenbolone in bovine urine based on immunoaffinity chromatographic clean-up and off-line high-performance liquid chromatography-thin-layer chromatography, *J.Chromatogr.*, **1988**, *445*, 385–392.

SAMPLE
Matrix: urine
Sample preparation: Add urine to an Amberlite XAD-2 column, elute with MeOH, add eluate to a column of neutral alumina (activity 1, Merck), elute with EtOH:water 96:4 (unconjugated), elute with water (sulfates), elute with 40 mM pH 6.0 citrate-phosphate buffer (glucuronides). Hydrolyze conjugates with β-glucuronidase/sulfatase (Helix pomatia, Serva), extract with ethyl acetate, evaporate the organic layer under reduced pressure, reconstitute the residue with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: Gradient. A was MeOH:water 10:90. B was MeCN. A:B from 85:15 to 63:37 over 10 min (concave gradient, Waters curve 8), maintain at 63:37 for 10 min, to 0:100 over 5 min.
Flow rate: 1
Detector: UV 340 or radioactivity

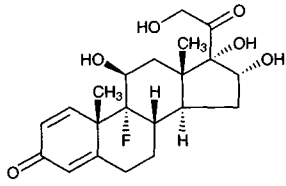
CHROMATOGRAM
Retention time: 33 (17β-trenbolone), 35 (17α-trenbolone)

OTHER SUBSTANCES
Extracted: metabolites, trendione

KEY WORDS
tritium labeled; SPE

REFERENCE
Spranger,B.; Metzler,M. Disposition of 17β-trenbolone in humans, *J.Chromatogr.*, **1991**, *564*, 485–492.

Triamcinolone



Molecular formula: $C_{21}H_{27}FO_6$
Molecular weight: 394.44
CAS Registry No.: 124-94-7, 76-25-5 (acetonide), 1997-15-5 (acetonide disodium phosphate), 31002-79-6 (benetonide), 5611-51-8 (hexacetonide), 67-78-7 (diacetate), 4989-94-0 (furetonide), 989-96-8 (21-(dihydrogen phosphate)
Merck Index: 9727
Lednicer No.: 1 201; 2 302

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL MeOH and 50 μL 1 μg/mL fluocortolone in MeOH to 1 mL plasma. Add 500 μL 100 mM NaOH and 2 mL dichloromethane, shake for 10 min, centrifuge at 2500 g for 10 min, evaporate a 1.9 mL aliquot of the supernatant under a stream of nitrogen at 45°. Reconstitute the residue in 50 μL MeOH, inject 17 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm LiChrospher RP 18
Column: 250 × 4 5 μm Lichrospher RP 18
Mobile phase: MeOH:THF:water 110:2.5:100
Flow rate: 1

Injection volume: 17
Detector: UV 252

CHROMATOGRAM
Internal standard: fluocortolone
Limit of quantitation: 600 pg/mL

OTHER SUBSTANCES
Extracted: hydrocortisone

KEY WORDS
plasma

REFERENCE
Doppenschmitt,S.A.; Scheidel,B.; Harrison,F.; Surmann,J.P. Simultaneous determination of triamcinolone acetonide and hydrocortisone in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *682*, 79–88.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 2.5 mg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:water 36:64
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 6.4 (triamcinolone acetonide)

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Cavina,G.; Alimenti,R.; Gallinella,B.; Valvo,L. The identification of related substances in triamcinolone acetonide by means of high-performance liquid chromatography with diode array detector and mass spectrometry, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 685–692.

SAMPLE
Matrix: formulations
Sample preparation: Dermatological patch (2 cm × 2 cm) + 2 mL hexane, shake mechanically for 10 min, add 8 mL mobile phase, mix thoroughly, centrifuge at 2500 rpm for 10 min, remove 1 mL of lower phase, inject a 10 μL aliquot of this solution.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C8
Mobile phase: MeOH:water 70:30
Flow rate: 0.5
Injection volume: 10
Detector: UV 240

KEY WORDS
for triamcinolone acetonide; dermatological patches; stability-indicating

REFERENCE
Edwardson,P.A.D.; Gardner,R.S. Problems associated with the extraction and analysis of triamcinolone acetonide in dermatological patches, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 935–938.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out amount containing 10 mg triamcinolone acetonide, make up to 50 mL with mobile phase. Remove a 2 mL aliquot and dilute it to 10 mL with mobile phase, filter, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water:96% acetic acid 55:44:1, pH 3.0
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.02 (triamcinolone acetonide)

OTHER SUBSTANCES
Simultaneous: salicylic acid

KEY WORDS
topical solution

REFERENCE
Kedor-Hackmann,E.R.M.; Gianotto,E.A.S.; Santoro,M.I.R.M. Determination of triamcinolone acetonide and salicylic acid in pharmaceutical formulations by high performance liquid chromatography, *Pharmazie*, **1996**, *51*, 63–63.

SAMPLE
Matrix: formulations, solutions
Sample preparation: Ointment. 1 g Ointment + 5 mL MeOH + 5 mL water + 800 μL 1 mg/mL hydrocortisone in EtOH, stir until a clear solution forms, make up to 25 mL with water, inject a 20 μL aliquot. Solutions. 8 mL Solution + 800 μL 1 mg/mL hydrocortisone in EtOH + 5 mL MeOH, make up to 25 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:200 mM KH_2PO_4 32:68, pH 4.2
Flow rate: 3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8 (triamcinolone acetonide)
Internal standard: hydrocortisone (4)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
ointment; stability-indicating

REFERENCE
Das Gupta,V. Stability of triamcinolone acetonide solutions as determined by high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 1453–1456.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak ODS

Mobile phase: MeCN:water 30:70
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 12 (triamcinolone acetonide)
Internal standard: fluoxymesterone (10)

REFERENCE
Kirschbaum,J. High-pressure liquid chromatography of triamcinolone acetonide: effect of different octadecylsilane columns on mobility, *J.Pharm.Sci.*, **1980**, *69*, 481–482.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

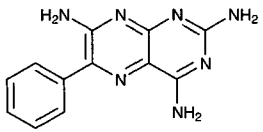
CHROMATOGRAM
Retention time: 10 (triamcinolone acetonide)
Limit of detection: 100 ppb

OTHER SUBSTANCES
Extracted: dexamethasone, diethylstilbestrol, medroxyprogesterone, melengestrol acetate, trenbolone, zeranol

KEY WORDS
cow; muscle; liver; SFE

REFERENCE
Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

Triamterene



Molecular formula: $C_{12}H_{11}N_7$
Molecular weight: 253.27
CAS Registry No.: 396-01-0
Merck Index: 9731
Lednicer No.: 1 427

SAMPLE

Matrix: blood

Sample preparation: Add 1.5 mL MeCN to 500 μL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 μL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 μm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 μm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)

Mobile phase: MeOH:20 mM pH 4 phosphate buffer 38:62

Column temperature: 40

Flow rate: 1

Injection volume: 200

Detector: UV 245, F ex 270 em 389

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

Extracted: trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 215.8

CHROMATOGRAM

Retention time: 8.705

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Pulverize tablets, add MeOH, shake for 30 min, sonicate for 5 min, filter (Albet 242 paper), wash solid with MeOH, make up filtrate to 50 mL with MeOH, inject a 20 μL aliquot. Urine. Adjust pH of 2 mL urine to 10.0 with 2 M KOH, add 1.5 mg NaCl, add 4 mL ethyl acetate, shake for 10 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL mobile phase, sonicate, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 30:70 containing 5 mM KH_2PO_4/K_2HPO_4, pH adjusted to 5.5
Flow rate: 1
Injection volume: 20
Detector: E, EG&G Princeton Applied Research PAR Model 400, glassy carbon working electrode +1300 mV, Ag/AgCl reference electrode (At the end of each day clean electrode with mobile phase of MeOH at 1.5 mL/min, -800 mV for 2 min then +1600 mV for 5 min.)

CHROMATOGRAM
Retention time: 5.01
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: furosemide

KEY WORDS
tablets; pharmacokinetics

REFERENCE
Barroso,M.B.; Alonso,R.M.; Jiménez,R.M. Simultaneous determination of the diuretics triamterene and furosemide in pharmaceutical formulations and urine by HPLC-EC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 231–246.

SAMPLE
Matrix: urine
Sample preparation: Inject 5 μL urine onto column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: A 50 mM pH 3 phosphate buffer; B MeCN:50 mM pH 3 phosphate buffer 60:40 (Prepare buffer as follows. Dissolve 3.45 g NaH_2PO_4 monohydrate in 500 mL water containing 750 μL propylamine hydrochloride, adjust to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 254, F ex 365 em 440

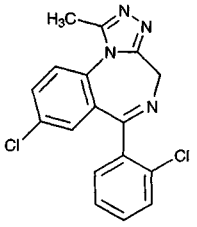
CHROMATOGRAM
Retention time: 5.5
Limit of detection: 10 pg/mL

OTHER SUBSTANCES
Extracted: amiloride, bumetanide, furosemide

KEY WORDS
column-switching

REFERENCE
Campins-Falcó,P.; Herráez-Hernández,R.; Pastor-Navarro,M.D. Analysis of diuretics in urine by column-switching chromatography and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1867–1885.

Triazolam



Molecular formula: $C_{17}H_{12}Cl_2N_4$
Molecular weight: 343.21
CAS Registry No.: 28911-01-5
Merck Index: 9734
Lednicer No.: 1 368

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 20 µL 20 µg/mL IS + 200 µL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 µL mobile phase, inject a 20 µL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 × 4.6 2 µm TSK gel Super-ODS (A) or 100 × 4.6 5 µm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 13.7 (A), 43.5 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES

Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione
Interfering: alprazolam

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-µm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum or plasma + 100 µL 1 µg/mL IS in water + 0.5 mL water, vortex, extract with 10 mL toluene:isoamyl alcohol 99:1 for 10 min on a rotator, centrifuge for 5 min. Remove upper organic layer, evaporate under a stream of nitrogen at 37°, take up in 150 µL mobile phase, vortex for 2 min, add 0.5 mL hexane, vortex briefly, centrifuge for 5 min, discard upper hexane layer, inject a 100 µL aliquot of the lower layer.

HPLC VARIABLES

Column: 250 × 4 Bio-Sil ODS-10 (Bio-Rad)
Mobile phase: MeCN:pH 4.5 50 mM phosphate buffer 30:70 (Buffer was 6.9 g KH_2PO_4 in 1 L adjusted to pH 4.5 with orthophosphoric acid.)
Column temperature: 45
Flow rate: 2.5
Injection volume: 100
Detector: UV 202

CHROMATOGRAM
Retention time: 8.4
Internal standard: U-31485 (6.9)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: desipramine, protriptyline
Noninterfering: N-acetylprocainamide, amitriptyline, caffeine, chlordiazepoxide, chlorpromazine, diazepam, flurazepam, lorazepam, oxazepam, prazepam, procainamide, propranolol, thioridazine
Interfering: alprazolam, imipramine, nortriptyline

KEY WORDS
plasma; serum

REFERENCE
McCormick,S.R.; Nielsen,J.; Jatlow,P. Quantification of alprazolam in serum or plasma by liquid chromatography, *Clin.Chem.*, **1984**, *30*, 1652–1655.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 2 mL water + 2 mL 100 mM NaOH, mix gently, add 8 mL diethyl ether, shake for 15 min, centrifuge at 2500 rpm for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 Shim-pack FLC-C8 (Shimadzu)
Mobile phase: MeOH:buffer 53:47 (Buffer was 5 mM Na_2HPO_4 adjusted to pH 6.0 with phosphoric acid.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Extracted: diazepam, nordiazepam, clorazepate, temazepam, oxazepam
Simultaneous: sulpride, bromazepam, nitrazepam, flunitrazepam
Noninterfering: haloperidol, trihexyphenidyl
Interfering: estazolam

KEY WORDS
serum

REFERENCE
Tada,K.; Moroji,T.; Sekiguchi,R.; Motomura,H.; Noguchi,T. Liquid-chromatographic assay of diazepam and its major metabolites in serum, and application to pharmacokinetic study of high doses of diazepam in schizophrenics, *Clin.Chem.*, **1985**, *31*, 1712–1715.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 4 μg/mL triazolam in methanol + 0.5 mL pH 9.12 saturated solution of sodium borate + 4.0 mL ethyl acetate:heptane 85:15, shake 10 min, centrifuge at 220 g for 10 min. Remove organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm C18 (IBM)
Mobile phase: MeCN:50 mM pH 6 potassium phosphate 30:70

Flow rate: 1.5
Detector: UV 214

CHROMATOGRAM
Retention time: 13.2
Internal standard: triazolam

OTHER SUBSTANCES
Extracted: alprazolam
Simultaneous: nitrazepam

KEY WORDS
plasma; between injections wash column with 10 mL MeCN:water 70:30 then 10 mL mobile phase; triazolam is IS

REFERENCE
Miller,R.L.; DeVane,C.L. Alprazolam, α-hydroxy- and 4-hydroxyalprazolam analysis in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 180–186.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 20 μL 2.5 μg/mL norprazepam in MeOH + 50 μL buffer + 6 mL diethyl ether:dichloromethane 2:1, agitate, centrifuge. Remove the organic phase and evaporate to dryness under vacuum at 45°, dissolve the residue in 50 μL MeOH, inject a 20 μL aliquot. (Prepare buffer as follows. Solution A was 6.18 g boric acid + 7.46 g KCl in 100 mL water. Solution B was 10.6 g sodium carbonate in 100 mL water. Mix 63 mL solution A and 37 mL solution B and adjust pH to 9.5.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nova Pak C18
Mobile phase: MeCN:MeOH:buffer 23:13:64 (Buffer was 94 mL 200 mM NaH_2PO_4 + 6 mL 200 mM Na_2HPO_4, adjusted to pH 5.0 with 100 mM HCl.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 14.9
Internal standard: norprazepam (18.6)
Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES
Simultaneous: alprazolam, bromazepam, chlordiazepoxide, clonazepam, diazepam, estazolam, flumazenil, flunitrazepam, loflazepate, lorazepam, nitrazepam, norflunitrazepam, oxazepam
Noninterfering: acepromazine, aceprometazine, amylobarbital, aprobarbital, barbital, brallobarbital, butalbital, caffeine, carbamazepine, chlorpromazine, cyclobarbital, ethosuximide, heptabarbital, hexobarbital, loprazolam, medazepam, midazolam, pentobarbital, phenobarbital, phenytoin, prazepam, secobarbital, theophylline, thiopental, vinylbarbital
Interfering: tofizopam, clobazam

KEY WORDS
plasma

REFERENCE
Boukhabza,A.; Lugnier,A.A.; Kintz,P.; Mangin,P. Simultaneous HPLC analysis of the hypnotic benzodiazepines nitrazepam, estazolam, flunitrazepam, and triazolam in plasma, *J.Anal.Toxicol.*, **1991**, *15*, 319–322.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 25 μL 1 μg/mL triazolam in toluene + 75 μL 0.1% ammonium hydroxide, vortex 30 s, add 5 mL methylene chloride + 5 mL toluene, shake 15 min,

centrifuge at 177 g for 10 min. Remove aqueous layer and freeze residual aqueous layer in dry ice-acetone for 30 s. Decant organic layer, dry under nitrogen at 50°, vortex residue with 200 μL mobile phase, inject a 125 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm C18 (Supelco)
Mobile phase: MeOH:buffer 40:60 (Buffer was 1 mM phosphate and 3 mM hexyltriethylammonium phosphate in water at pH 7.4.)
Column temperature: 35
Flow rate: 2
Injection volume: 125
Detector: UV 221

CHROMATOGRAM
Retention time: 30
Internal standard: triazolam
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: alprazolam

KEY WORDS
serum; triazolam is IS

REFERENCE
Schmith,V.D.; Cox,S.R.; Zemaitis,M.A.; Kroboth,P.D. New high-performance liquid chromatographic method for the determination of alprazolam and its metabolites in serum: instability of 4-hydroxyalprazolam, *J.Chromatogr.*, **1991**, *568*, 253–260.

SAMPLE
Matrix: blood
Sample preparation: 3 mL Plasma + 30 μL 10 μg/mL triazolam in water, mix 1 min, allow to stand for 15 min at room temperature, add to 3 mL Extrelut SPE cartridge and allow to soak in for 10 min, elute with 20 mL dichloromethane. Evaporate eluant at 30° under reduced pressure, take up residue in 1 mL MeCN:water 5:95, stand for 15 min, centrifuge at 14000 g for 2 min, remove supernatant. Inject a 250 μL aliquot of the supernatant onto column A with mobile phase A and elute to waste, after 7 min forward flush the contents of column A onto column B with mobile phase B, after 0.47 min remove column A from circuit and elute column B with mobile phase B, monitor the effluent from column B. When not in use flush column A with mobile phase A. Between injections clean column A with two injections of 250 μL MeCN.

HPLC VARIABLES
Column: A 30 × 2.1 10 μm MPLC cartridge PRP-1 (Kontron); B 100 × 4.6 MPLC cartridge 5 μm RP-8 Spheri-5 (Kontron)
Mobile phase: A 1 L water + 20 mL MeCN + 50 μL phosphoric acid (pH 3.2); B MeCN:buffer 40:60 (Buffer was 1 L water + 20 mL MeCN + 50 μL phosphoric acid (pH 3.2).)
Flow rate: A 0.3; B 1
Injection volume: 250
Detector: UV 230

CHROMATOGRAM
Retention time: 7.0
Internal standard: triazolam

OTHER SUBSTANCES
Extracted: alprazolam
Simultaneous: bromazepam, oxazepam, lorazepam, diazepam
Interfering: clobazam

KEY WORDS
plasma; SPE; column-switching; triazolam is IS

REFERENCE
Rieck,W.; Platt,D. High-performance liquid chromatographic method for the determination of alprazolam in plasma using the column-switching technique, *J.Chromatogr.*, **1992**, *578*, 259–263.

SAMPLE
Matrix: blood
Sample preparation: Inject 100-200 μL plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

HPLC VARIABLES
Column: A 45 × 4 12 μm TSK-gel G 3 PW (Tosohass); B 75 × 4.6 Ultrasphere ODS C18 3 μm
Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.
Flow rate: 1
Injection volume: 100-200
Detector: UV 230

CHROMATOGRAM
Retention time: 23.3

OTHER SUBSTANCES
Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, desmethylclobazam, desmethyldiazepam, diazepam, estazolam, loflazepate, lorazepam, medazepam, nitrazepam, oxazepam, prazepam, temazepam, tetrazepam, tofisopam
Noninterfering: carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid
Interfering: flunitrazepam

KEY WORDS
plasma; column-switching

REFERENCE
Lacroix,C.; Wojciechowski,F.; Danger,P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *617*, 285–290.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 25 μL 0.5 μg/mL demoxepam in water + 100 μL 1 M pH 9.0 borate buffer, mix well, add 2 mL diethyl ether, vortex for 40 s, centrifuge at 1100 g for 5 min. Remove ether layer and evaporate it at 40° under nitrogen. Take up residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 2 5μm Ultrasphere C18
Mobile phase: MeCN:MeOH:43 mM pH 2.4 sodium acetate buffer 8:45:47
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: k' 6.05
Internal standard: demoxepam (4, k' 2.73)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, alprazolam

Simultaneous: chlorpromazine, clonazepam, diazepam, flurazepam, hexobarbital, oxazepam, phenobarbital, temazepam
Noninterfering: amphetamine, buspirone, chlordiazepoxide, cocaine, cocathylene, flumazenil, midazolam, norcocaine

KEY WORDS
serum; rat

REFERENCE
Jin,L.; Lau,C.E. Determination of alprazolam and its major metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1994**, *654*, 77–83.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 20 μL 25 ng/mL diazepam in MeOH + 100 μL 5 M NaOH + 5 mL hexane:dichloromethane 50:50, shake mechanically for 10 min, centrifuge at 1300 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, sonicate, inject an aliquot. Urine. Adjust pH of 10 mL urine to 5 with acetic acid, add 2.5 mL 500 mM pH 5.0 acetate buffer, add 3000 U β-glucuronidase (type IX, Sigma), heat at 37° for 24 h, centrifuge at 1300 g for 10 min. 1 mL Supernatant + 20 μL 25 ng/mL diazepam in MeOH + 100 μL 5 M NaOH + 5 mL hexane: dichloromethane 50:50, shake mechanically for 10 min, centrifuge at 1300 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, sonicate, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-HG-5
Mobile phase: Gradient. MeOH:50 mM pH 4.0 ammonium acetate from 50:50 to 100:0 over 15 min.
Injection volume: 200
Detector: MS, Hitachi M-1200H, APCI, positive ion, nebulizer 170°, desolvation 400°, needle-electrode 2700 V

CHROMATOGRAM
Retention time: 9
Internal standard: diazepam (12)
Limit of quantitation: 20 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; LC-MS

REFERENCE
Senda,N.; Kohta,K.; Takahashi,T.; Hizukuishi,K.; Mimura,T.; Fujita,T.; Nakayama,M. A highly sensitive method to quantify triazolam and its metabolites with liquid chromatography--mass spectrometry, *Biomed.Chromatogr.*, **1995**, *9*, 48–51.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM

Retention time: 3.88
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 17.353

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Cool microsomal incubation on ice, add 100 μL MeCN, add phenacetin, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 3.9 NovaPak C18
Mobile phase: MeCN:MeOH:50 mM phosphate buffer 22.5:10:67.5
Flow rate: 1.3
Detector: UV 220

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
von Moltke,L.L.; Greenblatt,D.J.; Harmatz,J.S.; Duan,S.X.; Harrel,L.M.; Cotreau-Bibbo,M.M.; Pritchard,G.A.; Wright,C.E.; Shader,R.I. Triazolam biotransformation by human liver microsomes in vitro: Effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 370–379.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.8 5 μm Nova-Pak C18
Mobile phase: MeCN:water:85% phosphoric acid:7.6 mM tetramethylammonium chloride 30:70:0.2:0.075, final apparent pH 6.7 (adjusted with 1 M NaOH)
Flow rate: 1
Injection volume: 15-30
Detector: UV 198 (nitrogen purged)

CHROMATOGRAM
Retention time: 11.3
Internal standard: triazolam

OTHER SUBSTANCES
Simultaneous: mezlocillin, phenobarbital, diazepam, oxazepam, clindamycin, clindamycin B
Noninterfering: cefoperazone, cefotaxime, cephalothin, ticarcillin

REFERENCE
La Follette,G.; Gambertoglio,J.; White,J.A.; Knuth,D.W.; Lin,E.T. Determination of clindamycin in plasma or serum by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1988**, *431*, 379–388.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 6.43 (A), 6.65 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Heat 5 mL urine + 1 mL temazepam in MeOH with 1 mL β-glucuronidase at 37° for 2.5 h, cool, adjust to pH 8.5 with saturated Na_2CO_3, extract with 10 mL dichloromethane. Evaporate, take up the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Brownlee 5 μm RP-8
Mobile phase: MeCN:10 mM KH_2PO_4:n-nonylamine 450:550:0.6 adjusted to pH 3,2 with phosphoric acid
Flow rate: 1.6
Detector: UV 225

CHROMATOGRAM
Retention time: 8
Internal standard: temazepam (7)

OTHER SUBSTANCES
Extracted: metabolites
Interfering: alprazolam

REFERENCE
Fraser,A.D. Urinary screening for alprazolam, triazolam, and their metabolites with the EMIT d.a.u. benzodiazepine metabolite assay, *J.Anal.Toxicol.*, **1987**, *11*, 263–266.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 10 mL dichloromethane:MeOH 9:1, 5 mL MeOH, and 10 mL water. Condition a Sep-Pak Silica SPE cartridge with 20 mL dichloromethane, 20 mL dichloromethane:MeOH 9:1, and 20 mL dichloromethane, then air dry. 10 mL Urine adjusted to pH 5 with acetic acid, add 2.5 mL 500 mM pH 5.0 acetate buffer, add 3000 U β-glucuronidase, incubate at 37° for 24 h, make alkaline with ammonia, centrifuge at 1200 g for 15 min, add the supernatant to the C18 SPE cartridge with 100 μL 5 μg/mL etizolam in MeOH, wash with 5 mL water, wash with 5 mL MeOH:water 20:80, wash with 2 mL water, elute with 7 mL dichloromethane:MeOH 9:1. Evaporate the eluate to dryness under vacuum, dissolve the residue in 5 mL dichloromethane:MeOH 99:1, add to the silica SPE cartridge, wash with 20 mL dichloromethane, wash with 25 mL dichloromethane:MeOH 99:1, elute with 20 mL dichloromethane:MeOH 9:1. Evaporate the eluate to dryness under vacuum, dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (MeOH for silica SPE cartridge was distilled and dried over 3 Å molecular sieve, then 0.1% water added just before use.)

HPLC VARIABLES
Column: 100 × 8 10 μm Radial-Pak C18
Mobile phase: MeOH:10 mM pH 8.0 phosphate buffer 65:35
Flow rate: 1
Injection volume: 20
Detector: UV 220

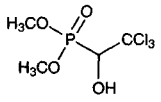
CHROMATOGRAM
Retention time: 14
Internal standard: etizolam (17)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
SPE

REFERENCE
Inoue,T.; Suzuki,S.-I. High-performance liquid chromatographic determination of triazolam and its metabolites in human urine, *J.Chromatogr.*, **1987**, *422*, 197–204.

Trichlorfon



Molecular formula: $C_4H_8Cl_3O_4P$
Molecular weight: 257.44
CAS Registry No.: 52-68-6
Merck Index: 9753

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 5 mL chloroform, vortex for 15 s, centrifuge at 0-5° at 800 g for 15 min, remove a 4.5 mL aliquot of the organic layer and add it to 3.5 mL 5 M HCl, vortex for 15 s, centrifuge at 0-5° at 800 g for 15 min, remove a 4 mL aliquot of the organic layer and add it to 200 mg anhydrous calcium sulfate (Drierite), vortex for 15 s, centrifuge at 0-5° at 800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen (in an acid-washed tube), reconstitute the residue in 120 µL water, vortex, centrifuge at 0-5° at 11000 g for 5 min, inject a 100 µL aliquot of the supernatant. (Trichlorfon may degrade to dichlorvos during sample preparation unless whole blood is immediately acidified with phosphoric acid (J.Chromatogr. 1993,612, 336).)

HPLC VARIABLES
Guard column: 37-50 µm C18/Corasil
Column: 200 × 3.9 10 µm C18 (Waters)
Mobile phase: MeOH:THF:water 10:0.1:89.9 containing 1 mM octanesulfonic acid, pH 3.0
Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 10.8
Limit of detection: 1 µg/mL

KEY WORDS
plasma

REFERENCE
Unni,L.K.; Hannant,M.E.; Becker,R.E. High-performance liquid chromatographic method using ultraviolet detection for measuring metrifonate and dichlorvos levels in human plasma, *J.Chromatogr.*, **1992**, *573*, 99–103.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 5 mL chloroform, vortex for about 15 s, centrifuge at 0-5° at 800 g for 15 min. Remove the organic layer and add it to 3.5 mL 5 M HCl, vortex for 15 s, centrifuge at 0-5° at 800 g for 15 min. Remove a 4 mL aliquot of the organic layer and dry it over 200 mg anhydrous calcium sulfate (Drierite), vortex, centrifuge at 0-5° at 800 g for 5 min. Remove a 3.8 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature in an acid-washed vial, reconstitute the residue in 120 µL water, vortex, centrifuge at 0-5° at 11000 g for 5 min, inject a 100 µL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 37-50 µm C18/Corasil
Column: 300 × 3.9 10 µm C18 (Waters)
Mobile phase: MeOH:THF:water 10:0.1:89.9 containing 1 mM sodium 1-octanesulfonate, pH adjusted to 3.0

Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 10.8
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Noninterfering: dichlorvos

KEY WORDS
plasma

REFERENCE
Unni,L.K.; Hannant,M.E.; Becker,R.E. High-performance liquid chromatographic method using ultraviolet detection for measuring metrifonate and dichlorvos levels in human plasma, *J.Chromatogr.*, **1992**, *573*, 99–103.

SAMPLE
Matrix: formulations
Sample preparation: 1 g Paste + 75 mL MeOH, sonicate for 15 min, shake for 1 min, sonicate for 5 min, shake for 30 s, cool to room temperature, make up to 100 mL with MeOH, centrifuge an aliquot at 2000 rpm for 10 min. Mix 2 mL supernatant with 2 mL 200 μg/mL methyl paraben in MeOH, make up to 25 mL with MeCN:water:phosphoric acid 20:80:1, filter (Millipore 0.6 μm polyvic), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:PELL ODS
Column: 250 × 4.6 Partisil-5 ODS-3
Mobile phase: MeCN:buffer 20:80 (Buffer was 720 mL 1.38 g/L $NaH_2PO_4.H_2O$ + 80 mL 1.42 g/L Na_2HPO_4.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 200

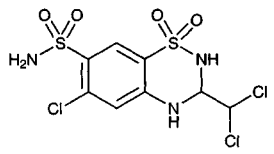
CHROMATOGRAM
Retention time: 6.69
Internal standard: methyl paraben (15.71)

OTHER SUBSTANCES
Simultaneous: oxfendazole

KEY WORDS
horse; paste

REFERENCE
Fleitman,J.; Neu,D.; Benjamin,E. Analysis of pharmaceutical dosage forms for oxfendazole: II. Simultaneous liquid chromatographic determination of oxfendazole and trichlorfon in equine paste, *J.Assoc.Off. Anal.Chem.*, **1986**, *69*, 24–28.

Trichlormethiazide



Molecular formula: $C_8H_8Cl_3N_3O_4S_2$
Molecular weight: 380.66
CAS Registry No.: 133-67-5
Merck Index: 9754
Lednicer No.: 1 359

SAMPLE

Matrix: blood
Sample preparation: 3 mL Plasma + 1 mL 10 mM NaOH + 1 mL 2 μg/mL bendroflumethiazide in 10 mM NaOH + 2 mL 10 mM HCl, mix, add 10 mL diethyl ether, shake gently on a platform shaker for 15 min, centrifuge at -10° at 2200 g for 15 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70° for 45 min (these conditions are required to remove residual benzyl alcohol that is present as a preservative in the heparin), reconstitute the residue in 50 μL 10 mM NaOH, vortex for 25 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Isopropanol:water:acetic acid 17:82:1
Flow rate: 2
Injection volume: 20
Detector: UV 269

CHROMATOGRAM

Retention time: 3.7
Internal standard: bendroflumethiazide (14.4)
Limit of detection: 10 ng/mL

KEY WORDS

plasma; silanize glassware; pharmacokinetics

REFERENCE

Meyer,M.C.; Hwang,P.T.R. Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *223*, 466–472.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 225.2

CHROMATOGRAM
Retention time: 14.907

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetra-

cycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 500 mg solid sodium bicarbonate, mix, add 1 mL 10 mM NaOH, add 1 mL 2 μg/mL bendroflumethiazide in 10 mM NaOH, mix, add 10 mL diethyl ether, shake gently on a platform shaker for 15 min, centrifuge at -10° at 2200 g for 15 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70° for 45 min (these conditions are required to remove residual benzyl alcohol that is present as a preservative in the heparin), reconstitute the residue in 100 μL MeOH, vortex for 25 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 5:35:59:1
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 3.7
Internal standard: bendroflumethiazide (10.7)
Limit of detection: 50 ng/mL

KEY WORDS

silanize glassware; pharmacokinetics

REFERENCE

Meyer,M.C.; Hwang,P.T.R. Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *223*, 466–472.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + 1 mL 10 mM pH 7.0 phosphate buffer + 3 g NaCl + 9 mL ether, shake vigorously for 10 min, centrifuge at 2500 rpm for 10 min. Remove 7 mL of the organic layer and evaporate it to dryness using a centrifugal evaporator at 50°, reconstitute the residue in 100 μL 50 μg/mL methyl p-hydroxybenzoate in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Zorbax ODS
Mobile phase: MeCN:10 mM pH 7.0 phosphate buffer 20:80
Column temperature: 35
Flow rate: 0.8
Injection volume: 20
Detector: UV 274

CHROMATOGRAM

Internal standard: methyl p-hydroxybenzoate
Limit of detection: 2 μg/mL

KEY WORDS

pharmacokinetics

REFERENCE

Takahashi,H.; Watanabe,Y.; Shimamura,H.; Sugito,K. Effects of magnesium oxide on trichlormethiazide bioavailability, *J.Pharm.Sci.*, **1985**, *74*, 862–865.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 11.1 (A), 12.0 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 15.6

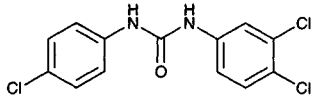
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

Triclocarban



Molecular formula: $C_{13}H_9Cl3N_2O$
Molecular weight: 315.59
CAS Registry No.: 101-20-2
Merck Index: 9786

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

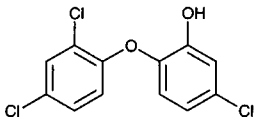
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 264.1

CHROMATOGRAM
Retention time: 25.573

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Triclosan



Molecular formula: $C_{12}H_{17}Cl_3O_2$
Molecular weight: 289.54
CAS Registry No.: 3380-34-5
Merck Index: 9790

SAMPLE

Matrix: formulations
Sample preparation: 2 g Dentifrice + 25 mL MeCN:water 60:40 + four 4 mm glass beads, vortex for 4 min, centrifuge at 10-20° at 13000 rpm for 15 min, repeat extraction twice. Combine the supernatants and make up to 100 mL with MeCN:water 60:40, vortex. Dilute 1:20, vortex, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: Nova-Pak C18 Guard-Pak
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:water 60:40
Flow rate: 1.5
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 4.5

OTHER SUBSTANCES

Noninterfering: excipients

KEY WORDS

dentifrice; toothpaste

REFERENCE

Demkowicz,M.P.; Chauhan,V.; Stern,D.A.; Vasquez,F.G. Simultaneous determination of anions and triclosan in dentifrices by gradient ion chromatography and isocratic high-performance liquid chromatography interfaced with conductivity and ultraviolet detection, *J.Chromatogr.A*, **1994**, *671*, 351–357.

SAMPLE

Matrix: textiles
Sample preparation: Reflux 1 g fabric with 20 mL MeOH:acetic acid 90:10 for 30 min, filter (glass, 3G2), wash solid with 50 mL MeOH. Combine filtrate and washings and evaporate them to 5 mL under reduced pressure, add 10 mL 100 mM HCl, extract three times with 10 mL portions of diethyl ether. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 2 mL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 TSK gel ODS-80TM
Mobile phase: MeCN:water:acetic acid 60:40:0.1
Column temperature: 37
Flow rate: 1
Detector: UV 240

CHROMATOGRAM

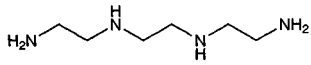
Retention time: 15

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE
Kanetoshi,A.; Ogawa,H.; Katsura,E.; Kaneshima,H. Chlorination of Irgasan DP300 and formation of dioxins from its chlorinated derivatives, *J.Chromatogr.*, **1987**, *389*, 139–153.

Trientine



Molecular formula: $C_6H_{18}N_4$
Molecular weight: 146.24
CAS Registry No.: 112-24-3, 38260-01-4 (HCl)
Merck Index: 9796

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SCX SPE cartridge with 5 mL water. 500 μL Plasma + 100 μL water + 1 mL MeCN, vortex briefly, centrifuge at 1000 g for 5 min. Add 1.2 mL of the supernatant to the SPE cartridge, wash with 3 mL water, wash with 2 mL 1 M KCl, wash with 3 mL 2 M KCl, elute with 1 mL 4 M KCl. Remove a 200 μL aliquot of the eluate and add it to 600 μL 100 mM pH 9.5 sodium phosphate buffer and 100 μL 0.15 mM trisodium EDTA in 100 mM pH 9.5 sodium phosphate buffer, mix, add 100 μL 10 mM fluorescamine in MeCN, vortex vigorously for 1 min, let stand for 20 min, add 50 μL 0.25 mM α-naphthylamine (Caution! α-Naphthylamine is a carcinogen!) in MeOH, inject a 20-50 μL aliquot.

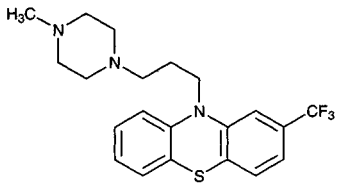
HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 5-CN
Mobile phase: MeCN:buffer 27:73 , pH adjusted to 6.0 with 2 M NaOH (Buffer was 140 mM ammonium chloride containing 48 mM sodium benzenesulfonate and 9.2 mM acetic acid.)
Column temperature: 40
Flow rate: 0.5
Injection volume: 20-50
Detector: F ex 380 em 485

CHROMATOGRAM
Retention time: 9.5
Internal standard: α-naphthylamine (13)
Limit of detection: 100 ng/mL

KEY WORDS
plasma; derivatization; SPE; rat; human; pharmacokinetics

REFERENCE
Miyazaki,K.; Kishino,S.; Kobayashi,M.; Arashima,S.; Matsumoto,S.; Arita,T. Determination of triethylenetetramine in plasma of patients by high-performance liquid chromatography, *Chem.Pharm.Bull.(Tokyo)*, **1990**, *38*, 1035–1038.

Trifluoperazine

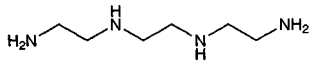


Molecular formula: $C_{21}H_{24}F_3N_3S$
Molecular weight: 407.50
CAS Registry No.: 117-89-5, 440-17-5 (di HCl)
Merck Index: 9811

SAMPLE
Matrix: blood

REFERENCE
Kanetoshi,A.; Ogawa,H.; Katsura,E.; Kaneshima,H. Chlorination of Irgasan DP300 and formation of dioxins from its chlorinated derivatives, *J.Chromatogr.*, **1987**, *389*, 139–153.

Trientine



Molecular formula: $C_6H_{18}N_4$
Molecular weight: 146.24
CAS Registry No.: 112-24-3, 38260-01-4 (HCl)
Merck Index: 9796

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SCX SPE cartridge with 5 mL water. 500 µL Plasma + 100 µL water + 1 mL MeCN, vortex briefly, centrifuge at 1000 g for 5 min. Add 1.2 mL of the supernatant to the SPE cartridge, wash with 3 mL water, wash with 2 mL 1 M KCl, wash with 3 mL 2 M KCl, elute with 1 mL 4 M KCl. Remove a 200 µL aliquot of the eluate and add it to 600 µL 100 mM pH 9.5 sodium phosphate buffer and 100 µL 0.15 mM trisodium EDTA in 100 mM pH 9.5 sodium phosphate buffer, mix, add 100 µL 10 mM fluorescamine in MeCN, vortex vigorously for 1 min, let stand for 20 min, add 50 µL 0.25 mM α-naphthylamine (Caution! α-Naphthylamine is a carcinogen!) in MeOH, inject a 20-50 µL aliquot.

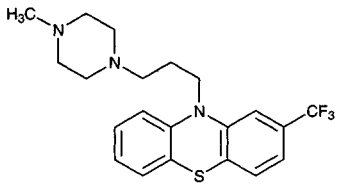
HPLC VARIABLES
Column: 250 × 4.6 5 µm Nucleosil 5-CN
Mobile phase: MeCN:buffer 27:73 , pH adjusted to 6.0 with 2 M NaOH (Buffer was 140 mM ammonium chloride containing 48 mM sodium benzenesulfonate and 9.2 mM acetic acid.)
Column temperature: 40
Flow rate: 0.5
Injection volume: 20-50
Detector: F ex 380 em 485

CHROMATOGRAM
Retention time: 9.5
Internal standard: α-naphthylamine (13)
Limit of detection: 100 ng/mL

KEY WORDS
plasma; derivatization; SPE; rat; human; pharmacokinetics

REFERENCE
Miyazaki,K.; Kishino,S.; Kobayashi,M.; Arashima,S.; Matsumoto,S.; Arita,T. Determination of triethylenetetramine in plasma of patients by high-performance liquid chromatography, *Chem.Pharm.Bull.(Tokyo)*, **1990**, *38*, 1035–1038.

Trifluoperazine



Molecular formula: $C_{21}H_{24}F_3N_3S$
Molecular weight: 407.50
CAS Registry No.: 117-89-5, 440-17-5 (di HCl)
Merck Index: 9811

SAMPLE
Matrix: blood

Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 9.3
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: amitriptyline, butaperazine, carphenazine, chlorpromazine, fluphenazine, promazine, promethazine, trimeprazine
Simultaneous: acetophenazine, benztropine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, thiothixene, trifluprom azine, trihexyphenidyl

KEY WORDS

plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood
Sample preparation: 1-5 mL Plasma + 1 mL 1 M NaOH + hexanes, extract for 30 min, centrifuge. Remove a 9 mL aliquot of the organic phase and evaporate it to dryness at 30° under a stream of nitrogen. Dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:5 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 20.1
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, fluphenazine, promethazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, chlorpromazine, thiothixene, thioridazine, trifluprom azine, trihexyphenidyl, trimeprazine, metabolites

KEY WORDS

plasma

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 50 mM HCl + 200 μL concentrated ammonium hydroxide + 7 mL n-pentane:isopropanol 95:5, shake horizontally for 30 min, centrifuge at 2000 g. Remove the top organic layer and add it to 2 mL 100 mM perchloric acid, agitate for 10 min, centrifuge. Remove the aqueous layer and add it to 200 μL concentrated ammonium hydroxide, add 6 mL n-pentane:isopropanol 95:5, agitate for 30 min, centrifuge. Remove the top organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μL MeCN, inject a 20-40 μL aliquot. (Clean glassware scrupulously by soaking overnight in 50 mL/L Contrad (Curtin Matheson), rinse several times with water, and air dry.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano

Mobile phase: MeCN:10 mM pH 2.5 KH_2PO_4 60:40

Flow rate: 2.5

Injection volume: 20-40

Detector: E, Environmental Science Associates Coulochem Model 5100A, Model 5100 guard cell +0.85 V (between pump and injector), Model 5010 analytical cell +0.8 V, preanalytical cell +0.3 V

CHROMATOGRAM

Retention time: 8.2

Internal standard: trifluoperazine dihydrochloride

OTHER SUBSTANCES

Extracted: thiothixene

Simultaneous: amitriptyline, amoxapine, chlorpromazine, desipramine, doxepin, haloperidol, imipramine, loxapine, mesoridazine, nortriptyline, pheniramine, phenylephrine, prochlorperazine, promazine, promethazine, trazodone, trimeprazine, tripelennamine

Noninterfering: diazepam, diphenhydramine, ethopropazine, fluoxetine, nordiazepam, oxazepam, phenylpropanolamine, pseudoephedrine

Interfering: fluphenazine, perphenazine, thioridazine, triflupromazine

KEY WORDS

plasma; trifluoperazine is IS

REFERENCE

Hariharan,M.; VanNoord,T.; Kindt,E.K.; Tandon,R. A simple, sensitive liquid chromatographic assay of cis-thiothixene in plasma with coulometric detection, *Ther.Drug Monit.*, **1991**, *13*, 79–85.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 260

CHROMATOGRAM

Retention time: 19.78

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 258.2

CHROMATOGRAM
Retention time: 17.747

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Add 5 mL water to powdered tablets containing 4 mg trifluoperazine, heat on a water-bath for 3 min. Cool, add 50 mL water, shake for 15 min and make up to 100 mL with MeOH. Filter, remove a 5 mL aliquot, add IS to a concentration of 3 μg/mL, make up to 50 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Novapak-phenyl-4
Mobile phase: MeOH:15 mM pH 6.5 sodium acetate buffer 81:19
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: alprenolol (3.4)
Limit of detection: 150 pg

OTHER SUBSTANCES
Simultaneous: degradation products, triflupromazine

KEY WORDS
tablets

REFERENCE
Al-Obaid,A.M.; Hagga,M.E.M.; El-Khawad,I.E.; El-Mahi,O.H.M. Simultaneous quantitation of some phenothiazine drug substances and their monosulphoxide degrades by high performance liquid chromatography (HPLC), *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1369–1389.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablet or capsule, to 2 mg amitriptyline add 20 mL MeOH, shake 30 min, centrifuge at 2000 rpm for 5 min, to 5 mL supernatant add 4 mL 1.25 mg/mL norephedrine.HCl in MeOH, dilute to 10 mL with MeOH.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:MeOH:25 mM pH 4.8 sodium acetate-acetic acid buffer 35:45:20
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.4
Internal standard: norephedrine (2.7)

OTHER SUBSTANCES
Also analyzed: chlorpromazine, amitriptyline, imipramine, thioridazine

KEY WORDS
tablets; capsules

REFERENCE
Lovering,E.G.; Beaulieu,N.; Lawrence,R.C.; Sears,R.W. Liquid chromatographic method for identity, assay, and content uniformity of five tricyclic drugs, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 168–171.

SAMPLE
Matrix: formulations
Sample preparation: Measure out syrup or injection, make up to 50 mL with MeOH, mix, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:MeOH:25 mM pH 4.5 acetate buffer 40:30:30
Flow rate: 2.5
Injection volume: 10
Detector: UV 229

CHROMATOGRAM
Retention time: 6
Internal standard: trifluoperazine

OTHER SUBSTANCES
Simultaneous: perphenazine

KEY WORDS
syrup; injections; trifluoperazine is IS

REFERENCE
Beaulieu,N.; Lovering,E.G. Liquid chromatographic method for perphenazine and its sulfoxide in pharmaceutical dosage forms for determination of stability, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 167–169.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Zorbax ODS
Mobile phase: MeOH containing 0.5 g/L sodium acetate
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 4.30

OTHER SUBSTANCES
Simultaneous: chlorpromazine, trihexyphenidyl

REFERENCE
Pradas,T.N.V.; Sivakumar,M. HPLC quantification of a tricomponent psychiatric formulation containing chlorpromazine, trifluoperazine and trihexyphenidyl, *Pharmazie*, **1992**, *47*, 231–231.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Lichrosorb SI60
Mobile phase: MeCN:MeOH:ammonium hydroxide 250:55:13
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 3.1

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, amitriptyline, butaperazine, chlorimipramine, chlorpromazine, codeine, desipramine, dimethacrine, diphenhydramine, disopyramide, doxepin, hydroquinidine, maprotiline, melitracene, mesoridazine, nortriptyline, perazine, perphenazine, procainamide, prochlorperazine, prothipendyl, protriptyline, quinidine, thiethylperazine, thioperazine
Noninterfering: acenocoumaron, acetaminophen, acetophenetidine, aspirin, benzodiazepines, bibenzepin, butriptyline, caffeine, chlorprothixene, clopenthixol, clothiapine, dixyrazine, droperidol, fluphenazine, haloperidol, hydroxyzine, isoniazid, methotrimeprazine, metopimazine, moperone, noxiptyline, orphenadrine, pericyazine, phenprocoumon, pipothiazine, promethazine, salicylic acid, theophylline, thiopropazate, trimeprazine, trimipramine
Interfering: imipramine, opipramol, pipamperone, promazine, thioridazine, thiothixene

REFERENCE
Edelbroek,P.M.; de Haas,E.J.M.; de Wolff,F.A. Liquid-chromatographic determination of amitryptyline and its metabolites in serum, with adsorption onto glass minimized, *Clin.Chem.*, **1982**, *28*, 2143–2148.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak CN
Mobile phase: MeCN:MeOH:5 mM phosphate buffer 60:15:25, adjusted to pH 7.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.96

OTHER SUBSTANCES
Simultaneous: acetaminophen, amitriptyline, caffeine, chlordiazepoxide, chlorpromazine, desipramine, desmethyldoxepin, diazepam, disopyramide, doxepin, imipramine, maprotiline, methaqualone, nortriptyline, procainamide, propoxyphene, propranolol, protriptyline, salicylic acid, theophylline, thioridazine
Interfering: trimipramine

REFERENCE
Koteel,P.; Mullins,R.E.; Gadsden,R.H. Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum, *Clin.Chem.*, **1982**, *28*, 462–466.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)
Flow rate: 2

Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 6.0
Internal standard: promazine (5.2)

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, amitriptyline, amoxapine, amphetamine, buprion, chlordiazepoxide, chlorimipramine, chlorpheniramine, chlorpromazine, cocaine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldoxepin, dextropropoxyphene, diazepam, disopyramide, doxepin, fluphenazine, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, iprindole, loxepin, maprotiline, meperidine, methadone, mianserin, nortriptyline, norzimeldine, oxapam, oxaprotiline, perphenazine, phentermine, procainamide, prochlorperazine, prolixin, promethazine, propoxyphene, protriptyline, pyrilamine, quinidine, thioridazine, triflupromazine, trimeprazine, trimipramine
Noninterfering: thiopropazine
Interfering: codeine, desmethyldisopyramide, morphine, zimeldine

KEY WORDS
normal phase

REFERENCE
Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine,

methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Retention time: 5.6
Internal standard: N-propionylprocainamide (6)

OTHER SUBSTANCES

Simultaneous: amitriptyline, atropine, butalbital, chlorpromazine, desipramine, desmethylmaprotiline, doxepin, imipramine, maprotiline, methadone, norpropoxyphene, nortriptyline, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, protriptyline, trimipramine

Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil

Interfering: quinidine, trimeprazine

REFERENCE

Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 65 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb S5W
Mobile phase: MeOH:50 mM pH 9.9 ammonium acetate buffer 85:15
Flow rate: 1.4
Injection volume: 65
Detector: UV 261

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: amitriptyline, carbamazepine, chlorprothixene, clomipramine, clozapine, imipramine, levomepromazine, mianserine, nortriptyline, perphenazine, zuclopenthixol

REFERENCE
Olesen,O.V.; Poulsen,B. On-line fully automated determination of clozapine and desmethylclozapine in human serum by solid-phase extraction on exchangeable cartridges and liquid chromatography using a methanol buffer mobile phase on unmodified silica, *J.Chromatogr.*, **1993**, *622*, 39–46.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 15.8

OTHER SUBSTANCES
Simultaneous: mesoridazine, promazine, thiothixene, chlorpromazine, thioridazine
Also analyzed: amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 RP-8
Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.32

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

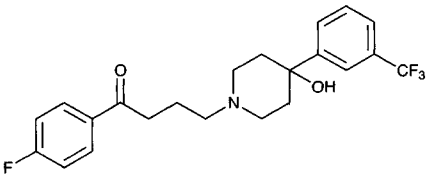
KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Trifluperidol



Molecular formula: $C_{22}H_{23}F_4NO_2$
Molecular weight: 409.42
CAS Registry No.: 749-13-3, 2062-77-3 (HCl)
Merck Index: 9813
Lednicer No.: 1 306

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 247

CHROMATOGRAM

Retention time: 6.61
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 21.638

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.0

OTHER SUBSTANCES

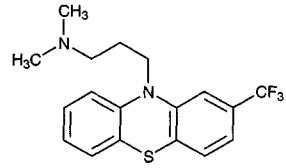
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine,

phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Trifluprom azine



Molecular formula: $C_{18}H_{19}F_3N_2S$
Molecular weight: 352.42
CAS Registry No.: 146-54-3, 1098-60-8 (HCl)
Merck Index: 9814
Lednicer No.: 1 380

SAMPLE

Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 6.6
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, trihexyphenidyl
Interfering: carphenazine, trimeprazine

KEY WORDS

plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg Phenomenex C2 SPE cartridge (cat. no. AHO-0857) with 1 mL MeOH then 1 mL water (add analyte within 2-3 min). 500 μL Serum + 300 μL 2 M pH 4.5 sodium acetate, vortex for 5-10 s, add to SPE cartridge, wash with 1 mL water, 1 mL MeOH:water 1:1, 1 mL MeCN:water 1:1, elute with 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 Vydac C18 reverse phase (cat. no. 201SC)
Column: 150 × 4.6 5 μm UltraCarb 5 octadecylsilyl (cat. no. OOF-0351-EO)
Mobile phase: 875 mL MeOH:MeCN 1:1 + 125 mL 30 mM pH 4.0 ammonium acetate
Column temperature: 40
Flow rate: 1.5
Injection volume: 50
Detector: UV 242

CHROMATOGRAM

Retention time: 2.2
Internal standard: triflupromazine
Limit of quantitation: 160 ng/mL

OTHER SUBSTANCES

Simultaneous: amiodarone

Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, caffeine, carbamazepine, chloramphenicol, clonazepam, cyclosporine, desipramine, digoxin, disopyramide, ethosuximide, flecainide, gentamicin, haloperidol, imipramine, kanamycin, lidocvaine, methotrexate, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, propranolol, propoxyphene, quinidine, salicylic acid, streptomycin, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS

serum; SPE; triflupromazine is IS

REFERENCE

Jandreski,M.A.; Vanderslice,W.E. Clinical measurement of serum amiodarone and desethylamiodarone by using solid-phase extraction followed by HPLC with a high-carbon reversed-phase column, *Clin.Chem.*, **1993**, *39*, 496–500.

SAMPLE

Matrix: formulations

Sample preparation: Add 5 mL water to powdered tablets containing 4 mg triflupromazine, heat on a water-bath for 3 min. Cool, add 50 mL water, shake for 15 min and make up to 100 mL with MeOH. Filter, remove a 5 mL aliquot, add IS to a concentration of 3 μg/mL, make up to 50 mL with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Novapak-phenyl-4
Mobile phase: MeOH:15 mM pH 6.5 sodium acetate buffer 81:19
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 6.1
Internal standard: alprenolol (3.2)

Limit of detection: 100 pg

OTHER SUBSTANCES
Simultaneous: degradation products, trifluperazine

KEY WORDS
tablets

REFERENCE
Al-Obaid,A.M.; Hagga,M.E.M.; El-Khawad,I.E.; El-Mahi,O.H.M. Simultaneous quantitation of some phenothiazine drug substances and their monosulphoxide degrades by high performance liquid chromatography (HPLC), *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1369–1389.

SAMPLE
Matrix: solutions
Sample preparation: Add 50 μL of a solution in ethyl acetate to 25 μL trichloroethyl chloroformate, vortex, heat at 120° for 20 min, cool. Evaporate to dryness under a stream of air at 40°, reconstitute the residue in 100 μL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 MCH-10 reversed-phase (Varian)
Mobile phase: MeOH:water 84:16
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: chlorpromazine, chlorprothixene, phenothiazine, phenothiazine-5-oxide, promazine, promethazine, trimeprazine

KEY WORDS
derivatization

REFERENCE
Wallace,J.E.; Shimek,E.L.,Jr.; Harris,S.C.; Stavchansky,S. Determination of promethazine in serum by liquid chromatography, *Clin.Chem.*, **1981**, *27*, 253–255.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 3.0
Internal standard: promazine (5.2)

OTHER SUBSTANCES

Simultaneous: N-acetylprocainamide, amoxapine, amphetamine, buprion, chlordiazepoxide, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, disopyramide, doxepin, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, iprindole, maprotiline, meperidine, mianserin, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, procainamide, prochlorperazine, prolixin, promethazine, propoxyphene, protriptyline, pyrilamine, quinidine, thioridazine, trifluoperazine, trimeprazine, trimipramine, zimeldine
Noninterfering: thiopropazine
Interfering: amitriptyline, chlorimipramine, fluphenazine, loxepin, methadone, perphenazine, phentermine

KEY WORDS

normal phase

REFERENCE

Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pi-

renzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 1.47

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl

Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03

Column temperature: 50

Flow rate: 3

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 2.6

OTHER SUBSTANCES

Simultaneous: carphenazine, methotrimeprazine, promazine, perphenazine, chlorprothixene, deserpidine, thiothixene, reserpine

Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

KEY WORDS

SFC; pressure 200 bar

REFERENCE

Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

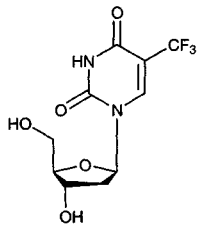
CHROMATOGRAM
Retention time: 17.28 (A), 8.93 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Trifluridine



Molecular formula: $C_{10}H_{11}F_3N_2O_5$
Molecular weight: 296.20
CAS Registry No.: 70-00-8
Merck Index: 9816

SAMPLE

Matrix: aqueous humor, tissue, vitreous humor
Sample preparation: Minced cornea or 100 μL aqueous humor or vitreous humor + 75 μL 200 mM pH 3.88 sodium acetate buffer + 25 μL 8.2 μg/mL 3-methylthymidine in water, mix, add 1.5 mL ethyl acetate, swirl-mix for 1.5 min, centrifuge at 300 g for 5 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:10 mM pH 3.88 acetate buffer 12.5:87.5 containing 1 mM sodium hexanesulfonate
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.1
Internal standard: 3-methylthymidine (4.1)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: bacitracin, chloramphenicol, flurbiprofen, scopolamine, sulfamethoxazole
Noninterfering: antazoline, atropine, cyclopentolate, dexamethasone, dipivefrin, epinephrine, epinephryl borate, erythromycin, fluorometholone, homatropine,hydrocortisone acetate, naphazoline, neomycin, phospholine, polymyxin B, procaine, proparacaine, tetracycline, timolol, tropicamide
Interfering: sulfacetamide

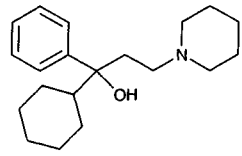
KEY WORDS

rabbit; cornea

REFERENCE

Riegel,M.R.; Ellis,P.P. Determination of trifluorothymidine in the eye using high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *568*, 467–474.

Trihexyphenidyl



Molecular formula: $C_{20}H_{31}NO$
Molecular weight: 301.47
CAS Registry No.: 144-11-6, 52-49-3 (HCl)
Merck Index: 9823
Lednicer No.: 1 47

SAMPLE

Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 7.3
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trimeprazine
Interfering: acetophenazine

KEY WORDS

plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane:dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)

Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM
Retention time: 20.1
Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES
Extracted: chlorpromazine, clomipramine, cyamemazine, desipramine, droperidol, flunitrazepam, haloperidol, pipamperone, risperidone
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid
Interfering: imipramine

KEY WORDS
plasma; SPE

REFERENCE
Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 18.96
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.298

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Zorbax ODS
Mobile phase: MeOH containing 0.5 g/L sodium acetate
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 3.60

OTHER SUBSTANCES
Simultaneous: chlorpromazine, trifluoperazine

REFERENCE
Pradas,T.N.V.; Sivakumar,M. HPLC quantification of a tricomponent psychiatric formulation containing chlorpromazine, trifluoperazine and trihexyphenidyl, *Pharmazie*, **1992**, *47*, 231–231.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Zorbax Rx C8
Mobile phase: MeCN:water 60:40, pH 5.5
Flow rate: 1.0
Detector: UV 257

REFERENCE
Dayan,N.; Touitou,E. Transdermal delivery of trihexyphenidyl HCl from a novel vesicular carrier (Abstract 2302), *Pharm.Res.*, **1997**, *14*, S318.

SAMPLE
Matrix: solutions

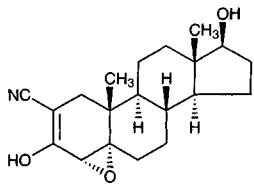
HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sul-

fadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Trilostane



Molecular formula: $C_{20}H_{27}NO_3$
Molecular weight: 329.44
CAS Registry No.: 13647-35-3
Merck Index: 9827
Lednicer No.: 2 158

SAMPLE
Matrix: blood
Sample preparation: 100 µL Plasma + 200 µL 200 mM pH 4.3 acetate buffer, vortex for 1 min, add 10 mL 2.5 ng/mL ethisterone in diethyl ether, mix on a rotary shaker, centrifuge at 10000 g for 10 min. Remove the organic layer and add it to 1-2 g anhydrous magnesium sulfate, mix on a rotary shaker, centrifuge, evaporate the organic layer to dryness under reduced pressure at 35-40°, reconstitute the residue in 150 µL mobile phase, sonicate for 5 min, inject a 100 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.9 Spherisorb S5 ODS2
Mobile phase: Dioxane:pH 5.0 Sorenson's buffer 52:48 (Caution! Dioxane is a carcinogen!)
Flow rate: 1
Injection volume: 100
Detector: UV 255

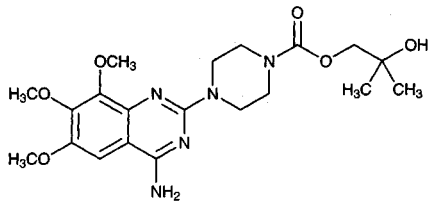
CHROMATOGRAM
Retention time: 5.6
Internal standard: ethisterone (12)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: ketotrilostane

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
McGee,J.P.; Palin,K.J.; Shaw,P.N.; Potter,C. High-performance liquid chromatographic analysis of trilostane and ketotrilostane in rat plasma, *J.Chromatogr.*, **1991**, *567*, 282–287.

Trimazosin



Molecular formula: $C_{20}H_{29}N_5O_6$
Molecular weight: 435.48
CAS Registry No.: 35795-16-5, 53746-46-6 (HCl monohydrate)
Merck Index: 9828
Lednicer No.: 2 382

SAMPLE

Matrix: blood
Sample preparation: Place 50 µL of a 100 µg/mL solution of doxazosin in MeOH into the bottom of a tube, evaporate to dryness under a stream of nitrogen at 37°, add 1 mL whole blood, mix thoroughly, add 5 mL diethyl ether, shake for 10 min, centrifuge at 2000 rpm for 5 min, freeze in acetone/dry ice. Remove the organic layer and add it to 100 µL 50 mM sulfuric acid, shake for 10 min, centrifuge at 2000 rpm for 5 min, inject a 20 µL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4 5 µm Spherisorb ODS
Mobile phase: MeOH:water 55:45 containing 10 mM pentane sodium sulfate and 9 mM tetramethylammonium chloride, adjusted to pH 3.4 with glacial acetic acid
Flow rate: 1.8
Injection volume: 20
Detector: F ex 254 em 400 (cut-off filter)

CHROMATOGRAM

Retention time: 5.5
Internal standard: doxazosin (9)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

whole blood; pharmacokinetics

REFERENCE

Hughes,M.A.; Meredith,P.A.; Elliott,H.L. The determination of trimazosin and its metabolite CP23445 in whole blood by high performance liquid chromatography using fluorescence detection, *J.Pharmacol.Methods*, **1984**, *12*, 29–34.

SAMPLE

Matrix: solutions

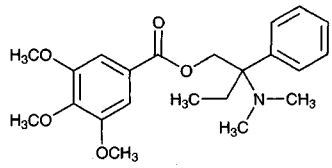
HPLC VARIABLES

Column: 125 × 5 5 µm Spherisorb C8
Mobile phase: MeCN:water 25:45 containing 5 mM dibutylamine
Flow rate: 2
Detector: F ex 346 em 340 (filter)

REFERENCE

Ferry,D.G.; Caplan,N.B.; Cubeddu,L.X. Interaction between antidepressants and α 1-adrenergic receptor antagonists on the binding to α 1-acid glycoprotein, *J.Pharm.Sci.*, **1986**, *75*, 146–149.

Trimebutine



Molecular formula: $C_{22}H_{29}NO_5$
Molecular weight: 387.48
CAS Registry No.: 39133-31-8, 34140-59-5 (maleate)
Merck Index: 9829

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM

Retention time: 14.588

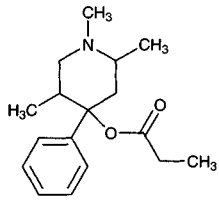
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Trimeperidine



Molecular formula: $C_{17}H_{25}NO_2$
Molecular weight: 275.39
CAS Registry No.: 64-39-1
Merck Index: 7968

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.8

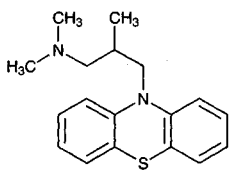
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Trimeprazine



Molecular formula: $C_{18}H_{22}N_2S$
Molecular weight: 298.45
CAS Registry No.: 84-96-8, 4330-99-8 (tartrate)
Merck Index: 9834
Lednicer No.: 1 378

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.8

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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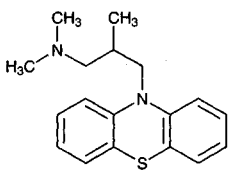
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Trimeprazine



Molecular formula: $C_{18}H_{22}N_2S$
Molecular weight: 298.45
CAS Registry No.: 84-96-8, 4330-99-8 (tartrate)
Merck Index: 9834
Lednicer No.: 1 378

SAMPLE
Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Homogenize tissue with 12 volumes water. 1 mL Whole blood, urine, bile, gastric contents, or tissue homogenate + 5 mL n-heptane:isoamyl alcohol 98.5:1.5 + 500 μL pH 8.5 saturated sodium carbonate buffer + 20 μL 100 μg/mL prochlorperazine in MeOH, agitate, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL MeCN, inject a 70 μL aliquot.

HPLC VARIABLES
Column: 250 × 3.2 5 μm Spherisorb CN
Mobile phase: MeCN:15 mM pH 6.5 sodium acetate buffer 95:5
Flow rate: 1
Injection volume: 70
Detector: UV 254

CHROMATOGRAM
Internal standard: prochlorperazine
Limit of detection: 20 ng/mL

KEY WORDS
whole blood; brain; liver; kidney; heart; muscle

REFERENCE
Kintz,P.; Berthault,F.; Tracqui,A.; Mangin,P. A fatal case of alimemazine poisoning, *J.Anal.Toxicol.*, **1995**, *19*, 591–594.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254 or E, Bioanalytical Systems LC4A, glassy carbon electrode +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.6
Limit of detection: 0.1 ng/mL (electrochemical)

OTHER SUBSTANCES
Extracted: amitriptyline, butaperazine, chlorpromazine, fluphenazine, promazine, promethazine, thioridazine, trifluoperazine
Simultaneous: acetophenazine, benztropine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, thiothixene, trihexyphenidyl
Interfering: carphenazine, triflupromazine

KEY WORDS
plasma; whole blood

REFERENCE
Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1-5 mL Plasma + 1 mL 1 M NaOH, extract with mixed hexanes for 30 min, centrifuge. Remove a 9 mL aliquot of the hexane layer and evaporate it to dryness under a stream of nitrogen at 30°, dissolve residue in 100 μL mobile phase, inject a 50 μL aliquot. Whole blood. 10 mL Whole blood + 1 mL 1 M NaOH, extract with 15 mL mixed hexanes for 1 h. Remove an aliquot of the hexane layer and evaporate it to dryness, reconstitute the residue in 1 mL 100 mM HCl, extract with 5 mL chloroform by vortexing for 1 min, centrifuge. Remove a 4.5 mL aliquot of the chloroform layer, evaporate to dryness, dissolve in 10 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 10 μm Micropak CN
Mobile phase: MeCN:5 mM ammonium acetate 90:10 (vary ammonium acetate concentration to achieve best separation)
Flow rate: 2.5
Injection volume: 10-50
Detector: UV 254 or E, Bioanalytical Systems LC-4A, glassy carbon electrode +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 17.0
Limit of detection: 0.1 ng/mL (E), 10 ng/mL (UV)

OTHER SUBSTANCES
Extracted: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, piperacetazine, orphenadrine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl

KEY WORDS
plasma; whole blood

REFERENCE
Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 1 mL 100 ng/mL prochlorperazine in water + 500 μL saturated sodium carbonate solution, vortex for 5 s, add 5 mL pentane:isopropanol 97:3, shake for 15 min, centrifuge at 1725 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness at 65°, reconstitute the residue in 200 μL MeCN, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:100 mM ammonium acetate buffer 90:10
Flow rate: 4
Injection volume: 100
Detector: E, Bioanalytical Systems, +0.9 V

CHROMATOGRAM
Retention time: 2.74
Internal standard: prochlorperazine (5.56)
Limit of detection: 0.125 ng/mL
Limit of quantitation: 0.25 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
McKay,G.; Cooper,J.K.; Midha,K.K.; Hall,K.; Hawes,E.M. Simple and sensitive high-performance liquid chromatographic procedure with electrochemical detection for the determination of plasma concentrations of trimeprazine following single oral doses, *J.Chromatogr.*, **1982**, *233*, 417–422.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Whole blood + 1 mL 1 M NaOH + 10 mL hexanes, extract for 30 min, repeat extraction. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, shake gently for 10 min or vortex for 1 min, centrifuge. Remove 4.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 10 μL mobile phase, inject an aliquot (J. Chromatogr. 1982, 231, 361).

HPLC VARIABLES
Column: 10 μm Micropak CN
Mobile phase: MeCN:100 mM ammonium acetate 90:10
Flow rate: 2
Detector: E, Bioanalytical Systems LC-4A, glassy carbon electrode +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4.4
Internal standard: imipramine (6.8)
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, butaperazine, chlorpromazine, fluphenazine, promazine, thioridazine, trifluoperazine
Simultaneous: acetophenazine, benztropine, carphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, thiothixene
Interfering: promethazine, triflupromazine, trihexyphenidyl

KEY WORDS
whole blood

REFERENCE
Hu,O.Y.; Gfeller,E.; Perrin,J.H.; Curry,S.H. Relative bioavailability of trimeprazine tablets investigated in man using HPLC with electrochemical detection, *J.Pharm.Pharmacol.*, **1986**, *38*, 172–176.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood + 5 mL chloroform:isopropanol:n-heptane 60:14:26 + 1.5 mL saturated ammonium chloride solution (pH 9.5), agitate horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:THF:10 mM pH 2.6 KH_2PO_4 65:5:30
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 5.87
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
whole blood

REFERENCE
Kintz,P.; Berthault,F.; Tracqui,A.; Mangin,P. A fatal case of alimemazine poisoning, *J.Anal.Toxicol.*, **1995**, *19*, 591–594.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 255

CHROMATOGRAM
Retention time: 8.35
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil;

lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 7.53

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimethoprim

KEY WORDS

plasma; serum; column-switching

REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 253.5

CHROMATOGRAM
Retention time: 15.257

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 2.6
Internal standard: promazine (5.2)

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, amitriptyline, amoxapine, amphetamine, buprion, chlordiazepoxide, chlorimipramine, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, disopyramide, doxepin, fluphenazine, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, iprindole, maprotiline, meperidine, methadone, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, perphenazine, phentermine, procainamide, prochlorperazine, prolixin, promethazine, propoxyphene, protriptyline, pyrilamine, quinidine, thioridazine, trifluoperazine, triflupromazine, trimipramine, zimeldine
Noninterfering: thiopropazine
Interfering: loxepin, mianserin

KEY WORDS
normal phase

REFERENCE
Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.07

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 5.8

OTHER SUBSTANCES
Simultaneous: amitriptyline, atropine, butalbital, chlorpromazine, desipramine, desmethylmaprotiline, doxepin, imipramine, maprotiline, methadone, norpropoxyphene, nortriptyline, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, protriptyline, trimipramine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil
Interfering: N-propionylprocainamide, quinidine, trifluoperazine

REFERENCE
Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a 20 mg/mL solution. Collect eluted enantiomers by switching column effluent onto one of two 100 × 4.6 25-40 μm Lichroprep RP18 columns. Wash these columns with 2.5 mL water, wash with 5 mL MeCN:water 10:90, elute with MeCN: 0.14% trifluoroacetic acid in water 70:30, evaporate eluate to give the enantiomers as their trifluoroacetate salts.)

HPLC VARIABLES
Column: 100 × 4 5 μm SGE-100GLC4-C8-30/5 octylsilica (Scientific Glass Engineering)
Mobile phase: MeCN:buffer 10:90 containing 9 g/L β-cyclodextrin (Buffer was 0.8% triethylamine adjusted to pH 4 with glacial acetic acid.)
Injection volume: 50
Detector: UV

CHROMATOGRAM
Retention time: 8.5, 10 (enantiomers)

KEY WORDS
chiral; preparative

REFERENCE

Cooper,A.D.; Jefferies,T.M. On-line recovery of trimeprazine enantiomers following chiral separation by reversed-phase high-performance liquid chromatography using a β-cyclodextrin-containing mobile phase, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 847–851.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 14.91 (A), 7.07 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions
Sample preparation: Make up a 500 ng/mL solution in mobile phase, inject an aliquot.

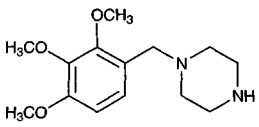
HPLC VARIABLES
Column: 250 × 4.6 5 μm Sumchiral OA-4700 (S)-tert-leucine-(R)-1-(α-naphthyl)ethylamine (YMC)
Mobile phase: Hexane:1,2-dichloroethane:EtOH:trifluoroacetic acid 800:150:100:1
Flow rate: 1
Injection volume: 100
Detector: F ex 254 em 280 (filter)

CHROMATOGRAM
Retention time: 5.73, 6.26 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ponder,G.W.; Butram,S.L.; Adams,A.G.; Ramanathan,C.S.; Stewart,J.T. Resolution of promethazine, ethopropazine, trimeprazine and trimipramine enantiomers on selected chiral stationary phases using high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *692*, 173–182.

Trimetazidine



Molecular formula: $C_{14}H_{22}N_2O_3$
Molecular weight: 266.34
CAS Registry No.: 5011-34-7, 13171-25-0 (2.HCl)
Merck Index: 9835

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

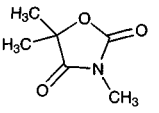
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 6.06

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Trimethadione



Molecular formula: $C_6H_9NO_3$
Molecular weight: 143.14
CAS Registry No.: 127-48-0
Merck Index: 9836
Lednicer No.: 1 232

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL α-methyl-α-propylsuccinimide in MeOH, shake for 2 min, centrifuge at 1500 g for 5 min, inject a 10-20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Shodex ODSpak F-411A
Mobile phase: MeCN:water:PIC-B5 (low UV) 15:85:3.5
Injection volume: 10-20
Detector: UV 200

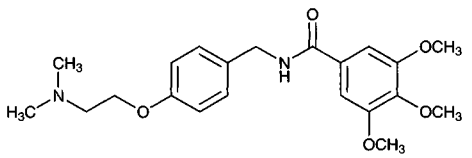
CHROMATOGRAM
Retention time: 5.5
Internal standard: α-methyl-α-propylsuccinimide (12.7)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetazolamide, carbamazepine, pentobarbital, phenobarbital, phenytoin, primidone

KEY WORDS
serum; rat; human; pharmacokinetics

REFERENCE
Tanaka,E.; Hagino,S.; Yoshida,T.; Kuroiwa,Y. Simultaneous determination of trimethadione and its metabolite in rat and human serum by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *308*, 393–397.

Trimethobenzamide



Molecular formula: $C_{21}H_{28}N_2O_5$
Molecular weight: 388.46
CAS Registry No.: 138-56-7, 554-92-7 (HCl)
Merck Index: 9839
Lednicer No.: 1 110

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum or plasma + 100 μL 10 μg/mL trimethobenzamide in MeOH + 1 mL buffer + 10 mL n-butyl chloride:isopropanol 95:5, shake for 10 min, centrifuge at 500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL chloroform and 200 μL 25 mM HCl, vortex for 10 s, centrifuge at 500 g for 3-5 min, inject a 50-60 μL aliquot of the upper aqueous layer. (Buffer was 630 mL of a solution containing 1 M boric acid and 1 M KCl + 370 mL 1 M sodium carbonate, adjust pH to 9.0.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyanopropyldimethylsilyl (PCN) (Supelco)
Mobile phase: MeCN:0.06% phosphoric acid 15:85 containing 0.01% octylamine (After analysis wash out system with MeCN:water 20:80.)
Flow rate: 2
Injection volume: 50-60
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: trimethobenzamide

OTHER SUBSTANCES
Extracted: encainide
Simultaneous: dipyridamole, oxazepam
Noninterfering: amiodarone, caffeine, chloral hydrate, chlordiazepoxide, diazepam, ethosuximide, flecainide, lidocaine, methadone, mexiletine, nicotine, phenobarbital, phenytoin, primidone, procainamide, propranolol, quinidine, tocainide, tricyclic antidepressants

KEY WORDS
plasma; serum; trimethobenzamide is IS

REFERENCE
Dasgupta,A.; Rosenzweig,I.B.; Turgeon,J.; Raisys,V.A. Encainide and metabolites analysis in serum or plasma using a reversed-phase high-performance liquid chromatographic technique, *J.Chromatogr.*, **1990**, *526*, 260–265.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.9

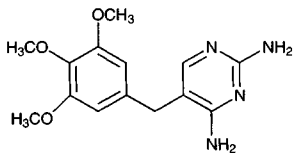
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine,

methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Trimethoprim



Molecular formula: $C_{14}H_{18}N_4O_3$
Molecular weight: 290.32
CAS Registry No.: 738-70-5
Merck Index: 9840
Lednicer No.: 1 262

SAMPLE

Matrix: blood
Sample preparation: Add 1.5 mL MeCN to 500 µL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 µL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 µm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 µm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
Mobile phase: MeOH:20 mM pH 4 phosphate buffer 38:62
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 245, F ex 270 em 389

CHROMATOGRAM

Retention time: 1.9

OTHER SUBSTANCES

Extracted: triamterene

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 75 μL acetone:10% trichloroacetic acid 1:2, vortex for 5 s, centrifuge for 4 min. Remove 62.5 μL of the supernatant and add it to 62.5 μL 50 mM KH_2PO_4, add 250 μL diethyl ether, vortex for 10 s, centrifuge for 5 min, filter (0.45 μm) the lower aqueous layer, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 75 × 4.6 TSK gel ODS-80TM (Tosoh)
Mobile phase: MeCN:50 mM pH 6.0 KH_2PO_4 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 30
Internal standard: trimethoprim

OTHER SUBSTANCES
Extracted: vancomycin

KEY WORDS
serum; trimethoprim is IS

REFERENCE
Morishige,H.; Shuto,H.; Ieiri,I.; Otsubo,K.; Oishi,R. Instability of standard calibrators may be involved in overestimating vancomycin concentrations determined by fluorescence polarization immunoassay, *Ther.Drug Monit.*, **1996**, *18*, 80–85.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 8.282

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 31

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

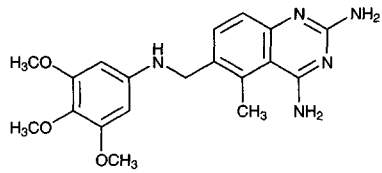
CHROMATOGRAM
Retention time: 27.5

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

Trimetrexate



Molecular formula: $C_{19}H_{23}N_5O_3$
Molecular weight: 369.42
CAS Registry No.: 52128-35-5, 82952-64-5 (glucuronate)
Merck Index: 9851
Lednicer No.: 4 149

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C2 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of water, do not allow to go dry. 1 mL Plasma + 160 ng IS, vortex, add to the SPE cartridge at 0.5 mL/min, wash with two 1 mL portions of water, dry under vacuum for 1 min, wash with 250 μL MeCN, dry under vacuum for 20 s, elute with 500 μL MeOH:water 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 400 μL mobile phase, vortex for 30 s, centrifuge at 1800 g for 15 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 7 μm Zorbax TMS
Column: 250 × 4.6 5 μm Zorbax TMS
Mobile phase: MeCN:buffer 23:77 (Buffer was 50 mM $(NH_4)H_2PO_4$ containing 0.8% triethylamine and 0.2% phosphoric acid, pH 4.5.)
Column temperature: 45
Flow rate: 1.2
Injection volume: 100
Detector: UV 241

CHROMATOGRAM

Retention time: 9.4
Internal standard: N6-phenylmethyl-2,4,6-quinazolinetriamine (12.0)
Limit of quantitation: 2.4 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites, cisplatin, cytarabine, doxorubicin, etoposide, 5-fluorouracil, folinic acid (leucovorin), lomustine, methotrexate, morphine, prednisolone, ribavirin, sulfamethoxazole, 6-thioguanine, trimethoprim, vinblastine, vincristine, zidovudine
Interfering: chlorambucil, melphalan

KEY WORDS

SPE; plasma; pharmacokinetics

REFERENCE

Bullen,W.W.; Chang,T.; Whitfield,L.R. High-performance liquid chromatographic assay for trimetrexate in human plasma, *J.Chromatogr.*, **1990**, *526*, 266–272.

SAMPLE

Matrix: blood, feces, urine
Sample preparation: Plasma. Condition a SPICE ODS SPE cartridge (Analtech) with 7.5 mL MeOH and 7.5 mL buffer. 1 mL Plasma + 1 mL buffer, add to SPE cartridge, wash with two 2.5 mL portions of buffer, dry under vacuum, elute with two 2.5 mL portions of MeOH:triethylamine 99:1. Evaporate the eluate to dryness under reduced pressure, reconstitute with 1 mL mobile phase, inject a 200 μL aliquot. Feces. Condition a SPICE ODS SPE cartridge (Analtech) with 7.5 mL MeOH and 7.5 mL water. Add dithiothreitol to feces (final concentration 1 mM), add an equal volume of water, add to SPE cartridge, wash with two 2.5 mL portions of MeOH:water 12.5:87.5, dry under vacuum, elute with two 2.5 mL portions of MeOH:triethylamine 99:1. Evaporate the eluate to dryness under reduced pressure, reconstitute with 1 mL mobile phase, add dithiothreitol (final concentration 1 mM), inject a 200 μL aliquot. Urine. Dilute 1:10 to 1:100 with 15 μM IS in mobile phase, inject an aliquot. (Buffer was 1.5% acetic acid adjusted to pH 5.5 with ammonium hydroxide.)

HPLC VARIABLES
Guard column: 5 μm ODS (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:50 mM NaH_2PO_4:acetic acid 17:82.2:0.8 adjusted to pH 5.5 with triethylamine
Column temperature: 20
Flow rate: 1
Injection volume: 200
Detector: E, Bioanalytical Systems LC-4A, 0.6 V or UV 254

CHROMATOGRAM
Retention time: 40
Internal standard: N,N-diethyl-5,7-dimethoxytryptamine hydrogen oxalate (35)
Limit of quantitation: 20 nM (feces), 50 nM (plasma, urine)

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Lin,J.T.; Cashmore,A.R.; Baker,M.; Dreyer,R.N.; Ernstoff,M.; Marsh,J.C.; Bertino,J.R.; Whitfield,L.R.; Delap,R.; Grillo-Lopez,A. Phase I studies with trimetrexate: clinical pharmacology, analytical methodology, and pharmacokinetics, *Cancer Res.*, **1987**, *47*, 609–616.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 500 mg C18 Bond Elut SPE cartridge with three column volumes of MeOH and three column volumes of water. Plasma, serum. Centrifuge plasma for 3-5 min. Add 1 mL serum or plasma to the SPE cartridge, wash with 6 mL water, wash with 1.5 mL MeCN, wash with 500 μL water, elute with 1 mL MeOH:80 mM sodium citrate 95:5. Filter (0.45 μm) the eluate, inject a 10 μL aliquot of the filtrate. Urine. Add 1 mL urine to the SPE cartridge, wash with 6 mL water, wash with 1 mL MeCN, wash with 1 mL MeOH:20 mM pH 4.5 sodium acetate 25:75, elute with 1.25 mL MeOH:80 mM sodium citrate 95:5. Filter (0.45 μm) the eluate, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 30 × 4.6 Spherisorb RP-18
Column: 300 × 3.9 10 μ μBondapak C18
Mobile phase: MeCN:Buffer 40:60 (Buffer was water containing 0.02% phosphoric acid and 0.08% triethylamine.)
Flow rate: 2
Injection volume: 10
Detector: UV 241

CHROMATOGRAM
Retention time: 4.5
Limit of quantitation: 50 ng/mL (urine), 20 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; serum; SPE; human; mouse

REFERENCE
Ackerly,C.C.; Hartshorn,J.; Tong,W.P.; McCormack,J.J. A rapid and sensitive method for determination of trimetrexate from biological fluids, *J.Liq.Chromatogr.*, **1985**, *8*, 125–134.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 500 mg C18 Bond Elut SPE cartridge with three column volumes of MeOH and three column volumes of water. Plasma, serum. Centrifuge plasma for

3-5 min. Add 1 mL serum or plasma to the SPE cartridge, wash with 6 mL water, wash with 1.5 mL MeCN, wash with 500 μL water, elute with 1 mL MeOH:80 mM sodium citrate 95:5. Filter (0.45 μm) the eluate, inject a 10 μL aliquot of the filtrate. Urine. Add 1 mL urine to the SPE cartridge, wash with 6 mL water, wash with 1 mL MeCN, wash with 1 mL MeOH:20 mM pH 4.5 sodium acetate 25:75, elute with 1.25 mL MeOH:80 mM sodium citrate 95:5. Filter (0.45 μm) the eluate, inject a 10 μL aliquot of the filtrate (J.Liq.Chromatogr. 1985, 8, 125).

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Aquapore C18
Column: 100 × 4.6 5 μm Spherisorb ODS
Mobile phase: Gradient. MeCN:buffer from 15:85 to 40:60 over 10 min, re-equilibrate at initial conditions for 10 min. (Buffer was 0.08% triethylamine containing 0.04% phosphoric acid.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 241

CHROMATOGRAM
Retention time: 7.6
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; serum; SPE

REFERENCE
Hudes,G.R.; LaCreta,F.; DeLap,R.J.; Grillo-Lopez,A.J.; Catalano,R.; Comis,R.L. Phase I clinical and pharmacologic trial of trimetrexate in combination with 5-fluorouracil, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 117–122.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injection 1:5 with MeOH:water 50:50, inject a 2 μL aliquot.

HPLC VARIABLES
Column: 100 × 2 5 μm MOS C8 (Hewlett-Packard)
Mobile phase: MeCN:MeOH:50 mM $(NH_4)H_2PO_4$ 18:12:70
Flow rate: 0.5
Injection volume: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 2.03

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; water

REFERENCE
Stetson,P.L.; Shukla,U.A.; Ensminger,W.D. Stability of trimetrexate, a new non-classical antifolate, in infusion solutions, *J.Chromatogr.*, **1989**, *464*, 163–171.

SAMPLE
Matrix: urine
Sample preparation: Condition a 1 mL 100 mg Bond Elut CN SPE cartridge with 3 mL MeCN and 3 mL water. 1 mL Urine + 100 μL 20 μg/mL trimethoprim in water + 1 mL water, add to the SPE cartridge, wash with 3 mL water, elute with 1 mL MeCN:water 15:85 containing 0.75% triethylamine and 0.375% phosphoric acid (85%), inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Aquapore C18 (Brownlee)

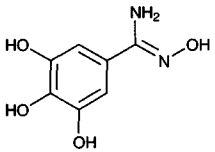
Column: 100 × 4.6 5 μm Hypersil ODS C18
Mobile phase: Gradient. MeCN:buffer from 9:91 to 35:65, re-equilibrate at initial conditions for 5 min. Buffer was 0.16% triethylamine containing 0.08% orthophosphoric acid (85%), pH 4.2.)
Flow rate: 1.5
Injection volume: 200
Detector: UV 241

CHROMATOGRAM
Retention time: 11.0
Internal standard: trimethoprim (5.8)
Limit of quantitation: 100 ng/mL

KEY WORDS
SPE

REFERENCE
Tinsley,P.W.; LaCreta,F.P. Improved chromatographic method for the determination of trimetrexate in urine, *J.Chromatogr.*, **1990**, *529*, 468–472.

Trimidox



Molecular formula: $C_7H_8N_2O_4$
Molecular weight: 184.15

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 0.1 mM solution in 10 mM KH_2PO_4, adjust pH to 6 with a few drops 5 M KOH or phosphoric acid, inject a 20 μL aliquot.

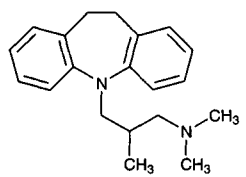
HPLC VARIABLES
Column: 150 × 4.6 3 μm Supelcosil LC18
Mobile phase: MeOH:buffer 5:95 (Buffer was 0.05% triethylamine adjusted to pH 6 with 50 mM phosphoric acid.)
Flow rate: 0.5
Injection volume: 20
Detector: UV 255

OTHER SUBSTANCES
Simultaneous: amidox, didox

KEY WORDS
comparison with DC polarography and UV spectrophotometry

REFERENCE
Romanova,D.; Vachalkova,A.; Szekeres,T.; Elford,H.L.; Novotny,L. The new inhibitors of ribonucleotide reductase -comparison of some physico-chemical properties, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 951–956.

Trimipramine



Molecular formula: $C_{20}H_{26}N_2$
Molecular weight: 294.44
CAS Registry No.: 739-71-9, 521-78-8 (maleate)
Merck Index: 9852

SAMPLE
Matrix: blood
Sample preparation: Condition a 15 mg 3 mL PLUS.MPI (Ansys, USA) SPE disc with 200 μL MeOH and 200 μL 100 mM pH 6.0 potassium phosphate monobasic, do not allow to dry. Mix 1 mL serum with 30 μL 10 μg/mL IS in water, add 1 mL 100 mM pH 6.0 potassium phosphate monobasic buffer, mix well. Add the sample to the SPE disc, wash with 500 μL 1 M acetic acid, wash with 500 μL MeOH, dry under vacuum for 5 min. Elute with two 300 μL portions of MeCN:triethylamine 100:2. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 800 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 1 opti-guard column RP C8
Column: 250 × 4.6 10 μm Chiralcel OD-R (Optimize Technologies, USA)
Mobile phase: MeCN:300 mM aqueous sodium perchlorate 42:58
Flow rate: 0.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 20.2 (R), 22.9 (S)
Internal standard: diphenhydramine (13.8)
Limit of detection: 10 ng/mL
Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE; chiral

REFERENCE
Liu,J.; Stewart,J.T. Quantitation of trimipramine enantiomers in human serum by enantioselective high-performance liquid chromatography and mixed-mode disc solid-phase extraction, *J.Chromatogr.B*, **1997**, *700*, 175–182.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 800 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 μL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: μBondapak/Porasil
Column: μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Internal standard: clomipramine
Limit of detection: 3 ng

KEY WORDS
plasma

REFERENCE
Wong,S.H.; Stolarun,S.L. Liquid-chromatographic analysis of trimipramine in plasma, *Clin.Chem.*, **1981**, *27*, 1101.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 μL 10 μg/mL protriptyline in water + 200 μL 80 g/L $NaHCO_3$ + 5 mL hexane, vortex for 15 s, centrifuge for 5 min. Remove the hexane layer and evaporate it in a stream of nitrogen at 60°. Reconstitute in 100 μL mobile phase, vortex for 15 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak CN
Mobile phase: MeCN:MeOH:5 mM phosphate buffer 60:15:25, adjusted to pH 7.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 2.33
Internal standard: protriptyline (12.20)
Limit of detection: 6 ng/mL

OTHER SUBSTANCES
Extracted: imipramine, doxepin, amitriptyline, desmethyldoxepin, nortriptyline, desipramine, chlorpromazine, procainamide, thioridazine, propranolol, propoxyphene, disopyramide, maprotiline
Noninterfering: caffeine, theophylline, salicylic acid, chlordiazepoxide, methaqualone, diazepam, acetaminophen
Interfering: trifluoperazine

KEY WORDS
serum

REFERENCE
Koteel,P.; Mullins,R.E.; Gadsden,R.H. Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum, *Clin.Chem.*, **1982**, *28*, 462–466.

SAMPLE
Matrix: blood
Sample preparation: Evaporate 200 μL 1 μg/mL clomipramine in MeOH into a tube, add 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:50:5
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.10
Internal standard: clomipramine (13)
Limit of detection: 4-5 ng/mL

OTHER SUBSTANCES
Extracted: imipramine, desipramine
Noninterfering: norclobazam, triazolam, monodesmethyltrimipramine, flunitrazepam, alimemazine, alprazolam, amineptine, caffeine, carbamazepine, citalopram, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, indalpine, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine

Interfering: amitriptyline, clobazam, levomepromazine

KEY WORDS
plasma

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500 μL Serum + 50 μL 1 μg/mL N-propionylprocainamide in 2.5 mM HCl, add to SPE cartridge, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with 1 volume MeOH:2.5 mM HCl 10:90. Add 200 μL 10 mM acetic acid and 5 mM diethylamine in MeOH to column, let stand 1 min, elute under vacuum, repeat, evaporate eluents to dryness under nitrogen at room temperature, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 6.9
Internal standard: N-propionylprocainamide (6)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, doxepin, imipramine, nortriptyline, protriptyline
Simultaneous: atropine, butalbital, chlorpromazine, maprotiline, methadone, norpropoxyphene, phenylpropanolamine, prochlorperazine, promethazine, propranolol, quinidine, trifluoperazine, trimeprazine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil
Interfering: hydroxyamitriptyline, procainamide

KEY WORDS
serum; SPE

REFERENCE
Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE
Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES
Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 19
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, amoxapine, clomipramine, desipramine, doxepin, imipramine, maprotiline, nortriptyline

KEY WORDS
serum; column-switching; use gradient to determine metabolites

REFERENCE
Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 20 ng chlorpromazine + 10 mL hexane:isoamyl alcohol 98:2, vortex, shake for 15 min, centrifuge at 840 g at 0-2° for 15 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb nitrile
Mobile phase: MeCN:MeOH:buffer 1:1:1 (Buffer was K_2HPO_4 adjusted to pH 6.5 with orthophosphoric acid.)
Column temperature: 40
Flow rate: 0.8
Detector: E, ESA Coulochem Model 5100A, electrode 1 +0.3 V. electrode 2 +0.85 V

CHROMATOGRAM
Retention time: 15
Internal standard: chlorpromazine (17)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Gulaid,A.A.; Jahn,G.A.; Maslen,C.; Dennis,M.J. Simultaneous determination of trimipramine and its major metabolites by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *566*, 228–233.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL protriptyline in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL

MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 9.6
Internal standard: protriptyline (6.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, maprotiline, nortriptyline

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum or plasma + 200 μL 0.33 M NaOH, shake 5 s, add 7 mL n-hexane:iso-amyl alcohol 985:15, shake 20 min, centrifuge at 2100 g for 5 min. Remove organic phase and add 200 μL 0.1 M HCl to it, shake for 1 min, discard organic phase, inject 30 μL of aqueous phase.

HPLC VARIABLES
Guard column: 10 mm 10 μm Bischoff C18
Column: 125 × 4 5 μm Ecotube Nucleosil C8
Mobile phase: MeCN:water:diethylamine:PicB5 370:630:0.4:25 (PicB5 is water-MeOH-1-pentanesulfonic acid.)
Column temperature: 55
Flow rate: 1.7
Injection volume: 30
Detector: UV 230

CHROMATOGRAM
Retention time: 8.4
Internal standard: trimipramine
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: fluoxetine, norfluoxetine
Noninterfering: alprazolam, bromazepam, clorazepate, diazepam, flunitrazepam, lorazepam, oxazepam, triazolam, amitriptyline, clomipramine, desipramine, imipramine, fluvoxamine, nortriptyline

KEY WORDS
serum; plasma; trimipramine is IS

REFERENCE

el Maanni,A.; Combourieu,I.; Bonini,M.; Creppy,E.E. Fluoxetine, an antidepressant, and norfluoxetine, its metabolite, determined by HPLC with a C_8 column and ultraviolet detection, *Clin.Chem.*, **1993**, *39*, 1749–1750.

SAMPLE

Matrix: blood

Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 5.9
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: acetazolamide, amitriptyline

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood

Sample preparation: 990 μL Serum + 10 μL 14 μg/mL trimipramine in MeOH. Inject onto column A and elute with mobile phase A for 15 min then elute contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 10 μm Hypersil MOS C8; B 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:5778:235 (sic, perhaps 188:577:235 ?)
Detector: UV 214

CHROMATOGRAM
Internal standard: trimipramine

KEY WORDS
serum; trimipramine is IS; column-switching

REFERENCE
Rao,M.L.; Staberock,U.; Baumann,P.; Hiemke,C.; Deister,A.; Cuendet,C.; Amey,M.; Härtter,S.; Kraemer,M. Monitoring tricyclic antidepressant concentrations in serum by fluorescence polarization immunoassay compared with gas chromatography and HPLC, *Clin.Chem.*, **1994**, *40*, 929–933.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma or serum at 3000 g for 5 min, inject a 100 μL aliquot on to column A and elute to waste withy mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 3 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4 10 μm Hypersil CPS; B 20 × 4.6 5 μm Spherisorb CN + 250 × 4.6 5 μm Spherisorb CN
Mobile phase: A MeCN:water 5:95; B MeCN:MeOH:8 mM pH 6.2 phosphate buffer 58:19:23
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 15
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Noninterfering: biperiden, chlorprothixene, diazepam, flunitrazepam, fluvoxamine, haloperidol, lorazepam, maprotiline, moclobemide, paroxetine, perazine, risperidone

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Hermes,B.; Hiemke,C. Automated determination of trimipramine and N-desmethyltrimipramine in human plasma or serum by HPLC with on-line solid phase extraction, *J.Liq.Chromatogr.*, **1995**, *18*, 3495–3505.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 251

CHROMATOGRAM

Retention time: 9.78
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur--compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 22.0
Internal standard: cianopramine (8.93)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: promethazine, benztropine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL MeOH + 1 mL 200 mM pH 9.8 carbonate buffer + 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 150 μL mobile phase, inject a 75 μL aliquot. Urine. 1 mL Urine + 1 mL β-glucuronidase (7200 Fishman Units) in 20 mM pH 6.5 phosphate buffer, heat at 37° for 24 h, add 100 μL 10 M NaOH, add 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 C18 base-deactivated silica (BDS) (Keystone)
Column: 50 × 4.6 5 μm C18 base-deactivated silica (BDS) (Keystone)
Mobile phase: MeCN:water 90:10 containing 0.1% formic acid and 10 mM ammonium acetate
Flow rate: 1
Injection volume: 50-75
Detector: MS, PE Sciex API III, heated nebulized interface, corona discharge needle +4 μA, nebulizer probe 500°, nebulizing gas was air at 2 L/min and 80 psi, curtain gas flow was nitrogen at 0.9 L/min, sampling orifice +45 V, dwell time 400 ms, interface heater 60°, electron multiplier–3.7 kV, collision gas was argon 355 × 10exp12 atoms/cm^2, first quadrupole filter admits m/z 276 (cyclobenzaprine) and 295 (trimipramine, collisional fragmentation at second filter, monitor m/z 215 (cyclobenzaprine) and 208 (trimipramine) at third quadrupole filter

CHROMATOGRAM

Retention time: 2.2
Internal standard: trimipramine

OTHER SUBSTANCES

Extracted: cyclobenzaprine

KEY WORDS
plasma; trimipramine is IS

REFERENCE
Constanzer,M.; Chavez,C.; Matuszewski,B. Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine, *J.Chromatogr.B*, **1995**, *666*, 117–126.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL MeOH + 1 mL 200 mM pH 9.8 carbonate buffer + 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL mobile phase, inject a 150 μL aliquot. Urine. 1 mL Urine + 1 mL β-glucuronidase (7200 Fishman Units) in 20 mM pH 6.5 phosphate buffer, heat at 37° for 24 h, add 100 μL 10 M NaOH, add 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL mobile phase, inject a 150 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long C18 base-deactivated silica (BDS) (Keystone)
Column: 250 × 4.6 5 μm C18 base-deactivated silica (BDS) (Keystone)
Mobile phase: MeCN:buffer 50:50 (plasma) or 43:57 (urine) (Buffer was 0.085% phosphoric acid adjusted to pH 6.5 with triethylamine.)
Flow rate: 1
Injection volume: 150
Detector: UV 229

CHROMATOGRAM
Retention time: 10.5 (plasma), 12.8 (urine)
Internal standard: trimipramine

OTHER SUBSTANCES
Extracted: cyclobenzaprine

KEY WORDS
plasma; trimipramine is IS

REFERENCE
Constanzer,M.; Chavez,C.; Matuszewski,B. Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine, *J.Chromatogr.B*, **1995**, *666*, 117–126.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.943

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quin-

idine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4 5 μm CHIRAL-AGP (ChromTech)
Mobile phase: Isopropanol:59 mM pH 4.0 acetate buffer 1:99
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM

Retention time: k' 4.43, 7.32 (enantiomers)

KEY WORDS

chiral

REFERENCE

Hermansson,J.; Grahn,A. Optimization of the separation of enantiomers of basic drugs. Retention mechanisms and dynamic modification of the chiral bonding properties on an α_1-acid glycoprotein column, *J.Chromatogr.A*, **1995**, *694*, 57–69.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES

Column: 100 × 4.7 7 μm Hypercarb (Shandon)
Mobile phase: MeOH containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH
Column temperature: 17
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: k' 21 (first enantiomer)

KEY WORDS

chiral; α = 1.19

REFERENCE

Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonylglyclyl-L-proline as counter ion in methanol, *J.Chromatogr.A*, **1995**, *705*, 275–287.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 15.49 (A), 7.66 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, meronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Make up a 500 ng/mL solution in mobile phase, inject an aliquot.

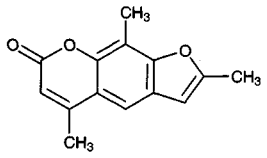
HPLC VARIABLES
Column: 250 × 4.6 5 μm Sumchiral OA-4700 (S)-tert-leucine-(R)-1-(α-naphthyl)ethylamine (YMC)
Mobile phase: Hexane:1,2-dichloroethane:EtOH:trifluoroacetic acid 1200:150:100:1
Flow rate: 1
Injection volume: 100
Detector: F ex 254 em 280 (filter)

CHROMATOGRAM
Retention time: 8.4, 9.3 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ponder,G.W.; Butram,S.L.; Adams,A.G.; Ramanathan,C.S.; Stewart,J.T. Resolution of promethazine, ethopropazine, trimeprazine and trimipramine enantiomers on selected chiral stationary phases using high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *692*, 173–182.

Trioxsalen



Molecular formula: $C_{14}H_{12}O_3$
Molecular weight: 228.25
CAS Registry No.: 3902-71-4
Merck Index: 9864
Lednicer No.: 1 334

SAMPLE
Matrix: aqueous humor, blood, tissue, vitreous humor
Sample preparation: Homogenize skin in 1 M pH 9.0 sodium borate buffer. 2 mL Whole blood, skin homogenate, aqueous humor, or vitreous humor + 5 mL 1 M pH 9.0 sodium borate buffer, mix, add 16 mL n-hexane:isopropanol 95:5, shake on a reciprocating shaker at 70-80 strokes/min for 30 min, centrifuge at 1020 g for 20 min. Remove 10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL EtOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 Micropak MCH-10 reverse-phase
Mobile phase: Gradient. MeCN:water (not otherwise specified)
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 16.5
Internal standard: 8-methoxypsoralen (8)
Limit of detection: 2 ng/mL

KEY WORDS
whole blood; guinea pig; skin; human

REFERENCE
Chakrabarti,S.G.; Grimes,P.E.; Minus,H.R.; Kenney,J.A.,Jr.; Pradhan,T.K. Determination of trimethylpsoralen in blood, ophthalmic fluids, and skin, *J.Invest.Dermatol.*, **1982**, *79*, 374–377.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + diazepam, extract with heptane:dichloromethane 80:20.

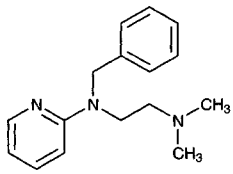
HPLC VARIABLES
Column: C18
Mobile phase: MeOH:water 70:30
Detector: UV 254 or F ex 360 em 430

CHROMATOGRAM
Limit of detection: 1 μg/mL (UV)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Stolk,L.; Siddiqui,A.H.; Cormane,R.H. Serum levels of trimethylpsoralen after oral administration, *Br.J.Dermatol.*, **1981**, *104*, 443–445.

Tripelennamine



Molecular formula: $C_{16}H_{21}N_3$
Molecular weight: 255.36
CAS Registry No.: 91-81-6, 6138-56-3 (citrate), 154-69-8 (HCl)
Merck Index: 9868
Lednicer No.: 1 51

SAMPLE
Matrix: blood, milk
Sample preparation: Centrifuge milk at 1200 g, remove the middle aqueous layer. 1 mL Plasma or milk + 50 μL MeOH:water 50:50 + 50 μL 4 μg/mL protriptyline in MeOH:water 50:50, mix, inject a 250 μL aliquot of this mixture on to column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 37-50 μm 10 × 1.5 Corasil RP C18; B 100 × 4 Techsphere 3CN (HPLC Technology)
Mobile phase: A water; B MeCN:50 mM pH 7.2 acetate buffer 70:30
Flow rate: A 0.8; B 0.9
Injection volume: 250
Detector: UV 246

CHROMATOGRAM
Retention time: 5.2
Internal standard: protriptyline (6.8)
Limit of detection: 2 ng/mL

KEY WORDS
column-switching; cow; plasma

REFERENCE
Dadgar,D.; Power,A. Applications of column-switching techniques in biopharmaceutical analysis. II. High-performance liquid chromatographic determination of tripelennamine in bovine plasma and milk, *J.Chromatogr.*, **1987**, *421*, 216–222.

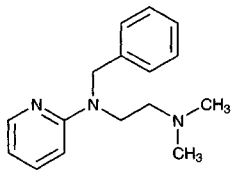
SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

KEY WORDS
serum; pharmacokinetics

REFERENCE
Stolk,L.; Siddiqui,A.H.; Cormane,R.H. Serum levels of trimethylpsoralen after oral administration, *Br.J.Dermatol.*, **1981**, *104*, 443–445.

Tripelennamine



Molecular formula: $C_{16}H_{21}N_3$
Molecular weight: 255.36
CAS Registry No.: 91-81-6, 6138-56-3 (citrate), 154-69-8 (HCl)
Merck Index: 9868
Lednicer No.: 1 51

SAMPLE
Matrix: blood, milk
Sample preparation: Centrifuge milk at 1200 g, remove the middle aqueous layer. 1 mL Plasma or milk + 50 μL MeOH:water 50:50 + 50 μL 4 μg/mL protriptyline in MeOH:water 50:50, mix, inject a 250 μL aliquot of this mixture on to column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 37-50 μm 10 × 1.5 Corasil RP C18; B 100 × 4 Techsphere 3CN (HPLC Technology)
Mobile phase: A water; B MeCN:50 mM pH 7.2 acetate buffer 70:30
Flow rate: A 0.8; B 0.9
Injection volume: 250
Detector: UV 246

CHROMATOGRAM
Retention time: 5.2
Internal standard: protriptyline (6.8)
Limit of detection: 2 ng/mL

KEY WORDS
column-switching; cow; plasma

REFERENCE
Dadgar,D.; Power,A. Applications of column-switching techniques in biopharmaceutical analysis. II. High-performance liquid chromatographic determination of tripelennamine in bovine plasma and milk, *J.Chromatogr.*, **1987**, *421*, 216–222.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.9

OTHER SUBSTANCES
Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrilamine, pyrimethamine

KEY WORDS
tablets; syrups; elixirs; injections

REFERENCE
Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: fungal incubations
Sample preparation: 40 mL Fungal incubation + 100 mL water, centrifuge, wash pellet with 50 mL MeOH. Combine the supernatant and the MeOH wash and extract three times with 150 mL portions of dichloromethane, filter the extracts through a plug of anhydrous sodium sulfate, evapporate the filtrate to dryness under reduced pressure at 50°, reconstitute with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 containing 20 mM trimethylamine, pH 7.0.)
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 17.9

OTHER SUBSTANCES
Extracted: metabolites, methapyrilene, thenyldiamine

REFERENCE
Cerniglia,C.E.; Hansen,E.B.,Jr.; Lambert,K.J.; Korfmacher,W.A.; Miller,D.W. Fungal transformations of antihistamines: metabolism of methapyrilene, thenyldiamine and tripelennamine to *N*-oxide and *N*-demethylated derivatives, *Xenobiotica*, **1988**, *18*, 301–312.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine,

buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, triprolidine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: thonzylamine, pheniramine, chlorpheniramine, brompheniramine, phenindamine, phenyltoxamine, clemizole

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REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM

Retention time: 7.3

OTHER SUBSTANCES

Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate
Interfering: tranylcypromine

KEY WORDS

details for purification of triethylamine in paper

REFERENCE

Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isox-

suprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac 201HS54 C18
Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min
Flow rate: 1.5
Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, triprolidine, cyclizine, methaphenilene, pyrrobutamine, meclizine, buclizine

REFERENCE

Vydac HPLC Catalog, 1994-5.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 7.71

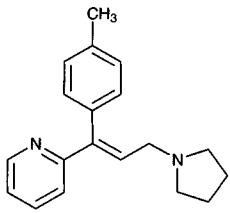
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Triprolidine



Molecular formula: $C_{19}H_{22}N_2$
Molecular weight: 278.40
CAS Registry No.: 468-12-4, 6138-79-0 (HCl monohydrate), 550-70-9 (HCl)
Merck Index: 9877
Lednicer No.: 1 78

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 250 µL 10% KOH + 5 mL ether, vortex, centrifuge. Remove ether layer and add it to 100 µL 0.5% phosphoric acid, vortex, centrifuge, remove most of ether layer and discard it, remove traces of ether by nitrogen at room temperature for 2-3 min, inject all of aqueous layer.

HPLC VARIABLES

Column: Waters CN reverse-phase radial compression
Mobile phase: MeCN:buffer 27:73 (Buffer was 75 mM pH 3.0 phosphate buffer containing 20 mM dibutylamine and 50 ng/mL triprolidine.)
Flow rate: 1
Injection volume: 100
Detector: UV 229

CHROMATOGRAM

Retention time: 3.6
Internal standard: triprolidine

OTHER SUBSTANCES

Simultaneous: hydroxyzine

KEY WORDS

serum; triprolidine is IS

REFERENCE

Simons,F.E.R.; Simons,K.J.; Frith,E.M. The pharmacokinetics and antihistaminic of the H_1 receptor antagonist hydroxyzine, *J.Allerg.Clin.Immunol.*, **1984**, *73*, 69–75.

SAMPLE

Matrix: blood
Sample preparation: Condition a 200 mg Bond Elut C8 SPE cartridge with 4 mL MeOH and 4 mL 100 mM pH 5 ammonium acetate. 1 mL Plasma + 1 mL 100 mM HCl, add to the SPE cartridge, wash with 2 mL MeOH:water 30:70, elute with 4 mL MeOH:1 M HCl in MeOH 97:3. Evaporate the eluate to dryness under a stream of nitrogen with gentle heating, reconstitute the residue in 200 µL mobile phase, filter (0.45 µm), inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm Spherisorb C18
Column: two 150 × 4.5 5 μm Econosphere C8 columns in series
Mobile phase: MeCN:buffer 22:78 (Buffer was 100 mM ammonium acetate containing 6 mM n-heptylamine, adjusted to pH 2.5 with perchloric acid).
Flow rate: 0.9
Detector: F ex 300 em 490

CHROMATOGRAM
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites (UV 290)

KEY WORDS
dog; plasma; SPE; pharmacokinetics

REFERENCE
McNulty,M.J.; Deal,D.L.; Page,T.L.; Chandrasurin,P.; Findlay,J.W.A. Disposition of triprolidine in the male beagle dog, *Drug Metab.Dispos.*, **1992**, *20*, 928–935.

SAMPLE
Matrix: blood
Sample preparation: 600 μL Serum + 100 μL 400 mM NaOH + 7 mL ethyl acetate, vortex for 1 min, centrifuge at 1000 g for 10 min. Remove the organic layer and add it to 150 μL 100 mM HCl, vortex for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb-C6
Mobile phase: MeCN:30 mM KH_2PO_4 45:55 containing 2 g sodium hexanesulfonate, pH adjusted to 2.7 with phosphoric acid
Column temperature: 30
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: triprolidine

OTHER SUBSTANCES
Extracted: clozapine

KEY WORDS
serum; triprolidine is IS

REFERENCE
Volpicelli,S.A.; Centorrino,F.; Puopolo,P.R.; Kando,J.; Frankenburg,F.R.; Baldessarini,R.J.; Flood,J.G. Determination of clozapine, norclozapine, and clozapine-N-oxide in serum by liquid chromatography, *Clin.Chem.*, **1993**, *39*, 1656–1659.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 5.34
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF
Sample preparation: Plasma. Centrifuge blood at 7000 rpm, decant 100 μL plasma. Mix 100 μL plasma with 200 μL acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot. CSF. Add 25 μL water to 25 μL CSF, mix with 50 μL acetone, centrifuge at 7000 rpm for 5 min, decant the supernatant, evaporate under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: Rainin C18

Mobile phase: MeOH:water 61.9:38.1 containing 0.2% diethylamine

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Chou,K.-J.; Donovan,M.D. Distribution of antihistamines into the CSF following intranasal delivery, *Biopharm.Drug Dispos.*, **1997**, *18*, 335–346.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 16.05
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Extracted: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.133

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: feces, urine
Sample preparation: Urine. Condition a 200 mg Bond Elut C8 SPE cartridge with 4 mL MeOH and 4 mL 100 mM pH 5 ammonium acetate. Dilute urine five-fold with 100 mM pH 5 ammonium acetate, add to the SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH:1 M HCl in MeOH 97:3. Evaporate the eluate to dryness under a stream of nitrogen with gentle heating, reconstitute the residue in 200 μL MeCN:100 mM ammonium acetate 5:95, vortex, sonicate, centrifuge, filter (0.45 μm), inject an aliquot. Feces. Lyophilize, grind to a powder, add 100-200 mg to 2 mL 2% ammonium hydroxide, heat at 37° for 30-60 min, add 8 mL MeOH, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen with gentle heating, reconstitute the residue in 300 μL MeOH:water 50:50, centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm Spherisorb C18
Column: two 150 × 4.5 5 μm Econosphere C8 columns in series
Mobile phase: Gradient. A was MeCN:buffer 10:90 (buffer was 100 mM ammonium acetate containing 10 mM n-heptylamine, adjusted to pH 2.5 with perchloric acid). B was MeCN:buffer 20:80 (buffer was 100 mM ammonium acetate containing 15 mM n-heptylamine, adjusted to pH 2.5 with perchloric acid). Urine: A:B 100:0 for 10 min, to 0:100 over 15 min. Feces: A:B 100:0 for 20 min, to 0:100 over 20 min.
Flow rate: 0.9
Detector: UV 290 or radioactivity

CHROMATOGRAM
Retention time: 36 (urine), 43 (feces)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
dog; SPE; pharmacokinetics

REFERENCE
McNulty,M.J.; Deal,D.L.; Page,T.L.; Chandrasurin,P.; Findlay,J.W.A. Disposition of triprolidine in the male beagle dog, *Drug Metab.Dispos.*, **1992**, *20*, 928–935.

SAMPLE
Matrix: fungal incubations
Sample preparation: Extract fungal incubation with dichloromethane, evaporate the extract to dryness, reconstitute with 5 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: cyano (Beckman/Altex)
Mobile phase: MeCN:buffer 35:65 (Buffer was 80 mM ammonium acetate containing 10 mM tripropylamine, adjusted to pH 6.5 with trifluoroacetic acid.)
Flow rate: 1.25
Injection volume: 20
Detector: MS, Delsi-Nermag R1010C quadrupole, Vestec thermospray interface, discharge-off, filament off, thermospray source block 268°, control temperature 101°, tip 217°, m/z 279

CHROMATOGRAM
Retention time: 19
Limit of detection: 10 μg

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Hansen,E.B.J.; Getek,T.A.; Korfmacher,W.A. Application of HPLC-thermospray ionization mass spectrometry for the analysis of triprolidine and its metabolite hydroxymethyltriprolidine in biological samples, *J.Anal.Toxicol.*, **1989**, *13*, 185–187.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metopro-

lol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 2.40

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 7.6 μg/mL solution, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: Supelguard LC-8-DB (Supelco)

Column: 50 × 4.6 Supelcosil LC-8-DB

Mobile phase: MeCN:buffer 10:90 containing 0.02% triethylamine (Buffer was KH_2PO_4 adjusted to pH 2.0 with phosphoric acid.)

Column temperature: 35

Flow rate: 2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Simultaneous: chlorpheniramine, methscopolamine, phenylpropanolamine, pseudoephedrine

REFERENCE
Supelco Catalog, **1992**, p. 179.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Supelguard (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH_2PO_4 containing 0.2% triethylamine, pH 2.5.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: chlorcyclizine, chlorpheniramine, clonidine, diphenhydramine, promethazine, pyrilamine

REFERENCE
Supelco Catalog, **1994**, p. 768.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, meth-

apyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac 201HS54 C18
Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min
Flow rate: 1.5
Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM

Retention time: 8.6

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, tripelennamine, cyclizine, methaphenilene, pyrrobutamine, meclizine, buclizine

REFERENCE

Vydac HPLC Catalog, 1994-5.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 12.13

OTHER SUBSTANCES

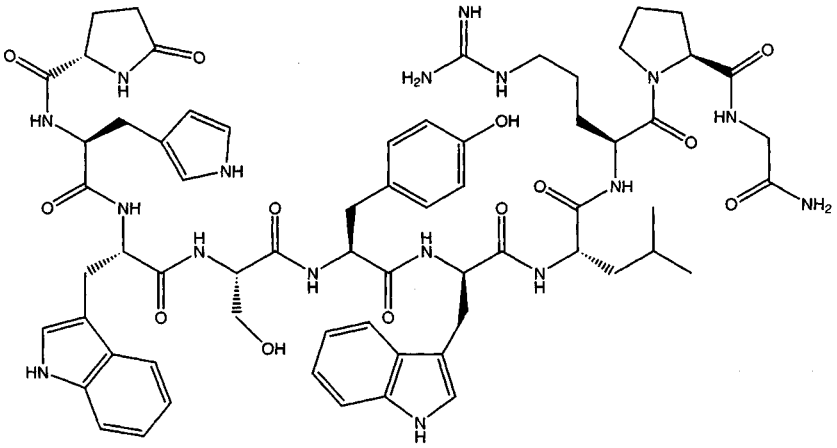
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Triptorelin

Molecular formula: $C_{64}H_{82}N_{18}O_{13}$
Molecular weight: 1311.47
CAS Registry No.: 57773-63-4, 140194-24-7 (acetate)
Merck Index: 9878



SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 4 5 μm Lichrosphere 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 22:78
Flow rate: 1
Detector: UV 214

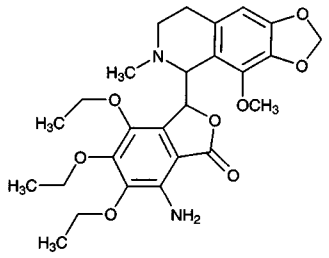
OTHER SUBSTANCES

Simultaneous: degradation products
Also analyzed: goserelin

REFERENCE

Hoitink,M.A.; Beijnen,J.H.; Boschma,M.U.S.; Bult,A.; Hop,E.; Nijholt,J.; Versluis,C.; Wiese,G.; Underberg,W.J.M. Identification of the degradation products of gonadorelin and three analogues in aqueous solution, *Anal.Chem.*, **1997**, *69*, 4972–4978.

Tritoqualine



Molecular formula: $C_{26}H_{32}N_2O_8$
Molecular weight: 500.55
CAS Registry No.: 14504-73-5
Merck Index: 9894

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM

Retention time: 20.968

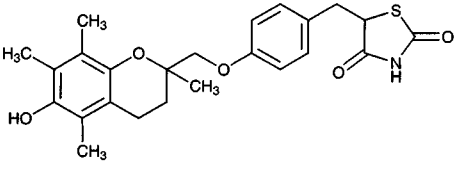
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Troglitazone



Molecular formula: $C_{24}H_{27}NO_5S$
Molecular weight: 441.55
CAS Registry No.: 97322-87-7
Merck Index: 9898

SAMPLE

Matrix: blood
Sample preparation: Add 100 µL 5 µg/mL IS and 500 µL PIC A (Waters) to 500 µL plasma add 5 mL ethyl acetate:hexane 90:10. Shake for 20 min, centrifuge at 1000 g for 5 min. Evap-

orate the organic layer to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue in 500 μL MeCN:water 50:50. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 6.0 5 μm ODS (YMC, USA)
Mobile phase: MeCN:water:phosphoric acid 60:40:0.08
Flow rate: 1.2
Injection volume: 50
Detector: UV 230

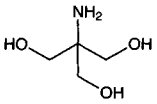
CHROMATOGRAM
Retention time: 23.2
Internal standard: 9-acetylanthracene (27.2)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Loi,C.-M.; Randinitis,E.J.; Vassos,A.B.; Kazierad,D.J.; Koup,J.R.; Sedman,A.J. Lack of effect of type II diabetes on the pharmacokinetics of troglitazone in a multiple-dose study, *J.Clin.Pharmacol.*, **1997**, *37*, 1114–1120.

Tromethamine



Molecular formula: $C_4H_{11}NO_3$
Molecular weight: 121.14
CAS Registry No.: 77-86-1
Merck Index: 9902

SAMPLE
Matrix: amniotic fluid, blood, CSF, urine
Sample preparation: Plasma. Condition a 100 mg Bond Elut SCX (propylbenzenesulfonic acid, H^+ form) SPE cartridge with 1 mL 50 mM HCl, 1 mL MeOH, 2 mL water, and 1 mL 50 mM HCl. 100 μL Plasma + 100 μL 250 μM norleucine in 100 mM HCl + 10 mg solid sulfosalicylic acid + 800 μL acetone or MeOH, mix, centrifuge, add a 50 μL aliquot to the SPE cartridge, wash with 2 mL water, elute with two 500 μL portions of MeOH:water:triethylamine 40:40:20, dry the eluate under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Dried blood. Add 25 μL 250 μM norleucine in 100 mM HCl to a 6 mm filter paper disc containing dried blood, add 100 μL MeCN, let stand for 30 min, centrifuge, remove a 75 μL aliquot of the supernatant, evaporate to dryness under reduced pressure, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 2 min, evaporate to dryness under vacuum, reconstitute with 50 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Amniotic fluid, CSF. Mix amniotic fluid or CSF with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 50 (CSF) or 100 (amniotic fluid) μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Urine. Dilute urine with water to a creatinine concentration of 1 mM, mix an aliquot with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a

50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 Pico-Tag amino acid column (Waters)
Mobile phase: Gradient. A was MeCN:70 mM pH 6.55 sodium acetate 2.5:97.5. B was MeCN:MeOH:water 45:15:40. A:B 100:0 for 13.5 min, to 97:3 (step gradient), to 94:6 over 10.5 min (Waters curve 8 (slightly concave)), to 91:9 over 6 min (Waters curve 5 (slightly convex)), to 66:34 over 20 min, maintain at 66:34 for 12 min, to 0:100 over 0.5 min, maintain at 0:100 for 4 min, return to initial conditions over 0.5 min.
Column temperature: 46
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 34.47
Internal standard: norleucine (55.07)

OTHER SUBSTANCES
Extracted: α-alanine, alanine, alloisoleucine, β-aminoadipic acid, 4-aminobenzoic acid, gamma-aminobutyric acid, β-amino-n-butyric acid, gamma-amino-n-butyric acid, 4-aminohippuric acid, β-aminoisobutyric acid, 4-aminophenylacetic acid, α-aminophenylacetic acid, 3-amino-3-phenylpropionic acid, δ-amino-n-valeric acid, ammonia, anserine, arginine, asparagine, aspartic acid, aspartylglucosamine, carnosine, citrulline, cystathionine, cysteic acid, cysteine, cysteine-homocysteine (mixed disulfide), cystine, ethanolamine, ethionine, ethylamine, galactosamine, glucosamine, glutamic acid, glutamine, glutathionine (oxidized), glycine, glycylglycine, glycylhistidine, glycylleucine, glycylphenylalanine, glycyltyrosine, histidine, homoarginine, homocitrulline, homoserine, homocystine, 3-hydroxyanthranilic acid, 3-hydroxykynurenine, hydroxyproline, isoleucine, kynurenine, leucine, levodopa, lysine, methionine sulfone, methionine, 3-methylhistidine, 1-methylhistidine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, sarcosine, serine, serotonin, taurine, threonine, tryptophan, tyrosine, valine
Noninterfering: cadaverine, 2-phenylethylamine

KEY WORDS
derivatization; SPE; ultrafiltrate; plasma; dried blood

REFERENCE
Davey,J.F.; Ersser,R.S. Amino acid analysis of physiological fluids by high-performance liquid chromatography with phenylisothiocyanate derivatization and comparison with ion-exchange chromatography, *J.Chromatogr.*, **1990**, *528*, 9–23.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 50 μL 5.027 mg/mL 2,3-butanediol in water + 1 mL 4 M NaOH + 200 μL benzoyl chloride, rotate for 10 min, add 2 drops 13.1 mg/mL sodium glycine salt in water, let stand for 2-3 min, add 8 mL hexane, rotate for 5 min, centrifuge. Remove the upper organic layer and evaporate it to dryness, reconstitute the residue in 300 μL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Column: reverse-phase 10 C ODS
Mobile phase: MeCN:MeOH water 25:50:25
Flow rate: 2
Injection volume: 50
Detector: UV 237

CHROMATOGRAM
Retention time: 4

Internal standard: 2,3-butanediol (3)
Limit of detection: 20 μg/mL

KEY WORDS
serum; derivatization

REFERENCE
Blanke,S.R.; Blanke,R.V. The Schotten-Baumann reaction as an aid to the analysis of polar compounds: application to the determination of tris(hydroxymethyl)aminomethane (THAM), *J.Anal.Toxicol.*, **1984**, *8*, 231–233.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 50 μL 750 μg/mL 2,3-butanediol in water + 200 μL 4 M NaOH, vortex briefly, add 40 μL benzoyl chloride, vortex for 3 min, add 8 mL MTBE:MeOH 99:1, vortex for 3 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness in a vortex evaporator at 55° for 30 min, cool to room temperature, reconstitute the residue in 500 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Ultrasphere octyl
Mobile phase: Gradient. MeCN:25 mM pH 6.5 potassium phosphate buffer 40:60 45:55 for 10 min, to 73:27 over 7 min (concave gradient), return to initial conditions over 1 min.
Flow rate: 3
Injection volume: 10
Detector: UV 237

CHROMATOGRAM
Retention time: 15.0
Internal standard: 2,3-butanediol (7.1)
Limit of detection: 282 ng/mL

KEY WORDS
plasma; derivatization

REFERENCE
Gumbhir,K.; Mason,W.D. High-performance liquid chromatographic method for the determination of tris(hydroxymethyl)aminomethane (tromethamine) in human plasma, *J.Chromatogr.*, **1992**, *583*, 99–104.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 100 μL 400 μg/mL 2-amino-2-ethyl-1,3-propanediol + 100 μL 7% perchloric acid, vortex, centrifuge at 2000 g for 5 min. Remove a 200 μL aliquot of the supernatant and add it to 300 μL 200 mM pH 9.2 borate buffer, vortex briefly, add 40 μL 4 mg/mL 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole in MeCN, vortex briefly, heat at 80° for 30 min, cool to room temperature in a water bath for 10 min, add 500 μL 5 M NaOH, vortex for 10 s, add 100 μL benzoyl chloride, vortex for 1 min, add 2 mL ethyl acetate:MeOH 90:10, rotate for 10 min, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under vacuum for 1 h, reconstitute the residue in 1 mL MeCN:10 mM phosphoric acid 80:20, inject a 50 μL aliquot. Urine. 200 μL Urine + 100 μL 200 μg/mL 2-amino-2-ethyl-1,3-propanediol + 100 μL water + 100 μL 10 mM pH 8.5 borate buffer + 200 μL 4 mg/mL 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole in MeCN, vortex briefly, heat at 80° for 30 min, cool to room temperature in a water bath for 10 min, add 500 μL 5 M NaOH, vortex for 10 s, add 100 μL benzoyl chloride, vortex for 1 min, add 2 mL ethyl acetate:MeOH 90:10, rotate for 10 min, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under vacuum for 1 h, reconstitute the residue in 1 mL MeCN:10 mM phosphoric acid 80:20, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelcosil octadecylsilane silica column
Column: 250 × 4.6 Supelcosil octadecylsilane
Mobile phase: MeCN:10 mM phosphoric acid 70:30, adjusted to pH 2.5 with 10 M KOH

Flow rate: 1
Injection volume: 50
Detector: F ex 460 em 520

CHROMATOGRAM
Retention time: 12
Internal standard: 2-amino-2-ethyl-1,3-propanediol (10)
Limit of quantitation: 5 μg/mL (urine), 1 μg/mL (plasma)

KEY WORDS
plasma; derivatization

REFERENCE
Morris,M.J.; Hsieh,J.Y.-K. Determination of tris(hydroxymethyl)aminomethane (tromethamine) in human plasma and urine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1993**, *622*, 87–92.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation with water to obtain a tromethamine concentration of 260 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 IonPac CS5 (Dionex)
Mobile phase: 10 mM HCl (If necessary regenerate column with MeCN:water 5:95 at 1 mL/min for 30 min and with 1 M HCl at 1 mL/min for 1 h.)
Flow rate: 1.5
Injection volume: 20
Detector: Conductivity

CHROMATOGRAM
Retention time: 5.4

OTHER SUBSTANCES
Simultaneous: sodium
Noninterfering: lodoxamide

REFERENCE
Hall,R.E.; Havner,G.D.; Good,R.; Dunn,D.L. Ion chromatographic method for rapid and quantitative determination of tromethamine, *J.Chromatogr.A*, **1995**, *718*, 305–308.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 20 × 4 5 μm Supelcosil LC-18
Column: 250 × 4 5 μm Supelcosil LC-18
Mobile phase: 5 mM pH 6.0 acetate buffer
Flow rate: 1.2
Detector: UV 270

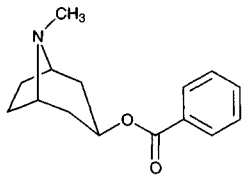
CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: guanine, xanthine

KEY WORDS
tromethamine is from Tris buffer

REFERENCE
Canepari,S.; Castellano,P.; Cernia,E.; Girelli,A.M.; Bozza,A. Determination of guanase activity in normal and pathological sera by high-performance liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 130–134.

Tropacocaine



Molecular formula: $C_{15}H_{19}NO_2$
Molecular weight: 245.32
CAS Registry No.: 537-26-8
Merck Index: 9904

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

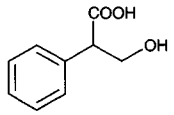
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine,

quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Tropic acid



Molecular formula: $C_9H_{10}O_3$
Molecular weight: 166.18
CAS Registry No.: 529-64-6
Merck Index: 9910

SAMPLE

Matrix: bulk
Sample preparation: Add a sample to 60 mg soy lecithin, make up to 50 mL with 0.1 mM HCl, sonicate, filter through a 0.2 μm nylon filter, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: Gradient. A was MeCN:buffer 20:80. B was MeCN:buffer 45:55. A:B 100:0 for 5 min, from 100:0 to 0:100 in 3 min, maintain at 0:100 for 4 min, from 0:100 to 100:0 in 1 min, maintain at 100:0 for 6 min. (Buffer was 100 mM KH_2PO_4 prepared by dissolving 27.2 g KH_2PO_4 in water, adjusting pH to 4.0 with 85% phosphoric acid and making up to 2 L with water.)
Column temperature: 35
Flow rate: 1 for 12 min, 2 for 7 min
Injection volume: 20
Detector: UV 210

CHROMATOGRAM

Retention time: 5.96

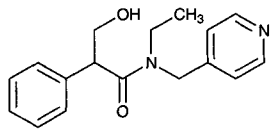
OTHER SUBSTANCES

Simultaneous: ipratropium bromide, N-isopropyl-nor-atropine, 8-s-ipratropium bromide, apoipratropium bromide

REFERENCE

Simms,P.J.; Towne,R.W.; Gross,C.S.; Miller,R.E. The separation of ipratropium bromide and its related compounds, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 841–849.

Tropicamide



Molecular formula: $C_{17}H_{20}N_2O_2$
Molecular weight: 284.36
CAS Registry No.: 1508-75-4
Merck Index: 9911

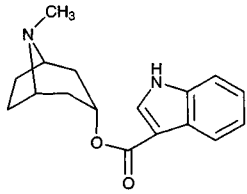
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3022 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 254

KEY WORDS
chiral; α = 1.10 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Tropisetron



Molecular formula: $C_{17}H_{20}N_2O_2$
Molecular weight: 284.36
CAS Registry No.: 89565-68-4
Merck Index: 9914

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma and 500 µL 50 mM NaOH, extract with diethyl ether. Remove the organic layer and add it to 200 µL 20 mM HCl, extract. Remove the aqueous layer and add it to 50 µL 1.5% triethylamine, inject a 150 µL aliquot.

HPLC VARIABLES
Column: Spherisorb RP8
Mobile phase: MeCN:0.3% triethylamine 65:35
Injection volume: 150
Detector: UV 283

CHROMATOGRAM
Limit of quantitation: 0.3 ng/mL

KEY WORDS
plasma

REFERENCE
Fischer,V.; Baldeck,J.-P.; Tse,F.L.S. Pharmacokinetics and metabolism of the 5-hydroxytryptamine antagonist tropisetron after single oral doses in humans, *Drug Metab.Dispos.*, **1992**, *20*, 603–607.

SAMPLE
Matrix: feces
Sample preparation: Exhaustively extract feces with MeOH, inject an aliquot of the extract.

HPLC VARIABLES
Column: 250 × 7 7 µm Lichrosorb RP18
Mobile phase: Gradient. A was 0.01% triethylamine adjusted to pH 8.4 with 330 mM phosphoric acid. B was MeCN. A:B 100:0 for 2 min, to 92:8 over 1 min, to 80:20 over 27 min, to 0:100 over 20 min.

Detector: UV 280 or radioactivity

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics

REFERENCE
Fischer,V.; Baldeck,J.-P.; Tse,F.L.S. Pharmacokinetics and metabolism of the 5-hydroxytryptamine antagonist tropisetron after single oral doses in humans, *Drug Metab.Dispos.*, **1992**, *20*, 603–607.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4 Nucleosil C18
Mobile phase: MeOH:THF:buffer 30:5:65 (Buffer was 100 mM triethylamine adjusted to pH 3.0 with nitric acid.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 10.10

OTHER SUBSTANCES
Simultaneous: granisetron, ondansetron

REFERENCE
Barbato,F.; Immacolata La Rotonda,M.; Quaglia,F. Retention behaviour of anti-emetic serotonin antagonists in reversed phase high performance liquid chromatography, *Farmaco*, **1995**, *50*, 875–880.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine at 3000 rpm for 15 min, inject an aliquot

HPLC VARIABLES
Column: 250 × 7 7 μm Lichrosorb RP18
Mobile phase: Gradient. MeCN:0.3% triethylamine (?) from 5:95 to 15:85 over 30 min, to 20:80 over 10 min, to 90:10 over 20 min
Flow rate: 3.1
Detector: UV 280 or radioactivity

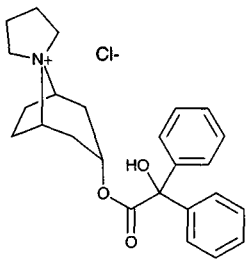
CHROMATOGRAM
Retention time: 60

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics

REFERENCE
Fischer,V.; Baldeck,J.-P.; Tse,F.L.S. Pharmacokinetics and metabolism of the 5-hydroxytryptamine antagonist tropisetron after single oral doses in humans, *Drug Metab.Dispos.*, **1992**, *20*, 603–607.

Trospium chloride



Molecular formula: $C_{25}H_{30}ClNO_3$
Molecular weight: 427.97
CAS Registry No.: 10405-02-4
Merck Index: 9918

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 5 mL Plasma + 1 mL 1 M NaOH, heat at 140-145° for 105 min, cool to 40°, add 200 μL 25% HCl, mix, centrifuge at 5000 g for 10 min, heat at 120° for 10 min, cool, centrifuge. Remove a 5 mL aliquot and add it to 500 μL reagent 1, mix. Remove a 5 mL aliquot and add it to 4.8 mL chloroform, shake vigorously for 3 min, centrifuge at 6000 g for 30 min. Remove a 4 mL aliquot of the organic phase and add it to 2.3 mL 100 mM HCl, shake vigorously, centrifuge at 5000 g for 10 min. Remove a 2 mL aliquot of the aqueous layer and add it to 2 mL MeOH, evaporate to dryness under reduced pressure, add MeOH, evaporate to dryness under a stream of nitrogen at 80-90°, repeat process several times with 10-11 mL MeOH total, add 200 μL 10 mg/mL benoxaprofen chloride in dry MeCN, heat at 140-145° for 30 min, evaporate to dryness under reduced pressure, add 1 mL ethyl acetate, add 1.2 mL water, shake mechanically for 5 min, centrifuge at 5000 g for 10 min, discard the organic layer, wash the aqueous phase again with 1 mL ethyl acetate. Evaporate the aqueous phase to dryness under reduced pressure, reconstitute with 100 μL MeCN:water 31:69, inject a 20 μL aliquot. Urine. 5 mL Urine + 1 mL 1 M NaOH, heat at 140-145° for 90 min, cool, add 200 μL 25% HCl, add 600 μL reagent 2, shake, centrifuge at 5000 g for 10 min. Remove a 5 mL aliquot and add it to 4.8 mL chloroform, shake vigorously for 3 min, centrifuge at 6000 g for 30 min. Remove a 4 mL aliquot of the organic phase and add it to 2.3 mL 100 mM HCl, shake vigorously, centrifuge at 5000 g for 10 min. Remove a 2 mL aliquot of the aqueous layer and add it to 2 mL MeOH, evaporate to dryness under reduced pressure, add MeOH, evaporate to dryness under a stream of nitrogen at 80-90°, repeat process several times with 10-11 mL MeOH total, add 200 μL 10 mg/mL benoxaprofen chloride in dry MeCN, heat at 140-145° for 30 min, evaporate to dryness under reduced pressure, add 1 mL ethyl acetate, add 1.2 mL water, shake mechanically for 5 min, centrifuge at 5000 g for 10 min, discard the organic layer, wash the aqueous phase again with 1 mL ethyl acetate. Evaporate the aqueous phase to dryness under reduced pressure, reconstitute with 100 μL MeCN:water 40:60, inject a 20 μL aliquot. (Trospium chloride is hydrolyzed to nortropane-8-spiro-1'-pyrrolidinium chloride (which is also a metabolite) and this compound is derivatized with benoxaprofen chloride. Reagent 1 was a mixture of 98.7 mg dipicrylamine containing 50% water, 10 mL 100 mM NaOH, and 600 mg anhydrous sodium carbonate. Reagent 2 was a mixture of 32.9 mg dipicrylamine containing 50% water and 10 mL 100 mM NaOH. Prepare dipicrylamine as follows (Caution! Dipicrylamine is potentially explosive and highly toxic, store moistened with 50% water!). Add 50 g 2,4-dinitrodiphenylamine to 420 g nitric acid (36° Bé., 52%, d 1.33) heated to 62° over 2 h, heat at 62-90° for another 3 h, cool, filter, wash the product until it is free of acid, dry to obtain 2,2',4,4'-tetranitrodiphenylamine as a yellow solid (mp 187.4°). Add 50 g tetranitrodiphenylamine over 1 h to 500 g of a mixture of equal parts 92% sulfuric acid and 93% nitric acid at room temperature, after 4.5 h add to a large volume of ice water, filter, recrystallize the product from acetone to obtain dipicrylamine (2,2',4,4',6,6'-hexanitrodiphenylamine) as yellow crystals (mp 242.9°) (J.Am.Chem.Soc. 1919, 41, 1013). Prepare benoxaprofen chloride as follows. Dissolve 600 mg benoxaprofen in 50 mL dry toluene, slowly add 5 mL thionyl chloride (freshly distilled from linseed oil), reflux for 30 min, evaporate to dryness, recrystallize from dichloromethane (if necessary) to give benoxaprofen chloride (mp 91.5°) (J. Chromatogr. 1984, 310, 167).)

HPLC VARIABLES

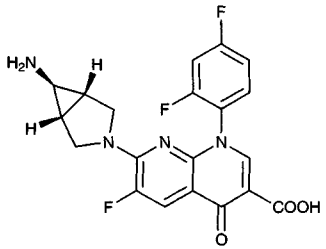
Column: 125 × 4.6 5 μm Nucleosil C8 (plasma) or 125 × 4.6 5 μm LiChrosorb RP-8 (urine)
Mobile phase: MeCN:water 31:69 (plasma) or 40:60 (urine) containing 80 mM NaCl, 31 mM choline chloride, and 10 mL/L 1 M HCl
Column temperature: 55 (plasma), 50 (urine)
Flow rate: 2
Injection volume: 20
Detector: F ex 313 em 370

CHROMATOGRAM
Retention time: 7.5 (plasma), 6 (urine)
Limit of quantitation: 0.5-1 ng/mL (plasma), 3 ng/mL (urine)

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Schladitz-Keil,G.; Spahn,H.; Mutschler,E. Fluorimetric determination of the quaternary compound trospium and its metabolite in biological material after derivatization with benoxaprofen chloride, *J.Chromatogr.*, **1985**, *345*, 99–110.

Trovafloxacin



Molecular formula: $C_{20}H_{15}F_3N_4O_3$
Molecular weight: 416.36
CAS Registry No.: 147059-72-1, 146961-34-4 (HCl), 147059-75-4 (monomethanesulfonate)
Merck Index: 9919

SAMPLE
Matrix: plasma
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 22:78 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 274

CHROMATOGRAM
Retention time: 8.57
Internal standard: fleroxacin (3.93)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

Trypsin

Molecular weight: 24000
CAS Registry No.: 9002-07-7
Merck Index: 9926

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak CN
Mobile phase: Gradient. MeCN containing 0.07% trifluoroacetic acid:0.1% trifluoroacetic acid in water from 0:100 to 60:40 over 30 min
Flow rate: 2
Detector: UV 206

CHROMATOGRAM
Retention time: 23.8

KEY WORDS
partial separation of α and β forms

REFERENCE
Titani,K.; Sasagawa,T.; Resing,K.; Walsh,K.A. A simple and rapid purification of commercial trypsin and chymotrypsin by reverse-phase high-performance liquid chromatography, *Anal.Biochem.*, **1982**, *123*, 408–412.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in 1 mM HCl, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 6 Asahipak GS-520-AHA-ABA (Prepare by suspending 10 g Asahipak-GS gel (Asahi Chemical Industry) in water, sonicate for 5 min, wash with 200 mL water, wash with 200 mL dioxane, suspend in 100 mL dioxane, add 3.24 g 1,1'-carbonyldiimidazole, stir gently for 15 min at room temperature, wash with 200 mL dioxane, suspend in 200 mL 1 M sodium bicarbonate containing 1 M 6-aminohexanoic acid, shake at 4° for 25 h, wash with 200 mL water, wash with 100 mL 1 M NaCl, wash with 200 mL water. Suspend 2 g of the gel in 15 mL 200 mM pH 4.752-(morpholino)ethanesulfonic acid/NaOH buffer, add 288 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide monohydrochloride, stir gently for 30 min, add 28.3 mg p-aminobenzamide monohydrochloride, adjust pH three times to 4.75 with 1 M HCl or 1 M NaOH at 30 min intervals, shake gently at room temperature for 24 h, wash with 150 mL water, wash with 100 mL 50 mM NaOH containing 1 M NaCl, wash with 100 mL 50 mM HCl containing 1 M NaCl, wash with water until washings are neutral. Caution! Dioxane is a carcinogen. It may be possible to use acetone instead.)
Mobile phase: Gradient. Buffer A, after 15 min buffer B. Buffer A was 50 mM pH 7.4 sodium phosphate buffer containing 100 mM NaCl. Buffer B was 50 mM pH 7.4 sodium phosphate buffer containing 100 mM NaCl and 20 mM pH 7.4 6-aminohexanoic acid.
Flow rate: 1
Injection volume: 20
Detector: F ex 285 em 340

CHROMATOGRAM
Retention time: 25

KEY WORDS
cow

REFERENCE
Ito,N.; Noguchi,K.; Shimura,K.; Kasai,K.-I. High-performance affinity chromatography of trypsins on Asahipak GS-gel coupled with *p*-aminobenzamidine, *J.Chromatogr.*, **1985**, *333*, 107–114.

SAMPLE
Matrix: solutions

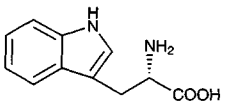
HPLC VARIABLES
Column: 150 × 4.1 10 μm PRP-3 (Hamilton)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in 50 mM NaOH. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 40:60 over 30 min.
Flow rate: 2
Detector: UV 220

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: cytochrome C, insulin, lysozyme, myoglobin, ribonuclease A

REFERENCE
Rainin Catalog 1991-2, p. 3.33, p. 3.33.

Tryptophan



Molecular formula: $C_{11}H_{12}N_2O_2$
Molecular weight: 204.23
CAS Registry No.: 73-22-3
Merck Index: 9929

SAMPLE
Matrix: solutions

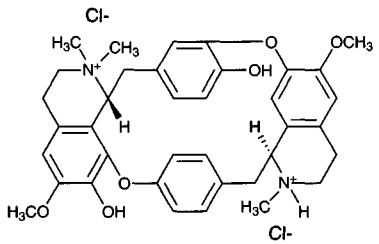
HPLC VARIABLES
Column: C18
Mobile phase: MeOH:MeCN:2.5 nM pH 3.0 ammonium dihydrogen phosphate buffer 17.5:17.5:65
Detector: UV 278

CHROMATOGRAM
Retention time: 4.2
Internal standard: tryptophan

KEY WORDS
tryptophan is IS

REFERENCE
Duggirala,S.M.; Mitra,A.K. Intravitreal pharmacokinetics of anti CMV agents ganciclovir and cidofovir -a comparison (Abstract 1119), *Pharm.Res.*, **1997**, *14*, S39.

Tubocurarine chloride



Molecular formula: $C_{37}H_{42}Cl_2N_2O_6$
Molecular weight: 681.66
CAS Registry No.: 57-94-3, 6989-98-6 (pentahydrate)
Merck Index: 9939

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 2 column volumes of THF, 2 volumes of MeOH, and 2 volumes of water. 1 mL Plasma + 100 µL 10 µg/mL metocurine iodide in 10 mM HCl, add to the SPE cartridge, wash with 2 volumes of water, elute with 250 µL mobile phase. Evaporate the eluate and reconstitute with 100 µL mobile phase, vortex, centrifuge at 12800 g for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm CN Guard-Pak (Waters)
Column: 10 μm Radial-Pak CN (Waters)
Mobile phase: MeCN:MeOH:water:1 M pH 2.5 dibutylamine phosphate 40:10:10:1
Flow rate: 2.4
Detector: UV 204

CHROMATOGRAM
Retention time: 5.9
Internal standard: metocurine iodide (13.2)
Limit of quantitation: 25 ng/mL

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Avram,M.J.; Shanks,C.A. Determination of D-tubocurarine chloride or metocurine iodide in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1984**, *306*, 398–404.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL water + 50 μL 10% sodium tungstate:335 mM sulfuric acid 50:50, vortex for 15 s, centrifuge at 12800 g for 2 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 50 mm long C18
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:7.5 mM tetrabutylammonium hydrogen sulfate 10:90
Flow rate: 1.8
Injection volume: 30
Detector: UV 229

CHROMATOGRAM
Retention time: 10.0
Internal standard: d-tubocurarine chloride

OTHER SUBSTANCES
Extracted: gallamine
Noninterfering: barbiturates, alcuronium, metocurine, neostigmine, edrophonium

KEY WORDS
serum; rat; tubocurarine is IS

REFERENCE
Ramzan,I.M. Determination of the neuromuscular blocking drug gallamine in rat serum using high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *417*, 428–433.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 250 μL picric acid (1:50 dilution of saturated picric acid solution) + 250 μL metocurine solution + 250 μL water + 2.5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 μL MeCN: water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 μPorasil
Mobile phase: MeCN:2 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100

Detector: UV 210

CHROMATOGRAM
Retention time: 3.7
Internal standard: metocurine (5.3)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Also analyzed: atracurium, alcuronium

KEY WORDS
plasma

REFERENCE
Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Blood + 1 mL 1 M pH 2.5 KH_2PO_4 + 1 mL 3% perchloric acid + 12 mL dichloromethane, rotate for 20 min, centrifuge at 1520 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 25 mm long CN guard column
Column: 250 × 4.6 Spherisorb S5 CN
Mobile phase: MeCN:100 mM pH 5 phosphate 50:50
Column temperature: 40
Flow rate: 1.5
Detector: UV 214 or E, BAS LC-4B, LC-17A thin-layer flow cell, working glassy carbon electrode (W1) +0.65 V, quantitating glassy carbon electrode (W2) +1.05 V, Ag/AgCl reference electrode and auxiliary electrode

CHROMATOGRAM
Internal standard: d-tubocurarine

OTHER SUBSTANCES
Extracted: vecuronium
Noninterfering: atropine, apresoline, haloperidol, fentanyl, labetalol, thiopental, atracurium, diazepam

KEY WORDS
tubocurarine is IS

REFERENCE
Hu,O.Y.; Chou,C.H.; Ho,W.; Ho,S.T. Determination of vecuronium in blood by HPLC with UV and electrochemical detection: a pilot study in man, *Proc.Natl.Sci.Counc.Repub.China.[B].*, **1991**, *15*, 186–190.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut CBA cation-exchange SPE cartridge with 3 mL MeOH, 3 mL water, 1 mL 50 mM pH 9.0 borate. Add 250 μL plasma to SPE cartridge, wash with 3 mL water, wash with 1 mL 50 mM pH 3.0 NaH_2PO_4, wash with 1 mL water, wash with two 500 μL portions of MeOH, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL MeOH:MeCN:water 30:15:55 adjusted to pH 3.4 with 1 M phosphoric acid, inject a 70 μL aliquot. (Acidified MeOH was 833 μL HCl in 100 mL MeOH.)

HPLC VARIABLES
Column: 100 × 4.9 5 μm octyl Spherisorb
Mobile phase: MeOH:MeCN:buffer 30:15:55 adjusted to pH 3.4 with 1 M phosphoric acid (Buffer was 10 mM sodium octanesulfonate and 1.5 mM dibutylamine.)
Flow rate: 2.5
Injection volume: 70
Detector: UV 272

CHROMATOGRAM
Retention time: 5
Internal standard: D-tubocurarine

OTHER SUBSTANCES
Extracted: bretylium

KEY WORDS
plasma; SPE; human; pig; tubocurarine is IS

REFERENCE
Théorêt,Y.; Varin,F. Simple, rapid and selective method using high-performance liquid chromatography for the determination of bretylium in plasma, *J.Chromatogr.*, **1992**, *575*, 162–166.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 100 mg Bond Elut SPE cartridge with 2 mL water and 1 mL MeOH. Add 1 mL plasma or 10 mL urine to the SPE cartridge, wash with 2 mL water, wash with 1 mL MeCN:water 50:50, elute with 300 μL MeCN:buffer 50:50, inject an aliquot. (Buffer was 12 g NaH_2PO_4 and 1.2 mL concentrated phosphoric acid in 100 mL water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Waters)
Mobile phase: MeOH:buffer 80:20 (Buffer was 1.44 g sodium lauryl sulfate and 2.5 mL glacial acetic acid in 1 L water.)
Flow rate: 1.4
Detector: UV 280

CHROMATOGRAM
Retention time: 7.2
Internal standard: d-tubocurarine

OTHER SUBSTANCES
Extracted: alcuronium

KEY WORDS
SPE; tubocurarine is IS; plasma

REFERENCE
deBros,F.; Okutani,R.; Inada,E.; Lawrence,K. Determination of alcuronium in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 449–454.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg silica gel SPE cartridge (Analytichem) with 2 mL ethyl acetate, 2 mL MeOH, and 2 mL water. Lyophilize 40 mL urine, reconstitute with 10 mL 100 mM pH 5.0 acetate buffer, centrifuge at 5000 g for 15 min. If desired, incubate a 3 mL aliquot with 10 μL of a solution containing 500 U/μL glucuronidase and 26.4 U/μL sulfatase (Sigma), heat at 37° overnight, freeze. Lyophilize 3 mL aliquots of hydrolyzed or unhydrolyzed solutions, reconstitute with 2 mL water, add 10 μL 2.8 mg/mL D-chondocurarine in water. Remove a 200 μL aliquot and add it to the SPE cartridge, wash with 2 mL water, wash with 500 μL MeOH, elute with two 1 mL portions of mobile phase, inject an aliquot of the eluate.

Alternatively add 10 μL 60 ng/mL D-chondocurarine in water to 1 mL urine, add this mixture to the SPE cartridge and proceed as above.

HPLC VARIABLES

Guard column: 20 × 4.6 10 μm C18 (Rainin)
Column: 150 × 4.6 5 μm ODS-IP (Beckman)
Mobile phase: MeOH:water:dibutylamine phosphate solution 19.8:79.1:0.1, adjust pH to 2.5 with 1 M NaOH (Prepare dibutylamine phosphate solution by cautiously adding 32 g dibutylamine to 250 mL concentrated orthophosphoric acid.)
Flow rate: 1.5
Injection volume: 200
Detector: UV 204

CHROMATOGRAM

Retention time: 7.5
Internal standard: D-chondocurarine (10)
Limit of detection: 15 ng/mL

KEY WORDS

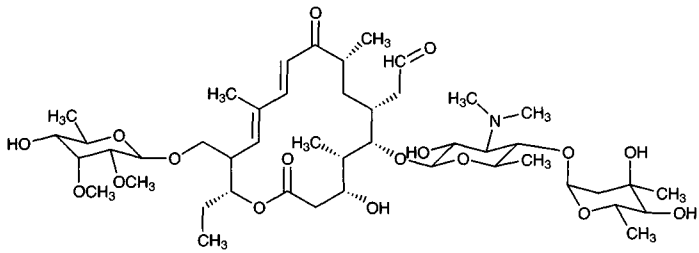
SPE

REFERENCE

Annan,R.S.; Kim,C.; Martyn,J. Measurement of D-tubocurarine chloride in human urine using solid-phase extraction and reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1990**, *526*, 228–234.

Tylosin

Molecular formula: $C_{46}H_{77}NO_{17}$
Molecular weight: 916.11
CAS Registry No.: 1401-69-0
Merck Index: 9963



SAMPLE

Matrix: blood, tissue
Sample preparation: Blend 25 g tissue with 3 volumes of water (muscle) or 200 mM pH 2.2 phosphate buffer (liver, kidney). Add 32 (tissue) or 30 (serum) mL MeCN slowly with vigorous swirling to 8 mL tissue homogenate or 10 mL serum, let stand for 1 min, decant through a glass wool plug. 20 mL Filtrate + 20 mL water + 30 mL dichloromethane, shake vigorously, repeat extraction. Combine the organic layers and evaporate them to near dryness under reduced pressure at 40-50°, reconstitute the residue in two 3 mL portions of MeOH and transfer to a small tube. Evaporate to dryness under reduced pressure, reconstitute with 1 mL MeCN and 1 drop of water, add 3 mL petroleum ether (bp 30-60°), vortex for 10 s, discard petroleum ether layer, repeat wash, remove traces of petroleum ether under reduced pressure, adjust volume to 0.2-1 mL with MeCN, inject an aliquot.

HPLC VARIABLES

Column: 300 × 4.6 10 μm Micropak MCH-10-N-Cap C18
Mobile phase: MeCN:MeOH:5 mM $(NH_4)H_2PO_4$ 60:30:10, 65:30:5, or 70:25:5 or MeCN:MeOH:4 mM $(NH_4)H_2PO_4$ 72:20:8 (tissue) or MeCN:MeOH:2 mM $(NH_4)H_2PO_4$ 75:20:5 or 75:18:7 (serum)
Flow rate: 1.5-2
Injection volume: 200
Detector: UV 280

CHROMATOGRAM

Limit of detection: <100 ppb

KEY WORDS
muscle; liver; kidney; pig; serum

REFERENCE
Moats,W.A.; Harris,E.W.; Steele,N.C. Comparison of liquid chromatographic and bioassay procedures for determining depletion of intramuscularly injected tylosin, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 413–416.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 1 mg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 8 μm RoSil C8 (Bio-Rad)
Mobile phase: MeCN:water:200 mM tetrabutylammonium hydrogen sulfate:200000 mM phosphoric acid 20:67:8:5
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 0.05% (of tylosin A)

OTHER SUBSTANCES
Simultaneous: impurities, demycinosyltylosin

REFERENCE
Roets,E.; Beirinckx,P.; Quintens,I.; Hoogmartens,J. Quantitative analysis of tylosin by column liquid chromatography, *J.Chromatogr.*, **1993**, *630*, 159–166.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 1 mg/mL solution in 50 mM pH 7.0 potassium phosphate buffer, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 8 μm 1000 Å PLRP-S (Polymer Laboratories)
Mobile phase: THF:water:200 mM pH 9.0 potassium phosphate buffer 20:75:5
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 37

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
protect from light

REFERENCE
Paesen,J.; Crommen,J.; de Beer,J.; Shaohong,J.; Porqueras,E.; Van Overbeke,A.; Violon,C.; Hoogmartens,J. Collaborative study of the analysis of tylosin by liquid chromatography on wide-pore poly(styrene-divinylbenzene), *J.Liq.Chromatogr.*, **1995**, *18*, 1195–1205.

SAMPLE
Matrix: bulk

Sample preparation: Prepare a 1 mg/mL solution in 40 mM K_2HPO_4, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 8 μm 1000 Å PLRP-S poly(styrene-divinylbenzene) (Polymer Labs)
Mobile phase: THF:water:200 mM pH 9.0 potassium phosphate buffer 20:75:5
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 29 (tylosin A)
Limit of detection: 0.06%

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Paesen,J.; Claeys,P.; Cypers,W.; Roets,E.; Hoogmartens,J. Liquid chromatography of tylosin A and related substances on poly(styrene-divinylbenzene), *J.Chromatogr.A*, **1995**, *699*, 93–97.

SAMPLE

Matrix: feed
Sample preparation: Mix 25 g feed with 100 mL MeOH:100 mM pH 4.0 phosphate buffer 50:50, shake for 1 h, centrifuge at 2500 rpm for 5 min, gently add several mL of the supernatant to the top of a dry column containing 3 mL acidic alumina (J.T. Baker), discard the first 2 mL and collect the next 2-4 mL, inject a 20 μL aliquot. (Prepare 100 mM pH 4.0 phosphate buffer as follows. Dissolve 16.73 g anhydrous K_2HPO_4 and 523 mg anhydrous KH_2PO_4 in water and dilute to 1L with water.)

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Econosphere C8
Column: 150 × 4.6 5 μm Econosphere C8
Mobile phase: Gradient. MeOH:buffer from 40:60 to 60:40 over 12 min, maintain at 60:40 until no other peaks elute (ca. 2 min), re-equilibrate at 40:60 for 5 min. (Prepare buffer as follows. Dissolve 5 g tetramethylammonium chloride in water, add 5 mL glacial acetic acid, make up to 1 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 7.4 (tylosin B), 9.3 (tylosin-urea adduct), 10.7 (tylosin A),
Limit of quantitation: 4 μg/g

OTHER SUBSTANCES

Noninterfering: monensin, sulfamethazine

KEY WORDS

feed

REFERENCE

Houglum,J.E.; Tasler,M.K. Liquid chromatographic assay of tylosin in animal feeds, *J.AOAC Int.*, **1996**, *79*, 369–374.

SAMPLE

Matrix: feed
Sample preparation: 10 g Ground feed + 100 mL methanolic NaCl, sonicate for 1 h, cool to room temperature, filter through a glass wool pad, wash with three 20 mL portions of methanolic NaCl, make up filtrate to 200 mL with methanolic NaCl, mix. Add a 25 mL aliquot of the filtrate to 10 mL 550 mg/mL KI in water, mix, add 50 mL chloroform, shake vigorously for

10 s, repeat extraction. Combine the chloroform layers and add them to 25 mL 100 mM NaOH, shake vigorously for 10 s. Remove the organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 10 mL mobile phase, inject a 25 μL aliquot. (Prepare methanolic NaCl by mixing 1 L 100 g/L NaCl in water and 1 L MeOH, prepare fresh daily.)

HPLC VARIABLES
Guard column: 400 mg Corasil II
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeCN:acetic acid:water:diethylamine 94:2.5:2.5:1
Flow rate: 1.6
Injection volume: 25
Detector: UV 313

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: pyrantel
Noninterfering: carbadox, lincomycin

KEY WORDS
protect from light

REFERENCE
Goras,J.T. High performance liquid chromatographic method for pyrantel tartrate in swine feeds and supplements, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 1291–1296.

SAMPLE
Matrix: formulations, premix
Sample preparation: Premixes, powders. Shake with MeCN:water 50:50 so as to produce a 0.02% solution, inject a 20 μL aliquot. Tablets. Powder tablets, weigh out amount equivalent to 200 mg tylosin, add 50 mL MeOH, shake, filter. Remove a 5 mL aliquot of the filtrate and make up to 100 mL with MeCN:water 50:50, inject a 20 μL aliquot. Injections. Dilute 1 mL injection to 250 mL with MeCN:water 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeCN:850 mM sodium perchlorate 40:60, adjusted to pH 2.5 with 1 M HCl
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 14 (tylosin A)

OTHER SUBSTANCES
Simultaneous: impurities, desmycosin, desmycinosyl tylosin, macrocin, relomycin

KEY WORDS
powders; tablets; injections

REFERENCE
Fish,B.J.; Carr,G.P.R. Pharmacopoeial procedure for the determination of tylosin factors by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *353*, 39–50.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Adjust pH of 500 μL enzyme reaction mixture in 100 mM pH 7.8 potassium phosphate buffer to 2.6 with trichloroacetic acid, add 5.6 nmoles relomycin, adjust pH to 12.9 with NaOH, add 1 mL ethyl acetate, shake at 60° for 10 min, centrifuge at 300 g for 5 min, inject a 125 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 4.6 6 μm Zorbax C8
Mobile phase: MeCN:THF:buffer 20:15:65 (Buffer was 1% acetic acid containing 0.025% 1-pentanesulfonic acid.)
Column temperature: 55
Flow rate: 2
Injection volume: 125
Detector: UV 285

CHROMATOGRAM
Retention time: 6.5
Internal standard: relomycin (5)
Limit of quantitation: 200 pmoles

OTHER SUBSTANCES
Simultaneous: macrocin

REFERENCE
Yeh,W.K.; Bauer,N.J.; Dotzlaf,J.E. High-performance liquid chromatographic assay for S-adenosyl-L-methionine:macrocin O-methyltransferase, *J.Chromatogr.*, **1984**, *288*, 157–165.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 90:10, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18 end-capped
Mobile phase: MeCN:MeOH:4 mM $(NH_4)H_2PO_4$ 70:25:5
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 10

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 8 μm 1000 Å PLRP-S (Polymer Laboratories)
Mobile phase: THF:200 mM pH 9.0 potassium phosphate buffer:water 20:5:75
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 28 (tylosin A)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Paesen,J.; Cypers,W.; Pauwels,K.; Roets,E.; Hoogmartens,J. Study of he stability of tylosin A in aqueous solutions, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1153–1159.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm C18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
Flow rate: 0.5
Injection volume: 200
Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 562.3-916.5 in NCI and 916.5-773.4 in PCI

CHROMATOGRAM
Retention time: 6.1
Limit of detection: 50 μg/kg

OTHER SUBSTANCES
Extracted: erythromycin, josamycin, spiramycin, tilmicosin

KEY WORDS
muscle; cow; SPE

REFERENCE
Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut SCX SPE cartridge (Varian) with 5 mL MeOH and 10 mL 100 mM pH 4.4 KH_2PO_4 buffer. Homogenize 5 g tissue with 100 mL MeOH: 0.3% metaphosphoric acid 30:70 at high speed for 2 min, filter through 2 mm Hyflo Super-Cel coated on a suction funnel (when filtering liver or kidney add several grams of Hyflo Super-Cel to the homogenized solution before filtration). Evaporate the filtrate to ca. 20 mL under reduced pressure at 45°, add to the SPE cartridge, wash with 10 mL distilled water and 5 mL 100 mM pH 8.9 K_2HPO_4 buffer, elute with 10 mL MeOH, evaporate the eluate to dryness under reduced pressure at 45°, dissolve the residue in 1 mL MeCN:50 mM pH 4.5 NaH_2PO_4 buffer 30:70, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Puresil 5C18 (Waters)
Mobile phase: Gradient. A:B from 60:40 to 0:100 over 16 min. A was buffer. B was MeCN:buffer 40:60 (Buffer was 2.5 g KH_2PO_4 dihydrate and 0.65 mL 85% phosphoric acid dissolved in 1 L distilled water, pH 2.5.)
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 232 for 9 min, UV 287 for 2 min, UV 232 for 4 min

CHROMATOGRAM
Retention time: 10.2
Limit of detection: 50 ng/g

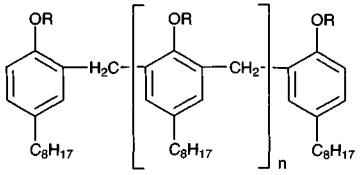
OTHER SUBSTANCES
Extracted: josamycin, leucomycin (kitasamycin), mirosamicin, spiramycin

KEY WORDS
meat; SPE

REFERENCE
Horie,M.; Saito,K.; Ishii,R.; Yoshida,T.; Haramaki,Y.; Nakazawa,H. Simultaneous determination of five macrolide antibiotics in meat by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 295–302.

Tyloxapol

CAS Registry No.: 25301-02-4
Merck Index: 9964



R = $CH_2CH_2O(CH_2CH_2O)_mCH_2CH_2OH$;
m is 6 to 8; n is not more than 5

SAMPLE
Matrix: formulations
Sample preparation: Condition a 1 mL Supelcoclean cyano SPE cartridge with 2 mL MeCN and 2 mL water. Add 4 mL formulation to the SPE cartridge, wash with 2 mL MeCN:buffer 30:70, elute with 5 mL mobile phase, make up eluate to 10 mL with water, inject a 100 µL aliquot. (Buffer was 6 mL concentrated phosphoric acid in 1950 mL water, adjust pH to 5.0 with 50% NaOH, make up to 2 L with water.).

HPLC VARIABLES
Column: 150 × 4.6 5 µm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 6 mL concentrated phosphoric acid in 1950 mL water, adjust pH to 5.0 with 50% NaOH, make up to 2 L with water.)
Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Also analyzed: benzalkonium C10-C18

KEY WORDS
ophthalmic solutions; eye; SPE

REFERENCE
Fan,T.Y.; Wall,G.M. Determination of benzalkonium chloride in opthalmic solutions containing tyloxapol by solid-phase extraction and reversed-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1993**, *82*, 1172–1174.

SAMPLE
Matrix: solutions

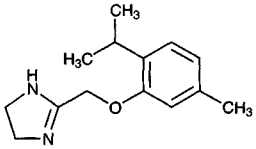
HPLC VARIABLES
Column: 250 × 4.6 diol (Supelco)

Mobile phase: Gradient. A was dichloromethane:MeOH 90:10. B was hexane. A:B from 10:90 to 90:10 over 18 min, return to initial conditions over 2 min.
Flow rate: 1
Detector: UV 280

REFERENCE

DeAngelis,R.L.; Kearney,M.F.; Barnes,E.R.; Shockcor,J.P.; Findlay,J.W.A. Balance/excretion of ^{3}H- and ^{14}C-tyloxapol in the male rabbit after intratracheal administration, *Xenobiotica*, **1995**, *25*, 521–530.

Tymazoline



Molecular formula: $C_{14}H_{20}N_2O$
Molecular weight: 232.33
CAS Registry No.: 24243-97-8
Merck Index: 9965

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 16.00

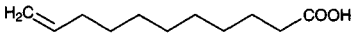
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Undecylenic acid



Molecular formula: $C_{11}H_{20}O_2$
Molecular weight: 184.28
CAS Registry No.: 112-38-9, 557-08-4 (Zn salt)
Merck Index: 9983

SAMPLE

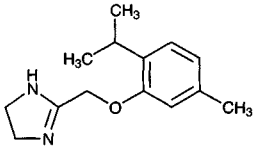
Matrix: blood
Sample preparation: Perform all operations with the exclusion of light. Evaporate 240 μL derivatization solution into a vial, add 400 μL 50 mM pH 7.0 phosphate buffer, add 100 μL

Mobile phase: Gradient. A was dichloromethane:MeOH 90:10. B was hexane. A:B from 10:90 to 90:10 over 18 min, return to initial conditions over 2 min.
Flow rate: 1
Detector: UV 280

REFERENCE

DeAngelis,R.L.; Kearney,M.F.; Barnes,E.R.; Shockcor,J.P.; Findlay,J.W.A. Balance/excretion of ^{3}H- and ^{14}C-tyloxapol in the male rabbit after intratracheal administration, *Xenobiotica*, **1995**, *25*, 521–530.

Tymazoline



Molecular formula: $C_{14}H_{20}N_2O$
Molecular weight: 232.33
CAS Registry No.: 24243-97-8
Merck Index: 9965

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 16.00

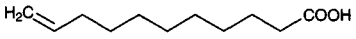
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Undecylenic acid



Molecular formula: $C_{11}H_{20}O_2$
Molecular weight: 184.28
CAS Registry No.: 112-38-9, 557-08-4 (Zn salt)
Merck Index: 9983

SAMPLE

Matrix: blood
Sample preparation: Perform all operations with the exclusion of light. Evaporate 240 μL derivatization solution into a vial, add 400 μL 50 mM pH 7.0 phosphate buffer, add 100 μL

plasma, vortex for 5 s, heat at 70° for 40 min, add 500 μL MeCN, centrifuge at 3000 g for 5 min, inject a 20 μL aliquot. (Derivatization solution was 1.65 g Arkopal N-130 (a non-ionic surfactant, nonylphenol/13 unit chain polyoxyethylene) + 650 mg tetrahexylammonium bromide + 60 mg 4-bromomethyl-7-methoxycoumarin in 20 mL acetone.)

HPLC VARIABLES
Guard column: 10 × 3 5-20 μm LiChroprep RP-8
Column: 100 × 3 5 μm Chromspher C18
Mobile phase: Gradient. MeOH:water 80:20 for 3 min, then to 100:0 over 6 min, then held at 100:0 for 4 min.
Injection volume: 20
Detector: F ex 330 em 395

CHROMATOGRAM
Retention time: 7
Internal standard: undecylenic acid
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: valproic acid
Noninterfering: phenobarbital, phenytoin, carbamazepine

KEY WORDS
plasma; metabolites; undecylenic acid is IS; derivatization

REFERENCE
van der Horst,F.A.; Eikelboom,G.G.; Holthuis,J.J. High-performance liquid chromatographic determination of valproic acid in plasma using a micelle-mediated pre-column derivatization, *J.Chromatogr.*, **1988**, *456*, 191–199.

SAMPLE
Matrix: blood
Sample preparation: Prepare ultrafiltrate from serum with an Amicon Centifree unit by centrifuging at 700 g for 10 min. 25 μL Ultrafiltrate + 475 μL, centrifuge. Remove 50 μL supernatant, add 100 μL 18-crown-6 solution, add 50 μL 1 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, let stand in the dark at 65° for 30 min, inject a 5 μL aliquot. (Prepare 18-crown-6 solution by dissolving 100 mg potassium carbonate in 50 μL water, add 5 mL 20 mM 18-crown-6 in MeCN, sonicate for 30 min, add 5 mL MeCN.)

HPLC VARIABLES
Column: 100 × 2.1 5 μm HP Hypersil-ODS
Mobile phase: MeOH:water 80:20
Column temperature: 40
Flow rate: 0.3
Injection volume: 5
Detector: F ex 322 em 695

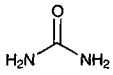
CHROMATOGRAM
Retention time: 4.5
Internal standard: undecylenic acid

OTHER SUBSTANCES
Simultaneous: valproic acid
Noninterfering: phenobarbital, phenytoin, carbamazepine

KEY WORDS
serum; undecylenic acid is IS; derivatization

REFERENCE
Liu,H.; Forman,L.J.; Montoya,J.; Eggers,C.; Barham,C.; Delgado,M. Determination of valproic acid by high-performance liquid chromatography with photodiode-array and fluorescence detection, *J.Chromatogr.*, **1992**, *576*, 163–169.

Urea



Molecular formula: CH_4N_2O
Molecular weight: 60.06
CAS Registry No.: 57-13-6
Merck Index: 10005

SAMPLE
Matrix: blood
Sample preparation: Dilute serum 200-fold with water. Add 50 μL 40 μg/mL uracil solution to 1 mL diluted serum, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 6.0 5 μm Sumipax ODS C-212 (Sumika Chemical Analysis Service, Japan)
Mobile phase: MeOH:water 15:85
Flow rate: 1
Injection volume: 10
Detector: MS, Finnigan MAT Model TSQ 700, APCI source 250°, vaporizer 400°, discharge current 5 μA, m/z 61, 62 and 113

CHROMATOGRAM
Retention time: 3.0 (^{12}C-urea, ^{13}C-urea)
Internal standard: uracil (3.7)
Limit of quantitation: 200 μg/mL (^{12}C-urea), 2 μg/mL (^{13}C-urea)

KEY WORDS
serum

REFERENCE
Tanigawa,T.; Mizo-oku,Y.; Moriguchi,K.; Suzuki,T.; Osumi,T.; Odomi,M. Simple and rapid quantitative assay of 13C-labelled urea in human serum using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.B*, **1996**, *683*, 135–142.

SAMPLE
Matrix: blood
Sample preparation: Inject a 500 μL aliquot of serum ultrafiltrate onto a 50 × 4.6 column packed with immobilized urease, let stand at room temperature for 10-15 min, inject another 100 μL ultrafiltrate to force ultrafiltrate into the 10 μL sample loop, inject this aliquot. (Prepare the urease column by suspending 1 g Eupergit C (epoxyacrylic resin granules, Röhm Pharma, Weiterstadt, Germany) in 2.5 mL 4 mg/mL urease (EC 3.5.1.5, Type IV, Jack Beans, Sigma) in water, filter after 1 h (Analyst 1984, 109, 147), suspend in water, slurry pack in a 50 × 4.6 column. The enzyme converts serum urea to ammonium ions which are then detected by the HPLC system. Wash column with pH 7 phosphate buffer after use, store in the refrigerator.)

HPLC VARIABLES
Column: 250 × 4.6 Wescan cation-exchange (Bio-Rad)
Mobile phase: Dilute phosphoric acid, pH 2.28
Flow rate: 1
Injection volume: 10
Detector: Conductivity

CHROMATOGRAM
Retention time: 4

KEY WORDS
derivatization; horse; serum; ultrafiltrate

REFERENCE
Shintani,H.; Ube,S. Simultaneous determination of serum cations, anions and uremic toxins by ion chromatography using an immobilized enzyme, *J.Chromatogr.*, **1985**, *344*, 145–156.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bond Elut SCX strong cation exchange (SO3H type) SPE cartridge with 2 mL MeOH and 2 mL 100 mM HCl. Filter (Amicon Centricon, cut-off 10000 daltons) while centrifuging at 4000 rpm, acidify ultrafiltrate to pH 3 with HCl, add 1 mL to the SPE cartridge, wash with 2 mL water, elute with 2 mL 1 M HCl at 0.3 mL/min, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 MCI CK 08S strong cation exchange SDB polymer base (SO3H type) (Mitsubishi Kasei)
Mobile phase: 1.5 mM HCl
Flow rate: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 24

KEY WORDS
whole blood; SPE; ultrafiltrate

REFERENCE
Shintani,H. Selection of columns for analysis of blood urea, *J.Liq.Chromatogr.*, **1994**, *17*, 1737–1742.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg 0.6 mL BondElut SCX SPE cartridge with 2 mL MeOH and 2 mL 100 mM HCl. Filter (Amicon Centricon, cut-off 10000 daltons) while centrifuging at 4000 rpm. Acidify the ultrafiltrate to pH 3 with HCl, add 1 mL to the SPE cartridge, wash with 2 mL water, elute with 2 mL 1 M HCl at 0.3 mL/min, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 11-14 μm Mitsubishi Kasei MCI CK 08S strong cation exchange (SO3H type on 8% crosslinked DVB, 1.9 meq/mL)
Mobile phase: 1.5 mM HCl
Flow rate: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 24

KEY WORDS
whole blood; SPE; ultrafiltrate

REFERENCE
Shintani,H. Solid phase extraction (SPE) of blood urea compared with liquid-liquid extraction regarding artifact formation, *J.Liq.Chromatogr.*, **1995**, *18*, 2167–2174.

SAMPLE
Matrix: blood
Sample preparation: Condition a 2.8 mL 500 mg Bond Elut SCX H type cation-exchange SPE cartridge with 3 mL MeOH and 3 mL water at 3 mL/min. Add blood, wash with 1 mL water at 3 mL/min, elute with 4 mL 5% phosphoric acid at 1 mL/min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 11-14 μm MCI GEL CK 08S polymer-based strong cation exchange (Na type) (Mitsubishi)
Mobile phase: 1 mM HCl
Column temperature: 35
Flow rate: 1

Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 6

KEY WORDS
SPE

REFERENCE
Shintani,H. Comparison of solid-phase extraction and dialysis on pretreatment efficiency of blood urea analysis, *J.Chromatogr.Sci.*, **1996**, *34*, 92–94.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute 100 (serum) or 1000 (urine) fold with water, filter (0.2 μm), adjust pH to 10-11 with KOH, add a 2 mL aliquot to the SPE column, discard the first 1.5 mL eluate, inject a 10 μL aliquot of the next eluate fraction. (Prepare the SPE column by adding 50-100 mesh Dowex 1-X2 strongly basic anion exchange resin to a Pasteur pipette (40 mm bed depth), add a few mL water to the column.)

HPLC VARIABLES
Guard column: 60 × 2 C18
Column: 150 × 3 5 μm Spherisorb ODS-2
Mobile phase: 50 mM pH 6.9 potassium phosphate buffer containing 5 mM sodium octylsulfonate
Flow rate: 0.5
Injection volume: 10
Detector: F ex 340 em 455 following post-column reaction. The column effluent passed through a 60 × 2 immobilized urease solid-phase reactor and then mixed with the reagent pumped at 0.5 mL/min. The mixture flowed through a coil of 0.2 mm i.d. PTFE tubing (volume 600 μL) to the detector. (Prepare reagent by dissolving 24.7 g boric acid in 1 L water, adjust pH to 10.2 with KOH, add 10 mL 80 mg/mL o-phthalaldehyde in EtOH, add 1 mL mercaptoethanol, store under nitrogen at 4°, stable for at least 1 week. Prepare the solid-phase reactor by treating 10 μm LiChrospher SI 500 with 3-aminopropyltriethoxysilane, couple urease (urea amidohydrolase EC 3.5.1.5, U-2000 (Sigma)) to the silica using 25% glutaraldehyde solution. Slurry the urease-silica in water and pack the reactor with 10 mM pH 6.9 potassium phosphate buffer at 110 bar for 15 min (J. Chromatogr. 1985, 325, 255). To maintain reactor performance flush system for 30 min with 50 mM pH 6.9 potassium phosphate buffer containing 5 mM EDTA every 2 weeks. Store reactor at 4° when not in use.)

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 30 ppb

OTHER SUBSTANCES
Simultaneous: ammonia
Noninterfering: amino acids

KEY WORDS
serum; SPE; post-column reaction

REFERENCE
Jansen,H.; van der Velde,E.G.; Brinkman,U.A.T.; Frei,R.W. Liquid chromatographic determination of urea and ammonia in body fluids using a post-column enzymatic reactor, *J.Chromatogr.*, **1986**, *378*, 215–221.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 100 mg carbamide peroxide formulation, make up to 50 mL with water, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 6.5 CHO-620 Carbohydrate (Interaction Chemicals Inc., Mountain View, CA)
Mobile phase: 50 μg/mL Calcium disodium EDTA in water
Column temperature: 85
Flow rate: 0.6
Injection volume: 20
Detector: UV 200, UV 210

CHROMATOGRAM
Retention time: 15
Limit of detection: 1 μg/g
Limit of quantitation: 15 μg/g

OTHER SUBSTANCES
Simultaneous: excipients

REFERENCE
Walker,T.A. A liquid chromatographic assay for urea in over-the-counter carbamide peroxide products, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1277–1282.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron for >10 mg; Kontes micro-ultrasonic cell disrupter for <10 mg) tissue with 40 volumes 25 μg/mL β-aminoisobutyric acid in EtOH:water:glacial acetic acid 75:20:5, centrifuge at 4° at 25000 g for 20 min. Remove a 50 μL aliquot of the supernatant and evaporate it to dryness under reduced pressure, suspend the residue in 100 μL 100 mM sodium bicarbonate by sonicating or vortexing, add 200 μL 1.25 mg/mL dansyl chloride in acetone, vortex, heat at 90° for 30 min, centrifuge at 5000 g for 20 min, inject a 4 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:water:phosphoric acid 13:87:0.15
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.45
Internal standard: β-aminoisobutyric acid (k' 9.25)
Limit of quantitation: 10 pmole

OTHER SUBSTANCES
Extracted: amino acids, taurine
Interfering: arginine, hydroxyproline, homocarnosine

KEY WORDS
rat; brain; derivatization

REFERENCE
Saller,C.F.; Czupryna,M.J. γ-Aminobutyric acid, glutamate, glycine and taurine analysis using reversed-phase high-performance liquid chromatography and ultraviolet detection of dansyl chloride derivatives, *J.Chromatogr.*, **1989**, *487*, 167–172.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 500 mg washed and mixed Duolite ion-exchange resin (BDH), vortex for 10 s, centrifuge at 3000 g for 10 min, filter (0.2 μm) the supernatant, inject an aliquot.

HPLC VARIABLES
Guard column: Direct-Connect polymeric guard column (Alltech)
Column: 250 × 4.6 5 μm Kromasil NH2 (Alltech)
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: lactulose, mannitol, L-rhamnose

REFERENCE
Miki,K.; Butler,R.; Moore,D.; Davidson,G. Rapid and simultaneous quantification of rhamnose, mannitol, and lactulose in urine by HPLC for estimating intestinal permeability in pediatric practice, *Clin.Chem.*, **1996**, *42*, 71–75.

Urokinase

Molecular weight: 30000-50000
CAS Registry No.: 9039-53-6
Merck Index: 10024

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 7.5 TSK G3000SW (Alltech)
Mobile phase: 100 mM KH_2PO_4 containing 100 mM NaCl and 5 mM sodium azide, adjusted to pH 6.0 with 10 M NaOH
Flow rate: 0.5
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 19

REFERENCE
Cox,R.A.; McFarland,K.N.; Sackett,P.H.; Short,M.T. Correlation of urokinase activity from biopotency and high-performance liquid chromatographic assays, *J.Chromatogr.*, **1986**, *370*, 495–500.

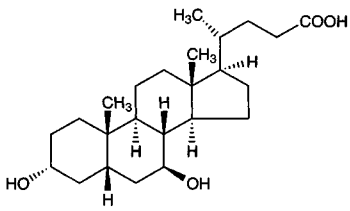
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Bakerbond C4
Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 0:100 to 60:40 over 90 min
Flow rate: 1
Injection volume: 200
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: alteplase, streptokinase, anistreplase

REFERENCE
Werner,R.G.; Bassarab,S.; Hoffmann,H.; Schlüter,M. Quality aspects of fibrinolytic agents based on biochemical characterization, *Arzneimittelforschung*, **1991**, *41*, 1196–1200.

Ursodiol



Molecular formula: $C_{24}H_{40}O_4$
Molecular weight: 392.58
CAS Registry No.: 128-13-2
Merck Index: 10026

SAMPLE
Matrix: bile
Sample preparation: Extract bile with 20 volumes EtOH, boil on a hot water bath, cool, let stand overnight, filter (Toyo Roshi 5A paper), filter (0.45 μm), add 200 μg/mL testosterone acetate in EtOH (final IS concentration 100 μg/mL), inject a 5-10 μL aliquot.

HPLC VARIABLES
Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:30 mM phosphate buffer 10:60:30, pH 3.40
Flow rate: 0.5
Injection volume: 5-10
Detector: UV 200

CHROMATOGRAM
Retention time: 12 (taurine conjugate), 13 (glycine conjugate)
Internal standard: testosterone acetate (39)
Limit of detection: 50 ng

OTHER SUBSTANCES
Extracted: chenodiol, conjugates, bile acids, deoxycholic acid

REFERENCE
Nakayama,F.; Nakagaki,M. Quantitative determination of bile acids in bile with reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 287–293.

SAMPLE
Matrix: bile, blood
Sample preparation: Serum. 100-200 μL Serum + 1 mL MeOH, mix, sonicate for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute with 1 mL 50 mM pH 7.0 phosphate buffer, add to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot. Bile. Mix 10 μL bile with 10 mL 50 mM pH 7.0 phosphate buffer, add a 1 mL aliquot to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 10 μm Model RCM-100 Radial-Pak A (Waters)

Mobile phase: Gradient. MeCN:MeOH:water 100:50:40 for 30 min then 100:50:20 (step gradient).
Flow rate: 2
Injection volume: 8
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 26
Internal standard: lauric acid (56)
Limit of detection: 10 pmole
Limit of quantitation: 50 pmole

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid

KEY WORDS

derivatization; serum; SPE

REFERENCE

Kamada,S.; Maeda,M.; Tsuji,A. Fluorescence high-performance liquid chromatographic determination of free and conjugated bile acids in serum and bile using 1-bromoacetylpyrene as a pre-labeling reagent, *J.Chromatogr.*, **1983**, *272*, 29–41.

SAMPLE

Matrix: bile, blood, feces, gastric contents, tissue
Sample preparation: Condition a Sep-Pak C18 cartridge with 2 mL 720 mM MeOH in water and 6 mL 100 mM pH 7.0 potassium phosphate buffer. Serum. 200 μL Serum + 1 mL MeCN, mix, sonicate for 10 min, centrifuge at 17000 g for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Liver. Homogenize (glass homogenizer) liver in 1 mL 720 mM EtOH in water, add 2 mL 720 mM EtOH in water, heat at 75° for 15 min, centrifuge at 17000 g for 10 min, remove the supernatant, extract the residue twice more. Combine the supernatants and evaporate them to dryness at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Bile. Dilute 20 μL bile with 10 mL 100 mM pH 7.0 potassium phosphate buffer. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Gastric juice. Dilute 1 mL gastric juice with 9 mL 100 mM pH 7.0 potassium phosphate buffer, sonicate for 10 min. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Feces. Dilute 1 g feces with 9 mL MeOH, mix thoroughly, sonicate for 10 min, centrifuge at 17000 g for 10 min. Remove a 1 mL aliquot of the supernatant and evaporate it to dryness, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, recon-

stitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere I.P. C18
Mobile phase: Gradient. A was MeCN:MeOH:water 100:50:75. B was MeCN:MeOH 100:50. A:B 100:0 for 7 min, to 70:30 over 0.5 min, maintain at 70:30 for 5 min, to 50:50 over 0.5 min, maintain at 50:50 over 7 min, to 25:75 over 1 min, maintain at 25:75 for 7 min.
Column temperature: 35
Flow rate: 1.7
Injection volume: 100
Detector: F

CHROMATOGRAM

Retention time: 15
Internal standard: lauric acid (24.5)
Limit of detection: 0.5 pmole

OTHER SUBSTANCES

Extracted: chenodiol (chenodeoxycholic acid), cholic acid, deoxycholic acid, glycinechenodeoxycholic acid, glycinecholic acid, glycinedeoxycholic acid, glycinelithocholic acid, glycineursodeoxycholic acid, lithocholic acid

KEY WORDS

derivatization; SPE; liver; serum

REFERENCE

Güldütuna,S.; You,T.; Kurts,W.; Leuschner,U. High performance liquid chromatographic determination of free and conjugated bile acids in serum, liver biopsies, bile, gastric juice and feces by fluorescence labeling, *Clin.Chim.Acta*, **1993**, *214*, 195–207.

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Urine. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-200 μL urine 1:4 with 100 mM NaOH, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Serum. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-500 μL serum with 3.5 mL 100 mM NaOH, heat at 64° for 30 min, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Bile. Dilute 1:500 to 1:1000 with mobile phase, filter (0.22 μm), inject an aliquot.

HPLC VARIABLES

Column: 70 × 4.6 3 μm Ultrasphere XL C18
Mobile phase: MeOH:15 mM ammonium acetate 80:20, apparent pH 6.0 ± 0.1
Flow rate: 0.3
Detector: MS, electrospray, Fisons VG TRIO 2000 quadrupole (6% of the mobile phase was diverted to the MS detector) or evaporative light scattering detector (Varex)

CHROMATOGRAM

Retention time: 7.75
Limit of detection: 15 pg

OTHER SUBSTANCES

Extracted: chenodiol, deoxycholic acid, bile acids, cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycoursodeoxycholic acid, lithocholic acid, taurochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, tauroursodeoxycholic acid

KEY WORDS

serum; SPE; hamster; human; LC-MS

REFERENCE

Roda,A.; Gioacchini,A.M.; Cerrè,C.; Baraldini,M. High-performance liquid chromatographic-electrospray mass spectrometric analysis of bile acids in biological fluids, *J.Chromatogr.B*, **1995**, *665*, 281–294.

SAMPLE

Matrix: bile, formulations

Sample preparation: Bile. Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL MeOH. 50 μL Bile + 5 mL 50 mM pH 7.5 phosphate buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH:40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge (J. Pharm. Biomed. Anal. 1990, 8, 235). Evaporate to dryness under a stream of nitrogen, reconstitute with 2 mL MeOH, sonicate at 40° for 3 min, filter (0.2 μm). Add a 500 μL aliquot of the filtrate to 50 μL 0.01% KOH in MeOH, evaporate to dryness, reconstitute with 200 μL MeOH:water 10:90, sonicate at 40° for 3 min, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. Formulations. Powder capsule contents, weigh out amount containing about 25 mg compound, add 100 mL MeOH (water for bile acid salts), stir for 10 min, filter, dilute the filtrate 10-fold with water (or MeOH:water 10:90 for bile acid salts). Evaporate 50 μL 0.01% KOH in MeOH in to a tube, add a 200 μL aliquot of the diluted filtrate, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform: petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil RP-18

Mobile phase: Gradient. For bile use MeCN:water 60:40 for 10 min, to 80:20 over 10 min, maintain at 80:20 for 25 min, return to initial conditions over 5 min. For formulations use isocratic MeCN:water 78:22.

Flow rate: 1

Injection volume: 50

Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 18 (gradient), 7 (isocratic)

Internal standard: 6-methoxynaphthacyl ester of valproic acid (23 (gradient), 10.5 (isocratic))

Limit of detection: 1-2 pmole

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid

KEY WORDS

derivatization; capsules; SPE

REFERENCE

Cavrini,V.; Gatti,R.; Roda,A.; Cerrè,C.; Roveri,P. HPLC-fluorescence determination of bile acids in pharmaceuticals and bile after derivatization with 2-bromoacetyl-6-methoxynaphthalene, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 761–770.

SAMPLE

Matrix: bile, gastric contents

Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL MeOH. Mix 50 μL bile or 500 μL gastric juice with 5 mL 50 mM pH 7.5 phosphate

buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH:40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge. Evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL initial mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeOH:30 mM sodium acetate 65:35, adjusted to pH 4.3 with phosphoric acid. B was MeOH:70 mM sodium acetate 90:10, adjusted to pH 4.3 with phosphoric acid. A:B 85:15 for 10 min, to 10:90 over 25 min, maintain at 10:90 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Extracted: cholic acid, chenodiol, deoxycholic acid, lithocholic acid

KEY WORDS
SPE

REFERENCE
Scalia,S. Group separation of free and conjugated bile acids by pre-packed anion-exchange cartridges, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 235–241.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH, 10 mL water, and 2 mL 100 mM pH 8.0 Tris-HCl buffer. 5-7 mL Serum + 19 volumes 100 mM pH 8.0 Tris-HCl buffer, sonicate for 10 min, add to the SPE cartridge, wash with 15 mL water, elute with 6-7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, dissolve residue in water, filter (Millipore GS 0.22 μm), wash filter, evaporate filtrates to dryness, reconstitute in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:20 mM KH_2PO_4 65:35, adjust pH to 5.3
Flow rate: 1.4
Injection volume: 30
Detector: UV 210

CHROMATOGRAM
Retention time: 6 (taurine conjugate), 7 (glycine conjugate)

OTHER SUBSTANCES
Extracted: conjugates, chenodiol, bile acids, deoxycholic acid

KEY WORDS
serum; SPE

REFERENCE
Linnet,K. A high-pressure liquid chromatogaphic-enzymatic assay for glycine and taurine conjugates of cholic, chenodeoxycholic and deoxycholic acid in serum, *Scand.J.Clin.Lab.Invest.*, **1982**, *42*, 455–460.

SAMPLE
Matrix: blood
Sample preparation: Condition a BondElut SPE cartridge with 5 mL EtOH and 5 mL water. 100 μL Serum + 250 ng deoxycholic acid 12-propionate + 1 mL 500 mM pH 7.0 phosphate

buffer, mix, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL 1.5% EtOH, elute with 2 mL 90% EtOH. Evaporate a 400 μL aliquot of the eluate, add 100 μL 2 mg/mL 1-anthroyl nitrile in MeCN, add 0.16% quinuclidine in MeCN, heat at 60° for 20 min, add 50 μL MeOH, evaporate under nitrogen. Dissolve the residue in 1 mL 90% EtOH, add to a 18 × 6 100 mg column of PHP-LH-20 Sephadex at 0.2 mL/min, wash with 1 mL 90% EtOH, elute with 5 mL 100 mM acetic acid in 90% EtOH (free bile acids), elute with 5 mL 200 mM formic acid in 90% EtOH (glycine-conjugated bile acids), elute with 5 mL 300 mM pH 6.3 acetic acid-potassium acetate in 90% EtOH (taurine-conjugated bile acids). Evaporate each fraction, dissolve the residue in 100-200 μL MeOH, inject a 5-10 μL aliquot. (Preparation of PHP-LH-20 Sephadex is as follows. Suspend 75.7 g Sephadex LH-20 in 200 mL dichloromethane using a glass stirring rod (not a magnetic stirrer) for 30 min, add 19 mL boron trifluoride ethyl etherate, after 15 min add 50 mL 35% epichlorohydrin in dichloromethane at 1-2 mL/min (Caution! Epichlorohydrin is a carcinogen!), stir for another 30 min, filter, wash with EtOH, dry chlorohydroxypropyl Sephadex LH-20 at 50° (J.Chromatogr. 1971, 59, 45). Stir 27.2 g chlorohydroxypropyl Sephadex LH-20 in 100.5 mL piperidine at room temperature for 30 min, add 5.74 g KOH in 302 mL MeOH, heat at 50-60° for 3 h with occasional shaking, filter, wash with EtOH:water 50:50, wash with 200 mM acetic acid in EtOH:water 70:30, wash with EtOH:water 90:10 until washings become neutral, store in EtOH:water 90:10 (Clin. Chim. Acta 1978 87 141). Prepare 1-anthroyl nitrile as follows. Dissolve 50 g benzanthrone in 500 mL concentrated sulfuric acid with gentle warming, pour this solution cautiously into 4 L hot water with vigorous stirring. Boil the suspension and slowly add 200 g chromium(VI) oxide (Caution! Chromium oxide is a carcinogen and highly corrosive!), after 6 h cool the mixture, filter, wash the precipitate with hot water. Dissolve the precipitate in dilute ammonia and precipitate with acid, crystallize from boiling concentrated nitric acid to give anthraquinone-1-carboxylic acid (Ber. 1924, 57, 1775). Warm, on a water bath, anthraquinone-1-carboxylic acid in dilute ammonia with twice the amount of zinc dust, when the reaction has ceased (30 min ?) filter the reaction the reaction mixture, add HCl to the filtrate to obtain anthracene-1-carboxylic acid as yellow needles, recrystallize from EtOH (mp 245°) (Ber 1897, 30, 1118). Stir 1 g anthracene-1-carboxylic acid in 15 mL anhydrous dichloromethane, add 2 mL oxalyl chloride, reflux for 1 h, evaporate to give 1-anthroyl chloride as an oily residue. Dissolve 1-anthroyl chloride in 15 mL dichloromethane, add 3 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, recrystallize from hexane/dichloromethane to give 1-anthroyl nitrile as orange-yellow needles (mp 164-5°) (Anal.Chim.Acta 1983, 147, 397).)

HPLC VARIABLES

Column: 150 × 4 5 μm Cosmosil 5C18
Mobile phase: MeOH:0.3% pH 6.0 potassium phosphate buffer 5:1
Flow rate: 1.8
Injection volume: 10
Detector: F ex 370 em 470

CHROMATOGRAM

Retention time: 10
Internal standard: deoxycholic acid 12-propionate (20)
Limit of detection: 50 nM

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid deoxycholic acid, conjugates

KEY WORDS

serum; SPE; derivatization

REFERENCE

Goto,J.; Saito,M.; Chikai,T.; Goto,N.; Nambara,T. Studies on Steroids. CLXXXVII. Determination of serum bile acids by high-performance liquid chromatography with fluorescence labeling, *J.Chromatogr.*, **1983**, *276*, 289–300.

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL serum to a Waters C18 SPE cartridge, wash with two 4 mL portions of water, wash with two 2 mL portions of MeOH:water 10:90, wash with two 2 mL portions of MeOH:water 20:80, wash with two 2 mL portions of MeOH:water 30:70, wash with two 2 mL portions of MeOH:water 50:50, elute with 3 mL MeOH. Evaporate the eluate to

dryness under a stream of nitrogen at 80°, reconstitute the residue in 50 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Lichrosorb RP 18
Mobile phase: MeOH:30 mM KH_2PO_4 76:24
Flow rate: 1.2
Injection volume: 20
Detector: UV 201

CHROMATOGRAM
Retention time: 6
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: chenodiol (chenodeoxycholic acid)

KEY WORDS
serum; SPE

REFERENCE
Baillet-Guffroy,A.; Baylocq,D.; Rabaron,A.; Pellerin,F. Nuclear magnetic resonance spectrometry and liquid chromatography of two bile acid epimers: ursodeoxycholic and chenodeoxycholic acid, *J.Pharm.Sci.*, **1984**, *73*, 847–849.

SAMPLE
Matrix: blood
Sample preparation: Deproteinize 20 μL serum with a pretreatment column (Autoserumout, Sekisui), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Medipola Bile column (Sekisui)
Mobile phase: Gradient. A was MeCN:MeOH:30 mM ammonium acetate 20:20:60. B was MeCN:MeOH:30 mM ammonium acetate 30:30:40. A:B from 100:0 to 80:20 over 10 min, to 0:100 over 27 min, maintain at 0:100 for 30 min.
Flow rate: 1
Detector: F ex 340 em 460 following post-column reaction detection. The effluent from the column was mixed with reagent pumped at 1 mL/min, the mixture flowed through a 20 × 4 3α-HSD column (Sekisui) containing bound 3α-hydroxysteroid dehydrogenase to the detector. (The reagent was 1.36 g/L KH_2PO_4, 372 mg/L disodium EDTA, 140 mg/L βNAD, and 450 μL/L 2-mercaptoethanol in water adjusted to pH 7.8 with 5 M KOH.)

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Extracted: chenodiol, deoxycholic acid, bile acids

KEY WORDS
post-column reaction; immobilized enzyme reactor; serum

REFERENCE
Adachi,Y.; Nanno,T.; Itoh,T.; Kurumi,Y.; Yamazaki,K.; Sawada,Y.; Yamamoto,T. Determination of individual serum bile acids in chronic liver diseases: fasting levels and results of oral chenodeoxycholic acid tolerance test, *Gastroenterol.Jpn.*, **1988**, *23*, 401–407.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Dilute 100-200 μL serum with 4 mL 400 mM sodium bicarbonate, add to the SPE cartridge,

wash with 20 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, add 400 μg sodium carbonate, add 50 μL 20 mg/mL 18-crown-6 in MeCN, heat at 40° for 1 h, make up to 500 μL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak ODS
Mobile phase: Gradient. A was MeCN:MeOH:water 15:13.8:71.2. B was MeCN. A:B from 100:0 to 37:63 over 47 min (Waters convex curve + 2), to 0:100 over 0.1 min (Waters curve +9), maintain at 0:100 for 7.9 min, re-equilibrate at initial conditions for 6 min.
Flow rate: 1 for 47 min then 1.5
Injection volume: 10
Detector: F ex 320 em 385

CHROMATOGRAM
Retention time: 36.43
Limit of detection: 50 nM

OTHER SUBSTANCES
Extracted: chenodiol, cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid

KEY WORDS
derivatization; serum; SPE

REFERENCE
Wang,G.F.; Stacey,N.H.; Earl,J. Determination of individual bile acids in serum by high performance liquid chromatography, *Biomed.Chromatogr.*, **1990**, *4*, 136–140.

SAMPLE
Matrix: bulk
Sample preparation: Add 5 mL of a 39.2 mg/mL solution in dry MeCN to 120 mg 1-(2,5-dihydroxyphenyl)-2-bromoethane and 100 μL triethylamine, heat at 70° for 2 h, dilute with 20 mL water, extract 3 times with diethyl ether. Combine the extracts and wash them with saturated sodium bicarbonate and water, dry over anhydrous sodium sulfate, evaporate, reconstitute, inject a 5 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethane is as follows. Slowly add 2.5 g phenyltrimethylammonium tribromide to a solution of 2',5'-dihydroxyacetophenone in 20 mL dry THF, stir at room temperature overnight (check by TLC with cyclohexane:ethyl acetate 70:30). Remove the precipitate by filtration and dry under reduced pressure, chromatograph using cyclohexane:ethyl acetate 70:30 to give 1-(2,5-dihydroxyphenyl)-2-bromoethane.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm 5 μm Hypersyl ODS RP-18
Column: 100 × 4.6 3 μm Adsorbosphere
Mobile phase: MeCN:MeOH:100 mM pH 6.5 sodium acetate buffer 20:60:20
Flow rate: 1
Injection volume: 5
Detector: E, ESA Coulochem Model 5100A, Model 5010 analytical cell, porous graphite electrodes +0.6 V

CHROMATOGRAM
Retention time: 4.07
Limit of detection: 0.88 nM

OTHER SUBSTANCES
Simultaneous: chenodiol

KEY WORDS
derivatization

REFERENCE

Bousquet,E.; Santagati,N.A.; Tirendi,S. Determination of chenodeoxycholic acid in pharmaceutical preparations of ursodeoxycholic acid by high performance liquid chromatography with coulometric electrochemical detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 757–770.

SAMPLE

Matrix: formulations

Sample preparation: Mix a 750 μL aliquot of the liquid formulation with 3 mL MeOH, shake vigorously for 15 s, centrifuge at 1000 rpm for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Altima C18 (Alltech)

Mobile phase: MeCN:buffer 55:45 (Buffer was 10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: UV 201

KEY WORDS

liquid formulations

REFERENCE

Mallett,M.S.; Hagan,R.L.; Peters,D.A. Stability of ursodiol 25 mg/mL in an extemporaneously prepared oral liquid, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 1401–1404.

SAMPLE

Matrix: formulations

Sample preparation: Dilute with MeOH to an ursodiol concentration of 1.5 mg/mL, filter (0.22 μm), inject a 15 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spheri-5 ODS C18

Mobile phase: MeOH:10 mM potassium phosphate buffer 75:25, pH adjusted to 5.25 with dilute phosphoric acid

Flow rate: 1.2

Injection volume: 15

Detector: UV 201

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

syrup; suspensions; stability-indicating

REFERENCE

Johnson,C.E.; Nesbitt,J. Stability of ursodiol in an extemporaneously compounded oral liquid, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1798–1800.

SAMPLE

Matrix: solutions

Sample preparation: Mix an aliquot of solution (or hydrolyzed bile) with a 50% molar excess of triethylamine in MeCN, warm briefly, add a 50% molar excess of 100 mM 2-bromoacetophenone in MeCN, heat at 80-90° for 45-60 min, evaporate to dryness, reconstitute with dioxane (Caution! Dioxane is a carcinogen!), filter (0.47 μm), inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10/25 ODS

Mobile phase: Gradient. n-Heptane:dioxane 90:10 for 3 min then n-heptane:dioxane:isopropanol 70:25:5 (step gradient). (Caution! Dioxane is a carcinogen!)
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 19
Limit of quantitation: 5 pmole

OTHER SUBSTANCES
Simultaneous: chenodiol, cholic acid, deoxycholic acid, hyodeoxycholic acid, lithocholic acid

KEY WORDS
derivatization

REFERENCE
Stellaard,F.; Hachey,D.L.; Klein,P.D. Separation of bile acids as their phenacyl esters by high-pressure liquid chromatography, *Anal.Biochem.*, **1978**, *87*, 359–366.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 1 mg/mL.

HPLC VARIABLES
Column: 100 mm long 5 μm C18
Mobile phase: MeOH:10 mM KH_2PO_4 65:35, pH 7.0
Flow rate: 1
Injection volume: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: k' = 3.66

OTHER SUBSTANCES
Simultaneous: chenodiol

REFERENCE
Roda,A.; Minutello,A.; Angellotti,M.A.; Fini,A. Bile acid structure-activity relationship: evaluation of bile acid lipophilicity using 1-octanol/water partition coefficient and reverse phase HPLC, *J.Lipid Res.*, **1990**, *31*, 1433–1443.

SAMPLE
Matrix: solutions
Sample preparation: Sample + 400 μL 5 mM DBD-PZ + 70 mM diethylphosphorocyanidate in MeCN, react for 6 h, inject a 1 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10°

(use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM
Retention time: 17
Limit of detection: 13 fmol

OTHER SUBSTANCES
Simultaneous: dehydrocholic acid

KEY WORDS
derivatization

REFERENCE
Toyo'oka,T.; Ishibashi,M.; Takeda,Y.; Nakashima,K.; Akiyama,S.; Uzu,S.; Imai,K. Precolumn fluorescence tagging reagent for carboxylic acids in high-performance liquid chromatography: 4-substituted-7-aminoalkylamino-2,1,3-benzoxadiazoles, *J.Chromatogr.*, **1991**, *588*, 61–71.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:0.8 M NaOH 8:92, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: CarboPac PA-100 (Dionex)
Column: 250 × 4 8.5 μm CarboPac PA-100 (Dionex)
Mobile phase: MeCN:water 15:85 containing 900 mM sodium acetate and 100 mM NaOH
Flow rate: 0.8
Injection volume: 25
Detector: E, Dionex PAD-2 pulsed amperometric detector, gold working electrode, V1 + 0.05 V, t1 480 ms, V2 + 0.60 V, t2 120 ms, V3 -0.60 V, t3 60 ms

CHROMATOGRAM
Retention time: 5.87

OTHER SUBSTANCES
Simultaneous: chenodiol, deoxycholic acid, cholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, glycodeoxychenodeoxycholic acid, ursodeoxycholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid, glycolithocholic acid, lithocholic acid, taurolithocholic acid

REFERENCE
Chaplin,M.F. Analysis of bile acids and their conjugates using high-pH anion-exchange chromatography with pulsed amperometric detection, *J.Chromatogr.B*, **1995**, *664*, 431–434.

SAMPLE
Matrix: solutions
Sample preparation: Mix 200 μL of a solution of bile acids with 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, add 300 μL 10 mM tetrakis(decyl)ammonium bromide in 100 mM pH 7.0 phosphate buffer, heat at 40° for with sonication 10 min, add 300 μL 5.1 μM IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES
Column: 250 × 4.6 Ultracarb 5 ODS
Mobile phase: Gradient. A was water. B was MeCN:MeOH 60:40. A:B 55:45 for 20 min, to 30:70 over 10 min, maintain at 30:70 for 25 min, return to initial conditions over 5 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM
Retention time: 13
Internal standard: 6-methoxynaphthacyl ester of lauric acid (36)
Limit of detection: 1-2 pmole

OTHER SUBSTANCES
Simultaneous: chenodiol, cholic acid, deoxycholic acid, lithocholic acid

KEY WORDS
derivatization

REFERENCE
Gatti,R.; Roda,A.; Cerre,C.; Bonazzi,D.; Cavrini,V. HPLC-fluorescence determination of individual free and conjugated bile acids in human serum, *Biomed.Chromatogr.*, **1997**, *11*, 11–15.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine, pass 40 mL urine through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 10 mL 100 mM pH 5.0 sodium acetate buffer, add 100 μg β-glucuronidase, add 100 μg cholylglycine hydrolase, heat at 37° for 36 h, pass the mixture through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 1 mL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 100 × 85 μm μBondapak C18 radial pack
Mobile phase: MeCN:MeOH:water:acetic acid 70:20:70:1
Flow rate: 2
Injection volume: 50
Detector: RI

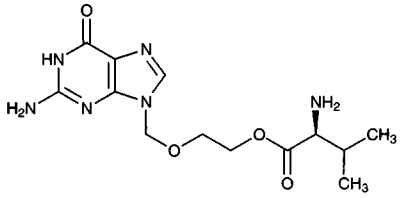
CHROMATOGRAM
Retention time: 11
Limit of detection: 1000 ng

OTHER SUBSTANCES
Extracted: chenodiol, bile acids, deoxycholic acid

KEY WORDS
SPE

REFERENCE
Batta,A.K.; Shefer,S.; Batta,M.; Salen,G. Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography, *J.Lipid Res.*, **1985**, *26*, 690–698.

Valacyclovir



Molecular formula: $C_{13}H_{20}N_6O_4$
Molecular weight: 324.34
CAS Registry No.: 124832-26-4, 124832-27-5 (HCl)
Merck Index: 10039

SAMPLE
Matrix: tissue
Sample preparation: Blend 20% intestinal tissue in Ringers buffer at high speed for 2 min. Centrifuge the homogenate at 12000 g for 15 min at 4°. Remove a 50 μL aliquot of the supernatant, add 450 μL stopping solution. Centrifuge at 12000 g for 5 min at 4°, inject an aliquot of the supernatant. (Stopping solution was an ice cold mixture of 200 μL MeOH and 200 μL pH 6.5 buffer. Buffer was 15 mM 2-[N-morpholino]-ethanesulfonic acid (MES), 130 mM NaCl, 5 mM KCl, and 0.01% PEG-3350.)

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18S
Column: 250 × 4.6 Supelcosil LC-18S
Mobile phase: Water:buffer 20:80 (Buffer was MeOH:100 mM potassium phosphate monobasic 25:75, adjusted to pH 6.7 with 1 M NaOH. Water:buffer ratio may be adjusted to ensure separation of other compounds.)
Flow rate: 1
Detector: UV 252

CHROMATOGRAM
Limit of quantitation: 1 μM

OTHER SUBSTANCES
Simultaneous: p-aminohippuric acid sodium salt, amoxicillin, ampicillin, cefadroxil, cephradine, formycin B, quinine, stavudine, thymidine, valine

KEY WORDS
rat; intestine

REFERENCE
Sinko,P.J.; Balimane,P.V. Carrier-mediated intestinal absorption of valacyclovir, the L-valyl ester prodrug of acyclovir: 1. Interactions with peptides, organic anions and organic cations in rats, *Biopharm.Drug Dispos.*, **1998**, *19*, 209–217.

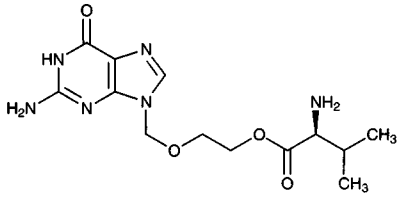
CHROMATOGRAM
Retention time: 11
Limit of detection: 1000 ng

OTHER SUBSTANCES
Extracted: chenodiol, bile acids, deoxycholic acid

KEY WORDS
SPE

REFERENCE
Batta,A.K.; Shefer,S.; Batta,M.; Salen,G. Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography, *J.Lipid Res.*, **1985**, *26*, 690–698.

Valacyclovir



Molecular formula: $C_{13}H_{20}N_6O_4$
Molecular weight: 324.34
CAS Registry No.: 124832-26-4, 124832-27-5 (HCl)
Merck Index: 10039

SAMPLE
Matrix: tissue
Sample preparation: Blend 20% intestinal tissue in Ringers buffer at high speed for 2 min. Centrifuge the homogenate at 12000 g for 15 min at 4°. Remove a 50 μL aliquot of the supernatant, add 450 μL stopping solution. Centrifuge at 12000 g for 5 min at 4°, inject an aliquot of the supernatant. (Stopping solution was an ice cold mixture of 200 μL MeOH and 200 μL pH 6.5 buffer. Buffer was 15 mM 2-[N-morpholino]-ethanesulfonic acid (MES), 130 mM NaCl, 5 mM KCl, and 0.01% PEG-3350.)

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18S
Column: 250 × 4.6 Supelcosil LC-18S
Mobile phase: Water:buffer 20:80 (Buffer was MeOH:100 mM potassium phosphate monobasic 25:75, adjusted to pH 6.7 with 1 M NaOH. Water:buffer ratio may be adjusted to ensure separation of other compounds.)
Flow rate: 1
Detector: UV 252

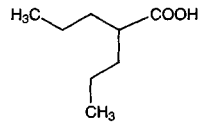
CHROMATOGRAM
Limit of quantitation: 1 μM

OTHER SUBSTANCES
Simultaneous: p-aminohippuric acid sodium salt, amoxicillin, ampicillin, cefadroxil, cephradine, formycin B, quinine, stavudine, thymidine, valine

KEY WORDS
rat; intestine

REFERENCE
Sinko,P.J.; Balimane,P.V. Carrier-mediated intestinal absorption of valacyclovir, the L-valyl ester prodrug of acyclovir: 1. Interactions with peptides, organic anions and organic cations in rats, *Biopharm.Drug Dispos.*, **1998**, *19*, 209–217.

Valproic acid



Molecular formula: $C_8H_{16}O_2$
Molecular weight: 144.21
CAS Registry No.: 99-66-1, 1069-66-5 (sodium salt), 76584-70-8 (divalproex, 1:1 mixture of valproic acid and sodium valproate)
Merck Index: 10049

SAMPLE

Matrix: blood
Sample preparation: Mix 20μL serum with 100 μg/mL IS, add 400 μL 300 mM pH 2.5 citrate/phosphate buffer, stir the mixture, add it to a 350 mg Extrelut SPE cartridge, adsorb for 20 min, elute with 12 mL chloroform. Add 25 μL 0.1% NaOH in MeOH to the eluate and concentrate it in vacuum. Mix the residue with 25 μL each 2% thionyl chloride in chloroform and 8% triethylamine in chloroform, stir at room temperature for 30 s, add 150 μL 10 mg/mL 9-aminophenanthrene in chloroform, stir at -10 to -5° for 90 min. Inject a 10μL aliquot of the reaction mixture directly. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 150 × 6 5 μm Shimpack CLC-ODS
Mobile phase: MeCN:MeOH:water 11:23:6
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 303 em 376

CHROMATOGRAM

Retention time: 15.2
Internal standard: cyclohexane carboxylic acid (10.8)
Limit of detection: 9.4 pg
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES

Noninterfering: phenobarbital, phenytoin, carbamazepine

KEY WORDS

serum; derivatization; SPE

REFERENCE

Nakajima,M.; Sato,A.; Shimada,K. Determination of serum valproate by high-performance liquid chromatography using fluorescence labeling with 9-aminophenanthrene, *Anal.Sci.*, **1988**, *4*, 385–388.

SAMPLE

Matrix: blood
Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 13.5
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: ethosuximide, primidone, phenobarbital, phenytoin, carbamazepine

KEY WORDS
plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: formulations

Sample preparation: Capsules. Dissolve 10 broken capsules in 200 mL acetone. Remove a 20 mL aliquot and make it up to 100 mL with acetone:water 45:55. Remove a 1 mL aliquot and make it up to 10 mL with acetone, mix. Remove a 1 mL aliquot and add it to 1 mL IS solution, add 50 μL 12.8 mg/mL 2-bromoacetophenone (phenacyl bromide) in acetone, add 50 μL 10 mg/mL triethylamine in acetone, mix with gentle swirling, heat at 50° for 2 h, cool to room temperature, inject an aliquot. Syrup. Dilute 5 mL syrup to 100 mL with acetone:water 45:55. Remove a 1 mL aliquot and make it up to 10 mL with acetone, mix. Remove a 1 mL aliquot and add it to 1 mL IS solution, add 50 μL 12.8 mg/mL 2-bromoacetophenone (phenacyl bromide) in acetone, add 50 μL 10 mg/mL triethylamine in acetone, mix with gentle swirling, heat at 50° for 2 h, cool to room temperature, inject an aliquot. Tablets. Weigh out powdered tablets equivalent to 250 mg valproic acid, add 50 mL acetone:water 45:55, sonicate for 10 min, make up to 100 mL with acetone:water 45:55, sonicate for 10 min, mix, centrifuge an aliquot of the suspension at 4000 rpm for 5 min. Remove a 1 mL aliquot of the supernatant and make it up to 10 mL with acetone, mix. Remove a 1 mL aliquot and add it to 1 mL IS solution, add 50 μL 12.8 mg/mL 2-bromoacetophenone (phenacyl bromide) in acetone, add 50 μL 10 mg/mL triethylamine in acetone, mix with gentle swirling, heat at 50° for 2 h, cool to room temperature, inject an aliquot. (Prepare IS solution by diluting a 400 μg/mL solution of sodium caproate in acetone:water 45:55 with an equal volume of acetone.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb-MV C18
Mobile phase: MeCN:MeOH:water 50:20:30
Flow rate: 2
Injection volume: 50
Detector: UV 245

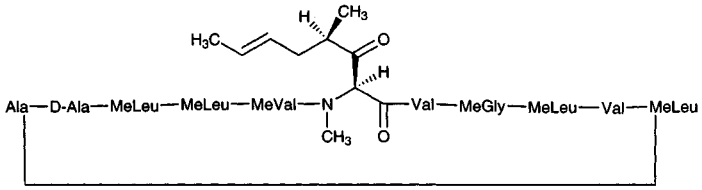
CHROMATOGRAM
Retention time: 8.5
Internal standard: caproic acid (4.5)

KEY WORDS
derivatization; capsules; syrup; tablets

REFERENCE
Lau-Cam,C.A.; Roos,R.W. HPLC method with precolumn phenacylation for the assay of valproic acid and its salts in pharmaceutical dosage forms, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2075–2087.

Valspodar

Molecular formula: $C_{63}H_{111}N_{11}O_{12}$
Molecular weight: 1214.65
CAS Registry No.: 121584-18-7



SAMPLE

Matrix: blood
Sample preparation: Mix whole blood with pH 9.0 borate buffer and saturated aqueous NaCl, add MTBE:ethyl acetate 50:50, extract, evaporated the extract under vacuum, reconstitute the residue in MeCN:water 40:60, inject an aliquot.

HPLC VARIABLES

Column: phenyl-bonded silica gel
Mobile phase: MeCN:water:MTBE 45:40:15
Column temperature: 70
Detector: UV 210

CHROMATOGRAM

Limit of quantitation: 30 ng/mL

KEY WORDS

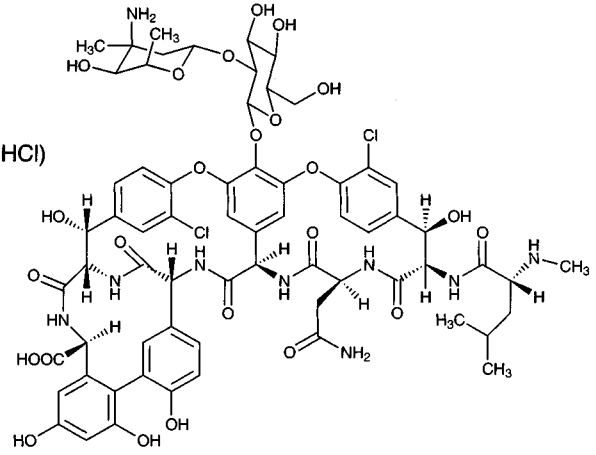
pharmacokinetics; whole blood;

REFERENCE

Mueller,E.A.; Kovarik,J.M.; üresin,Y.; Preisig-Flückiger,S.S.; Hensel,S.; Lücker,P.W.; Holt,B. Optimizing the absorption of valspodar, a P-glycoprotein modulator, PartI: Selecting an oral formulation and exploring its clinical pharmacokinetics/dynamics, *J.Clin.Pharmacol.*, **1997**, *37*, 1001–1008.

Vancomycin

Molecular formula: $C_{66}H_{75}Cl_2N_9O_{24}$
Molecular weight: 1449.27
CAS Registry No.: 1404-90-6, 1404-93-9 (HCl)
Merck Index: 10066



SAMPLE

Matrix: blood
Sample preparation: Condition a 800 µL 500 mg Sep-Pak Vac 3cc C18 SPE cartridge twice with 800 µL MeOH and with 800 µL water. 200 µL Serum + 200 µL water + 50 µL 100 mg/mL IS, vortex. Add to the SPE cartridge. Wash twice with 800 µL water. Elute twice with 400 µL MeCN:50 mM KH_2PO_4 70:30 and twice with 400 µL MeCN:water 50:50. Evaporate the eluate under a stream of nitrogen at 50° for 10 min. Cool, centrifuge at 1450-1475 g for 4 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: KGCQ-324C (YMC, Wilmington, NC)
Column: 250 × 4.6 5 µm YMC pack ODS-AQ (YMC, Wilmington, NC)
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 6 phosphate buffer 5:4:91. B was MeCN:MeOH:50 mM pH 6 phosphate buffer 8:8:84. A:B 100:0 for 2 min, to 0:100 over 9 min, maintain at 0:100 for 14 min, to 100:0 over 5 min, maintain at 100:0 for 5 min (Prepare buffer as follows. Dissolve 11.94 g KH_2PO_4 and 2.14 g K_2HPO_4 in 2 L water.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 18
Internal standard: cefazolin (27)
Limit of quantitation: 1 µg/mL

OTHER SUBSTANCES

Extracted: degradation products
Simultaneous: acetaminophen, salicylates, theophylline

KEY WORDS

serum; SPE

REFERENCE

Backes,D.W.; Aboleneen,H.I.; Simpson,J.A. Quantitation of vancomycin and its crystalline degradation product (CDP-1) in human serum by high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1281–1287.

SAMPLE

Matrix: blood
Sample preparation: 50 µL Serum + 9 µL 125 µg/mL trimethoprim + 75 µL acetone:10% trichloroacetic acid 1:2, vortex for 5 s, centrifuge for 4 min. Remove 62.5 µL of the supernatant

and add it to 62.5 μL 50 mM KH_2PO_4, add 250 μL diethyl ether, vortex for 10 s, centrifuge for 5 min, filter (0.45 μm) the lower aqueous layer, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 75 × 4.6 TSK gel ODS-80TM (Tosoh)
Mobile phase: MeCN:50 mM pH 6.0 KH_2PO_4 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 12
Internal standard: trimethoprim (30)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
serum; comparison with fluorescence polarization immunoassay

REFERENCE
Morishige,H.; Shuto,H.; Ieiri,I.; Otsubo,K.; Oishi,R. Instability of standard calibrators may be involved in overestimating vancomycin concentrations determined by fluorescence polarization immunoassay, *Ther.Drug Monit.*, **1996**, *18*, 80–85.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 5 μm C18 reverse-phase
Mobile phase: MeCN:5 mM KH_2PO_4 22:78
Flow rate: 1.5
Detector: UV 229

CHROMATOGRAM
Retention time: 1.32
Internal standard: salicylic acid (3.94)

KEY WORDS
stability-indicating; ophthalmic solutions

REFERENCE
Tse,M.M.; Liu,C.-M.; Wu,J.; Gee,W.L.; Lin,E.T. HPLC analysis of omeprazole in human plasma (Abstract APQ 1018), *Pharm.Res.*, **1996**, *13*, S7.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute vancomycin injection with water to a drug concentration of 50 mg/mL, dilute with artificial tears solution (Alcon) to a concentration of 31 mg/mL. Dilute 1000-fold with water and inject a 200 μL aliquot.

HPLC VARIABLES
Column: Microsorb MV C18 (Rainin)
Mobile phase: MeCN:5 mM pH 2.8 Na_3PO_4 78:22
Flow rate: 1.5
Injection volume: 200
Detector: UV 229

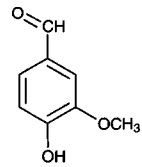
CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
ophthalmic solution; artificial tears; powder; stability-indicating

REFERENCE
Fuhrman,L.C.,Jr.; Stroman,R.T. Stability of vancomycin in an extemporaneously compounded ophthalmic solution, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1386–1388.

Vanillin



Molecular formula: $C_8H_8O_3$
Molecular weight: 152.15
CAS Registry No.: 121-33-5
Merck Index: 10069

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapack C18
Mobile phase: Gradient. A was 0.5% formic acid in water. B was 0.5% formic acid in MeOH. A: B 95:5 for 5 min, to 70:30 over 15 min (Waters curve 9), to 60:40 over 10 min (Waters curve 9), to 55:45 over 10 min (Waters curve 9), to 50:50 over 10 min (Waters curve 9) (J.Chromatogr.A, 1994, 683, 31).
Flow rate: 1
Injection volume: 10
Detector: F following post-column reaction. The column effluent flowed through a 10 m × 0.33 mm ID coil of PTFE tubing irradiated with five 8 w low-pressure mercury lamp to the detector. The design of the photoreactor is detailed in the paper.

CHROMATOGRAM
Retention time: 4.5 (protocatechualdehyde), 6.5 (2,5-dihydroxybenzaldehyde), 7.5 (4-hydroxybenzaldehyde), 8.5 (3-hydroxybenzaldehyde), 12.5 (salicaldehyde), 15 (vanillin), 17 (isovanillin), 24 (o-vanillin), 31.5 (m-anisaldehyde), 40 (veratraldehyde), 42.5 (2,4-dimethoxybenzaldehyde), 43.5 (3,5-dimethoxybenzaldehyde)

KEY WORDS
post-column photochemical derivatization

REFERENCE
Lores,M.; Garcia,C.M.; Cela,R. Selectable-power photoreactor for flow-injection analysis systems and high-performance liquid chromatography post-column photochemical derivatization, *J.Chromatogr.A*, **1996**, *724*, 55–65.

Vasopressin

Molecular formula: $C_{46}H_{65}N_{15}O_{12}S_2$
Molecular weight: 1084.25
CAS Registry No.: 9034-50-8
Merck Index: 10073

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:buffer 50:50, inject a 5-75 μL aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) [Procedure was not necessarily validated for this compound.]

HPLC VARIABLES

Column: 250 × 2 5 μm Ultrasphere octyl
Mobile phase: Gradient. A was MeOH containing 10 mM sodium octanesulfonate. B was buffer containing 10 mM sodium octanesulfonate. A:B from 45:55 to 70:30 over 30 min, maintain at 70:30 for 1 h. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)
Column temperature: 60
Flow rate: 0.3
Injection volume: 5-75
Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 400 mM NaOH pumped at 0.15 mL/min and 0.05% ninhydrin pumped at 0.05 mL/min and the mixture flowed through a 12 m × 0.33 mm i.d. reaction coil at 70° to the detector.

CHROMATOGRAM

Retention time: 17
Limit of detection: 100 fmole

OTHER SUBSTANCES

Extracted: adrenocorticotropin, angiotensin I, angiotensin II, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, gonadorelin (LHRH), somatoliberin

KEY WORDS

plasma; SPE; post-column reaction

REFERENCE

Rhodes,G.R.; Boppana,V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J.Chromatogr.*, **1988**, *444*, 123–131.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C8 SPE cartridge with 4 mL MeOH and 4 mL water, do not allow to run dry. Immediately after preparation add 12 μL 8 M acetic acid to each 1 mL of plasma. 1 mL Acidified plasma + 4 mL 100 mM HCl, add to the SPE cartridge, wash with 4 mL MeCN:0.1% trifluoroacetic acid 10:90, elute with 1.5 mL MeCN:0.1% trifluoroacetic acid 60:40. Evaporate the eluate to dryness under reduced pressure, reconstitute with 10 mM ammonium acetate containing 0.1% bovine serum albumin, inject a 500 μL aliquot (of pooled extracts).

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18

Mobile phase: Gradient. MeOH:10 mM pH 4.15 ammonium acetate from 15:85 to 55:45 over 40 (?) min
Flow rate: 1
Injection volume: 500
Detector: UV 210

CHROMATOGRAM
Retention time: 23

KEY WORDS
rat; plasma; SPE

REFERENCE
Van de Heijning,B.J.M.; Koekkoek-van den Herik,I.; Iványi,T.; Van Wimersma Greidanus,T.B. Solid-phase extraction of plasma vasopressin: evaluation, validation and application, *J.Chromatogr.*, **1991**, *565*, 159–171.

SAMPLE
Matrix: blood, tissue
Sample preparation: Condition a Sep-Pak ODS SPE cartridge with MeOH. Homogenize 500 mg tissue with 6 mL 100 mM pH 7.4 Tris buffer. Acidify a 2 mL aliquot of plasma or tissue homogenate with 200 μL 1 M HCl, add to the SPE cartridge, elute with 3 mL MeOH over 3 min, elute with 2 mL over 1 min. Evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 280 × 5 Dynamax 300-A C8 (Rainin)
Mobile phase: MeCN:water 20:80 containing 0.1% trifluoroacetic acid, 50 mM heptanesulfonic acid and 30 mM triethylamine, pH adjusted to 2.5 with Na_2HPO_4
Flow rate: 1
Injection volume: 20
Detector: UV 200-400

CHROMATOGRAM
Retention time: 6.89 (arginine vasopressin)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: lypressin, oxytocin

KEY WORDS
pig; plasma; SPE; heart

REFERENCE
Rao,P.S.; Weinstein,G.S.; Wilson,D.W.; Rujikarn,N.; Tyras,D.H. Isocratic high-performance liquid chromatography-photodiode-array detection method for determination of lysine- and arginine-vasopressins and oxytocin in biological samples, *J.Chromatogr.*, **1991**, *536*, 137–142.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10-20 μL aliquot of a 500 μg/mL solution in water.

HPLC VARIABLES
Column: 250 × 5 10 μm Nucleosil 10 C18
Mobile phase: MeOH:50 mM pH 6.5 ammonium acetate 39:61
Flow rate: 2
Injection volume: 10-20
Detector: UV 220

OTHER SUBSTANCES
Simultaneous: vasopressin analogs

REFERENCE
Lindeberg,G. Separation of vasopressin analogues by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *193*, 427–431.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 5 Nova-Pak C18
Mobile phase: Gradient. MeCN:10 mM pH 5.0 acetate buffer 15:85 for 10 min, then to 18:82 over 5 min, maintain at 18:82 for 25 min.
Flow rate: 1
Detector: UV 214, UV 280

CHROMATOGRAM
Retention time: 9.9 (arginine vasopressin)

OTHER SUBSTANCES
Simultaneous: lypressin, oxytocin

KEY WORDS
hippopotamus

REFERENCE
Rouille,Y.; Chauvet,M.T.; Chauvet,J.; Acher,R.; Hadley,M.E. The distribution of lysine vasopressin (lysipressin) in placental mammals: a reinvestigation of the Hippopotamidae (*Hippopotamus amphibius*) and Tayassuidae (*Tayassu angulatus*) families, *Gen.Comp.Endocrinol.*, **1988**, *71*, 475–483.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Kromasil (Alltech)
Mobile phase: MeCN:pH 7 phosphate buffer 20:80
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 3 (8-arginine vasopressin)

OTHER SUBSTANCES
Simultaneous: oxytocin

REFERENCE
Supelco Catalog, **1993**, p. 525.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 mL water and 3 mL MeCN (in this order ?). Homogenize 400 mg brain tissue with 2 mL 100 mM HCl, add 20 μL 10 μM IS, add 2 mL acetone, mix, centrifuge at 2450 g for 15 min. Remove the supernatant and add it to 220 μL 1 M sodium bicarbonate and 500 μL 100 mM disodium EDTA, centrifuge at 2450 g for 15 min. Remove the supernatant and evaporate it to remove the acetone, dilute the aqueous residue with 2 mL water, add to the SPE cartridge. wash with 1 mL water, wash with 3 mL 100 mM HCl, wash with two 3 mL portions of dichloromethane, wash with 1 mL water, wash with 2 mL 100 mM pH 8.0 phosphate buffer, wash with 2 mL water, elute with 2 mL MeCN:100 mM pH 2.3 phosphate buffer 70:30. Evaporate the eluate under reduced pressure, make up to 400 μL with water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 200 × 4 5 μm TSKgel ODS-120T (Tosoh)
Mobile phase: Gradient. A was MeCN:300 mM pH 2.3 sodium phosphate buffer:water 1:20:79. B was MeCN:300 mM pH 2.3 sodium phosphate buffer:water 60:20:20. A:B from 90:10 to 55:45 over 33 min, maintain at 55:45 for 7 min, to 0:100 (step gradient), maintain at 0:100.
Flow rate: 1
Injection volume: 100
Detector: F ex 325 em 435 following post-column reaction. The column effluent mixed with 2 mM benzoin in 1.6 M KOH containing 700 mM 2-mercaptoethanol and this mixture flowed through a 15 m × 0.33 mm ID PTFE coil at 76 ± 1°. The effluent from this coil mixed with 500 mM Tris containing 2.1 M HCl pumped at 0.4 mL/min and this mixture flowed to the detector.

CHROMATOGRAM
Retention time: 19.1
Internal standard: [D-Phe^{11}]-neurotensin (40.0)
Limit of detection: 4.4 pmole

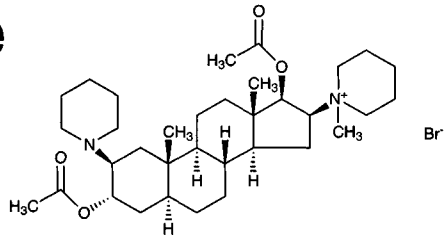
OTHER SUBSTANCES
Extracted: bradykinin, dynorphin 1-8, gonadorelin, kallidin, leucine enkephalin-Arg, methionine enkephalin-Arg-Gly-Leu, methionine enkephalin-Arg-Phe, α-neoendorphin, β-neoendorphin, neurotensin, substance P

KEY WORDS
post-column reaction; rat; brain; SPE

REFERENCE
Ohno,M.; Kai,M.; Ohkura,Y. High-performance liquid chromatographic determination of substance P-like arginine-containing peptide in rat brain by on-line post-column fluorescence derivatization with benzoin, *J.Chromatogr.*, **1989**, *490*, 301–310.

Vecuronium bromide

Molecular formula: $C_{34}H_{57}BrN_2O_4$
Molecular weight: 637.74
CAS Registry No.: 50700-72-6
Merck Index: 10075



SAMPLE
Matrix: bile, blood, perfusate, tissue, urine
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL MeOH:MeCN 2:1 and 1 mL water. Acidify 1 mL plasma with 150 μL 1 M NaH_2PO_4. Homogenize liver, centrifuge. Dilute 1 μL urine or bile to 1 mL with 100 mM pH 3 NaH_2PO_4. Add 20-200 ng IS to 1 mL acidified plasma, liver homogenate supernatant, diluted urine, diluted bile, or liver perfusate, add to the SPE cartridge, wash with 1 mL water, wash with 1 mL 100 mM pH 3 NaH_2PO_4, elute with 400 μL mobile phase, discard first 100 μL eluate, inject a 200 μL aliquot of the remaining eluate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Dioxane:water 20:80 containing 100 mM NaH_2PO_4 and 0.44 mM 9,10-dimethoxyanthracene-2-sulfonate, pH adjusted to 3 with phosphoric acid. (Caution! Dioxane is a carcinogen!) (After each series of analyses flush column with 200 mL MeOH then re-equilibrate with 120 mL mobile phase.)
Flow rate: 1
Injection volume: 200

Detector: F ex 380 em 452 following post-column extraction. The column effluent mixed with dichloroethane pumped at 1.6 mL/min and the mixture flowed through a 1 m × 0.25 mm i.d. stainless steel coil to a phase separator (Anal. Chim. Acta 1987, 192, 267) then the organic phase flowed through the detector.

CHROMATOGRAM
Retention time: 13
Internal standard: 1-(3α,17β-diacetoxy-2β-piperidino-5α-androstan-16β,5α-yl)piperidine(16)
Limit of detection: 5 ng/mL

KEY WORDS
SPE; pharmacokinetics; rat; dog; human; cat; plasma; liver; post-column reaction; post-column extraction

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REFERENCE
Paanakker,J.E.; Thio,J.M.; Van den Wildenberg,H.M.; Kaspersen,F.M. Assay of vecuronium in plasma using solid-phase extraction, high-performance liquid chromatography and post-column ion-pair extraction with fluorimetric detection, *J.Chromatogr.*, **1987**, *421*, 327–335.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 250 μL picric acid (1:10 dilution of saturated picric acid solution) + 250 μL pancuronium solution + 250 μL water + 5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 μL MeCN: water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 μPorasil
Mobile phase: MeCN:2 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100
Detector: conductivity 2500 nS full scale

CHROMATOGRAM
Retention time: 4.7
Internal standard: pancuronium (5.7)
Limit of detection: 10 ng/mL

KEY WORDS
plasma

REFERENCE
Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Blood + 1 mL 1 M pH 2.5 KH_2PO_4 + 50 μL 6 μg/mL d-tubocurarine + 1 mL 3% perchloric acid + 12 mL dichloromethane, rotate for 20 min, centrifuge at 1520 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 25 mm long CN guard column
Column: 250 × 4.6 Spherisorb S5 CN
Mobile phase: MeCN:100 mM pH 5 phosphate 50:50
Column temperature: 40
Flow rate: 1.5

Detector: UV 214 or E, BAS LC-4B, LC-17A thin-layer flow cell, working glassy carbon electrode (W1) +0.65 V, quantitating glassy carbon electrode (W2) +1.05 V, Ag/AgCl reference electrode and auxiliary electrode

CHROMATOGRAM
Retention time: 1 (relative retention time)
Internal standard: d-tubocurarine (relative retention time = 0.5)
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Noninterfering: atropine, apresoline, haloperidol, fentanyl, labetalol, thiopental, atracurium, diazepam

REFERENCE
Hu,O.Y.; Chou,C.H.; Ho,W.; Ho,S.T. Determination of vecuronium in blood by HPLC with UV and electrochemical detection: a pilot study in man, *Proc.Natl.Sci.Counc.Repub.China.[B].*, **1991**, *15*, 186–190.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C1 SPE cartridge with two 3 mL portions of MeOH and two 3 mL portions of water. Add 1 mL plasma, 1 mL water, and 100 μL 15 μg/mL IS in 100 mM pH 5.0 $(NH_4)H_2PO_4$ to the SPE cartridge. Wash with 3 mL water, wash with 3 mL MeCN, wash with 3 mL MeOH, elute with two 500 μL portions of 10 mM sodium perchlorate in MeOH under gravity for 3 min then under vacuum. Evaporate the eluate to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:33 mM phosphoric acid 40:60, adjusted to pH 5.55 with ammonium hydroxide
Flow rate: 2
Injection volume: 30
Detector: E, Environmental Science Associates Coulochem 5100A, 5010 analytical cell, screen electrode +0.4 V (detector 1), working electrode +0.8 V (detector 2)

CHROMATOGRAM
Retention time: 7.3
Internal standard: Org 7465 (dipropionyl ester of vecuronium) (9.8)
Limit of quantitation: 3.9 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; SPE

REFERENCE
Ducharme,J.; Varin,F.; Bevan,D.R.; Donati,F.; Théorêt,Y. High-performance liquid chromatography-electrochemical detection of vecuronium and its metabolites in human plasma, *J.Chromatogr.*, **1992**, *573*, 79–86.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 0.5% solution in the mobile phase, inject a 20 μL aliquot.

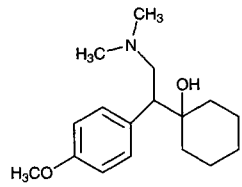
HPLC VARIABLES
Column: 250 × 4 5 μm SI 100 (Bio Separation Technologies)
Mobile phase: MeCN:100 mM sodium perchlorate 96:4
Flow rate: 1
Injection volume: 20
Detector: UV 213

CHROMATOGRAM
Retention time: 4.2

OTHER SUBSTANCES
Simultaneous: pipecuronium
Interfering: pancuronium

REFERENCE
Gazdag,M.; Babják,M.; Kemenes-Bakos,P.; Görög,S. Analysis of steroids. XLI. Ion-pair high-performance liquid chromatographic separation of quaternary ammonium steroids on silica, *J.Chromatogr.*, **1991**, *550*, 639–644.

Venlafaxine



Molecular formula: $C_{17}H_{27}NO_2$
Molecular weight: 277.41
CAS Registry No.: 93413-69-5, 99300-78-4 (HCl)
Merck Index: 10079
Lednicer No.: 4 26

SAMPLE
Matrix: blood
Sample preparation: Mix 50 μL 100 ng/mL maprotiline with 1 mL plasma and 400 μL 700 mM pH 9.7 bicarbonate buffer, vortex for 1 min, add 1.5 mL isoamyl alcohol:hexane 7.5:92.5, shake at 300 rpm for 30 min. Centrifuge at 1500 g for 15 min, freeze at -20° overnight, decant the organic layer into a tube containing 200 μL 0.05% orthophosphoric acid. Shake vigorously at 300 rpm for 45 min, centrifuge at 1500 g for 15 min, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 13 × 4.3 5 μm C4/E butyl-bonded reversed-phase (MetaChem Technologies)
Column: 150 × 4.6 5 μm C4/E butyl-bonded reversed-phase(MetaChem Technologies)
Mobile phase: MeCN:40 mM pH 6.8 sodium phosphate buffer 50:50
Flow rate: 1.5
Injection volume: 100
Detector: F ex 276 em 598

CHROMATOGRAM
Retention time: 7
Internal standard: maprotiline (10)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Vu,R.L.; Helmeste,D.; Albers,L.; Reist,C. Rapid determination of venlafaxine and O-desmethylvenlafaxine in human plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1997**, *703*, 195–201.

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Carboxymethyl Isolute SPE cartridge with 1 mL MeOH and 1 mL 25 mM pH 6.8 phosphate buffer, dry under vacuum. 500 μL Plasma + 20 ng IS, add

to the SPE cartridge, wash with two 1 mL portions of 25 mM pH 6.8 phosphate buffer, dry under vacuum, elute with 1 mL 1% ammonia in MeOH, evaporate to dryness under vacuum at 40°, reconstitute the residue in 100 μL MeOH, vortex, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS/CN
Mobile phase: MeOH:50 mM pH 4.8 potassium phosphate buffer 70:30
Flow rate: 1
Injection volume: 25
Detector: E, ESA, Model 5100 A, Model 5010 analytical cell +650 mV on channel 1, +950 mV on channel 2, Model 5020 guard cell +980 mV

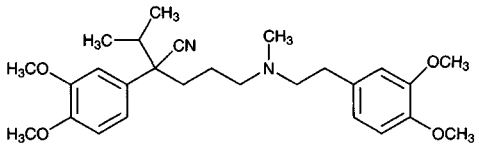
CHROMATOGRAM
Retention time: 8.8
Internal standard: paroxetine (9.6), desipramine (11.5)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Interfering: m-chlorophenylpiperazine

KEY WORDS
plasma; SPE

REFERENCE
Clement,E.M.; Odontiadis,J.; Franklin,M. Simultaneous measurement of venlafaxine and its major metabolite, oxydesmethylvenlafaxine, in human plasma by high-performance liquid chromatography with coulometric detection and utilisation of solid-phase extraction, *J.Chromatogr.B*, **1998**, *705*, 303–308.

Verapamil



Molecular formula: $C_{27}H_{38}N_2O_4$
Molecular weight: 454.61
CAS Registry No.: 52-53-9, 152-11-4 (HCl)
Merck Index: 10083
Lednicer No.: 4 34

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 M NaOH + IS, extract with 5 mL pentane: dichloromethane 2:1. Remove the organic layer and evaporate it to dryness under a gentle stream of nitrogen, reconstitute the residue in mobile phase, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 10 μm Chiralpak AD (Chiral Technologies, Exton, PA)
Mobile phase: n-Hexane:isopropanol:diethylamine 92.5:7.5:0.1
Column temperature: 32
Flow rate: 0.2
Injection volume: 1
Detector: MS, SCIEX API 300 tandem mass, positive ion mode, nebulizer 440°, scan 455.0/165.0

CHROMATOGRAM
Retention time: 4.04 (S), 4.75 (R)
Internal standard: (+)-glaucine (5.49)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral; small-bore

REFERENCE
Alebic-Kolbah,T.; Zavitsanos,A.P. Chiral bioanalysis by normal phase high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry, *J.Chromatogr.A*, **1997**, *759*, 65–77.

SAMPLE
Matrix: blood
Sample preparation: Condition column A with two 995 μL portions of mobile phase at 3 mL/min and then with 995 μL solution B. Wash the donor channel of the dialyzer (Gilson ASTED XL fitted with a Cuprophan cellulose acetate membrane with a molecular mass cut-off of 15000) with 2 mL solution A at 3 mL/min and wash the acceptor channel with 2 mL solution B. Add 50 μL 1.2 μg/mL gallopamil in water to 450 μL plasma, mix by bubbling. Dialyze a 370 μL aliquot against 9 mL solution B is pumped through the acceptor channel at 1 mL/min. Pass the dialysate through column A, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B. (Solution A was 10 mM pH 3 acetate buffer containing 0.01% Triton X-100 and 50 μg/mL sodium azide. Solution B was 10 mM pH 3 acetate buffer containing 50 μg/mL sodium azide.)

HPLC VARIABLES
Column: A 30 μm Nucleosil CN; B 5 μm LiChrospher 100 RP-18 guard column + 4 μm Superspher 100 RP-18
Mobile phase: MeCN:2-aminoheptane:buffer 25:0.5:75 (Buffer was 10 mM sodium acetate adjusted to pH 3.0 with acetic acid.)
Column temperature: 35
Flow rate: 0.9
Detector: F ex 275 em 310

CHROMATOGRAM
Retention time: 12.5
Internal standard: gallopamil (15)
Limit of detection: 1.3 ng/mL
Limit of quantitation: 4.3 ng/mL

OTHER SUBSTANCES
Extracted: norverapamil

KEY WORDS
dialysate; column-switching; plasma

REFERENCE
Ceccato,A.; Chiap,P.; Hubert,P.; Toussaint,B.; Crommen,J. Automated determination of verapamil and norverapamil in human plasma with on-line coupling of dialysis to high-performance liquid chromatography and fluorimetric detection, *J.Chromatogr.A*, **1996**, *750*, 351–360.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 100 μL 200 ng/mL R-(+)-propranolol in mobile phase, 100 μL 1 M NaOH, and 5 mL diethyl ether. Shake for 10 min and centrifuge at 1000 g for 10 min. Evaporate 4 mL of the diethyl ether layer to dryness under nitrogen at 40°, reconstitute the residue with 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralpak AD (Daicel)
Mobile phase: Hexane:2-propanol:diethylamine 93.9:6:0.1
Flow rate: 1.2
Injection volume: 50
Detector: F ex 272 em 312

CHROMATOGRAM
Retention time: 10 (S-(-)), 12 (R-(+))

Internal standard: R-(+)-propranolol (7.8)
Limit of detection: 500 pg/mL (S-(-)), 1.3 ng/mL (R-(+))

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
chiral; pharmacokinetics; plasma

REFERENCE
Hashiguchi,M.; Ogata,H.; Maeda,A.; Hirashima,Y.; Ishii,S.; Mori,Y.; Amamoto,T.; Handa,T.; Otsuka,N.; Irie,S.; Urae,A.; Urae,R.; Kimura,R. No effect of high-protein food on the stereoselective bioavailability and pharmacokinetics of verapamil, *J.Clin.Pharmacol.*, **1996**, *36*, 1022–1028.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

CHROMATOGRAM
Retention time: 15.365

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cell culture media, microsomal incubations
Sample preparation: 250 µL Sample + 20 µL 50 µg/mL IS + 6 mL diethyl ether, vortex in shaker for 20 min, centrifuge at 4000 g for 20 min. Remove a 5 mL aliquot of the organic layer, add 250 µL 100 mM HCl, vortex in an overhead shaker for 20 min, centrifuge at 4000 g for 20 min, discard the organic phase, inject a 40 µL of the aqueous phase.

HPLC VARIABLES
Column: 150 × 8 4 µm Nucleosil 100-3 C8
Mobile phase: MeCN:MeOH:buffer 28:5:67, adjusted to pH 4.2 with 1 M HCl (Prepare the buffer by dissolving 0.71 g NaH_2PO_4 and 2 g 1-heptanesulfonic acid sodium salt in 1 L water.)
Column temperature: 51
Flow rate: 1.7

Injection volume: 40
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 19
Internal standard: norverapamil (7)
Limit of quantitation: 200 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Fischer,U.; Wacke,R.; Stange,J.; Nitschke,F.-P.; Adam,U.; Drewelow,B. Rapid HPLC assay for verapamil and its metabolites: use for application to in vitro studies, *Pharmazie*, **1996**, *51*, 220–223.

SAMPLE
Matrix: cells
Sample preparation: Add 20 μL 33% silver nitrate solution to a suspension of 2×10^6 cells, agitate for 10 s, sonicate for 20 min (Bransonic 52, Vel, Belgium), add 140 μL MeCN, vortex for 5 min, cool at 4° for 30 min, centrifuge at 10000 g for 30 s, add 200 μL 200 mM pH 3 phosphate buffer, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Hibar LiChrocart RP 18 (Merck)
Mobile phase: MeCN:buffer 35:65 (Buffer was 200 mM KH_2PO_4 containing 0.2% triethylamine, adjusted to pH 3.0 with 200 mM orthophosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 237

CHROMATOGRAM
Retention time: 12.4
Internal standard: altretamine (6.2)
Limit of detection: 8 pmol
Limit of quantitation: 27 pmol

OTHER SUBSTANCES
Extracted: daunorubicin, doxorubicin, vincristine, S 9788

KEY WORDS
human; cells; epidermoid carcinoma

REFERENCE
Tassin,J.P.; Dubois,J.; Atassi,G.; Hanocq,M. Simultaneous determination of cytotoxic (adriamycin, vincristine) and modulator of resistance (verapamil, S 9788) drugs in human cells by high-performance liquid chromatography and ultraviolet detection, *J.Chromatogr.B*, **1997**, *691*, 449–456.

SAMPLE
Matrix: hepatocyte cultures, microsomal incubations
Sample preparation: Add 20 μL 50 μg/mL IS and 6 mL diethyl ether to 250 μL hepatocyte culture or microsomal incubation, shake for 20 min, centrifuge at 4000 g for 20 min. Remove 5 mL of the organic phase and add it to 250 μL 100 mM HCl. Shake for 20 min and centrifuge at 4000 g for 20 min. Inject a 40 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 150 × 8 4 μm Nucleosil 100-3 C8

Mobile phase: MeCN:MeOH:buffer 28:5:67, adjusted to pH 4.2 with 1 M HCl (Buffer was 710 mg sodium dihydrogen phosphate and 2.0 g 1-heptanesulfonic acid sodium salt in 1 L distilled water.)
Column temperature: 51
Flow rate: 1.7
Injection volume: 40
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 18
Internal standard: D 600 (Knoll AG, Germany) (16.5)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver; rat

REFERENCE
Fischer,U.; Rohde,B.; Wacke,R.; Stange,J.; Nitschke,F.P.; Adam,U.; Drewelow,B. Prediction of in vivo drug interaction from in vitro systems exemplified by interaction between verapamil and cimetidine using human liver microsomes and primary hepatocytes, *J.Clin.Pharmacol.*, **1997**, *37*, 1150–1159.

SAMPLE
Matrix: perfusate
Sample preparation: Dilute perfusate with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Chiral AGP (Chromotech, Sweden)
Column: 150 × 4.0 Chiral AGP (Chromotech, Sweden)
Mobile phase: MeCN: pH 7.6 phosphate buffer (I=0.01) 22:78
Column temperature: 30
Flow rate: 1
Injection volume: 50
Detector: F ex 232 em 310

CHROMATOGRAM
Limit of detection: 2.9 ng/mL
Limit of quantitation: 5.5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
chiral; intestine

REFERENCE
Sandström,R.; Karlsson,A.; Knutson,L.; Lennernäs,H. Jejeunal absorption and metabolism of R/S-verapamil in humans, *Pharm.Res.*, **1998**, *15*, 856–862.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in water, mix a 1 mL aliquot with 100 μL 13.36 μg/mL IS, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.0 5 μm Chiral-AGP (Baker)
Mobile phase: MeCN:pH 6.8 (I=0.01) ammonium acetate 11:89 (Buffer was adjusted to pH 6.8 with ammonium hydroxide or acetic acid.)
Column temperature: 22

Flow rate: 0.9
Injection volume: 20
Detector: UV 225; MS, Finnigan MAT SSQ 710A, interface particle beam, desolvation chamber 45°, nebulizing gas helium, electron impact mode 70 eV, source 250°, filament current 200 μA, electron multiplier 1500 V, m/z 45-400

CHROMATOGRAM
Retention time: 9.74 ((2R)-(+)), 12.02 ((2S)-(-))
Internal standard: procaine hydrochloride (5.09)
Limit of detection: 109 ng/mL ((2R)-(+)), 114 ng/mL ((2S)-(-))

OTHER SUBSTANCES
Also analyzed: gallopamil

KEY WORDS
chiral

REFERENCE
Rustichelli,C.; Ferioli,V.; Gamberini,G. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry, *Chromatographia*, **1997**, *44*, 477–483.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 7.8

OTHER SUBSTANCES
Simultaneous: cimetidine, clomipramine, halofantrine, haloperidol, minoxidil, reserpine

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in water, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.0 5 μm Chiral-AGP (Baker)
Mobile phase: Gradient. A was MeCN. B was isopropanol. C was pH 6.8 (I = 0.01) ammonium acetate adjusted to pH 6.8 with ammonium hydroxide or acetic acid. A:B:C from 11:1:88 to 7:1:92 over 1.5 min, maintain at 7:1:92 for 35 min, to 11:1:88 over 5 min
Column temperature: 22
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 14 ((2R)-(+)), 19 ((2S)-(-))

OTHER SUBSTANCES
Simultaneous: gallopamil

KEY WORDS
chiral

REFERENCE
Rustichelli,C.; Ferioli,V.; Gamberini,G. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry, *Chromatographia*, **1997**, *44*, 477–483.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 10.96

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: Condition a 7 mm/3 mL 3M Empore C8 SPE disc (Varian Associates, CA) with 500 μL MeOH and two 500 μL portions of water. Mix 500 μL urine, 50 μL 400 ng/mL IS in MeOH:water 50:50, and 500 μL 30 mM pH 10 phosphate buffer. Add to the SPE disc. Wash twice with 500 μL water, twice with 500 μL MeCN:water 30:70, elute with two 500 μL portions of MeOH, apply full vacuum for a few seconds. Evaporate to dryness at 60°. Reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: NewGuard C18
Column: 250 × 4.6 10μm Chiralcel OD-R CSP
Mobile phase: MeCN:200 mM sodium perchlorate 40:60
Flow rate: 0.8
Injection volume: 100
Detector: F ex 230 em 312

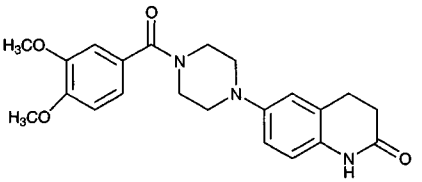
CHROMATOGRAM
Retention time: 21.8 (R-), 24.2 (S-)
Internal standard: (+)-glaucine (16.5)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
chiral; SPE

REFERENCE
Asafu-Adjaye,E.B.; Shiu,G.K. Solid-phase extraction-high-performance liquid chromatography determination of verapamil and norverapamil enantiomers in urine, *J.Chromatogr.B*, **1998**, *707*, 161–167.

Vesnarinone



Molecular formula: $C_{22}H_{25}N_3O_4$
Molecular weight: 395.46
CAS Registry No.: 81840-15-5
Merck Index: 10105
Lednicer No.: 5 122

SAMPLE
Matrix: blood, microsomal incubations, urine
Sample preparation: Condition a 3 mL 200 mg UCT C18 (United Chemical Technologies, Bristol PA) SPE cartridge with 2 mL MeOH, 2 mL 10 mM ascorbic acid, and 2 mL 80 mM ammonium acetate solution. 500 μL Microsomal incubation + 500 μL MeOH, mix, centrifuge at 9500 g for 10 min. Add 100 μL supernatant from the microsomal incubation, 500 μL plasma, or 50 μL urine to 50 μL MeOH and 50 μL 1 μg/mL IS solution, vortex, add 1 mL 50 mM ammonium acetate, vortex for 30 s, centrifuge at 3000 rpm for 10 min. Add the mixture to the SPE cartridge, wash with 2 mL 50 mM ammonium acetate and 2 mL n-butyl chloride. Dry the cartridge under a light vacuum, elute with three 1 mL portions of MeOH:6 M acetic acid:10 mM ascorbic acid 95:2:3. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue with 500 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 45 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:0.1% acetic acid 2.5:97.5. B was MeCN:0.1% acetic acid 80:20. A:B from 100:0 to 0:100 over 7 min.
Detector: MS, PE Sciex API III, turbo ion spray interface in MS/MS mode, liquid nitrogen nebulizer auxiliary gas, ultrahigh purity nitrogen, argon curtain gas and collision gas

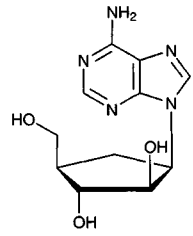
CHROMATOGRAM
Internal standard: OPC-8192 (Otsuka Pharmaceutical, Japan)
Limit of detection: 200 ng/mL (plasma), 500 ng/mL (urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Wandel,C.; Lang,C.C.; Cowart,D.C.; Girard,A.F.; Bramer,S.; Flockhart,D.A.; Wood,A.J.J. Effect of CYP3A inhibition on vesnarinone metabolism in humans, *Clin.Pharmacol.Ther.*, **1998**, *63*, 506–511.

Vidarabine



Molecular formula: $C_{10}H_{13}N_5O_4$
Molecular weight: 267.24
CAS Registry No.: 5536-17-4, 24356-66-9 (monohydrate), 29984-33-6 (phosphate), 71002-10-3 (sodium phosphate)
Merck Index: 10113
Lednicer No.: 4 122

SAMPLE

Matrix: aqueous humor, blood, urine
Sample preparation: Plasma. Filter (Amicon CF25 or CF50A Centriflo membrane cones) plasma while centrifuging at 100 g for 1 h. 160 μL Ultrafiltrate + 20 μL 20 μg/mL IS in water + 20 μL 100 mM pH 6.3 sodium acetate buffer, mix, inject a 10 μL aliquot. Urine. Filter (Schleicher and Schüll paper No. 497) urine, dilute the filtrate with water, inject an aliquot. Aqueous humor. Inject directly.

HPLC VARIABLES

Column: 150 × 3.7 Aminex A-28 (equilibrate in 2 M sodium acetate for 24 h before use) (Bio-Rad)
Mobile phase: 7.5 mM pH 6.4 Sodium borate buffer containing 2.5 mM sodium acetate
Column temperature: 60
Flow rate: 0.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 6.5
Internal standard: 8-aminoarabinosyladenine (9.0)

OTHER SUBSTANCES

Extracted: metabolites, adenosine, 9-β-D-arabinofuranosylhypoxanthine

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Schneider,H.G.; Glazko,A.J. High-performance liquid chromatography of adenine and hypoxanthine arabinosides, *J.Chromatogr.*, **1977**, *139*, 370–375.

SAMPLE

Matrix: blood
Sample preparation: Prepare plasma rapidly, keep at 4°. Mix 250 μL plasma and 50 μL water at 4°, vortex for 5 s, add 1 mL MeCN with vortexing, centrifuge at 4° at 13000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 400 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 45 × 4.6 5 μm Ultrasphere Octadecyl
Column: 250 × 4.6 5 μm Ultrasphere Octadecyl
Mobile phase: MeCN:buffer 5:95 (Buffer was 50 mM $(NH_4)H_2PO_4$ adjusted to pH 6.0 with 100 mM NaOH.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 12
Internal standard: vidarabine

OTHER SUBSTANCES
Extracted: sinefungin

KEY WORDS
rat; plasma; vidarabine is IS

REFERENCE
Tharasse-Bloch,C.; Brasseur,P.; Favennec,L.; Marchand,J. Determination of sinefungin in rat plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *674*, 247–252.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Filter (Amicon Centriflo CF25) plasma, urine, or CSF while centrifuging at 1100 g for 20 min, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 10 mM KH_2PO_4. B was MeOH:10 mM KH_2PO_4 30:70, pH 4.9. A:B from 90:10 to 70:30 over 15 min. (At the end of each day flush column with 20-30 mL B. If column becomes contaminated wash with water, 1 mM phosphoric acid, water, MeOH:water 70:30, and water for 15-30 min.)
Flow rate: 1
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM
Retention time: 17.24
Limit of detection: 10 pmole (100 μL injection)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: adenine, adenosine, arabinosylhypoxanthine, 5-bromodeoxyuridine, deoxyadenosine, deoxycoformycin, deoxycytidine, deoxyguanosine, deoxyinosine, guanosine, hypoxanthine, inosine, N^1-methyladenosine, thymidine, uric acid

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Agarwal,R.P.; Major,P.P.; Kufe,D.W. Simple and rapid high-performance liquid chromatographic method for analysis of nucleosides in biological fluids, *J.Chromatogr.*, **1982**, *231*, 418–424.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Add pentostatin to blood samples to prevent deamination. Dilute urine 1:10 with water. 200 μL Serum, CSF, or diluted urine + 20 μL isoamyl alcohol + 50 μL chloroform, vortex for 30 s, centrifuge at 20931 g for 10 min. Remove the aqueous layer and add it to 1 mL cold acetone (0°), vortex for 10 s, centrifuge for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb RP-8
Mobile phase: MeCN:buffer 4:96 (Buffer was 5 mM sodium pentanesulfonate, pH 7.2.)
Column temperature: 40
Flow rate: 1
Detector: UV 250

CHROMATOGRAM
Retention time: 6.65
Limit of detection: 2.5 μg/mL (urine), 500 ng/mL (serum, CSF)

OTHER SUBSTANCES
Extracted: metabolites, pentostatin
Noninterfering: chlorothiazide, cytosine arabinoside, guanine arabinoside, hydroxyzine, kanamycin, metaproterenol, nystatin, penicillin G, phenobarbital, prednisone, sulfamethoxazole, theophylline, trimethoprim, uracil arabinoside

KEY WORDS
serum

REFERENCE
Bowman,D.B.; Kauffman,R.E. Reversed-phase high-performance liquid chromatographic method to determine vidarabine and hypoxanthine arabinoside in biological fluids, *J.Chromatogr.*, **1982**, *229*, 487–491.

SAMPLE
Matrix: blood, urine
Sample preparation: Centrifuge plasma or urine at 1000 g at 4° for 10 min. Add 1 mL supernatant to 1 mL 12% trichloroacetic acid (in an ice bath), mix thoroughly, let stand for 15 min, centrifuge at 4° at 1000 g for 30 min. Remove the supernatant and extract it four times with water-saturated diethyl ether, discard the ether extracts. Remove traces of ether from the aqueous layer by heating it at 65° with intermittent suction, cool. Remove 500 μL of the aqueous layer and add it to 200 μL 40 mM chloroacetaldehyde in 28 mM pH 5.1 sodium acetate buffer. Heat at 80° for 40 min, cool, extract four times with 1 mL portions of water-saturated diethyl ether, discard the ether extracts. Heat the aqueous layer at 65° with intermittent suction to remove traces of ether, cool. Dilute urine preparation 1:9, do not dilute plasma preparations, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4 μBondapak C18/Corasil
Column: 250 × 4.6 Spherisorb S5 ODSII octadecyl
Mobile phase: Gradient. A was 20 mM sodium tetraborate decahydrate adjusted to pH 7.7 with 4.4 N phosphoric acid. B was MeOH:20 mM sodium tetraborate decahydrate adjusted to pH 7.7 with 4.4 N phosphoric acid 30:70. A:B from 100:0 to 0:100 over 15 min, maintain at 0:100 for 5 min, return to initial conditions, re-equilibrate for 5 min.
Flow rate: 1
Injection volume: 50
Detector: F ex 315 em 415

CHROMATOGRAM
Retention time: 15.2
Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES
Extracted: Ara-AMP, ara-H (UV detection)

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
McCann,W.P.; Hall,L.M.; Siler,W.; Barton,N.; Whitley,R.J. High-pressure liquid chromatographic methods for determining arabinosyladenine-5'-monophosphate, arabinosyladenine, and arabinosylhypoxanthine in plasma and urine, *Antimicrob.Agents Chemother.*, **1985**, *28*, 265–273.

SAMPLE
Matrix: blood, urine
Sample preparation: Filter urine (0.45 μm). Filter plasma (Centrifree Micropartition, Amicon) while centrifuging, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: Gradient. MeCN:50 mM pH 4.8 ammonium acetate from 0.5:99.5 to 12:88 over 40 min

Flow rate: 1
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 29

OTHER SUBSTANCES
Extracted: ara-H, ara-MAP, ara-DMAP

KEY WORDS
plasma; rat; monkey; pharmacokinetics; ultrafiltrate

REFERENCE
Lambe,C.U.; Resetar,A.ctor,T.; Koszalka,G.W.; Nelson,D.J. Metabolism and pharmacokinetics of the anti-varicella-zoster virus agent 6-dimethylaminopurine arabinoside, *Antimicrob.Agents Chemother.*, **1992**, *36*, 353–360.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 100 mg vidarabine in a little warm water, add 1 mL DMSO, make up to 100 mL with water. Remove a 125 μL aliquot, add 1.25 mL 100 μg/mL inosine in water, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: C18 (Brownlee)
Column: 250 × 4.6 Partisil 10/ODS-3
Mobile phase: MeCN:buffer 5:95 (Buffer was 10 mM KH_2PO_4 adjusted to pH 3.250 ± 0.005 with acetic acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: inosine (6.5)

OTHER SUBSTANCES
Simultaneous: impurities, adenosine, 2'-deoxyadenosine

REFERENCE
Schroeder,W.,III; Cupps,T.L.; Townsend,L.B. Quantitative high-performance liquid chromatography of structurally similar nucleosides, *J.Chromatogr.*, **1983**, *254*, 315–321.

SAMPLE
Matrix: fermentation solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Phenomenex C8
Mobile phase: MeCN:MeOH:50 mM $(HN_4)_2HPO_4$ 2.5:2.5:95 adjusted to pH 7.4 with phosphoric acid
Flow rate: 1.5
Detector: UV 258

CHROMATOGRAM
Retention time: 9.1

OTHER SUBSTANCES
Extracted: cytosine, coformycin, (8S)-pentostatin, 2'-deoxyguanosine, pentostatin

REFERENCE
Showalter,H.D.H.; Bunge,R.H.; French,J.C.; Hurley,T.R.; Leeds,R.L.; Leja,B.; McDonnell,P.D.; Edmunds,C.R. Improved production of pentostatin and identification of fermentation cometabolites, *J.Antibiot.(Tokyo)*, **1992**, *45*, 1914–1918.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 30 μL of the injection to 100 mL with buffer, add 50 μL 10 mg/mL 5-flucytosine in water, inject a 20 μL aliquot. (Buffer contained 1 g/L $Na_2HPO_4.2H_2O$ and 0.363 g/L KH_2PO_4, pH 7.4.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC 18
Mobile phase: 10 mM KH_2PO_4 adjusted to pH 6.8 with 25% KOH
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.5 (vidarabine phosphate)
Internal standard: 5-flucytosine (k' 0.9)

KEY WORDS
injections; water

REFERENCE
Kwee,M.S.L.; Stolk,L.M.L. Formulation of a stable vidarabine phosphate injection, *Pharm.Weekbl.[Sci.]*, **1984**, *6*, 101–104.

SAMPLE
Matrix: perfusate
Sample preparation: 1 mL Perfusate (Krebs solution) + 40 μL chloroacetaldehyde + 360 μL buffer, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4, 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80-85° which contains 1-1.15 M chloroacetaldehyde, store at 0° (Anal. Biochem. 1984, 137, 93).)

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Ultron N-phenyl (Shinwa, Kyoto)
Column: 150 × 4.6 5 μm Ultron N-phenyl (Shinwa, Kyoto)
Mobile phase: MeCN:buffer 1.5:98.5, adjusted to pH 4.5 with 2-diethylaminoethanol (Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4, pH 4.0.)
Flow rate: 1
Detector: F ex 305 em 420

CHROMATOGRAM
Retention time: 17
Internal standard: vidarabine

OTHER SUBSTANCES
Extracted: adenosine, adenosine diphosphate, adenosine monophosphate, adenosine triphosphate

KEY WORDS
derivatization; vidarabine is IS

REFERENCE
Mohri,K.; Takeuchi,K.; Shinozuka,K.; Bjur,R.A.; Westfall,D.P. Simultaneous determination of nerve-induced adenine nucleotides and nucleosides released from rabbit pulmonary artery, *Anal.Biochem.*, **1993**, *210*, 262–267.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 3 Partisil XS 10/25 C8
Mobile phase: MeCN:buffer 5:95 (Buffer was 800 mL 75 mM sodium borate and 200 mL 10 mM $(NH_4)H_2PO_4$, pH adjusted to 6.03 with boric acid.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: metabolites, adenosine, 9-β-D-arabinofuranosylhypoxanthine, 2'-deoxyadenosine, 2'-deoxyinosine, inosine

REFERENCE
Delia,T.J.; Kirt,D.D. Reversed-phase high-performance liquid chromatographic separation of ribosyl, 2'-deoxyribosyl and arabinosyl nucleosides of adenine and hypoxanthine, *J.Chromatogr.*, **1982**, *243*, 173–177.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 5:95 (Buffer was 50 mM pH 3.0 phosphate containing 0.4 mM sodium 1-heptanesulfonate.)
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 270

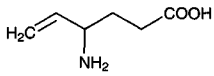
CHROMATOGRAM
Retention time: 9.4
Internal standard: vidarabine

OTHER SUBSTANCES
Simultaneous: cytarabine, uracil arabinoside
Noninterfering: allopurinol, cephalosporins, ciprofloxacin, diazepam, metoclopramide, mitoxantrone, ondansetron

KEY WORDS
vidarabine is IS

REFERENCE
Burk,M.; Volmer,M.; Fartash,K.; Schneider,W. Ion-pair liquid chromatography of cytarabine and uracil-arabinoside in human plasma, *Arzneimittelforschung*, **1995**, *45*, 616–619.

Vigabatrin



Molecular formula: $C_6H_{11}NO_2$
Molecular weight: 129.16
CAS Registry No.: 60643-86-9
Merck Index: 10114

SAMPLE

Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 100 μg/mL in MeOH:water 10:90 + 1 mL MeOH, vortex, centrifuge at 200 g. Mix 2 volumes of supernatant with 1 volume of reagent, let stand for 1 min, inject a 10 μL aliquot. (Reagent was 10 mL 30 mg/mL o-phthaldialdehyde and 200 μL 2-mercaptoethanol made up to 50 mL with 400 mM pH 9.5 borate buffer.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Microsorb C18
Mobile phase: MeCN:MeOH:10 mM orthophosphoric acid 30:10:60
Flow rate: 2
Injection volume: 10
Detector: F ex 370 em 418-700 (filter)

CHROMATOGRAM

Retention time: 5.6
Internal standard: gamma-phenyl-gamma-aminobutyric acid (13.1)
Limit of detection: 80 ng/mL
Limit of quantitation: 540 ng/mL

OTHER SUBSTANCES

Noninterfering: carbamazepine, carbamazepine epoxide, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid

KEY WORDS

serum; derivatization

REFERENCE

Tsanaclis,L.M.; Wicks,J.; Williams,J.; Richens,A. Determination of vigabatrin in plasma by reversed-phase high-performance liquid chromatography, *Ther.Drug Monit.*, **1991**, *13*, 251–253.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 500 μL IS solution + 1 mL MeCN, vortex for 5 min, centrifuge for 15 min. Mix 6 μL buffer, 6 μL reagent, and 6 μL supernatant, let stand for 1 min, inject the whole amount. (Prepare IS solution by dissolving 100 mg gamma-phenyl-gamma-aminobutyric acid and 10 mg 1-(aminomethyl)cycloheptane acetic acid in 500 mL MeCN and 500 mL water. Prepare buffer by dissolving 15.5 mg boric acid in 500 mL water and adjusting to pH 9.5 with concentrated NaOH. Prepare reagent by mixing 100 mg o-phthaldialdehyde, 9 mL MeOH, 1 mL buffer, and 100 μL mercaptoethanol.)

HPLC VARIABLES

Column: 250 × 4 5 μm BANsil C18 (ASMT, Enger, Germany)
Mobile phase: Gradient. A was MeCN:MeOH:0.1% pH 2 phosphoric acid 10:10:80. B was MeCN:MeOH 50:50. A:B 90:10 for 1 min, to 30:70 over 25 min, maintain at 30:70 for 3 min, return to initial conditions over 0.1 min, re-equilibrate for 3.9 min.
Column temperature: 40
Flow rate: 1
Injection volume: 18
Detector: F ex 235 em 435

CHROMATOGRAM

Retention time: 19.9

Internal standard: gamma-phenyl-gamma-aminobutyric acid (Marion Merrel Dow) (23.4), 1-(aminomethyl)cycloheptane acetic acid (Gö-3609, Parke Davis) (28.3)
Limit of detection: 100 ng/mL
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Extracted: gabapentin

KEY WORDS
derivatization; serum; degas mobile phase continuously with helium

REFERENCE
Juergens,U.H.; May,T.W.; Rambeck,B. Simultaneous HPLC determination of vigabatrin and gabapentin in serum with automated pre-injection derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1459–1471.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 50 μL serum or urine (diluted between 1:50 and 1:200) to 1 mL MeOH containing 3.4 μg γ-phenyl-γ-amino-n-butyric acid, vortex for 15 s, centrifuge at 2000 g for 10 min, mix 6 μL of the supernatant with 3 μL reagent, inject an aliquot. (Reagent was 10 mL 30 mg/mL o-phthaldialdehyde and 200 μL 2-mercaptoethanol made up to 50 mL with 400 mM pH 9.5 borate buffer (Ther. Drug Monit. 1991, 13, 251).)

HPLC VARIABLES
Column: 125 × 3 5 μm Superspher 60 RP-Select B (Merck)
Mobile phase: Gradient. A was MeCN. B was 20 mM KH_2PO_4 buffer. A:B from 22:78 to 37:63 in 12 min, from 37:63 to 55:45 in 6 min, from 55:45 to 80:20 in 1.5 min, maintain at 80:20 for 2 min
Column temperature: 35
Flow rate: 0.7
Detector: F ex 230 em 455

CHROMATOGRAM
Retention time: 10.3
Internal standard: γ-phenyl-γ-amino-n-butyric acid (14.8)
Limit of detection: 500 nM

OTHER SUBSTANCES
Extracted: gabapentin

KEY WORDS
derivatization; serum

REFERENCE
Wad,N.; Krämer,G. Sensitive high-performance liquid chromatographic method with fluorometric detection for the simultaneous determination of gabapentin and vigabatrin in serum and urine, *J.Chromatogr.B*, **1998**, *705*, 154–158.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 20 μL 400 μg/mL IS in water + 200 μL MeCN + 100 μL 30 μM copper chloride, centrifuge at 800 g for 15 min. Remove the supernatant and add it to 200 μL buffer 1 and 200 μL 2 mg/mL dansyl chloride in MeCN, vortex for 5 s, heat at 50° for 15 min, cool to room temperature, wash with 1 mL diethyl ether. Extract the aqueous phase with ethyl acetate. Wash the ethyl acetate layer with 1 mL water. Evaporate the ethyl acetate layer to dryness under a stream of nitrogen at 35°, reconstitute the residue in 2-4 mL mobile phase, inject a 50-100 μL aliquot. Urine. 10 μL Urine + 100 μL water + 20 μL 400 μg/mL IS in water + 200 μL MeCN + 100 μL 15 μM copper chloride, vortex for 5 s, add 200 μL buffer 2, add 200 μL 2 mg/mL dansyl chloride in MeCN, vortex for 5 s, heat at 50° for 15 min, cool to room temperature, wash with 1 mL diethyl ether. Extract the aqueous phase with ethyl acetate. Wash the ethyl acetate layer with 1 mL water. Evaporate the ethyl acetate layer to

dryness under a stream of nitrogen at 35°, reconstitute the residue in 2-4 mL mobile phase, inject a 50-100 μL aliquot. (Buffer 1 was 25 mL 200 mM boric acid and 20 mL 50 mM sodium borate made up to 100 mL, pH 8.45 ± 0.05. Buffer 2 was 50 mL 400 mM boric acid and 20 mL 125 mM sodium borate made up to 100 mL, pH 8.05 ± 0.05.)

HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: MeCN:dioxane:500 mM orthophosphoric acid 35:15:50 (Caution! Dioxane is a carcinogen!)
Flow rate: 1
Injection volume: 50-100
Detector: F ex 345 em 418 (cut-off filter)

CHROMATOGRAM
Retention time: 8.8
Internal standard: gamma-aminobenzenebutanoic acid (13.5)
Limit of detection: 10 μg/mL (urine), 500 ng/mL (plasma)

KEY WORDS
plasma; derivatization; dog; pharmacokinetics

REFERENCE
Smithers,J.A.; Lang,J.F.; Okerholm,R.A. Quantitative analysis of vigabatrin in plasma and urine by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *341*, 232–238.

SAMPLE
Matrix: bulk
Sample preparation: 2.6 mg Vigabatrin + 250 μL 200 mM sodium bicarbonate, stir for 2 min, add 250 μL 65 mg/mL N-(tert-butoxycarbonyl)-L-leucine-N-hydroxysuccinimide ester (N-t-Boc-L-leucine N-hydroxysuccinimide ester) in THF, stir for 30 min, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL trifluoroacetic acid, let stand at room temperature for 5 min, evaporate to dryness under a stream of nitrogen, reconstitute with 10 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-8
Mobile phase: MeCN:50 mM pH 7 phosphate buffer 4:96
Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 4 (R-(-)), 17.5 (S-(+))
Limit of detection: 0.1% (of major enantiomer)

KEY WORDS
chiral; derivatization

REFERENCE
Chen,T.-M.; Contario,J.J. High-performance liquid chromatographic resolution of enantiomers of γ-vinyl-γ-aminobutyric acid, *J.Chromatogr.*, **1984**, *314*, 495–498.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet to a fine powder, add 90 mL mobile phase, stir for 10 min, make up to 100 mL with mobile phase, mix thoroughly, filter (Whatman GF/F). Remove a 10 mL aliquot of the filtrate and make up to 25 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil SCX
Mobile phase: MeCN:MeOH:25 mM pH 2.8 potassium phosphate buffer 0.4:4:100

Flow rate: 1.5
Injection volume: 20
Detector: UV 210

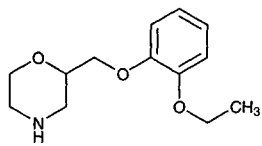
CHROMATOGRAM
Retention time: 6.4

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
tablets; stability-indicating

REFERENCE
Chen,T.-M.; Contario,J.J.; Fike,R.R. High-performance liquid chromatographic assay for vigabatrin and its primary degradation product in a pharmaceutical tablet formulation, *J.Chromatogr.*, **1987**, *398*, 351–354.

Viloxazine



Molecular formula: $C_{13}H_{19}NO_3$
Molecular weight: 237.30
CAS Registry No.: 46817-91-8, 35604-67-2 (HCl)
Merck Index: 10116
Lednicer No.: 2 306

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM
Retention time: 4.49
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam;

tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 10.993

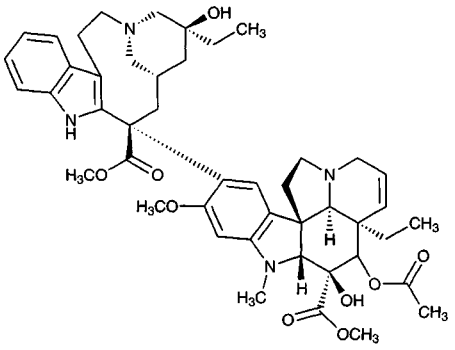
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Vinblastine



Molecular formula: $C_{46}H_{58}N_4O_9$
Molecular weight: 810.99
CAS Registry No.: 865-21-4, 143-67-9 (sulfate)
Merck Index: 10119

SAMPLE

Matrix: blood

Sample preparation: 1.2 mL Serum or plasma + 100 μL water, mix, centrifuge at 1500 g for 5 min, inject a 1 mL aliquot of the clear supernatant on to column A with mobile phase A and elute to waste, after 10 min backflush the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 3 30-38 μm pellicular ODS (Whatman) (replace daily); B 100 × 4.6 3 μm Microspher C18 (Chrompack)

Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:25 mM pH 7.0 phosphate buffer 20:48:32

Flow rate: 1.25

Injection volume: 1000

Detector: UV 300 or E, ANTEC VT-03, wall-jet glassy carbon working electrode + 0.83 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 4.9

Internal standard: vinblastine

OTHER SUBSTANCES

Extracted: vincristine

KEY WORDS

serum; plasma; column-switching; human; cow; vinblastine is IS

REFERENCE

Bloemhof,H.; Van Dijk,K.N.; De Graaf,S.S.N.; Vendrig,D.E.M.M.; Uges,D.R.A. Sensitive method for the determination of vincristine in human serum by high-performance liquid chromatography after on-line column-extraction, *J.Chromatogr.*, **1991**, *572*, 171–179.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 268

CHROMATOGRAM

Retention time: 5.75
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 6 mL ether, agitate slowly for 45 min, centrifuge. Remove the organic layer and evaporate it to 1 mL, add 220 μL pH 2.7 phosphate buffer, agitate slowly for 45 min, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 60:40 (Buffer 75 mM pH 2.70 phosphate buffer containing 100 mg/mL sodium dodecyl sulfate.)
Flow rate: 0.9
Injection volume: 200
Detector: F ex 280 em 360

CHROMATOGRAM
Internal standard: vinblastine

OTHER SUBSTANCES
Extracted: vinorelbine

KEY WORDS
plasma; vinblastine is IS

REFERENCE
Robieux,I.; Sorio,R.; Borsatti,E.; Cannizzaro,R.; Vitali,V.; Aita,P.; Freschi,A.; Galligoni,E.; Monfardini,S. Pharmacokinetics of vinorelbine in patients with liver metastases, *Clin.Pharmacol.Ther.*, **1996**, *59*, 32–40.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 8 mL diethyl ether, agitate slowly for 45 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to 1 mL, add 220 μL 75 mM pH 2.7 phosphate buffer, agitate slowly for 45 min, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: Novapak C18
Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 60:40 (Prepare buffer by mixing 75 mM phosphoric acid and 75 mM KH_2PO_4 in a 5:1 ratio, add sodium dodecyl sulfate to a final concentration of 100 mg/L, adjust pH to 2.80 with 2.5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 200
Detector: F ex 280 em 360

CHROMATOGRAM
Retention time: 8
Internal standard: vinblastine

OTHER SUBSTANCES
Extracted: vinorelbine

KEY WORDS
plasma; vinblastine is IS

REFERENCE
Robieux,I.; Vitali,V.; Aita,P.; Freschi,A.; Lazzarini,R.; Sorio,R. Sensitive high-performance liquid chromatographic method with fluorescence detection for measurement of vinorelbine plasma concentrations, *J.Chromatogr.B*, **1996**, *675*, 183–187.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum or urine + 1 mL 66 mM pH 7 phosphate buffer + 3 (serum) or 5 (urine) mL diethyl ether, rotate at 20 rpm for 30 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 μL MeOH:pH 2 HCl 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm cyano (SGE)
Mobile phase: MeCN:water 55:45 containing 40 mM ammonium acetate, pH adjusted to 3 with HCl
Flow rate: 1
Injection volume: 50
Detector: UV 268

CHROMATOGRAM
Retention time: 4.4

Internal standard: vinblastine

OTHER SUBSTANCES
Extracted: vinorelbine (navelbine)
Noninterfering: aminoglycoside antibiotics, analgesics, carbamazepine, digitoxin, furosemide, glycopeptide antibiotics, β-lactam antibiotics, phenytoin, quinidine, quinolone antibiotics, salicylic acid, theophylline

KEY WORDS
vinblastine is IS; serum

REFERENCE
Jehl,F.; Debs,J.; Herlin,C.; Quoix,E.; Gallion,C.; Monteil,H. Determination of navelbine and desacetylnavelbine in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *525*, 225–233.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL MeOH + 8 mL diethyl ether, shake for 10 min, centrifuge at 900 g for 10 min, freeze at -20° for 45 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 250 μL mobile phase, wash twice with 1 mL hexane, inject a 100 μL aliquot of the aqueous phase. Urine. Centrifuge urine at 900 g. 1 mL Urine + 100 μL MeOH + 500 μL 100 mM pH 7.0 NaH_2PO_4 + 8 mL diethyl ether, shake for 10 min, centrifuge at 900 g for 10 min, freeze at -20° for 45 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 250 μL mobile phase, wash twice with 1 mL hexane, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer 8:50:42 (Buffer was 0.1% NaH_2PO_4 containing 400 mg/L heptanesulfonate and 300 mg/L EDTA, adjusted to pH 3.0 with 1 M phosphoric acid.)
Flow rate: 1.1
Injection volume: 100
Detector: E, Chromatofield Model Eldec 103, glassy carbon electrode 0.93 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4.5
Internal standard: vinblastine

OTHER SUBSTANCES
Extracted: vinorelbine (navelbine)
Simultaneous: acetaminophen, aminophylline, amiodarone, carbocysteine, dextropropoxyphene, diclofenac, doxycycline, glafenine, indomethacin, morphine, noramidopyrine, propoxyphene
Noninterfering: acetylcysteine, albuterol, alizapride, amitriptyline, amoxicillin, aspirin, bromazepam, bromhexine, caffeine, clavulanic acid, clomipramine, clorazepate, diazepam, diprophylline, floctafenine, loperamide, lorazepam, methylprednisolone, metoclopramide, prednisolone, ranitidine, theophylline

KEY WORDS
vinblastine is IS; plasma

REFERENCE
Nicot,G.; Lachatre,G.; Marquet,P.; Bonnaud,F.; Vallette,J.P.; Rocca,J.-L. High-performance liquid chromatographic determination of navelbine in human plasma and urine, *J.Chromatogr.*, **1990**, *528*, 258–266.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 10 μL 10 μg/mL vintriptol in MeCN + 2.5 mL 500 mM pH 4.0 phosphate buffer + 5 mL chloroform, mix for 10 min, centrifuge at 2500 g for

10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL MeCN, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 2 5 μm Spherisorb Si
Mobile phase: MeCN:10 mM pH 3.0 citrate buffer 85:15 containing 10 mM tetrabutylammonium bromide
Flow rate: 0.2
Injection volume: 80
Detector: F ex 270 em 320 (long-pass filter)

CHROMATOGRAM
Retention time: 9
Internal standard: vintriptol (8)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
van Tellingen,O.; Beijnen,J.H.; Baurain,R.; ten Bokkel Huinink,W.W.; van der Woude,H.R.; Nooyen,W.J. High-performance liquid chromatographic determination of vinblastine, 4-O-deacetylvinblastine and the potential metabolite 4-O-deacetylvinblastine-3-oic acid in biological fluids, *J.Chromatogr.*, **1991**, *553*, 47–53.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 8.37

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cells
Sample preparation: Centrifuge cell suspensions at 200 g for 10 min, wash the pellets twice with 2 mL portions of phosphate-buffered saline, add 200 μL EtOH acidified to pH 5.5 with sulfuric acid, vortex for 2 min, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 μm Novapak C18 + 150 × 3.9 4 μm Novapak C18 in series
Mobile phase: MeCN:buffer 60:40 containing 100 mg/L sodium dodecyl sulfate (Buffer was 25 mM phosphate adjusted to pH 2.7 with phosphoric acid.)
Flow rate: 0.9
Injection volume: 25
Detector: F ex 280 em 360 or UV 268

CHROMATOGRAM
Retention time: 5.6
Internal standard: vinblastine
Limit of detection: 12 pmole (UV), 2 pmole (F)

OTHER SUBSTANCES
Extracted: vinorelbine
Noninterfering: acetaminophen, aclacinomycin, amoxicillin, aspirin, daunorubicin, doxorubicin, doxycycline, heparin, insulin, methotrexate, noramidopyrine, rifamycin

KEY WORDS
vinblastine is IS

REFERENCE
Debal,V.; Morjani,H.; Millot,J.-M.; Angiboust,J.-F.; Gourdier,B.; Manfait,M. Determination of vinorelbine (Navelbine) in tumour cells by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *581*, 93–99.

SAMPLE
Matrix: plants
Sample preparation: Freeze leaves with liquid nitrogen, air dry, grind to a fine powder. Mix 0.5 g powder and 2 mL MeOH, sonicate for 30 min, allow to settle, decant the liquid, repeat extraction. Combine the extracts and filter (0.45 μm) them, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsorb C18
Mobile phase: Gradient. MeCN:buffer 15:85 for 2 min, to 40:60 over 58 min, maintain at 40:60 for 5 min, to 95:5 over 5 min, maintain at 95:5 over 5 min. (Prepare buffer by mixing 2 mL trifluoroacetic acid and 1 mL triethylamine in water, make up to 1 L with water, adjust pH to 2.4 with ammonium hydroxide.)
Injection volume: 10
Detector: UV 274

CHROMATOGRAM
Retention time: 45.8

OTHER SUBSTANCES
Extracted: ajmalacine, tetrahydroalstonine, tryptamine, vincamine, vincristine, yohimbine

KEY WORDS
leaves

REFERENCE
Bowman,R.N.; Gerber,R.E.; Terry,M.E. Analysis of anti-cancer alkaloids vincristine & vinblastine, *Rainin Chromatography Update (TB-13)*, **1996**, 1–2.

SAMPLE
Matrix: tissue

Sample preparation: Lyophilize tissue for 24 h, pulverize in a mortar, mix. Weigh out 25-50 mg tissue, add 5 mL 100 mM HCl, sonicate for 30 min, add 5 mL MeCN dropwise with continual vortexing, centrifuge for 30 min. Remove the supernatant and evaporate it under a stream of nitrogen at 60° to remove MeCN, add 10 mL buffer, ad 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 500 μL dichloromethane, inject a 100 μL aliquot. (Buffer was 400 mM pH 3 phosphate buffer containing 50 mM octylsulfate. Silanize all glassware with Surfasil (Pierce).)

HPLC VARIABLES

Guard column: 30 × 4 5 μm LiChrosorb CN
Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: MeCN:40 mM pH 3 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM

Retention time: 19.14
Limit of detection: 10 ng/g

OTHER SUBSTANCES

Extracted: vincristine

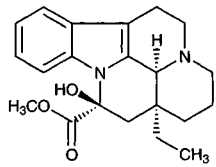
KEY WORDS

heart; kidney; lung; liver; muscle; tumor; mouse; pharmacokinetics

REFERENCE

Van Belle,S.J.-P.; de Smet,M.; De Neve,W.; Monsaert,C.; Storme,G.A.; Massart,D.L. Determination of vinca alkaloids in mouse tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *578*, 223–229.

Vincamine



Molecular formula: $C_{21}H_{26}N_2O_3$
Molecular weight: 354.45
CAS Registry No.: 1617-90-9, 10592-03-7 (HCl)
Merck Index: 10120

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 221.6

CHROMATOGRAM
Retention time: 12.08

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, sal-

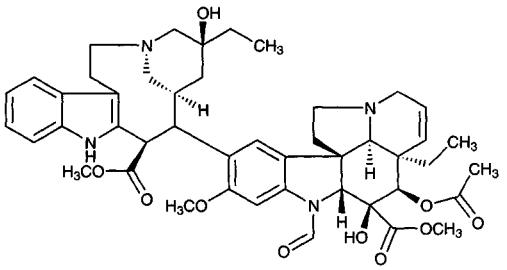
icylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Vincristine

Molecular formula: $C_{46}H_{56}N_4O_{10}$
Molecular weight: 824.97
CAS Registry No.: 57-22-7, 2068-78-2 (sulfate)
Merck Index: 10124



SAMPLE

Matrix: blood
Sample preparation: 1.2 mL Serum or plasma + 100 µL 2 µg/mL vinblastine in water, mix, centrifuge at 1500 g for 5 min, inject a 1 mL aliquot of the clear supernatant on to column A with mobile phase A and elute to waste, after 10 min backflush the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 3 30-38 µm pellicular ODS (Whatman) (replace daily); B 100 × 4.6 3 µm Microspher C18 (Chrompack)
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:25 mM pH 7.0 phosphate buffer 20:48:32
Flow rate: 1.25
Injection volume: 1000
Detector: UV 300 or E, ANTEC VT-03, wall-jet glassy carbon working electrode + 0.83 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 3.7
Internal standard: vinblastine (4.9)
Limit of detection: 1 ng/mL (UV), 0.3 ng/mL (E)

KEY WORDS

serum; plasma; column-switching; human; cow; pharmacokinetics

REFERENCE

Bloemhof,H.; Van Dijk,K.N.; De Graaf,S.S.N.; Vendrig,D.E.M.M.; Uges,D.R.A. Sensitive method for the determination of vincristine in human serum by high-performance liquid chromatography after on-line column-extraction, *J.Chromatogr.*, **1991**, *572*, 171–179.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute

the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 221

CHROMATOGRAM

Retention time: 5.06
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, cells
Sample preparation: Plasma. Add 8 mL MeCN dropwise with continuous vortexing to 4 mL plasma, centrifuge for 30 min. Remove the supernatant and evaporate it to dryness under a

stream of nitrogen at 60°, reconstitute the residue in 10 mL buffer and 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200 μL dichloromethane, inject a 100 μL aliquot. Cells. Centrifuge cell suspension, discard supernatant, add 4 mL 75 mM KCl to pellet, heat at 37° for 30 min, sonicate for 1 min, add 8 mL MeCN dropwise with continuous vortexing, centrifuge for 30 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 10 mL buffer and 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200 μL dichloromethane, inject a 100 μL aliquot. (Buffer was 400 mM pH 3 phosphate buffer containing 50 mM octylsulfate. Silanize all glassware with Surfasil (Pierce).)

HPLC VARIABLES
Guard column: 30 × 4 5 μm LiChrosorb CN
Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: MeCN:120 mM pH 3 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 15
Internal standard: vincristine

OTHER SUBSTANCES
Extracted: vinorelbine (navelbine)
Simultaneous: vinblastine, vindesine

KEY WORDS
plasma; vincristine is IS

REFERENCE
Van Belle,S.J.-P.; de Smet,M.; Monsaert,C.; Geerts,F.; Storme,G.A.; Massart,D.L. High-performance liquid chromatographic determination of navelbine in MO_4 mouse fibrosarcoma cells and biological fluids, *J.Chromatogr.*, **1992**, *576*, 351–357.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 13.765

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cells
Sample preparation: Add 20 μL 33% silver nitrate solution to a suspension of 2×10^6 cells, agitate for 10 s, sonicate for 20 min (Bransonic 52, Vel, Belgium), add 140 μL MeCN, vortex for 5 min, cool at 4° for 30 min, centrifuge at 10000 g for 30 s, add 200 μL 200 mM pH 3 phosphate buffer, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Hibar LiChrocart RP 18 (Merck)
Mobile phase: MeCN:buffer 35:65 (Buffer was 200 mM KH_2PO_4 containing 0.2% triethylamine, adjusted to pH 3.0 with 200 mM orthophosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 237

CHROMATOGRAM
Retention time: 4.9
Internal standard: daunorubicin (4.0)
Limit of detection: 4 pmol
Limit of quantitation: 13 pmol

OTHER SUBSTANCES
Extracted: altretamine, doxorubicin, verapamil, S 9788

KEY WORDS
human; cells; epidermoid carcinoma

REFERENCE
Tassin,J.P.; Dubois,J.; Atassi,G.; Hanocq,M. Simultaneous determination of cytotoxic (adriamycin, vincristine) and modulator of resistance (verapamil, S 9788) drugs in human cells by high-performance liquid chromatography and ultraviolet detection, *J.Chromatogr.B*, **1997**, *691*, 449–456.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot directly.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 100-5CN
Mobile phase: MeCN:MeOH:20 mM pH 4.5 ammonium dihydrogen phosphate in water 20:20:60, containing 10 mM sodium heptanesulfonate
Flow rate: 1.0
Injection volume: 25
Detector: UV 297

CHROMATOGRAM
Retention time: 11.8

OTHER SUBSTANCES
Simultaneous: degradation products, doxorubicin, methylparaben, propylparaben

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Nyhammar,E.K.; Johansson,S.G.; Seiving,B.E. Stability of doxorubicin hydrochloride and vincristine sulfate in two portable infusion-pump reservoirs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1171–1173.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:buffer 50:50 (Buffer was 20 mM KH_2PO_4 adjusted to pH 5.4 with 1 M NaOH
Flow rate: 1
Injection volume: 20
Detector: UV 233

CHROMATOGRAM
Retention time: 14.7
Limit of detection: 90 ng/mL

OTHER SUBSTANCES
Simultaneous: methyl paraben, ondansetron, doxorubicin, degradation products

KEY WORDS
injections; saline

REFERENCE
King,D.T.; Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of a vincristine, doxorubicin, and ondansetron mixture in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1994**, *17*, 1399–1411.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb RP8
Mobile phase: MeOH:buffer 70:30 (Prepare by adding 5 mL diethylamine to 295 mL water and making up to 1 L with MeOH.)
Flow rate: 1.75
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 4.01

OTHER SUBSTANCES
Simultaneous: granisetron

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: plants
Sample preparation: Freeze leaves with liquid nitrogen, air dry, grind to a fine powder. Mix 0.5 g powder and 2 mL MeOH, sonicate for 30 min, allow to settle, decant the liquid, repeat extraction. Combine the extracts and filter (0.45 μm) them, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsorb C18
Mobile phase: Gradient. MeCN:buffer 15:85 for 2 min, to 40:60 over 58 min, maintain at 40:60 for 5 min, to 95:5 over 5 min, maintain at 95:5 over 5 min. (Prepare buffer by mixing 2 mL trifluoroacetic acid and 1 mL triethylamine in water, make up to 1 L with water, adjust pH to 2.4 with ammonium hydroxide.)
Injection volume: 10
Detector: UV 274

CHROMATOGRAM
Retention time: 40.7

OTHER SUBSTANCES
Extracted: ajmalacine, tetrahydroalstonine, tryptamine, vinblastine, vincamine, yohimbine

KEY WORDS
leaves

REFERENCE
Bowman,R.N.; Gerber,R.E.; Terry,M.E. Analysis of anti-cancer alkaloids vincristine & vinblastine, *Rainin Chromatography Update (TB-13)*, **1996**, 1–2.

SAMPLE
Matrix: tissue
Sample preparation: Lyophilize tissue for 24 h, pulverize in a mortar, mix. Weigh out 25-50 mg tissue, add 5 mL 100 mM HCl, sonicate for 30 min, add 5 mL MeCN dropwise with continual vortexing, centrifuge for 30 min. Remove the supernatant and evaporate it under a stream of nitrogen at 60° to remove MeCN, add 10 mL buffer, ad 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 500 μL dichloromethane, inject a 100 μL aliquot. (Buffer was 400 mM pH 3 phosphate buffer containing 50 mM octylsulfate. Silanize all glassware with Surfasil (Pierce).)

HPLC VARIABLES
Guard column: 30 × 4 5 μm LiChrosorb CN
Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: MeCN:40 mM pH 3 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 220

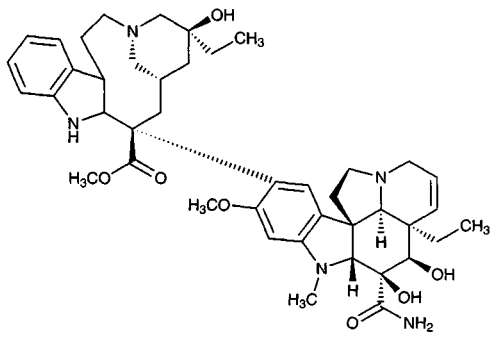
CHROMATOGRAM
Retention time: 15.60
Limit of detection: 10 ng/g

OTHER SUBSTANCES
Extracted: vinblastine

KEY WORDS
heart; kidney; lung; liver; muscle; tumor; mouse; pharmacokinetics

REFERENCE
Van Belle,S.J.-P.; de Smet,M.; De Neve,W.; Monsaert,C.; Storme,G.A.; Massart,D.L. Determination of vinca alkaloids in mouse tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *578*, 223–229.

Vindesine



Molecular formula: $C_{43}H_{55}N_5O_7$
Molecular weight: 753.94
CAS Registry No.: 53643-48-4, 59917-39-4 (sulfate salt)
Merck Index: 10125

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 4.92

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine;

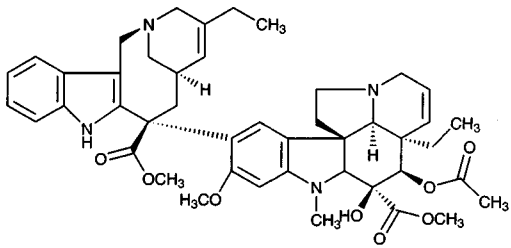
phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Vinorelbine

Molecular formula: $C_{45}H_{54}N_4O_8$
Molecular weight: 778.95
CAS Registry No.: 71486-22-1
Merck Index: 10127



SAMPLE

Matrix: blood
Sample preparation: 400 µL Plasma + 20 µL 10 µg/mL teniposide + 1.6 mL diethyl ether, vortex, centrifuge at 1000 g for 2 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 200 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm LiChrospher 100 RP-18
Mobile phase: MeCN:MeOH:buffer 30:20:50 (Buffer was 20 g/L NaH_2PO_4 containing 0.8 g/L heptanesulfonic acid, pH adjusted to 3.0 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: E, Environmental Sciences Coulochem 5100 A, guard cell +0.90 V (before injector), clean-up cell +0.40 V, detection cell +0.90 V

CHROMATOGRAM

Retention time: 15.5
Internal standard: teniposide (10.6)
Limit of detection: 1 ng/mL

KEY WORDS

plasma; rabbit; pharmacokinetics

REFERENCE

Mouchard-Delmas,C.; Gourdier,B.; Vistelle,R. Determination of vinorelbine in rabbit plasma by high-performance liquid chromatography with coulometric detection, *J.Chromatogr.B*, **1995**, *663*, 390–394.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 10 ng vinblastine + 6 mL ether, agitate slowly for 45 min, centrifuge. Remove the organic layer and evaporate it to 1 mL, add 220 µL pH 2.7 phosphate buffer, agitate slowly for 45 min, inject a 200 µL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 60:40 (Buffer 75 mM pH 2.70 phosphate buffer containing 100 mg/mL sodium dodecyl sulfate.)
Flow rate: 0.9
Injection volume: 200
Detector: F ex 280 em 360

CHROMATOGRAM
Internal standard: vinblastine
Limit of quantitation: 2 ng/mL

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Robieux,I.; Sorio,R.; Borsatti,E.; Cannizzaro,R.; Vitali,V.; Aita,P.; Freschi,A.; Galligoni,E.; Monfardini,S. Pharmacokinetics of vinorelbine in patients with liver metastases, *Clin.Pharmacol.Ther.*, **1996**, *59*, 32–40.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 20 μL 500 ng/mL vinblastine + 8 mL diethyl ether, agitate slowly for 45 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to 1 mL, add 220 μL 75 mM pH 2.7 phosphate buffer, agitate slowly for 45 min, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: Novapak C18
Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 60:40 (Prepare buffer by mixing 75 mM phosphoric acid and 75 mM KH_2PO_4 in a 5:1 ratio, add sodium dodecyl sulfate to a final concentration of 100 mg/L, adjust pH to 2.80 with 2.5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 200
Detector: F ex 280 em 360

CHROMATOGRAM
Retention time: 13
Internal standard: vinblastine (8)
Limit of detection: 1 ng/mL
Limit of quantitation: 2 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Robieux,I.; Vitali,V.; Aita,P.; Freschi,A.; Lazzarini,R.; Sorio,R. Sensitive high-performance liquid chromatographic method with fluorescence detection for measurement of vinorelbine plasma concentrations, *J.Chromatogr.B*, **1996**, *675*, 183–187.

SAMPLE
Matrix: blood, cells
Sample preparation: Plasma. Add 8 mL MeCN dropwise with continuous vortexing to 4 mL plasma, centrifuge for 30 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 10 mL buffer and 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200 μL dichloromethane, inject a 100 μL aliquot. Cells. Centrifuge cell suspension, discard supernatant, add 4 mL 75 mM KCl to pellet, heat at 37° for 30 min, sonicate for 1 min, add 100 μg vincristine, add 8 mL MeCN dropwise with continuous vortexing, centrifuge for 30 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 10 mL buffer and 5 mL

chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200 μL dichloromethane, inject a 100 μL aliquot. (Buffer was 400 mM pH 3 phosphate buffer containing 50 mM octylsulfate. Silanize all glassware with Surfasil (Pierce).)

HPLC VARIABLES
Guard column: 30 × 4 5 μm LiChrosorb CN
Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: MeCN:120 mM pH 3 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 29
Internal standard: vincristine (15)
Limit of detection: 1.25 ng/mL

OTHER SUBSTANCES
Simultaneous: vinblastine, vindesine

KEY WORDS
plasma

REFERENCE
Van Belle,S.J.-P.; de Smet,M.; Monsaert,C.; Geerts,F.; Storme,G.A.; Massart,D.L. High-performance liquid chromatographic determination of navelbine in MO_4 mouse fibrosarcoma cells and biological fluids, *J.Chromatogr.*, **1992**, *576*, 351–357.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum or urine + 100 μL 1 μg/mL vinblastine in water, vortex, add 1 mL 66 mM pH 7 phosphate buffer, add 3 (serum) or 5 (urine) mL diethyl ether, rotate at 20 rpm for 30 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 μL MeOH:pH 2 HCl 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm cyano (SGE)
Mobile phase: MeCN:water 55:45 containing 40 mM ammonium acetate, pH adjusted to 3 with HCl
Flow rate: 1
Injection volume: 50
Detector: UV 268

CHROMATOGRAM
Retention time: 5.6
Internal standard: vinblastine (4.4)
Limit of detection: 5 ng/mL (urine), 2.5 ng/mL (serum)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: aminoglycoside antibiotics, analgesics, carbamazepine, digitoxin, furosemide, glycopeptide antibiotics, β-lactam antibiotics, phenytoin, quinidine, quinolone antibiotics, salicylic acid, theophylline

KEY WORDS
serum; pharmacokinetics

REFERENCE
Jehl,F.; Debs,J.; Herlin,C.; Quoix,E.; Gallion,C.; Monteil,H. Determination of navelbine and desacetylnavelbine in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *525*, 225–233.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μL 1 μg/mL vinblastine in MeOH + 8 mL diethyl ether, shake for 10 min, centrifuge at 900 g for 10 min, freeze at -20° for 45 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 250 μL mobile phase, wash twice with 1 mL hexane, inject a 100 μL aliquot of the aqueous phase. Urine. Centrifuge urine at 900 g. 1 mL Urine + 100 μL 1 μg/mL vinblastine in MeOH + 500 μL 100 mM pH 7.0 NaH_2PO_4 + 8 mL diethyl ether, shake for 10 min, centrifuge at 900 g for 10 min, freeze at -20° for 45 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 250 μL mobile phase, wash twice with 1 mL hexane, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:MeOH:buffer 8:50:42 (Buffer was 0.1% NaH_2PO_4 containing 400 mg/L heptanesulfonate and 300 mg/L EDTA, adjusted to pH 3.0 with 1 M phosphoric acid.)

Flow rate: 1.1

Injection volume: 100

Detector: E, Chromatofield Model Eldec 103, glassy carbon electrode 0.93 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 6.8

Internal standard: vinblastine (4.5)

Limit of detection: 20 ng/mL (urine), 1 ng/mL (plasma)

OTHER SUBSTANCES

Simultaneous: acetaminophen, aminophylline, amiodarone, carbocysteine, dextropropoxyphene, diclofenac, doxycycline, glafenine, indomethacin, morphine, noramidopyrine, propoxyphene

Noninterfering: acetylcysteine, albuterol, alizapride, amitriptyline, amoxicillin, aspirin, bromazepam, bromhexine, caffeine, clavulanic acid, clomipramine, clorazepate, diazepam, diprophylline, floctafenine, loperamide, lorazepam, methylprednisolone, metoclopramide, prednisolone, ranitidine, theophylline

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Nicot,G.; Lachatre,G.; Marquet,P.; Bonnaud,F.; Vallette,J.P.; Rocca,J.-L. High-performance liquid chromatographic determination of navelbine in human plasma and urine, *J.Chromatogr.*, **1990**, *528*, 258–266.

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute 10-20 μL urine with 500 μL blank plasma. 500 μL Plasma or diluted urine + 50 μL 1 μg/mL desacetylvinblastine in MeCN + 4 mL diethyl ether, shake vigorously for 10 min, centrifuge at 4° at 1000 g for 10 min, freeze at -20° for 1 h. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL MeCN, sonicate for 5 min, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 250 × 2 5 μm Spherisorb Si

Mobile phase: MeCN:buffer 85:15 containing 10 mM tetrabutylammonium bromide (Buffer was 10 mM trisodium citrate adjusted to pH 3.0 with HCl.)

Flow rate: 0.2

Injection volume: 80

Detector: F ex 270 em 320 (filter)

CHROMATOGRAM

Retention time: 11

Internal standard: desacetylvinblastine (17)

Limit of detection: 25 ng/mL (urine), 1.5 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
van Tellingen,O.; Kuijpers,A.; Beijnen,J.H.; Baselier,M.R.P.; Burghouts,J.T.M.; Nooyen,W.J. Bio-analysis of vinorelbine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1992**, *573*, 328–332.

SAMPLE
Matrix: cells
Sample preparation: Centrifuge cell suspensions at 200 g for 10 min, wash the pellets twice with 2 mL portions of phosphate-buffered saline, add 20 μL 10 μM vinblastine in water, add 200 μL EtOH acidified to pH 5.5 with sulfuric acid, vortex for 2 min, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot.

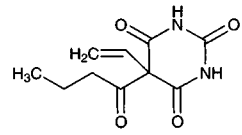
HPLC VARIABLES
Column: 300 × 3.9 4 μm Novapak C18 + 150 × 3.9 4 μm Novapak C18 in series
Mobile phase: MeCN:buffer 60:40 containing 100 mg/L sodium dodecyl sulfate (Buffer was 25 mM phosphate adjusted to pH 2.7 with phosphoric acid.)
Flow rate: 0.9
Injection volume: 25
Detector: F ex 280 em 360 or UV 268

CHROMATOGRAM
Retention time: 9.2
Internal standard: vinblastine (5.6)
Limit of detection: 13 pmole (UV), 8 pmole (F)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, aclacinomycin, amoxicillin, aspirin, daunorubicin, doxorubicin, doxycycline, heparin, insulin, methotrexate, noramidopyrine, rifamycin

REFERENCE
Debal,V.; Morjani,H.; Millot,J.-M.; Angiboust,J.-F.; Gourdier,B.; Manfait,M. Determination of vinorelbine (Navelbine) in tumour cells by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *581*, 93–99.

Vinylbital



Molecular formula: $C_{11}H_{16}N_2O_3$
Molecular weight: 224.26
CAS Registry No.: 2430-49-1
Merck Index: 10131

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

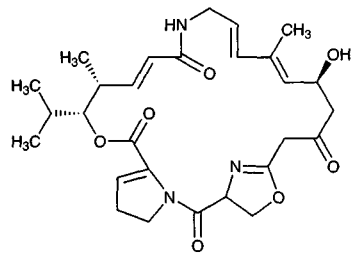
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.583

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Virginiamycin



Molecular formula: $C_{43}H_{49}N_7O_{10}$ (S_1), $C_{28}H_{35}N_3O_7$ (M_1)
Molecular weight: 823.91 (S_1), 525.61 (M_1)
CAS Registry No.: 21411-53-0 (M_1), 23152-29-6 (S_1)
Merck Index: 10142

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

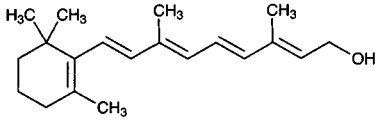
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 227.5

CHROMATOGRAM
Retention time: 17.21

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Vitamin A



Molecular formula: $C_{20}H_{30}O$
Molecular weight: 286.46
CAS Registry No.: 68-26-8
Merck Index: 10150

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL EtOH + 10 mL hexane, mix for 30 s, centrifuge at 3000 rpm for 5 min, store the hexane layer at 15°, repeat the extraction with 10 mL hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with 200 µL isopropanol, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm TSKgel ODS-80Ts
Mobile phase: Gradient. EtOH:water 80:20 for 11.5 min then 87:13 (step gradient)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 460

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Extracted: vitamin E (F ex 298 em 325)

KEY WORDS
serum

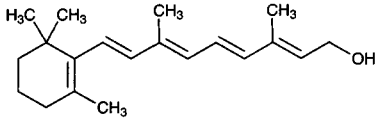
REFERENCE
Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE
Matrix: blood
Sample preparation: Dilute 100 µL serum with 900 µL 12.62 mg/mL pyrogallol in EtOH, filter (450 µm cellulose disk), cool at 15° in the autosampler, inject a 300 µL aliquot onto column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A onto column B with mobile phase B, after another 1 min remove column A from the circuit. Elute column B with mobile phase B for another 7.5 min then elute with mobile phase C. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 13 µm TSK BSA-80Ts; B 15 × 3.2 5 µm TSK ODS-80Ts + 150 × 4.6 5 µm TSKgel ODS-80Ts
Mobile phase: A 200 mM sodium dodecyl sulfate solution:EtOH 70:30 containing 200 mM ethylenediaminetetraacetic acid 4 sodium salt and 0.3% phosphoric acid; B EtOH:water 80:20; C EtOH:water 87:13
Column temperature: 40

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Vitamin A



Molecular formula: $C_{20}H_{30}O$
Molecular weight: 286.46
CAS Registry No.: 68-26-8
Merck Index: 10150

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL EtOH + 10 mL hexane, mix for 30 s, centrifuge at 3000 rpm for 5 min, store the hexane layer at 15°, repeat the extraction with 10 mL hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with 200 µL isopropanol, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm TSKgel ODS-80Ts
Mobile phase: Gradient. EtOH:water 80:20 for 11.5 min then 87:13 (step gradient)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 460

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Extracted: vitamin E (F ex 298 em 325)

KEY WORDS
serum

REFERENCE
Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE
Matrix: blood
Sample preparation: Dilute 100 µL serum with 900 µL 12.62 mg/mL pyrogallol in EtOH, filter (450 µm cellulose disk), cool at 15° in the autosampler, inject a 300 µL aliquot onto column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A onto column B with mobile phase B, after another 1 min remove column A from the circuit. Elute column B with mobile phase B for another 7.5 min then elute with mobile phase C. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 13 µm TSK BSA-80Ts; B 15 × 3.2 5 µm TSK ODS-80Ts + 150 × 4.6 5 µm TSKgel ODS-80Ts
Mobile phase: A 200 mM sodium dodecyl sulfate solution:EtOH 70:30 containing 200 mM ethylenediaminetetraacetic acid 4 sodium salt and 0.3% phosphoric acid; B EtOH:water 80:20; C EtOH:water 87:13
Column temperature: 40

Flow rate: A 1.5; B 1; C 1
Injection volume: 300
Detector: F ex 340 em 460

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 1.67 IU/dL

OTHER SUBSTANCES
Extracted: vitamin E (F ex 298 em 325)

KEY WORDS
serum; column-switching

REFERENCE
Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL serum with 200 μL MeCN and 10 μL 20 mM ascorbic acid, centrifuge at 16000 g for 5 min. Mix the supernatant with 200 μL water, inject a 2 μL aliquot. (Protect all solutions from light.)

HPLC VARIABLES
Column: 50 × 0.18 3 μm ODS-AQ (YMC, Wilmington, NC) (The separation capillary column was formed from fused-silica capillaries (Polymicro Technologies, Phoenix) by inserting a small 50 μm I.D. capillary ca. 15 mm into a larger 180 μm I.D. capillary and fixed by applying epoxy (No. 353ND, Epoxy Technology, Billerica MA). A glass filter paper frit (Whatman GF/A) was inserted into the larger capillary and forced against the smaller capillary with a stream of isopropanol. The stationary phase was suspended in 3 mL isopropanol and pumped into the larger capillary until a 50 mm bed was formed. The larger and smaller diameter capillaries extended no more than 100 and 16 mm from the frit, respectively.)
Mobile phase: MeCN:MeOH:water 65:2.5:32.5 containing 1% tetrabutylammonium perchlorate, adjusted to pH 5.0 with acetic acid and 174 mM sodium acetate
Flow rate: 0.004
Injection volume: 2
Detector: E, carbon-fiber working electrode +900 mV, Ag/AgCl reference electrode (details of preparation in paper)

CHROMATOGRAM
Retention time: 11
Limit of detection: 38 pg/mL
Limit of quantitation: 70 fmol

OTHER SUBSTANCES
Extracted: isotretinoin, retinaldehyde, tretinoin

KEY WORDS
cow; serum; capillary HPLC

REFERENCE
Hagen,J.J.; Washco,K.A.; Monnig,C.A. Determination of retinoids by reversed-phase capillary liquid chromatography with amperometric electrochemical detection, *J.Chromatogr.B*, **1996**, *677*, 225–231.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 837 ng/mL retinyl acetate in EtOH and 400 μL EtOH to 500 μL plasma, vortex for 30 s, add 2 mL hexane, vortex for 30 s, centrifuge at 1500 rpm for 5 min. Remove the upper hexane layer, add 2 mL hexane to the lower layer, reextract. Evaporate

the combined hexane layers to dryness under nitrogen at 40°, reconstitute the residue in 200 μL EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm 100 Å pore size Supelguard column (Supelco)
Column: 250 × 4.6 5 μm 100 Å pore size Suplex pKb-100 RP (Supelco)
Mobile phase: MeOH:MTBE:water 80:20:5
Column temperature: 0
Flow rate: 0.8
Injection volume: 50
Detector: UV 328

CHROMATOGRAM
Retention time: 6.7
Internal standard: retinyl acetate (7.8)

KEY WORDS
plasma

REFERENCE
Lane,J.R.; Webb,L.W.; Acuff,R.V. Concurrent liquid chromatographic separation and photodiode array detection of retinol, tocopherols, all-trans-α-carotene, all-trans-β-carotene and the mono-cis isomers of β-carotene in extracts of human plasma, *J.Chromatogr.A*, **1997**, *787*, 111–118.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge 200 μL serum, add 200 μL IS and 200 μL EtOH, mix on orbital shaker for 5 min, add 200 μL water and 500 μL hexane, mix for 10 min, centrifuge at 2000 g for 10 min at 17°, remove 300 μL upper organic layer. Re-extract with 300 μL hexane, mix for 10 min, centrifuge at 4000 g for 10 min at 17°, remove 300 μL upper organic layer. Combine the organic layers, and evaporate them to dryness under vacuum in 15 min. Reconstitute the residue with 300 μL MeOH:EtOH:hexane 88:10:2, vortex for 10 min, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Adsorbosphere HS C18 + 150 × 4.6 3 μm Adsorbosphere HS C18 in series
Mobile phase: Gradient. A was MeCN:MeOH 60:40 containing 0.05% acetic acid. B was MeCN:MeCN:dichloromethane 45.6:30.4:24 containing 0.04% acetic acid. A:B 100:0 for 7 min then 0:100 for 10.4 min (step gradient), re-equilibrate at initial conditions for 5.6 min.
Column temperature: 37
Flow rate: 0.9
Injection volume: 40
Detector: UV 325

CHROMATOGRAM
Retention time: 5.7
Internal standard: tocol (UV 292) (10.1), echinenone (UV 450) (12.8)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: canthaxanthine (UV 473), α-carotene (UV 450), β-carotene (UV 450), β-cryptoxanthine (UV 450), lutein (UV 450), lycopene (UV 473), vitamin E (UV 292), zeaxanthin (UV 450), nonidentified carotenoids

KEY WORDS
serum

REFERENCE
Steghens,J.-P.; van Kappel,A.L.; Riboli,E.; Collombel,C. Simultaneous measurement of seven carotenoids, retinol and α-tocopherol in serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *694*, 71–81.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 5% perchloric acid, mix rapidly, add 500 μL ethyl acetate, mix for 60-90 s, centrifuge at 13000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: Reversed-phase C18 (Waters or Bio-Rad)
Mobile phase: MeCN:1% ammonium acetate 75:25
Flow rate: 2.5
Detector: UV 340

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: isotretinoin, tretinoin

KEY WORDS
plasma; pharmacokinetics; protect from light

REFERENCE
Davis,T.P.; Peng,Y.-M.; Goodman,G.E.; Alberts,D.S. HPLC, MS, and pharmacokinetics of melphalan, bisantrene and 13-cis retinoic acid, *J.Chromatogr.Sci.*, **1982**, *20*, 511–516.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 5% perchloric acid, vortex for 30 s, add 500 μL ethyl acetate, whirl for 1 min, centrifuge at 13000 g for 1 min, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES
Guard column: present but not specified
Column: 250 × 4.6 5 μm Ultrasphere ODS + 300 × 4 10 μm μBondapak in series
Mobile phase: MeCN:1% ammonium acetate 95:5
Flow rate: 2.5
Injection volume: 50
Detector: UV 340, UV 365

CHROMATOGRAM
Retention time: 5.3
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: retinoic acid

KEY WORDS
protect from light; plasma

REFERENCE
Peng,Y.-M.; Xu,M.-J.; Alberts,D.S. Analysis and stability of retinol in plasma, *J.Natl.Cancer Inst.*, **1987**, *78*, 95–99.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL 0.5 μg/mL retinyl acetate , 1 μg/mL retinyl palmitate, and 25 μg/mL α-tocopheryl acetate in EtOH to 1 mL serum or plasma while continuously vortexing, add 3 mL hexane, vortex for 2 min, centrifuge at 2500 g for 2 min, remove the upper phase, add 2 mL hexane to the lower layer, repeat extraction. Combine the upper layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot,

HPLC VARIABLES
Guard column: C18 (Waters)
Column: 5 μm Biophase ODS C18 (Bioanalytical Systems)
Mobile phase: MeCN:chloroform:isopropanol:water 78:16:3.5:2.5
Flow rate: 2
Injection volume: 40
Detector: UV 460 (UV 292 for tocopherol)

CHROMATOGRAM
Retention time: 2.60
Internal standard: retinyl acetate (3.07), retinyl palmitate (18.66), α-tocopheryl acetate (8.33)

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), gamma-tocopherol, α-carotene, lycopene, cryptoxanthin

KEY WORDS
serum; plasma

REFERENCE
Kaplan,L.A.; Miller,J.A.; Stein,E.A.; Stampfer,M.J. Simultaneous, high-performance liquid chromatographic analysis of retinol, tocopherols, lycopene, and α- and β-carotene in serum and plasma, *Methods Enzymol.*, **1990**, *189*, 155–167.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 25 μL 80 μg/mL tocol in EtOH + 250 μL 20 μg/mL BHT (butylated hydroxytoluene) in EtOH + 1.5 mL hexane, vortex for 1 min, remove 1 mL of upper layer, add 500 μL hexane, vortex for 1 min, remove 300 μL of upper layer. Combine the hexane extracts, evaporate to dryness under a stream of inert gas. Reconstitute in 250 μL 20 μg/mL BHT in EtOH, sonicate, centrifuge if necessary, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201TP54 (wide pore, polymerically bonded C18)
Mobile phase: Gradient. A was MeOH:n-butanol:water 75:10:15 containing 50 mM ammonium acetate, pH 5.5. B was MeOH:n-butanol:water 88:10:2 containing 50 mM ammonium acetate, pH 5.5. A:B 100:0 for 3 min, to 0:100 over 15 min, maintain at 0:100 for 17 min
Injection volume: 25
Detector: UV 325 for 7 min, UV 295 for 13 min, UV 450 for 14 min or E, glassy carbon electrode, Ag/AgCl reference electrode +1050 mV for retinol, +900 mV for tocol, +750 mV for α-tocopherol, +700 mV for β-carotene

CHROMATOGRAM
Retention time: 5
Internal standard: tocol (13)
Limit of detection: 4.1 μg/mL (E), 6 μg/mL (UV)

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), gamma-tocopherol, lutein, zeaxanthin, cryptoxanthin, α-carotene, 9-cis-β-carotene

KEY WORDS
serum

REFERENCE
MacCrehan,W.A. Determination of retinol, α-tocopherol, and β-carotene in serum by liquid chromatography, *Methods Enzymol.*, **1990**, *189*, 172–181.

SAMPLE
Matrix: blood

Sample preparation: 200 μL Serum + 100 μL EtOH + 100 μL α-tocopheryl acetate in EtOH, vortex for 5 s, add 500 μL hexane, vortex for 2 min, centrifuge at 700 g for 5 min. Remove 250 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, mix for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm Spheri-10 RP18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:dichloromethane:MeOH 70:20:10
Flow rate: 1.2
Injection volume: 50
Detector: UV 325 for 3.5 min, UV 291 for 4.5 min, UV 450 for 6 min

CHROMATOGRAM
Retention time: 2.31
Internal standard: α-tocopheryl acetate (6.30)
Limit of detection: 16 nM

OTHER SUBSTANCES
Extracted: beta carotene, vitamin E

KEY WORDS
protect from light; serum

REFERENCE
Arnaud,J.; Fortis,I.; Blachier,S.; Kia,D.; Favier,A. Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *572*, 103–116.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma or serum + 100 μL 6 μg/mL 9-methylanthracene in MeOH + 1.5 mL MeCN + 100 μL 100 mM perchloric acid, flush headspace of vial with argon, vortex, centrifuge, inject a 50 μL aliquot of the supernatant. Sonicate serum, solvents, and mobile phase under vacuum before use. Use low actinic glassware and yellow light.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C18
Mobile phase: MeCN:0.5% acetic acid 85:15 containing 0.05% sodium hexanesulfonate
Flow rate: 2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 9
Internal standard: 9-methylanthracene (5)
Limit of detection: 12 ng/mL

OTHER SUBSTANCES
Simultaneous: 13-cis-retinoic acid, all-trans-retinoic acid, 4-oxo-13-cis-retinoic acid

KEY WORDS
plasma; serum

REFERENCE
Gadde,R.R.; Burton,F.W. Simple reversed-phase high-performance liquid chromatographic method for 13-cis-retinoic acid in serum, *J.Chromatogr.*, **1992**, *593*, 41–46.

SAMPLE
Matrix: blood

Sample preparation: 2.5 mL Plasma + 2.5 mL 18 ng/mL IS1 and 10 ng/mL IS2 in EtOH, shake vigorously for 20 s, centrifuge at 1200 g for 5 min, add 5 mL diethyl ether, shake vigorously, centrifuge for 5 min, extract twice more with 5 mL ether. Combine ether layers, wash with 15 mL 5% NaCl, dry over sodium sulfate, evaporate to dryness under vacuum at 35°. Dissolve residue in 1-2 mL dichloromethane, filter (0.45 μm). Evaporate to dryness under a stream of nitrogen, make up to 100 μL with MeCN:MeOH:dichloromethane:hexane 45:10:22.5:22.5, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Spheri-5-C18 (Brownlee)
Column: 250 × 4.6 5 μm Microsorb C18 (Rainin)
Mobile phase: Gradient. MeCN:MeOH:dichloromethane:hexane 85:10:2.5:2.5 for 10 min then to 45:10:22.5:22.5 over 30 min, re-equilibrate for 15 min
Flow rate: 0.7
Injection volume: 20
Detector: UV 325

CHROMATOGRAM

Retention time: 7
Internal standard: IS1 ethyl β-apo-8'-carotenate (18), IS2 (3R)-8'-apo-β-carotene-3,8'-diol (5)

OTHER SUBSTANCES

Extracted: carotenoids, β-carotene, vitamin E (α-tocopherol)

KEY WORDS

plasma; handle under yellow lights

REFERENCE

Khachik,F.; Beecher,G.R.; Goli,M.B.; Lusby,W.R.; Smith,J.C.,Jr. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma, *Anal.Chem.*, **1992**, *64*, 2111–2122.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 25 μg/mL tocopheryl acetate in EtOH, vortex, add 400 μL butanol:ethyl acetate 50:50, mix for 1 min, add 20 mg sodium sulfate, vortex for 1 min, let stand at -20° for 20 min, centrifuge at 15000 g for 2 min, inject a 10 μL aliquot of the upper organic layer.

HPLC VARIABLES

Guard column: 5 μm C18
Column: 110 × 4.7 5 μm Partisphere 5 C18 (Whatman)
Mobile phase: MeOH:butanol:water 89.5:5:5.5
Column temperature: 45
Flow rate: 1.5
Injection volume: 10
Detector: UV 340 for 3 min, UV 290 for 1.5 min, UV 280 for 10.5 min, UV 450 for 7 min

CHROMATOGRAM

Retention time: 1.7
Internal standard: tocopheryl acetate (5.3)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: α-carotene, β-carotene, lycopene, δ-tocopherol, gamma-tocopherol, vitamin E, xanthophyll

KEY WORDS

serum; plasma; protect from light

REFERENCE

Lee,B.L.; Chua,S.C.; Ong,H.Y.; Ong,C.N. High-performance liquid chromatographic method for routine determination of vitamins A and E and β-carotene in plasma, *J.Chromatogr.*, **1992**, *581*, 41–47.

SAMPLE
Matrix: blood
Sample preparation: Dilute 1 mL serum 0.5-5 times with saline. Add 1 mL 0.25 μg/mL IS in EtOH to 1 mL diluted serum dropwise while vortexing, add 1.5 mL n-heptane, vortex for 1 min, centrifuge at 3000 rpm (Labofuge) for 15 min. Remove 1.3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 40 μL MeCN:THF 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil
Mobile phase: MeCN:water:THF 81.3:5.7:13
Column temperature: 40
Flow rate: 0.4
Injection volume: 5
Detector: UV 326

CHROMATOGRAM
Retention time: 1.880
Internal standard: retinol acetate (2.216)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: vitamin E (α-tocopherol), probucol, gamma-tocopherol, lycopene, α-carotene, β-carotene, metabolites

KEY WORDS
serum

REFERENCE
Schäfer Elinder,L.; Walldius,G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants, *J.Lipid Res.*, **1992**, *33*, 131–137.

SAMPLE
Matrix: blood
Sample preparation: 20-500 μL Serum + 2 volumes EtOH + 1 mL ethyl acetate + 4-7 μL of a solution containing 16 mg/mL tocopheryl acetate, 2-3 μg/mL canthaxanthin, and 10 μg/mL retinoic acid, vortex for 30 s, centrifuge for 30 s, extract the pellet twice with 0.5-1 mL portions of ethyl acetate, extract the pellet with 0.5-1 mL hexane. Combine the supernatants, add 500 μL water, vortex, centrifuge. Remove the upper organic layer and evaporate it to dryness under a stream of argon, reconstitute the residue in 100 μL MeOH:dichloromethane 2:1, inject a 10-90 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 300 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:dichloromethane:MeOH:1-octanol 90:15:10:0.1
Flow rate: 1
Injection volume: 10-90
Detector: UV 325

CHROMATOGRAM
Retention time: 4
Internal standard: tocopheryl acetate, canthaxanthin, retinoic acid

OTHER SUBSTANCES
Extracted: beta carotene (UV 450), carotenoids (UV 450), vitamin E (UV 290)

KEY WORDS
protect from light; serum

REFERENCE

Barua,A.B.; Kostic,D.; Olson,J.A. New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum, *J.Chromatogr.*, **1993**, *617*, 257–264.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 100 μL retinyl hexanoate in MeOH, extract three times with 200 μL portions of hexane. Combine the hexane layers and evaporate them to dryness under a stream of argon, reconstitute the residue in 50 μL isopropanol:dichloromethane, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:dichloromethane:MeOH:n-butanol 90:15:10:0.1 containing 0.1% ammonium acetate
Flow rate: 1
Injection volume: 20
Detector: UV 300

CHROMATOGRAM

Internal standard: retinyl hexanoate

KEY WORDS

serum; comparison with capillary electrophoresis

REFERENCE

Ma,Y.; Wu,Z.; Furr,H.C.; Lammi-Keefe,C.; Craft,N.E. Fast minimicroassay of serum retinol (vitamin A) by capillary zone electrophoresis with laser-excited fluorescence detection, *J.Chromatogr.*, **1993**, *616*, 31–37.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum or plasma + 500 μL EtOH containing 4.27 μM retinyl acetate and 0.31 μM echinenone, rotamix for 30 s, add 2 mL n-hexane, rotamix for 30 s, centrifuge at 2000 g for 2 min, repeat extraction with 2 mL n-hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL THF, make up to 200 μL with EtOH, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 5 μm Spherisorb ODS1
Column: 250 × 4.6 5 μm Spherisorb ODS1
Mobile phase: Gradient. A was MeCN:MeOH 20:80 containing 100 mM ammonium acetate. B was 100 mM ammonium acetate in water. A:B from 90:10 to 100:0 over 12 min, maintain at 100:0 for 10 min, re-equilibrate at initial conditions for 5 min
Flow rate: 2
Injection volume: 50
Detector: UV 325 for 7.5 min, UV 292 for 5.5 min, then UV 450

CHROMATOGRAM

Retention time: 4.37
Internal standard: retinyl acetate (5.96), echinenone (15.15)
Limit of detection: 0.35 μM

OTHER SUBSTANCES

Extracted: β-carotene, cryptoxanthin, lutein, lycopene, vitamin E

KEY WORDS

plasma; protect from light; serum

REFERENCE

Zaman,Z.; Fielden,P.; Frost,P.G. Simultaneous determination of vitamins A and E and carotenoids in plasma by reversed-phase HPLC in elderly and younger subjects, *Clin.Chem.*, **1993**, *39*, 2229–2234.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 200 μL 850 ng/mL retinyl acetate in EtOH, mix for 1 min, add 1 mL 0.4 g/L BHT (2,6-di-tert-butyl-4-methylphenol) in n-hexane, shake on a mechanical shaker for 10 min, centrifuge at 2000 g for 5 min, remove 800 μL of the supernatant, evaporate to dryness at 40° under a stream of nitrogen, reconstitute in 100 μL MeCN:THF:MeOH 68:22:7, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Lichrosorb RP18
Column: 250 × 4.6 5 μm Nucleosil 100-5 C18
Mobile phase: MeCN:THF:MeOH 68:22:7 made up to 100 with 1% ammonium acetate
Flow rate: 1.5
Injection volume: 15
Detector: UV 325 for 3 min, UV 450 for 1.9 min, UV 290 for 2.5 min, UV 470 for 4.6 min, UV 450 for 3 min, then UV 325 for rest of run

CHROMATOGRAM
Retention time: 2.5
Internal standard: retinyl acetate (2.7)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), lutein, lycopene, α-carotene, zeaxanthin, trans β-carotene, δ-tocopherol

KEY WORDS
plasma; serum; protect from sunlight

REFERENCE
Bui,M.H. Simple determination of retinol, α-tocopherol and carotenoids (lutein, all-*trans*-lycopene, α- and β-carotenes) in human plasma by isocratic liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 129–133.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL IS in EtOH, vortex for 30 s, add 5 mL water, add 7.5 mL n-hexane, add 300 μL 2 M HCl, rotate for 10 min, centrifuge at 1250 g for 8 min. Remove the organic layer and evaporate it at room temperature under a stream of nitrogen. Dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb S5W
Mobile phase: n-Hexane:isopropanol:acetic acid 200:0.7:0.135
Flow rate: 0.9
Injection volume: 50
Detector: UV 350

CHROMATOGRAM
Retention time: 25
Internal standard: Ro 15-1570 (22)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: 13-cis-retinoic acid, trans-retinoic acid

KEY WORDS
plasma; normal phase

REFERENCE
Meyer,E.; Lambert,W.E.; De Leenheer,A.P. Simultaneous determination of endogenous retinoic acid isomers and retinol in human plasma by isocratic normal-phase HPLC with ultraviolet detection, *Clin.Chem.*, **1994**, *40*, 48–51.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL nonapreno-β-carotene and retinyl butyrate in EtOH, vortex for 10 s, add 1 mL hexane, vortex for 30 s, centrifuge at 1500 g for 5 min. Remove 900 μL of the hexane layer and evaporate it to a waxy or glassy consistency (not dryness) under vacuum, dissolve in 100 μL EtOH, add 100 μL MeCN, vortex, filter (0.45 μm), inject a 30 μL aliquot.

HPLC VARIABLES
Column: 1540 × 4.6 5 μm Ultramex C18 (Phenomenex)
Mobile phase: MeCN:EtOH 50:50 containing 0.1 mL/L diethylamine
Column temperature: 29
Flow rate: 0.9
Injection volume: 30
Detector: UV 300

CHROMATOGRAM
Retention time: 2.66
Internal standard: nonapreno-β-carotene (9.5, UV 450), retinyl butyrate (3.5, UV 300)
Limit of detection: 17 nM

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, retinyl linoleate, retinyl oleate, retinyl palmitate, retinyl stearate

KEY WORDS
serum; use gold fluorescent lamps; hold sample at 4° before injection

REFERENCE
Sowell,A.L.; Huff,D.L.; Yeager,P.R.; Caudill,S.P.; Gunter,E.W. Retinol, α-tocopherol, lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, trans-β-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection, *Clin.Chem.*, **1994**, *40*, 411–416.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 2 mL diluting agent (amber tube), mix for 10 s, add 5 mL n-hexane, vortex for 30 s, centrifuge at 1600 g at 4° for 5 min. Remove the organic phase and concentrate to < 1 mL under vacuum below 30°, evaporate the rest of the solvent under nitrogen, dissolve the residue in 25 μL MeCN:MeOH 2:1, mix for 30 s, centrifuge at 8000 g for 1 min, inject a 20 μL aliquot. (Diluting agent was MeCN:100 mM ammonium acetate 25:75, pH adjusted to 5.5 with acetic acid.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:MeOH:100 mM ammonium acetate 46.7:23.3:30, pH adjusted to 7.0
Column temperature: 50
Flow rate: 1
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 31.9
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: 13-cis-retinoic acid, all-trans-retinoic acid

KEY WORDS
serum

REFERENCE

Takeda,N.; Yamamoto,A. Simultaneous determination of 13-cis- and all-trans-retinoic acids and retinol in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *657*, 53–59.

SAMPLE

Matrix: blood

Sample preparation: 10 μL Serum + 30 μL 200-500 ng/mL retinyl acetate in isopropanol:dichloroethane 2:1 + 5 μL glacial acetic acid, vortex for 30 s, centrifuge for 1 min, inject a 10-20 μL aliquot.

HPLC VARIABLES

Guard column: C18 (Upchurch)

Column: 150 × 4.6 Ultracarb 5 ODS30 (Phenomenex)

Mobile phase: MeCN:dichloromethane:MeOH 85:12:3 containing 0.1% ammonium acetate (dissolve ammonium acetate in MeOH first)

Flow rate: 1

Injection volume: 10-20

Detector: UV 335

CHROMATOGRAM

Retention time: 4.5

Internal standard: retinyl acetate (5.5)

Limit of detection: 10 ng/mL

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: retinoic acid

KEY WORDS

protect from light; serum

REFERENCE

Barua,A.B.; Kostic,D.; Barua,M.; Olson,J.A. Determination of retinol and retinoic acid in capillary blood by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 1459–1471.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 2.5 mL EtOH, mix for 5 min, add 5 mL n-hexane, mix vigorously, centrifuge at 2000 g for 5 min, repeat extraction with 3 mL n-hexane. Combine the n-hexane layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in dichloromethane, inject an aliquot.

HPLC VARIABLES

Guard column: μBondapak C18 Guard-Pak + 50 mm long C18 ODS(4) (Shimadzu)

Column: 250 × 4.6 5 μm Vydac 201 TP 54 C18

Mobile phase: MeOH:MeCN 90:10 (Every 100 injections wash column with MeOH:MeCN:dichloromethane 8:1:1.)

Flow rate: 1

Detector: UV 324

CHROMATOGRAM

Retention time: 3.8, 3.85 (9-cis)

OTHER SUBSTANCES

Extracted: beta carotene (UV 451), vitamin E (UV 291)

KEY WORDS

serum

REFERENCE

Ben-Amotz,A. Simultaneous profiling and identification of carotenoids, retinols, and tocopherols by high performance liquid chromatography equipped with three-dimensional photodiode array detection, *J.Liq.Chromatogr.*, **1995**, *18*, 2813–2825.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 100 μL retinyl acetate in MeOH, mix, extract three times with 200 μL portions of hexane. Combine the extracts and evaporate them to dryness under a stream of argon, reconstitute with 25 μL isopropanol:dichloromethane, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:water 95:5
Flow rate: 1
Injection volume: 20
Detector: UV 325

KEY WORDS

comparison with capillary electrophoresis; serum

REFERENCE

Shi,H.; Ma,Y.; Humphrey,J.N.; Craft,N.E. Determination of vitamin A in dried human blood spots by high-performance capillary electrophoresis with laser-excited fluorescence detection, *J.Chromatogr.B*, **1995**, *665*, 89–96.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 300 μL MeCN, vortex for 1.5 min, centrifuge at 10500 g for 1 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 × 4.5 5 μm CLC-G-ODS C18 (Shimadzu)
Column: 250 × 4.6 5 μm CLC-ODS C18 (Shimadzu)
Mobile phase: MeCN:water 90:10
Column temperature: 37
Flow rate: 2
Injection volume: 100
Detector: UV 328

CHROMATOGRAM

Retention time: 5.5
Limit of detection: 10 ng/mL

KEY WORDS

protect from light; serum

REFERENCE

Siddiqui,F.Q.; Malik,F.; Fazli,F.R. Determination of serum retinol by reversed-phase, *J.Chromatogr.B*, **1995**, *666*, 342–346.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Serum + 200 μL 650 ng/mL tocopheryl acetate in MeOH, vortex for 30 s, add 200 μL n-hexane, shake for 15 min, centrifuge at 3000 rpm for 10 min. Remove 120 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 20 μL dichloromethane, add 100 μL MeCN:MeOH 50:50, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm Nucleosil C18

Mobile phase: MeCN:MeOH:dichloromethane 50:45:5
Flow rate: 0.7
Injection volume: 50
Detector: F ex 325 em 480 for 5 min, then ex 295 em 330 for 10 min, then ex 325 em 480

CHROMATOGRAM
Retention time: 2.9
Internal standard: tocopheryl acetate (9.3)

OTHER SUBSTANCES
Extracted: vitamin E, γ-tocopherol, retinyl palmitate, β-carotene (UV 450), α-carotene (UV 450)

KEY WORDS
serum

REFERENCE
Yakushina,L.; Taranova,A. Rapid HPLC simultaneous determination of fat-soluble vitamins, including carotenoids, in human serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 715–718.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 100 μL Plasma + 100 μL 0.9% NaCl + 200 μL MeOH, vortex for 30 s, let stand for 10 min, add 400 μL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot. Tissue. Homogenize (liver with Mikro-dismembrator II in liquid nitrogen; other tissue with Ultra-Turrax) tissue with 3 mL 1% acetic acid containing 1 mg/mL ascorbic acid and 10 mM EDTA, add 2 mL MeOH, vortex for 30 s, let stand for 10 min, add 4 mL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:dichloromethane:MeOH:water 70:10:15:5
Flow rate: 0.5 for 13 min, to 1 over 1 min, maintain at 1 for 10 min, to 1.5 over 1 min, maintain at 1.5 for 21 min, to 2 over 1 min, maintain at 2 for 10 min, return to 0.5 over 1 min, maintain at 0.5 for 2 min.
Injection volume: 50
Detector: UV 350

CHROMATOGRAM
Retention time: 6
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: beta carotene (UV 445), vitamin E (UV 292)

KEY WORDS
rat; protect from light; liver; plasma; lung

REFERENCE
Van Vliet,T.; Van Schaik,F.; Van Schoonhoven,J.; Schrijver,J. Determination of several retinoids, carotenoids and E vitamers by high-performance liquid chromatography. Application to plasma and tissues of rats fed a diet rich in either β-carotene or canthaxanthin, *J.Chromatogr.*, **1991**, *553*, 179–186.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, discard the effluent,

elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 3.5 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Spherisorb RP-18
Mobile phase: MeCN:MeOH 70:30
Flow rate: 1.5
Injection volume: 10
Detector: UV 290

CHROMATOGRAM
Retention time: 2.6
Internal standard: anthraquinone (1.89)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: vitamin E, vitamin E acetate

KEY WORDS
plasma; SPE

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: cheese
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70° for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10. Combine the organic layers and evaporate them to dryness, dissolve the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 325 em 475 (vitamin A), UV 450 (β-carotene), and F ex 325 em 475 for 3.5 min, ex 280 em 475 for 10.5 min, ex 325 em 475 for 9 min (others)

CHROMATOGRAM
Retention time: 18
Limit of detection: 0.32 ng

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), β-tocopherol, gamma-tocopherol, δ-tocopherol, 13-cis-retinol

KEY WORDS
normal phase; cheese

REFERENCE
Panfili,G.; Manzi,P.; Pizzoferrato,L. High-performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses, *Analyst*, **1994**, *119*, 1161–1165.

SAMPLE
Matrix: culture media
Sample preparation: 100 μL Culture media + 200 μL ice-cold EtOH, mix thoroughly, let stand for 15 min, centrifuge at 12000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: Whatman CO:PELL ODS guard column
Column: 100 × 8 5 μm Nova-Pak C18 (radial-packed)
Mobile phase: MeOH:100 mM pH 7.0 ammonium acetate 90:10
Flow rate: 1
Detector: UV 340

CHROMATOGRAM
Retention time: 12.50

OTHER SUBSTANCES
Extracted: isotretin, motretinid, acitretin, all-trans-retinoic acid, retinal, etretinate

REFERENCE
Kochhar,D.M.; Penner,J.D.; Minutella,L.M. Biotransformation of etretinate and developmental toxicity of etretin and other aromatic retinoids in teratogenesis bioassays, *Drug Metab.Dispos.*, **1989**, *17*, 618–624.

SAMPLE
Matrix: flour, milk
Sample preparation: Mix 5 g powdered milk or flour with 20 mL 500 g/L NaOH in water. Warm at 30° for 3 min, add 100 mL EtOH and 2 mL 200 g/L hydroquinone in EtOH. Heat at 80° for 30 min. Cool, add 100 mL water and 50 mL diethyl ether. Shake and add 50 mL petroleum ether, shake. Remove the organic layer and reextract the aqueous layer as above. Combine the organic layers, filter (cellulose), wash twice with 100 mL water, evaporate to dryness under reduced pressure, dissolve in 1 mL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Zorbax ODS
Column: 150 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 92:8
Column temperature: 25 ± 1
Flow rate: 1.5
Injection volume: 20
Detector: UV 330

CHROMATOGRAM
Retention time: 13.45
Limit of detection: 20 ng/g
Limit of quantitation: 120 ng/g

KEY WORDS
powdered milk

REFERENCE
Ake,M.; Mandrou,B.; Malan,A. Determination of vitamin A in milk and flour consumed by one- to four-year-old children in Côte d'Ivoire, *J.AOAC Int.*, **1998**, *81*, 111–114.

SAMPLE
Matrix: food
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70, for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10 (Analyst 1994, 119, 1161). Combine the organic layers and evaporate them to dryness, dissolve the residue in MeOH:dichloromethane 90:10, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Supelco C18
Mobile phase: MeOH
Flow rate: 2
Detector: F ex 325 em 475

CHROMATOGRAM
Retention time: 2.4
Limit of detection: 400 pg

OTHER SUBSTANCES
Extracted: vitamin E (α-tocopherol, β and γ-tocopherol, δ-tocopherol) (F ex 280 em 325), sterols (UV 208), carotenes (UV 450)

KEY WORDS
cheese

REFERENCE
Manzi,P.; Panfili,G.; Pizzoferrato,L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products, *Chromatographia*, **1996**, *43*, 89–91.

SAMPLE
Matrix: food
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70, for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10 (Analyst 1994, 119, 1161). Combine the organic layers and evaporate them to dryness, dissolve the residue in n-hexane:2-propanol 99:1, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si (Beckman)
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Detector: F ex 325 em 475

CHROMATOGRAM
Retention time: 15 (13-cis-retinol), 17.3 (all trans-retinols)
Limit of detection: 100 pg (13-cis-retinol), 300 pg (all trans-retinols)

OTHER SUBSTANCES
Extracted: vitamin E (α, β, γ, δ-tocopherol) (F ex 280 em 325), sterols (UV 208), carotenes (UV 450)

KEY WORDS
cheese; normal phase

REFERENCE
Manzi,P.; Panfili,G.; Pizzoferrato,L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products, *Chromatographia*, **1996**, *43*, 89–91.

SAMPLE
Matrix: food, milk, tissue
Sample preparation: 40 mL Fluid sample or 1-10 g solid sample homogenized with 30 mL water + 12 mL 60% KOH + 80 mL EtOH + 0.5 mL 1% BHT in EtOH + 0.5 g ascorbic acid, stir for 16 h under nitrogen, make up to 250 mL with water and EtOH so that the water:EtOH ratio is 50:50, add 20 mL to a 150 × 25 Kieselguhr SPE cartridge, let stand for 20 min, elute with 50 mL light petroleum, force out all the eluate with nitrogen, evaporate the eluate under reduced pressure, take up the residue in 2-50 mL isooctane, inject an aliquot.

HPLC VARIABLES
Guard column: 7 × 2 3 μm Spherisorb SW silica gel
Column: 100 × 2 3 μm Spherisorb SW silica gel
Mobile phase: n-Hexane:1-octanol 99.7:0.3
Flow rate: 0.4
Injection volume: 10
Detector: UV 325

CHROMATOGRAM
Retention time: 6.4 (11-cis), 7.1 (11,13-di-cis), 8.4 (13-cis), 9.3 (9,13-di-cis), 11.0 (9-cis), 11.6 (7-cis), 12.5 (all-trans)
Limit of detection: 140 ng/mL

KEY WORDS
normal phase; liver; sausage; sour cream; food; SPE

REFERENCE
Brinkmann,E.; Dehne,L.; Oei,H.B.; Tiebach,R.; Baltes,W. Separation of geometrical retinol isomers in food samples by using narrow-bore high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *693*, 271–279.

SAMPLE
Matrix: formula, reference material
Sample preparation: 10 g Zero-control reference material (ZRM) powder + 50 g hot water, mix. 6.5 g Reconstituted ZRM or 3.5 g concentrated commercial formula + 10 mL boiling isopropanol, mix, add 7.5 g anhydrous magnesium sulfate to the ZRM and 4 g to the concentrated commercial formula, mix thoroughly, add 25 mL hexane:ethyl acetate 85:15, add 1 mL 360 .micro g/mL BHT, mix, homogenize (Polytron) for 1 min, filter through 60 mL coarse-porosity fritted glass filter using vacuum, wash with two 15 mL portions of hexane:ethyl acetate 85:15. Re-extract with 20 mL hexane:ethyl acetate 85:15 and 5 mL isopropyl alcohol, homogenize for 1 min, filter through 60 mL coarse-porosity fritted glass filter using vacuum, wash with two 15 mL portions of hexane:ethyl acetate 85:15. Mix the combined filtrate with 500 mg anhydrous magnesium sulfate, evaporate to dryness, add 15 mL hexane to the residue, filter (0.45 μm nylon) using vacuum, wash with three 7 mL portions of hexane, evaporate to 1 mL with nitrogen at 45°, dilute to 10 mL with hexane, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: Hexane:isopropanol 99.875:0.125
Flow rate: 1
Injection volume: 50
Detector: F ex 325 em 470

CHROMATOGRAM
Retention time: 3.4 (vitamin A palmitate)
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Also analyzed: vitamin E

KEY WORDS
normal phase; soy-based infant formula

REFERENCE
Chase,G.W.,Jr.; Long,A.R.; Eitenmiller,R.R. Liquid chromatographic method for analysis of all-rac-α-tocopheryl acetate and retinyl palmitate in soy-based infant formula using a zero-control reference material (ZRM) as a method development tool, *J.AOAC Int.*, **1998**, *81*, 577–581.

SAMPLE
Matrix: formulations

Sample preparation: Reconstitute 28 g milk based infant formula with ca. 145 g 78-80° water, mix thoroughly. Add 15 mL boiling isopropanol to 6.5 g reconstituted infant formula, mix thoroughly. Add 7.5 g magnesium sulfate, 30 mL hexane:ethyl acetate 85:15 and 1 mL BHT. Homogenize mixture for 1 min, filter through a coarse porosity glass filter by vacuum, wash the magnesium sulfate cake with two 15 mL portions of hexane:ethyl acetate 85:15. Repeat the extraction with 5 mL isopropanol and 15 mL hexane:ethyl acetate 85:15. Add 1 g magnesium sulfate to the combined filtrate and evaporate it to dryness under nitrogen. Dissolve the residue in 10 mL hexane, filter (0.45 μm), evaporate to a volume of less than 5 mL at 45°. Dilute to 10 mL with mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: Hexane:isopropanol 99.5:0.5
Flow rate: 0.5
Injection volume: 50
Detector: F ex 325 em 470

CHROMATOGRAM

Retention time: 2.9
Limit of detection: 187 ng/mL

KEY WORDS

infant formula; normal phase

REFERENCE

Chase,Jr.,G.W.; Eitenmiller,R.R.; Long,A.R. Liquid chromatographic analysis of all-RAC-α-tocopheryl acetate, tocopherols, and retinyl palmitate in SRM 1846, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3317–3327.

SAMPLE

Matrix: formulations
Sample preparation: Tablets, capsules. Add 5 crushed tablets or the contents from 5 capsules to 10 mL DMSO, add 15 mL hexane, shake at 60° for 45 min, centrifuge at 3000 rpm for 10 min, remove hexane layer, add 15 mL hexane, vortex for 5 min at room temperature, remove hexane layer, repeat hexane extraction three more times, combine all hexane layers, filter, make up to 100 mL with hexane, dilute if necessary, inject a 100 μL aliquot. Syrup. 10 mL Syrup + 10 mL DMSO + 15 mL ether:hexane 10:90, vortex for 5 min, remove hexane layer, repeat extraction four more times, combine the extracts, evaporate with nitrogen until the ether is removed, filter, make up to 100 mL with hexane, dilute if necessary, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm amino bonded phase (Chromatography Sciences Co.)
Mobile phase: Hexane:isopropanol 99:1
Flow rate: 1-2
Injection volume: 100
Detector: UV 265

CHROMATOGRAM

Retention time: 12 (13-cis), 13 (9-cis), 14.5 (all-trans)

OTHER SUBSTANCES

Simultaneous: cholecalciferol (vitamin D3), ergocalciferol (vitamin D2)

KEY WORDS

work under subdued light; tablets; capsules; syrup

REFERENCE

Beaulieu,N.; Curran,N.M.; Gagné,C.; Gravelle,M.; Lovering,E.G. Liquid chromatographic methods for vitamins A and D in multivitamin-mineral formulations, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 247–254.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out a sample containing about 2500 UI of vitamin A, add 20 mL MeOH, protect from light, stir vigorously for 2-2.5 h, filter (0.45 μm), inject a 2 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pack C18
Mobile phase: MeCN:THF:water 55:37:8
Flow rate: 1.5
Injection volume: 2
Detector: UV 325

CHROMATOGRAM
Retention time: 1.08 (retinol), 1.25 (retinyl acetate)

KEY WORDS
protect from light; capsules; tablets

REFERENCE
Genestar,C.; Grases,F. Determination of vitamin A in pharmaceutical preparations by high-performance liquid chromatography with diode-array detection, *Chromatographia*, **1995**, *40*, 143–146.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder, weigh out 100-150 mg, mix with sea sand. Extract with supercritical carbon dioxide (Dionex) in the dynamic mode at 250 atmospheres and 40° for 15 min (restrictor 60°, gaseous flow rate 190-220 mL/min), collect in 6 mL THF at 0°, make up to 10 mL with THF, inject an aliquot. [Alternatively, add 5 crushed tablets or the contents from 5 capsules to 10 mL DMSO, add 15 mL hexane, shake at 60° for 45 min, centrifuge at 3000 rpm for 10 min, remove hexane layer, add 15 mL hexane, vortex for 5 min at room temperature, remove hexane layer, repeat hexane extraction three more times, combine all hexane layers, dilute with THF, inject a 25 μL aliquot. J.Assoc.Off.Anal.Chem. 1989, 72, 247.]

HPLC VARIABLES
Guard column: 4 × 4 5 μm (Merck)
Column: 250 × 4 5 μm Lichrospher CH-8
Mobile phase: MeOH:MeCN 75:25
Flow rate: 1.3
Injection volume: 50
Detector: UV 325

CHROMATOGRAM
Retention time: 9 (vitamin A palmitate)

OTHER SUBSTANCES
Simultaneous: vitamin E acetate (UV 280)

KEY WORDS
tablets; SFE

REFERENCE
Scalia,S.; Ruberto,G.; Bonina,F. Determination of vitamin A, vitamin E, and their esters in, *J.Pharm.Sci.*, **1995**, *84*, 433–436.

SAMPLE
Matrix: formulations
Sample preparation: Mix 100-150 mg of the formulation with celite and extract with supercritical carbon dioxide at 250 atmospheres at 40° at 190-220 mL/min with the restrictor at 100° (Dionex SFE-703), collect in 4 mL THF:MeOH 80:20 at 0°, make up to 5 mL, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH 25:75
Flow rate: 1.5
Detector: UV 325

CHROMATOGRAM
Retention time: 10 (vitamin A palmitate)

OTHER SUBSTANCES
Simultaneous: vitamin E acetate (UV 280)

KEY WORDS
SFE; cream; lotion; protect from light

REFERENCE
Scalia,S.; Renda,A.; Ruberto,G.; Bonina,F.; Menegatti,E. Assay of vitamin A palmitate and vitamin E acetate in cosmetic creams and lotions by supercritical fluid extraction and HPLC, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 273–277.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20 min. Extract twice with 10 mL n-hexane. Evaporate the extracts to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 4.45 (A), 4 (B)

OTHER SUBSTANCES
Extracted: retinal, isotretinoin, tretinoin, vitamin D2, vitamin D3, vitamin E, vitamin K1, vitamin K2

REFERENCE
Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: milk
Sample preparation: Add 500 mg ascorbic acid, 50 mL EtOH, and 10 mL 60% KOH to 25 g liquid or reconstituted powdered milk under a nitrogen stream, stir overnight at room temperature. Extract with three 50 mL portions of n-hexane and two 25 mL portions of n-hexane by shaking for 2 min each. Combine the n-hexane extracts, wash with 50 mL portions of water containing a few drops of phenolphthalein until the aqueous phase appears colorless, add 1 g butylated hydroxytoluene, filter through a Whatman No.1 filter containing 20 g anhydrous sodium sulfate, concentrate the filtrate under reduced pressure at 40°, reconstitute with 10 mL MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: Tracer Spherisorb ODS 2 C18
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeCN:MeOH:water 1:95:4
Injection volume: 20

Detector: UV 323 for 14 min then UV 292

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: vitamin E

REFERENCE
Albal-Hurtado,S.; Novella-Rodríguez,S.; Veciana-Nogués,M.; Mariné-Font,A. Determination of vitamins A and E in infant milk formulae by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 243–246.

SAMPLE
Matrix: milk
Sample preparation: 1 g Powdered milk or 25 mL liquid milk + alcoholic KOH, let stand overnight, extract with hexane. Remove the organic layer and evaporate it to dryness, reconstitute the residue in MeOH, filter, inject an aliquot. (Alcoholic KOH was prepared from 50 mL EtOH and 15 mL 60% KOH in water.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid/sodium acetate
Flow rate: 1.25
Injection volume: 10
Detector: E, EG & G PAR Model 400, MP 1304 glassy carbon series dual electrode, E1 (upstream) -1100 mV, E2 (downstream) +700 mV (Condition electrodes for 30 min at E1 -1200 mV and E2 +1500 mV at the start of each day.)

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.06 ng

OTHER SUBSTANCES
Extracted: vitamin E

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez Perez,A.; Gomez Perez,M.C.; Fernandez Moro,M.A.; Hernandez Mendez,J. Determination of vitamins A, E and K1 in milk by high-performance liquid chromatography with dual amperometric detection, *Analyst*, **1995**, *120*, 2489–2492.

SAMPLE
Matrix: milk
Sample preparation: Dilute milk to 30% with water, mix with reagent and pass through a 5 m × 0.5 mm i.d. PTFE tube knotted reactor at 1.25 mL/min, mix with 2.5 M acetic acid, pass onto a Sep-Pak Plus C18 SPE cartridge for 5 min, wash SPE cartridge with MeOH:water 40:60 for 4 min, elute SPE cartridge with MeOH for 4 min, inject the last 100 μL of the eluate. (Reagent was 50 mL EtOH + 15 mL 60% aqueous NaOH + 5 mL 10% ascorbic acid.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid-sodium acetate
Flow rate: 1
Injection volume: 100
Detector: E, glassy carbon working electrode +1300 mV

CHROMATOGRAM
Retention time: 6

Limit of detection: 34.9 nM

OTHER SUBSTANCES
Extracted: vitamin E, vitamin D3

KEY WORDS
SPE; derivatization

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez-Perez,A.; Gomez-Perez,M.C.; Hernandez-Mendez,J. Directly coupled sample treatment-high-performance liquid chromatography for on-line automatic determination of liposoluble vitamins in milk, *J.Chromatogr.A*, **1995**, *694*, 399–406.

SAMPLE
Matrix: silicone oils
Sample preparation: Condition a 1 g Si Bond-Elut SPE cartridge with 5 mL n-hexane. Mix 1 g silicone oil with 2 mL dichloromethane, vortex for 2 min, centrifuge at 3000 g. Withdrawn the supernatant, repeat this procedure twice, filter (0.45 μm), heat the filtrate at 50°, expose to a stream of helium for 30 min. Add 2.5 μg retinol acetate, 2.5 μg α-tocopherol acetate, and 25 μg BHT. Add the mixture to the SPE cartridge, elute with 500 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. A was MeCN:200 mM ammonium acetate 72:25. B was MeOH:water 95:5. A:B 100:0 for 10 min, to 0:100 over 1 min, maintain at 0:100 for 14 min
Flow rate: 2 for 10 min then 1.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 4
Internal standard: retinol acetate (9.5)
Limit of detection: 51.4 ng/mL
Limit of quantitation: 171.2 ng/mL

OTHER SUBSTANCES
Extracted: cholesterol (UV 210), retinal (UV 350), retinoic acid (UV 350), α-tocopherol acetate, vitamin E (UV 210)

KEY WORDS
ophthalmic silicone oils; SPE

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Marinero,P. Simultaneous HPLC determination of cholesterol, α-tocopherol, retinol, retinal and retinoic acid in silicone oils used as vitreous substitutes in eye surgery, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1151–1167.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 8 μm Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)
Mobile phase: MeOH:water 92:8
Flow rate: 1
Detector: UV 330

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: retinoic acid, retinol acetate, vitamin E

REFERENCE
Jedrejewski,P.T.; Taylor,L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1995**, *33*, 438–445.

SAMPLE
Matrix: tissue
Sample preparation: 50 mg Tissue + 50 μL 560 μg/mL vitamin K in EtOH, extract twice with 1 mL n-hexane using a sonicator, centrifuge at 2000 g for 5 min. Evaporate the supernatant to dryness, reconstitute it in 200 μL chloroform:MeOH 25:75, inject an aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeOH:water 96.5:3.5
Column temperature: 40
Flow rate: 2
Detector: UV 325

CHROMATOGRAM
Retention time: ca. 2
Internal standard: vitamin K (9)

OTHER SUBSTANCES
Extracted: vitamin A_p, vitamin E (F ex 295 em 350)

KEY WORDS
rat; liver; placenta; brain

REFERENCE
Barbas,C.; Castro,M.; Bonet,B.; Viana,M.; Herrera,E. Simultaneous determination of vitamins A and E in rat tissues by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 415–420.

SAMPLE
Matrix: tissue
Sample preparation: 100-120 mg Tadpole embryos + 100 mL isopropanol, sonicate on ice, vortex for 1 min, centrifuge at 4000 g at 4° for 20 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 4 10 μm LiChrosorb RB 18
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. MeOH:40 mM pH 7.3 ammonium acetate from 55:45 to 100:0 over 20 min
Flow rate: 1.6
Detector: UV 354

CHROMATOGRAM
Retention time: 15.2

OTHER SUBSTANCES
Extracted: isotretinoin, tretinoin, metabolites

KEY WORDS
handle under yellow light; tadpoles; embryos

REFERENCE
Creech Kraft,J.; Kimelman,D.; Juchau,M.R. *Xenopus Laevis*: A model system for the study of embryonic retinoid metabolism. I. Embryonic metabolism of 9-*cis*- and all-*trans*-retinals and retinols and their corresponding acid forms, *Drug Metab.Dispos.*, **1995**, *23*, 72–82.

SAMPLE
Matrix: tissue
Sample preparation: 100-120 mg Frog embryos + 100 μL isopropanol, sonicate on ice, vortex for 1 min, centrifuge at 4° at 4000 g for 20 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 4 10 μm LiChrosorb RB 18
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. MeOH:40 mM pH 7.3 ammonium acetate from 55:45 to 100:0 over 18 min.
Flow rate: 1.6
Detector: UV 354

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: retinoic acid

KEY WORDS
frog; embryo

REFERENCE
Creech Kraft,J.; Juchau,M.R. *Xenopus laevis*: A model system for the study of embryonic retinoid metabolism. III. Isomerization and metabolism of all-*trans*-retinoic acid and 9-*cis*-retinoic acid and their dysmorphogenic effects in embryos during neurulation, *Drug Metab.Dispos.*, **1995**, *23*, 1058–1071.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (7 mL Tenbroeck grinder, Bioblock) 2-10 mg liver and 1.5 mL 1% pyrogallol in EtOH, shake at 4° for 30 min, add 100 μL water, add 3 mL n-hexane, extract, centrifuge at 4° at 3000 g for 15 min, repeat extraction. Evaporate the hexane layers separately as rapidly as possible under a stream of nitrogen at 50°, reconstitute with 200 μL 30 μM retinyl acetate in MeOH, inject a 50 μL aliquot. (For total retinol homogenize liver with 1.5 mL 10% KOH in 95% EtOH, heat at 60° for 30 min, cool in an ice bath, add 800 μL water, extract twice with 1.9 mL n-hexane. Evaporate the hexane layers separately as rapidly as possible under a stream of nitrogen at 50°, reconstitute with 200 μL MeOH, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-8 (Brownlee)
Column: 250 × 4.6 5 μm Supelcosil LC-8
Mobile phase: MeOH:water 94:6
Flow rate: 1.5
Injection volume: 50
Detector: UV 325

CHROMATOGRAM
Retention time: 3
Internal standard: retinyl acetate (3.7)
Limit of quantitation: 6 nmole/g

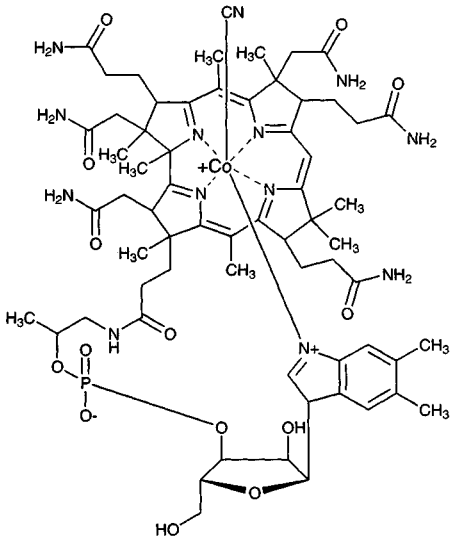
OTHER SUBSTANCES
Extracted: retinyl oleate, retinyl palmitate, retinyl stearate

KEY WORDS
liver; protect from light

REFERENCE
Got,L.; Gousson,T.; Delacoux,E. Simultaneous determination of retinyl esters and retinol in human livers by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *668*, 233–239.

Vitamin B12

Molecular formula: $C_{63}H_{88}CoN_{14}O_{14}P$
Molecular weight: 1355.38
CAS Registry No.: 68-19-9
Merck Index: 10152



SAMPLE

Matrix: blood, feces

Sample preparation: Feces. Condition a Sep-Pak C18 SPE cartridge with 2 mL MeCN and 6 mL water. 1 g Feces + 3 mL buffer, rotate at 4° for 24 h, centrifuge at 19000 g for 1 h. Remove 3 mL of the supernatant and add cadmium acetate to a concentration of 200 mM, let stand for 2 h, add four volumes of EtOH preheated to 80°, mix vigorously, heat at 80° for 20 min, cool in an ice bath, centrifuge at 2000 g for 10 min, remove the supernatant, mix the precipitate with two volumes of cold EtOH:water 80:20, centrifuge. Combine the supernatants and evaporate them to dryness under reduced pressure at 40°, reconstitute the residue in 2 mL water, add to the SPE cartridge, wash with 12 mL water, elute with 6 mL t-butanol:water 20:80, evaporate the eluate to dryness, reconstitute with 2 mL 1% acetic acid, add to the Amberlite XAD2 column, wash with 12 mL 1% acetic acid, wash with 12 mL MeOH:1% acetic acid 10:90, elute with 30 mL MeOH:1% acetic acid 50:50, lyophilize the eluate at -80°, reconstitute with 2 mL water, filter (0.2 μm), lyophilize, reconstitute with 300 μL 1% acetic acid, inject a 250 μL aliquot. Plasma. Condition a Sep-Pak C18 SPE cartridge with 2 mL MeCN and 6 mL water. Add cadmium acetate to a concentration of 200 mM, let stand for 2 h, add four volumes of EtOH preheated to 80°, mix vigorously, heat at 80° for 20 min, cool in an ice bath, centrifuge at 2000 g for 10 min, remove the supernatant, mix the precipitate with two volumes of cold EtOH:water 80:20, centrifuge. Combine the supernatants and evaporate them to dryness under reduced pressure at 40°, reconstitute the residue in 2 mL water, add to the SPE cartridge, wash with 12 mL water, elute with 6 mL t-butanol:water 20:80, evaporate the eluate to dryness, lyophilize the eluate at -80°, reconstitute with 2 mL water, filter (0.2 μm), lyophilize, reconstitute with 300 μL 1% acetic acid, inject a 250 μL aliquot (Meth. Enzymol. 1986, 123, 3). (Buffer was 100 mM pH 7.4 sodium phosphate buffer containing 5 U/mL aprotinin, 0.02 mM phenylmethylsulfonyl fluoride, 3 mM sodium azide, and 0.05% Triton X100. Prepare Amberlite XAD2 column as follows. Suspend 30 g resin in 50 mL acetone, filter, wash with 50 mL acetone, dry at 80°, suspend in 200 mL MeOH, allow to settle, discard the supernatant, repeat 2-4 times until supernatant is clear, suspend in 100 mL MeOH, fill a 330 × 8 glass column to a bed height of 40 mm (Meth. Enzymol. 1986, 123, 3).)

HPLC VARIABLES

Column: 250 × 5 5 μm LiChrospher RP18 glass column
Mobile phase: Gradient. A was 85 mM phosphoric acid adjusted to pH 3.0 with triethanolamine. B was MeCN. A:B from 90:10 to 50:50 over 20 min.
Flow rate: 0.5
Injection volume: 250
Detector: UV 365

CHROMATOGRAM

Retention time: 17.5

KEY WORDS
protect from light; plasma; SPE

REFERENCE
Djalali,M.; Gueant,J.-L.; Lambert,D.; el Kholty,S.; Saunier,M.; Nicolas,J.-P. High-performance liquid chromatographic separation and dual competitive binding assay of corrinoids in biological material, *J.Chromatogr.*, **1990**, *529*, 81–91.

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 17.12
Internal standard: xanthine (4.65)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: ascorbic acid, folic acid, niacin, niacinamide, riboflavin

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM
Retention time: 3.777

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations, premix
Sample preparation: Weigh out amount containing 20-100 μg vitamin B12, extract with DMSO: water 50:50 (or 0.5% ammonium pyrrolidine dithiocarbamate and 2% citric acid in DMSO: water 50:50 for samples containing ascorbic acid with iron or copper) by shaking at 55° for 45 min, centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 2540 × 4.6 μBondapak C18
Mobile phase: Gradient. MeOH:water from 15:85 to 50:50 over 15 min.
Detector: UV 546

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: riboflavin

REFERENCE
Hudson,T.S.; Subramanian,S.; Allen,R.J. Determination of pantothenic acid, biotin, and vitamin B12 in nutritional products, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 994–998.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 22
Limit of quantitation: ≤300 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, pyridoxine, folic acid, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 6 5 μm Capcell Pak C8 (Shiseido, Japan)
Mobile phase: MeOH:50 mM KH_2PO_4 containing 5 mM tetra-n-butylammonium phosphate 15:85, adjusted to pH 2.6 with 5% orthophosphoric acid (After one week of use, wash the column with water and MeOH:water 70:30 at 1 mL/min for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10-20
Detector: UV 215

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, dipotassium glycyrrizate, fumaric acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, maleic acid, neostigmine methylsulfate, pyridoxine, tetrahydrozoline
Noninterfering: chondroitin sulfate, lysozyme

KEY WORDS
ophthalmic solutions; ion-pair agents

REFERENCE
Yamato,S.; Nakajima,M.; Shimada,K. Simultaneous determination of chlorpheniramine and maleate by high-performance liquid chromatography using tetra-n-butylammonium phosphate as an ion-pair reagent, *J.Chromatogr.A*, **1996**, *731*, 346–350.

SAMPLE
Matrix: formulations
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeCN and 10 mL water. Dissolve 20 g powder formulation in 60 mL water, add 10 g NaCl, heat at 50° until completely dissolved, let stand at room temperature for 30 min, make up to 100 mL with water, wash with 10 mL hexane for 3 min. Add the aqueous layer to the SPE cartridge, elute with 8 mL MeCN:water 50:50, evaporate the eluate to dryness under reduced pressure at 50°, reconstitute with 4 mL water, inject a 2 mL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Capcellpak C18 (Shiseido)
Mobile phase: MeCN:water 13:87
Flow rate: 0.6
Injection volume: 2000
Detector: UV 550

CHROMATOGRAM
Retention time: 13
Limit of quantitation: 2.2 ng/g

KEY WORDS
SPE; powders

REFERENCE
Iwase,H. Ultramicrodetermination of cyanocobalamin in elemental diet by solid-phase extraction and high-performance liquid chromatography with visible detection, *J.Chromatogr.*, **1992**, *590*, 359–363.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 9.5

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, pyridoxine, riboflavin, saccharin, thiamine, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: solutions

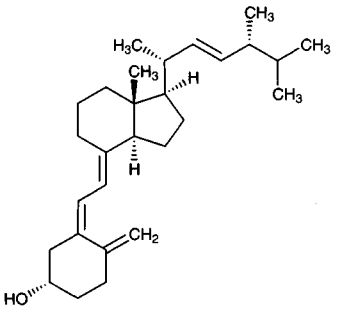
HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, pyridoxamine, thiamine, riboflavin, pyridoxine

REFERENCE
J & W Catalog, 1992-3, p. 277.

Vitamin D2



Molecular formula: $C_{28}H_{44}O$
Molecular weight: 396.66
CAS Registry No.: 50-14-6
Merck Index: 10156

SAMPLE

Matrix: blood

Sample preparation: 3-5 mL Plasma + 3 volumes ether, shake horizontally at 120 oscillations/min for 5 min, let stand for 1-2 min, freeze in dry ice/acetone, repeat ether extraction, extract aqueous layer with 4 volumes dichloromethane:MeOH 75:25, shake for 3-5 min, add 1 mL MeOH, shake for 15 s. Remove the organic layer and wash it twice with 100 mM pH 10.5 phosphate buffer, combine the dichloromethane and ether extracts and evaporate them to dryness under a stream of nitrogen, chromatograph on a 155 × 6 Sephadex LH-20 column with hexane:chloroform:MeOH 90:10:10, discard first 1 mL eluate, collect next 2.5 mL eluate. Evaporate to dryness under a stream of nitrogen, reconstitute with 500 µL hexane:chloroform 95:5 and chromatograph on a 145 × 6 Lipidex 5000 column (Packard) with hexane:chloroform 95:5, discard first 6 mL, collect next 4 mL. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.5 Zorbax Sil
Mobile phase: Dichloromethane:isopropanol 99.75:0.25
Flow rate: 2
Injection volume: 150
Detector: UV 254

CHROMATOGRAM

Retention time: 16

OTHER SUBSTANCES

Interfering: vitamin D3

KEY WORDS

plasma; normal phase; turkey; chicken; cow; pig; sheep; SPE

REFERENCE

Horst,R.L.; Littledike,E.T.; Riley,J.L.; Napoli,J.L. Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals, *Anal.Biochem.*, **1981**, *116*, 189–203.

SAMPLE

Matrix: blood

Sample preparation: 2-3 mL Serum + 3.75 volumes hexane:isopropanol 1:2, shake for 30 min, let stand for 5 min, add 1.25 volume hexane, shake for 5 min, centrifuge at 600 g for 5 min, remove the upper organic layer, extract the aqueous layer twice with 1.25 volumes of hexane. Combine all the organic layers and evaporate them to dryness under a stream of nitrogen at 35°, reconstitute the residue in 50 µL hexane:isopropanol 80:20 and inject on to a 200 × 1 column of Kieselgel Si-60, Size A (Merck) and elute with hexane:isopropanol 80:20 at 2 mL/min, monitor at UV 254 and collect the vitamin D fraction at 6.0-7.5 min, evaporate the eluate to dryness under a stream of nitrogen. Reconstitute with 10 µL hexane:isopropanol 95:5 and inject on to a 50 × 4.6 Polygosil (Macherey-Nagel) + 250 × 4.6 7 µm Nucleosil 50-7 silica column, elute with hexane:isopropanol 85:5 at 2 mL/min, monitor the effluent at UV 254 and

collect the vitamin D fraction at 4.5 min, evaporate the vitamin D fraction, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Vydac)
Column: 250 × 4.6 5 μm TP C18 (Vydac)
Mobile phase: MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Extracted: vitamin D3

KEY WORDS
serum; normal phase; reverse phase

REFERENCE
Parviainen,M.T.; Savolainen,K.E.; Korhonen,P.H.; Alhava,E.M.; Visakorpi,J.K. An improved method for routine determination of vitamin D and its hydroxylated metabolites in serum from children and adults, *Clin.Chim.Acta*, **1981**, *114*, 233–247.

SAMPLE
Matrix: formula
Sample preparation: Condition a 2.8 mL 500 mg silica SPE cartridge with 4 mL dichloromethane:isopropanol 80:20 and 5 mL dichloromethane:isopropanol 99.8:0.2. 15 mL Formula + 4 mL 46 ng/mL vitamin D_3 in EtOH + 15 mL ethanolic KOH, shake at 60° for 30 min, cool to room temperature, add 15 mL water, add 60 mL hexane, shake vigorously for 1.5 min, let stand for 10 min, discard aqueous layer. Wash the hexane layer with 15 mL water, add 15 mL water and 1 drop phenolphthalein to the hexane layer, add 10% acetic acid dropwise with shaking until the aqueous layer is colorless. Filter the hexane layer through anhydrous sodium sulfate, wash through with a few mL hexane, evaporate to dryness under reduced pressure at 40°, reconstitute with 2 mL dichloromethane:isopropanol 99.8:0.2, add to the SPE cartridge, rinse flask with 1 mL dichloromethane:isopropanol 99.8:0.2, add rinse to cartridge, wash with 2 mL dichloromethane:isopropanol 99.8:0.2, elute with 7 mL dichloromethane:isopropanol 99.8:0.2. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL MeCN, inject an aliquot. (Prepare ethanolic KOH by dissolving 140 g KOH in 310 mL EtOH, add 50 mL water. Prepare fresh each day.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 (not end-capped)
Mobile phase: Gradient. MeCN:MeOH:ethyl acetate 91:9:0 for 28 min, to 0:0:100 over 0.5 min, maintain at 0:0:100 for 2.5 min, return to initial conditions over 0.5 min, re-equilibrate for 2.5 min.
Column temperature: 27
Flow rate: 0.7 for 28 min, to 2.5 over 0.5 min, maintain at 2.5 for 4.5 min, return to initial conditions over 1 min
Injection volume: 250
Detector: UV 265

CHROMATOGRAM
Retention time: 19.5
Internal standard: vitamin D_3 (23)

KEY WORDS
protect from light and oxygen; SPE

REFERENCE
Sliva,M.G.; Sanders,J.K. Vitamin D in infant formula and enteral products by liquid chromatography: Collaborative study, *J.AOAC Int.*, **1996**, *79*, 73–80.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out powder equivalent to 200 IU vitamin D3, add 5 μg vitamin D2 and 10 mL EtOH:water 50:50. Extract with 15 mL hexane 3 times. Remove the organic layer and dry it under reduced pressure. Dissolve the residue in 1 mL MeOH:water 90:10 and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm TSK-gel ODS 80TM (TOSOH, Japan)
Mobile phase: MeOH:water 90:10
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M-1000, APCI interface, drift voltage 20 V, focus voltage 120 V, vaporizer 399°, desolvation chamber 200°, multiplier voltage 2 kV

CHROMATOGRAM
Retention time: 14.4
Internal standard: vitamin D2
Limit of detection: 400 pg

OTHER SUBSTANCES
Simultaneous: vitamin D3

KEY WORDS
tablets; vitamin D2 is IS

REFERENCE
Adachi,T.; Nishio,M.; Yunoki,N.; Hayashi,H. Determination of vitamin D_3 and D_2 in multi-vitamin tablets by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry, *Anal.Sci.*, **1994**, *10*, 457–460.

SAMPLE
Matrix: formulations
Sample preparation: Tablets, capsules. Add 5 crushed tablets or the contents from 5 capsules to 10 mL DMSO, add 15 mL hexane, shake at 60° for 45 min, centrifuge at 3000 rpm for 10 min, remove hexane layer, add 15 mL hexane, vortex for 5 min at room temperature, remove hexane layer, repeat hexane extraction three more times, combine all hexane layers, filter, make up to 100 mL with hexane, dilute if necessary, inject a 100 μL aliquot. Syrup. 10 mL Syrup + 10 mL DMSO + 15 mL ether:hexane 10:90, vortex for 5 min, remove hexane layer, repeat extraction four more times, combine the extracts, evaporate with nitrogen until the ether is removed, filter, make up to 100 mL with hexane, dilute if necessary, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm amino bonded phase (Chromatography Sciences Co.)
Mobile phase: Hexane:isopropanol 99:1
Flow rate: 1-2
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: vitamin A
Interfering: cholecalciferol (vitamin D3)

KEY WORDS
work under subdued light; tablets; capsules; syrup

REFERENCE
Beaulieu,N.; Curran,N.M.; Gagné,C.; Gravelle,M.; Lovering,E.G. Liquid chromatographic methods for vitamins A and D in multivitamin-mineral formulations, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 247–254.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20min. Extract the vitamins twice with 10 mL n-hexane. Evaporate to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5μm C18 (Alltech, CA, USA)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 8 (A), 9 (B)

OTHER SUBSTANCES
Extracted: isotretinoin, retinal, tretinoin, vitamin A, vitamin D3, vitamin E, vitamin K1, vitamin K2

REFERENCE
Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax PRO-10 C18
Mobile phase: MeOH:MeCN:hexane 95:3:2
Flow rate: 1
Injection volume: 20
Detector: UV 265

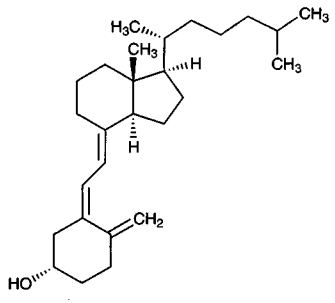
CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: cholecalciferol (vitamin D3), tachysterol

KEY WORDS
keep at 4° away from light; also preparative details

REFERENCE
Letter,W.S. Preparative isolation of vitamin D2 from previtamin D2 by recycle high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *590*, 169–173.

Vitamin D3



Molecular formula: $C_{27}H_{44}O$
Molecular weight: 384.65
CAS Registry No.: 67-97-0
Merck Index: 10157

SAMPLE

Matrix: blood

Sample preparation: 3-5 mL Plasma + 3 volumes ether, shake horizontally at 120 oscillations/min for 5 min, let stand for 1-2 min, freeze in dry ice/acetone, repeat ether extraction, extract aqueous layer with 4 volumes dichloromethane:MeOH 75:25, shake for 3-5 min, add 1 mL MeOH, shake for 15 s. Remove the organic layer and wash it twice with 100 mM pH 10.5 phosphate buffer, combine the dichloromethane and ether extracts and evaporate them to dryness under a stream of nitrogen, chromatograph on a 155 × 6 Sephadex LH-20 column with hexane:chloroform:MeOH 90:10:10, discard first 1 mL eluate, collect next 2.5 mL eluate. Evaporate to dryness under a stream of nitrogen, reconstitute with 500 µL hexane:chloroform 95:5 and chromatograph on a 145 × 6 Lipidex 5000 column (Packard) with hexane:chloroform 95:5, discard first 6 mL, collect next 4 mL. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.5 Zorbax Sil
Mobile phase: Dichloromethane:isopropanol 99.75:0.25
Flow rate: 2
Injection volume: 150
Detector: UV 254

CHROMATOGRAM

Retention time: 16

OTHER SUBSTANCES

Interfering: vitamin D2

KEY WORDS

plasma; normal phase; turkey; chicken; cow; pig; sheep; SPE

REFERENCE

Horst,R.L.; Littledike,E.T.; Riley,J.L.; Napoli,J.L. Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals, *Anal.Biochem.*, **1981**, *116*, 189–203.

SAMPLE

Matrix: blood

Sample preparation: 2-3 mL Serum + 3.75 volumes hexane:isopropanol 1:2, shake for 30 min, let stand for 5 min, add 1.25 volume hexane, shake for 5 min, centrifuge at 600 g for 5 min, remove the upper organic layer, extract the aqueous layer twice with 1.25 volumes of hexane. Combine all the organic layers and evaporate them to dryness under a stream of nitrogen at 35°, reconstitute the residue in 50 µL hexane:isopropanol 80:20 and inject on to a 200 × 1 column of Kieselgel Si-60, Size A (Merck) and elute with hexane:isopropanol 80:20 at 2 mL/min, monitor at UV 254 and collect the vitamin D fraction at 6.0-7.5 min, evaporate the eluate to dryness under a stream of nitrogen. Reconstitute with 10 µL hexane:isopropanol 95:5 and inject on to a 50 × 4.6 Polygosil (Macherey-Nagel) + 250 × 4.6 7 µm Nucleosil 50-7 silica column, elute with hexane:isopropanol 85:5 at 2 mL/min, monitor the effluent at UV 254 and collect the vitamin D fraction at 4.5 min, evaporate the vitamin D fraction, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Vydac)
Column: 250 × 4.6 5 μm TP C18 (Vydac)
Mobile phase: MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 6.0

OTHER SUBSTANCES
Extracted: vitamin D2

KEY WORDS
serum; normal phase; reverse phase

REFERENCE
Parviainen,M.T.; Savolainen,K.E.; Korhonen,P.H.; Alhava,E.M.; Visakorpi,J.K. An improved method for routine determination of vitamin D and its hydroxylated metabolites in serum from children and adults, *Clin.Chim.Acta*, **1981**, *114*, 233–247.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL EtOH, vortex for 3 min, add 3 mL hexane, vortex for 5 min, centrifuge at 1500 g for 10 min, repeat extraction with 3 mL hexane. Combine the hexane layers and wash with 2 mL MeOH:water 9:1. Remove the upper organic layer and filter (0.45 μm) it, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 33.4 μL EtOH, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: ODS
Column: 125 × 4 5 μm LiChrospher 100 RP-18 or 100 × 2.1 3 μm Spherisorb ODS-2
Mobile phase: Gradient. A was MeOH:water 99:1. B was MeOH:THF 70:30. A:B 100:0 for 2 min, 5:95 for 3.5 min, 0:100 for 4.5 min, re-equilibrate for 2 min (step gradients).
Flow rate: 1.5 (125 × 4 column) or 0.2 (100 × 2.1 column)
Injection volume: 5
Detector: UV 265

CHROMATOGRAM
Retention time: 4 (125 × 4 column), 4.5 (100 × 2.1 column)
Limit of detection: 0.44 ng (100 × 2.1 column), 19.8 ng (125 × 4 column)

OTHER SUBSTANCES
Extracted: retinyl palmitate (UV 328), vitamin E (α-tocopherol) (UV 284), vitamin K1 (UV 250)

KEY WORDS
cow; plasma; protect from light; degas stock solutions with helium; narrow bore

REFERENCE
Gomis,D.B.; Escotet Arias,V.J.; Fidalgo Alvarez,L.E.; Gutiérrez Alvarez,M.D. Simultaneous determination of vitamins D3, E and K1 and retinyl palmitate in cattle plasma by liquid chromatography with a narrow-bore column, *J.Chromatogr.B*, **1994**, *660*, 49–55.

SAMPLE
Matrix: formula
Sample preparation: Weigh out formula containing 12 IU vitamin D3, add 15 mL EtOH:isopropanol 95:5, add 400 mg ascorbic acid, shake vigorously for 5 s, add 7.5 g solid KOH, shake vigorously for 20 s, vent pressure, heat at 75° for 30 min, cool, rinse tube into separatory funnel with 5 mL EtOH:isopropanol 95:5, add 130 mL ethyl ether, shake vigorously for 30 s, add 130 mL petroleum ether, shake vigorously for 30 s, discard all but 1 mL of aqueous layer, add 50 mL water, shake vigorously for 15 s, repeat wash, add 15 mL EtOH:isopropanol 95:5, repeat

wash. Evaporate the organic layer to dryness under reduced pressure at <50°, reconstitute with 50 mL acetone, evaporate to dryness, transfer to small tube with three 10 mL portions of ether, evaporate to dryness, reconstitute with 1 mL hexane, vortex for 5 s, inject 320-370 μL on to a 30 × 4.6 Spheri-5 amino + 250 × 4.6 Partisil-5 PAC silica column and elute with hexane:amyl alcohol 99.2:0.8 at 2 mL/min, monitor at UV 254 and collect vitamin D3 fraction (elute peaks after vitamin D3 at 4 mL/min), evaporate collected fraction to dryness under a stream of nitrogen at 50°, reconstitute with 1 mL hexane, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Apex I silica (Jones Chromatography)
Mobile phase: Hexane:amyl alcohol 99.85:0.15
Flow rate: 3
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Retention time: 14-18

KEY WORDS
protect from light; normal phase

REFERENCE
Tanner,J.T.; Barnett,S.A.; Mountford,M.K. Analysis of milk-based infant formula. Phase IV. Iodide, linoleic acid, and vitamins D and K: U.S. Food and Drug Administration-Infant Formula Council: Collaborative study, *J.AOAC Int.*, **1993**, *76*, 1042–1056.

SAMPLE
Matrix: formula
Sample preparation: Condition a 2.8 mL 500 mg silica SPE cartridge with 4 mL dichloromethane:isopropanol 80:20 and 5 mL dichloromethane:isopropanol 99.8:0.2. 15 mL Formula + 4 mL 46 ng/mL vitamin D_2 in EtOH + 15 mL ethanolic KOH, shake at 60° for 30 min, cool to room temperature, add 15 mL water, add 60 mL hexane, shake vigorously for 1.5 min, let stand for 10 min, discard aqueous layer. Wash the hexane layer with 15 mL water, add 15 mL water and 1 drop phenolphthalein to the hexane layer, add 10% acetic acid dropwise with shaking until the aqueous layer is colorless. Filter the hexane layer through anhydrous sodium sulfate, wash through with a few mL hexane, evaporate to dryness under reduced pressure at 40°, reconstitute with 2 mL dichloromethane:isopropanol 99.8:0.2, add to the SPE cartridge, rinse flask with 1 mL dichloromethane:isopropanol 99.8:0.2, add rinse to cartridge, wash with 2 mL dichloromethane:isopropanol 99.8:0.2, elute with 7 mL dichloromethane:isopropanol 99.8:0.2. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL MeCN, inject an aliquot. (Prepare ethanolic KOH by dissolving 140 g KOH in 310 mL EtOH, add 50 mL water. Prepare fresh each day.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 (not end-capped)
Mobile phase: Gradient. MeCN:MeOH:ethyl acetate 91:9:0 for 28 min, to 0:0:100 over 0.5 min, maintain at 0:0:100 for 2.5 min, return to initial conditions over 0.5 min, re-equilibrate for 2.5 min.
Column temperature: 27
Flow rate: 0.7 for 28 min, to 2.5 over 0.5 min, maintain at 2.5 for 4.5 min, return to initial conditions over 1 min
Injection volume: 250
Detector: UV 265

CHROMATOGRAM
Retention time: 23
Internal standard: vitamin D_2 (19.5)

KEY WORDS
protect from light and oxygen; SPE

REFERENCE

Sliva,M.G.; Sanders,J.K. Vitamin D in infant formula and enteral products by liquid chromatography: Collaborative study, *J.AOAC Int.*, **1996**, *79*, 73–80.

SAMPLE

Matrix: formulations

Sample preparation: Powder tablets, weigh out powder equivalent to 200 IU vitamin D3, add 5 μg vitamin D2 and 10 mL EtOH:water 50:50. Extract with 15 mL hexane 3 times. Remove the organic layer and dry it under reduced pressure. Dissolve the residue in 1 mL MeOH:water 90:10 and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm TSK-gel ODS 80TM (TOSOH, Japan)
Mobile phase: MeOH:water 90:10
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M-1000, APCI interface, drift voltage 20 V, focus voltage 120 V, vaporizer 399°, desolvation chamber 200°, multiplier voltage 2 kV

CHROMATOGRAM

Retention time: 14.8
Internal standard: vitamin D2 (14.4)
Limit of detection: 400 pg

KEY WORDS

tablets

REFERENCE

Adachi,T.; Nishio,M.; Yunoki,N.; Hayashi,H. Determination of vitamin D_3 and D_2 in multi-vitamin tablets by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry, *Anal.Sci.*, **1994**, *10*, 457–460.

SAMPLE

Matrix: milk

Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20min. Extract twice with 10 mL n-hexane. Evaporate the extracts to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM

Retention time: 7.3 (A), 10 (B)

OTHER SUBSTANCES

Extracted: isotretinoin, retinal, tretinoin, vitamin A, vitamin D2, vitamin E, vitamin K1, vitamin K2

REFERENCE

Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE

Matrix: milk

Sample preparation: Dilute milk to 30% with water, mix with reagent and pass through a 5 m × 0.5 mm i.d. PTFE tube knotted reactor at 1.25 mL/min, mix with 2.5 M acetic acid, pass

onto column a Sep-Pak Plus C18 SPE cartridge for 5 min, wash column A with MeOH:water 40:60 for 4 min, elute column A with MeOH for 4 min, inject the last 100 μL aliquot of the eluate. (Reagent was 50 mL EtOH + 15 mL 60% aqueous NaOH + 5 mL 10% ascorbic acid.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid-sodium acetate
Flow rate: 1
Injection volume: 100
Detector: E, glassy carbon working electrode +1300 mV

CHROMATOGRAM
Retention time: 10
Limit of detection: 1.77 μM

OTHER SUBSTANCES
Extracted: vitamin A, vitamin E

KEY WORDS
SPE

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez-Perez,A.; Gomez-Perez,M.C.; Hernandez-Mendez,J. Directly coupled sample treatment-high-performance liquid chromatography for on-line automatic determination of liposoluble vitamins in milk, *J.Chromatogr.A*, **1995**, *694*, 399–406.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 μL 10 μg/mL 7-dehydrocholesterol in ethyl acetate with 100 μL ethyl acetate containing 20-50 equivalents 4-phenyl-1,2,4-triazoline-3,5-dione, after 30 min, inject an aliquot.

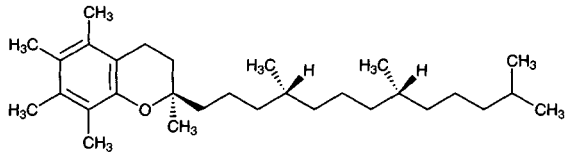
HPLC VARIABLES
Column: 150 × 4.6 5 μm YMC-Gel C8-120-S5
Mobile phase: MeCN:water 90:10
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 7

KEY WORDS
derivatization

REFERENCE
Shimada,K.; Oe,T.; Mizuguchi,T. Cookson-type reagents: highly sensitive derivatization reagents for conjugated dienes in high-performance liquid chromatography, *Analyst*, **1991**, *116*, 1393–1397.

Vitamin E



Molecular formula: $C_{29}H_{50}O_2$
Molecular weight: 430.71
CAS Registry No.: 59-02-9, 58-95-7 (acetate d-form), 52225-20-4 (acetate dl-form), 43119-47-7 (nicotinate)
Merck Index: 10159

SAMPLE

Matrix: blood
Sample preparation: Dilute 100 μL serum with 900 μL 12.62 mg/mL pyrogallol in EtOH, filter (450 μm cellulose disk), cool at 15° in the autosampler, inject a 300 μL aliquot onto column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A onto column B with mobile phase B, after another 1 min remove column A from the circuit. Elute column B with mobile phase B for another 7.5 min then elute with mobile phase C. Monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 4.6 13 μm TSK BSA-80Ts; B 15 × 3.2 5 μm TSK ODS-80Ts + 150 × 4.6 5 μm TSKgel ODS-80Ts
Mobile phase: A 200 mM sodium dodecyl sulfate solution:EtOH 70:30 containing 200 mM ethylenediaminetetraacetic acid 4 sodium salt and 0.3% phosphoric acid; B EtOH:water 80:20; C EtOH:water 87:13
Column temperature: 40
Flow rate: A 1.5; B 1; C 1
Injection volume: 300
Detector: F ex 298 em 325

CHROMATOGRAM

Retention time: 14
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: vitamin A (ex 340 em 460)

KEY WORDS

serum; column-switching

REFERENCE

Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE

Matrix: blood
Sample preparation: Suspend erythrocytes in pH 7.4 isotonic phosphate buffered saline (140 mM NaCl containing 10 mM phosphate buffer), centrifuge at 2900 g for 15 min at 4°. Homogenize erythrocyte precipitate with the same volume water, centrifuge at 725 g and 16260 g for 15 min to give cytosol component and membrane respectively. Filter samples (Millex HV13 0.45 μm) before injection.

HPLC VARIABLES

Column: 150 × 4.6 10 μm Pecosphere HS-5 HC ODS (Perkin-Elmer, USA)
Mobile phase: MeOH:water 93.5:6.5 containing 2.8 mm 1-octanesulfonic acid sodium salt
Flow rate: 1.6
Injection volume: 20

Detector: UV 282

OTHER SUBSTANCES
Also analyzed: tocopherol succinate, tocopherol succinate-3-glucose

KEY WORDS
erythrocytes; cow

REFERENCE
Bonina,F.; Lanza,M.; Montenegro,L.; Salerno,L.; Smeriglio,P.; Trombetta,D.; Saija,A. Transport of α-tocopherol and its derivatives through erythrocyte membranes, *Pharm.Res.*, **1996**, *13*, 1343–1347.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL EtOH + 10 mL hexane, mix for 30 s, centrifuge at 3000 rpm for 5 min, store the hexane layer at 15°, repeat the extraction with 10 mL hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with 200 μL isopropanol, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel ODS-80Ts
Mobile phase: Gradient. EtOH:water 80:20 for 11.5 min then 87:13 (step gradient)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 298 em 325

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Extracted: vitamin A (F ex 340 em 460)

KEY WORDS
serum

REFERENCE
Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 837 ng/mL retinyl acetate in EtOH and 400 μL EtOH to 500 μL plasma, vortex for 30 s, add 2 mL hexane, vortex for 30 s, centrifuge at 1500 rpm for 5 min. Remove the upper hexane layer, add 2 mL hexane to the remaining lower layer, reextract. Evaporate the combined hexane layers to dryness under nitrogen at 40°, reconstitute the residue in 200 μL 30.28 μg/mL (α-tocopheryl acetate in EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm 100 Å pore size Supelguard column (Supelco)
Column: 250 × 4.6 5 μm 100 Å pore size Suplex pKb-100 RP (Supelco)
Mobile phase: MeOH:MTBE:water 80:20:5
Column temperature: 0
Flow rate: 0.8
Injection volume: 50
Detector: UV 292

CHROMATOGRAM
Retention time: 19.9 (α-tocopherol)

Internal standard: α-tocopheryl acetate (16.6)

OTHER SUBSTANCES
Extracted: γ-tocopherol, retinyl acetate

KEY WORDS
plasma

REFERENCE
Lane,J.R.; Webb,L.W.; Acuff,R.V. Concurrent liquid chromatographic separation and photodiode array detection of retinol, tocopherols, all-trans-α-carotene, all-trans-β-carotene and the mono-cis isomers of β-carotene in extracts of human plasma, *J.Chromatogr.A*, **1997**, *787*, 111–118.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge 200 μL serum, add 200 μL IS and 200 μL EtOH, vortex on orbital shaker for 5 min, add 200 μL water and 500 μL hexane, vortex for 10 min, centrifuge at 2000 g for 10 min at 17°, remove 300 μL upper organic layer. Re-extract with 300 μL hexane, vortex for 10 min, centrifuge at 4000 g for 10 min at 17°, remove 300 μL upper organic layer. Combine organic layers, and evaporate to dryness under vacuum in 15 min. Reconstitute the residue with 300 μL MeOH:EtOH:hexane 88:10:2, vortex for 10 min, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Adsorbosphere HS C18 + 150 × 4.6 3 μm Adsorbosphere HS C18 in series
Mobile phase: Step gradient. A was MeCN:MeOH 60:40 containing 0.05% acetic acid. B was MeCN:MeCN:dichloromethane 45.6:30.4:24 containing 0.04% acetic acid. A:B 100:0 for 7 min, to 0:100 7.1 min after injection to 17.4 min, re-equilibrate with A for 5.6 min
Column temperature: 37
Flow rate: 0.9
Injection volume: 40
Detector: UV 292

CHROMATOGRAM
Retention time: 12.4
Internal standard: tocol (UV 292) (10.1), echinenone (UV 450) (12.8)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: canthaxanthine (UV 473), α-carotene (UV 450), β-carotene (UV 450), β-cryptoxanthine (UV 450), lutein (UV 450), lycopene (UV 473), vitamin A (UV 325), zeaxanthin (UV 450), nonidentified carotenoids

KEY WORDS
serum; protect from light

REFERENCE
Steghens,J.-P.; van Kappel,A.L.; Riboli,E.; Collombel,C. Simultaneous measurement of seven carotenoids, retinol and α-tocopherol in serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *694*, 71–81.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL 0.5 μg/mL retinyl acetate, 1 μg/mL retinyl palmitate, and 25 μg/mL α-tocopheryl acetate in EtOH to 1 mL serum or plasma while continuously vortexing, add 3 mL hexane, vortex for 2 min, centrifuge at 2500 g for 2 min, remove the upper phase, add 2 mL hexane to the lower layer, repeat extraction. Combine the upper layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot,

HPLC VARIABLES
Guard column: C18 (Waters)

Column: 5 μm Biophase ODS C18 (Bioanalytical Systems)
Mobile phase: MeCN:chloroform:isopropanol:water 78:16:3.5:2.5
Flow rate: 2
Injection volume: 40
Detector: UV 292 (UV 460 for carotenoids)

CHROMATOGRAM
Retention time: 6.74
Internal standard: retinyl acetate (3.07), retinyl palmitate (18.66), α-tocopheryl acetate (8.33)

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (retinol), gamma-tocopherol, α-carotene, lycopene, cryptoxanthin

KEY WORDS
serum; plasma

REFERENCE
Kaplan,L.A.; Miller,J.A.; Stein,E.A.; Stampfer,M.J. Simultaneous, high-performance liquid chromatographic analysis of retinol, tocopherols, lycopene, and α- and β-carotene in serum and plasma, *Methods Enzymol.*, **1990**, *189*, 155–167.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 25 μL 80 μg/mL tocol in EtOH + 250 μL 20 μg/mL BHT (butylated hydroxytoluene) in EtOH + 1.5 mL hexane, vortex for 1 min, remove 1 mL of upper layer, add 500 μL hexane, vortex for 1 min, remove 300 μL of upper layer. Combine the hexane extracts, evaporate to dryness under a stream of inert gas. Reconstitute in 250 μL 20 μg/mL BHT in EtOH, sonicate, centrifuge if necessary, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201TP54 (wide pore, polymerically bonded C18)
Mobile phase: Gradient. A was MeOH:n-butanol:water 75:10:15 containing 50 mM ammonium acetate, pH 5.5. B was MeOH:n-butanol:water 88:10:2 containing 50 mM ammonium acetate, pH 5.5. A:B 100:0 for 3 min, to 0:100 over 15 min, maintain at 0:100 for 17 min
Injection volume: 25
Detector: UV 325 for 7 min, UV 295 for 13 min, UV 450 for 14 min or E, glassy carbon electrode, Ag/AgCl reference electrode +1050 mV for retinol, +900 mV for tocol, +750 mV for α-tocopherol, +700 mV for β-carotene

CHROMATOGRAM
Retention time: 18.5
Internal standard: tocol (13)
Limit of detection: 650 ng/mL (E), 96 μg/mL (UV)

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (retinol), gamma-tocopherol, lutein, zeaxanthin, cryptoxanthin, α-carotene, 9-cis-β-carotene

KEY WORDS
serum

REFERENCE
MacCrehan,W.A. Determination of retinol, α-tocopherol, and β-carotene in serum by liquid chromatography, *Methods Enzymol.*, **1990**, *189*, 172–181.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL EtOH + 100 μL α-tocopheryl acetate in EtOH, vortex for 5 s, add 500 μL hexane, vortex for 2 min, centrifuge at 700 g for 5 min. Remove 250

μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, mix for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm Spheri-10 RP18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:dichloromethane:MeOH 70:20:10
Flow rate: 1.2
Injection volume: 50
Detector: UV 325 for 3.5 min, UV 291 for 4.5 min, UV 450 for 6 min

CHROMATOGRAM
Retention time: 5.31
Internal standard: α-tocopheryl acetate (6.30)
Limit of detection: 180 nM

OTHER SUBSTANCES
Extracted: beta carotene, vitamin A

KEY WORDS
protect from light; serum

REFERENCE
Arnaud,J.; Fortis,I.; Blachier,S.; Kia,D.; Favier,A. Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *572*, 103–116.

SAMPLE
Matrix: blood
Sample preparation: 2.5 mL Plasma + 2.5 mL 18 ng/mL IS1 and 10 ng/mL IS2 in EtOH, shake vigorously for 20 s, centrifuge at 1200 g for 5 min, add 5 mL diethyl ether, shake vigorously, centrifuge for 5 min, extract twice more with 5 mL ether. Combine ether layers, wash with 15 mL 5% NaCl, dry over sodium sulfate, evaporate to dryness under vacuum at 35°. Dissolve residue in 1-2 mL dichloromethane, filter (0.45 μm). Evaporate to dryness under a stream of nitrogen, make up to 100 μL with MeCN:MeOH:dichloromethane:hexane 45:10:22.5:22.5, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spheri-5-C18 (Brownlee)
Column: 250 × 4.6 5 μm Microsorb C18 (Rainin)
Mobile phase: Gradient. MeCN:MeOH:dichloromethane:hexane 85:10:2.5:2.5 for 10 min then to 45:10:22.5:22.5 over 30 min, re-equilibrate for 15 min
Flow rate: 0.7
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 24
Internal standard: IS1 ethyl β-apo-8'-carotenate (18), IS2 (3R)-8'-apo-β-carotene-3,8'-diol (5)

OTHER SUBSTANCES
Extracted: carotenoids, β-carotene, vitamin A (retinol)

KEY WORDS
plasma; handle under yellow lights

REFERENCE
Khachik,F.; Beecher,G.R.; Goli,M.B.; Lusby,W.R.; Smith,J.C.,Jr. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma, *Anal.Chem.*, **1992**, *64*, 2111–2122.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 25 μg/mL tocopheryl acetate in EtOH, vortex, add 400 μL butanol:ethyl acetate 50:50, mix for 1 min, add 20 mg sodium sulfate, vortex for 1 min, let stand at -20° for 20 min, centrifuge at 15000 g for 2 min, inject a 10 μL aliquot of the upper organic layer.

HPLC VARIABLES
Guard column: 5 μm C18
Column: 110 × 4.7 5 μm Partisphere 5 C18 (Whatman)
Mobile phase: MeOH:butanol:water 89.5:5:5.5
Column temperature: 45
Flow rate: 1.5
Injection volume: 10
Detector: UV 340 for 3 min, UV 290 for 1.5 min, UV 280 for 10.5 min, UV 450 for 7 min

CHROMATOGRAM
Retention time: 4.0
Internal standard: tocopheryl acetate (5.3)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: α-carotene, β-carotene, lycopene, δ-tocopherol, gamma-tocopherol, vitamin A, xanthophyll

KEY WORDS
serum; plasma; protect from light

REFERENCE
Lee,B.L.; Chua,S.C.; Ong,H.Y.; Ong,C.N. High-performance liquid chromatographic method for routine determination of vitamins A and E and β-carotene in plasma, *J.Chromatogr.*, **1992**, *581*, 41–47.

SAMPLE
Matrix: blood
Sample preparation: Dilute 1 mL serum 0.5-5 times with saline. Add 1 mL 10 μg/mL IS in EtOH to 1 mL diluted serum dropwise while vortexing, add 1.5 mL n-heptane, vortex for 1 min, centrifuge at 3000 rpm (Labofuge) for 15 min. Remove 1.3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 40 μL MeCN:THF 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil
Mobile phase: MeCN:water:THF 81.3:5.7:13
Column temperature: 40
Flow rate: 0.4
Injection volume: 5
Detector: UV 292

CHROMATOGRAM
Retention time: 5.585
Internal standard: α-tocopherol acetate (6.185)
Limit of detection: 15 ng

OTHER SUBSTANCES
Extracted: probucol, gamma-tocopherol, vitamin A (retinol), lycopene, α-carotene, β-carotene, metabolites

KEY WORDS
serum

REFERENCE
Schäfer Elinder,L.; Walldius,G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants, *J.Lipid Res.*, **1992**, *33*, 131–137.

SAMPLE
Matrix: blood
Sample preparation: 20-500 μL Serum + 2 volumes EtOH + 1 mL ethyl acetate + 4-7 μL of a solution containing 16 mg/mL tocopheryl acetate, 2-3 μg/mL canthaxanthin, and 10 μg/mL retinoic acid, vortex for 30 s, centrifuge for 30 s, extract the pellet twice with 0.5-1 mL portions of ethyl acetate, extract the pellet with 0.5-1 mL hexane. Combine the supernatants, add 500 μL water, vortex, centrifuge. Remove the upper organic layer and evaporate it to dryness under a stream of argon, reconstitute the residue in 100 μL MeOH:dichloromethane 2:1, inject a 10-90 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 300 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:dichloromethane:MeOH:1-octanol 90:15:10:0.1
Flow rate: 1
Injection volume: 10-90
Detector: UV 290

CHROMATOGRAM
Retention time: 10
Internal standard: tocopheryl acetate, canthaxanthin, retinoic acid

OTHER SUBSTANCES
Extracted: beta carotene (UV 450), carotenoids (UV 450), vitamin A (UV 325)

KEY WORDS
protect from light; serum

REFERENCE
Barua,A.B.; Kostic,D.; Olson,J.A. New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum, *J.Chromatogr.*, **1993**, *617*, 257–264.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 500 μL EtOH containing 4.27 μM retinyl acetate and 0.31 μM echinenone, rotamix for 30 s, add 2 mL n-hexane, rotamix for 30 s, centrifuge at 2000 g for 2 min, repeat extraction with 2 mL n-hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL THF, make up to 200 μL with EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Spherisorb ODS1
Column: 250 × 4.6 5 μm Spherisorb ODS1
Mobile phase: Gradient. A was MeCN:MeOH 20:80 containing 100 mM ammonium acetate. B was 100 mM ammonium acetate in water. A:B from 90:10 to 100:0 over 12 min, maintain at 100:0 for 10 min, re-equilibrate at initial conditions for 5 min
Flow rate: 2
Injection volume: 50
Detector: UV 325 for 7.5 min, UV 292 for 5.5 min, then UV 450

CHROMATOGRAM
Retention time: 11.87
Internal standard: retinyl acetate (5.96), echinenone (15.15)
Limit of detection: 5.80 μM

OTHER SUBSTANCES
Extracted: β-carotene, cryptoxanthin, lutein, lycopene, vitamin A

KEY WORDS
plasma; protect from light; serum

REFERENCE
Zaman,Z.; Fielden,P.; Frost,P.G. Simultaneous determination of vitamins A and E and carotenoids in plasma by reversed-phase HPLC in elderly and younger subjects, *Clin.Chem.*, **1993**, *39*, 2229–2234.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 200 μL 850 ng/mL retinyl acetate in EtOH, mix for 1 min, add 1 mL 0.4 g/L BHT (2,6-di-tert-butyl-4-methylphenol) in n-hexane, shake on a mechanical shaker for 10 min, centrifuge at 2000 g for 5 min, remove 800 μL of the supernatant, evaporate to dryness at 40° under a stream of nitrogen, reconstitute in 100 μL MeCN:THF:MeOH 68:22:7, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Lichrosorb RP18
Column: 250 × 4.6 5 μm Nucleosil 100-5 C18
Mobile phase: MeCN:THF:MeOH 68:22:7 made up to 100 with 1% ammonium acetate
Flow rate: 1.5
Injection volume: 15
Detector: UV 325 for 3 min, UV 450 for 1.9 min, UV 290 for 2.5 min, UV 470 for 4.6 min, UV 450 for 3 min, then UV 325 for rest of run

CHROMATOGRAM
Retention time: 6.3
Internal standard: retinyl acetate (2.7)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (retinol), lutein, lycopene, α-carotene, zeaxanthin, trans β-carotene, δ-tocopherol

KEY WORDS
plasma; serum; protect from sunlight

REFERENCE
Bui,M.H. Simple determination of retinol, α-tocopherol and carotenoids (lutein, all-*trans*-lycopene, α- and β-carotenes) in human plasma by isocratic liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 129–133.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL EtOH, vortex for 3 min, add 3 mL hexane, vortex for 5 min, centrifuge at 1500 g for 10 min, repeat extraction with 3 mL hexane. Combine the hexane layers and wash with 2 mL MeOH:water 9:1. Remove the upper organic layer and filter (0.45 μm) it, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 33.4 μL EtOH, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: ODS
Column: 125 × 4 5 μm LiChrospher 100 RP-18 or 100 × 2.1 3 μm Spherisorb ODS-2
Mobile phase: Gradient. A was MeOH:water 99:1. B was MeOH:THF 70:30. A:B 100:0 for 2 min, 5:95 for 3.5 min, 0:100 for 4.5 min, re-equilibrate for 2 min (step gradients).
Flow rate: 1.5 (125 × 4 column) or 0.2 (100 × 2.1 column)
Injection volume: 5
Detector: UV 284

CHROMATOGRAM
Retention time: 5 (125 × 4 column), 6 (100 × 2.1 column)
Limit of detection: 2.8 ng (100 × 2.1 column), 106.3 ng (125 × 4 column)

OTHER SUBSTANCES
Extracted: retinyl palmitate (UV 328), vitamin D3 (cholecalciferol) (UV 265), vitamin K1 (UV 250)

KEY WORDS
cow; plasma; protect from light; degas stock solutions with helium; narrow bore

REFERENCE
Gomis,D.B.; Escotet Arias,V.J.; Fidalgo Alvarez,L.E.; Gutiérrez Alvarez,M.D. Simultaneous determination of vitamins D3, E and K1 and retinyl palmitate in cattle plasma by liquid chromatography with a narrow-bore column, *J.Chromatogr.B*, **1994**, *660*, 49–55.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL nonapreno-β-carotene and retinyl butyrate in EtOH, vortex for 10 s, add 1 mL hexane, vortex for 30 s, centrifuge at 1500 g for 5 min. Remove 900 μL of the hexane layer and evaporate it to a waxy or glassy consistency (not dryness) under vacuum, dissolve in 100 μL EtOH, add 100 μL MeCN, vortex, filter (0.45 μm), inject a 30 μL aliquot.

HPLC VARIABLES
Column: 1540 × 4.6 5 μm Ultramex C18 (Phenomenex)
Mobile phase: MeCN:EtOH 50:50 containing 0.1 mL/L diethylamine
Column temperature: 29
Flow rate: 0.9
Injection volume: 30
Detector: UV 300

CHROMATOGRAM
Retention time: 4.60
Internal standard: nonapreno-β-carotene (9.5, UV 450), retinyl butyrate (3.5, UV 300)
Limit of detection: 460 nM

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (retinol), lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, retinyl linoleate, retinyl oleate, retinyl palmitate, retinyl stearate

KEY WORDS
serum; use gold fluorescent lamps; hold sample at 4° before injection

REFERENCE
Sowell,A.L.; Huff,D.L.; Yeager,P.R.; Caudill,S.P.; Gunter,E.W. Retinol, α-tocopherol, lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, trans-β-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection, *Clin.Chem.*, **1994**, *40*, 411–416.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 2.5 mL EtOH, mix for 5 min, add 5 mL n-hexane, mix vigorously, centrifuge at 2000 g for 5 min, repeat extraction with 3 mL n-hexane. Combine the n-hexane layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in dichloromethane, inject an aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak + 50 mm long C18 ODS(4) (Shimadzu)
Column: 250 × 4.6 5 μm Vydac 201 TP 54 C18
Mobile phase: MeOH:MeCN 90:10 (Every 100 injections wash column with MeOH:MeCN:dichloromethane 8:1:1.)
Flow rate: 1
Detector: UV 291

CHROMATOGRAM
Retention time: 6.9

OTHER SUBSTANCES
Extracted: beta carotene (UV 451), vitamin A (UV 324)

KEY WORDS
serum

REFERENCE
Ben-Amotz,A. Simultaneous profiling and identification of carotenoids, retinols, and tocopherols by high performance liquid chromatography equipped with three-dimensional photodiode array detection, *J.Liq.Chromatogr.*, **1995**, *18*, 2813–2825.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 2 mL MeOH, vortex for 5 min, centrifuge at 1200 g for 10 min, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 200 × 4.6 10 μm LiChrosorb RP-18
Mobile phase: MeOH
Flow rate: 1
Injection volume: 20
Detector: UV 292

CHROMATOGRAM
Retention time: 11.1 (vitamin E), 14.5 (vitamin E acetate)
Limit of detection: 750 ng/mL (vitamin E), 200 ng/mL (vitamin E acetate)

KEY WORDS
plasma; dog; pharmacokinetics

REFERENCE
Santiago Torrado,D.; Jimenez Caballero,E.; Cadorniga,R.; Torrado,J. A selective liquid chromatography assay for the determination of dl-α-tocopherol acetate on plasma samples, *J.Liq.Chromatogr.*, **1995**, *18*, 1251–1264.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 650 ng/mL tocopheryl acetate in MeOH, vortex for 30 s, add 200 μL n-hexane, shake for 15 min, centrifuge at 3000 rpm for 10 min. Remove 120 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 20 μL dichloromethane, add 100 μL MeCN:MeOH 50:50, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:dichloromethane 50:45:5
Flow rate: 0.7
Injection volume: 50
Detector: F ex 325 em 480 for 5 min, then ex 295 em 330 for 10 min, then ex 325 em 480

CHROMATOGRAM
Retention time: 7.3
Internal standard: tocopheryl acetate (9.3)

OTHER SUBSTANCES
Extracted: vitamin A, γ-tocopherol, retinyl palmitate, β-carotene (UV 450), α-carotene (UV 450)

KEY WORDS
serum

REFERENCE

Yakushina,L.; Taranova,A. Rapid HPLC simultaneous determination of fat-soluble vitamins, including carotenoids, in human serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 715–718.

SAMPLE

Matrix: blood

Sample preparation: Mix 500 μL plasma with 30 μL 500 mM sodium bisulfite, deoxygenate with nitrogen, add 500 μL EtOH (deoxygenated with nitrogen), mix vigorously for 3 min, add 2.5 mL n-hexane (deoxygenated with nitrogen), mix, centrifuge at 4° at 3000 rpm for 5 min. Remove the hexane layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase (deoxygenated with nitrogen), inject an aliquot

HPLC VARIABLES

Column: 80 × 4.6 3 μm MC MEDICAL C18 (MC Medical, Tokyo)

Mobile phase: MeOH:water 96:4 containing 40 mM sodium perchlorate (deoxygenate with nitrogen)

Column temperature: 35

Flow rate: 1

Injection volume: 100

Detector: E, ESA, Model 5100A, Model 5020 guard cell before injector -0.45 V, Model 5021 conditioning cell after column but before analytical cell -0.45 V, Model 5011 analytical cell at -0.45 V and +0.40 V (monitored)

CHROMATOGRAM

Retention time: 7.2

Limit of detection: 50 pg

OTHER SUBSTANCES

Extracted: β-tocopherol, gamma-tocopherol, δ-tocopherol, α-tocopherolquinone

KEY WORDS

recirculate mobile phase; rat; plasma

REFERENCE

Takeda,H.; Shibuya,T.; Yanagawa,K.; Kanoh,H.; Takasaki,M. Simultaneous determination of α-tocopherol and α-tocopherolquinone by high-performance liquid chromatography and coulometric detection in the redox mode, *J.Chromatogr.A*, **1996**, *722*, 287–294.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. 100 μL Plasma + 100 μL 0.9% NaCl + 200 μL MeOH, vortex for 30 s, let stand for 10 min, add 400 μL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot. Tissue. Homogenize (liver with Mikro-dismembrator II in liquid nitrogen; other tissue with Ultra-Turrax) tissue with 3 mL 1% acetic acid containing 1 mg/mL ascorbic acid and 10 mM EDTA, add 2 mL MeOH, vortex for 30 s, let stand for 10 min, add 4 mL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4.6 3 μm Hypersil ODS

Mobile phase: MeCN:dichloromethane:MeOH:water 70:10:15:5

Flow rate: 0.5 for 13 min, to 1 over 1 min, maintain at 1 for 10 min, to 1.5 over 1 min, maintain at 1.5 for 21 min, to 2 over 1 min, maintain at 2 for 10 min, return to 0.5 over 1 min, maintain at 0.5 for 2 min.

Injection volume: 50

Detector: UV 292

CHROMATOGRAM

Retention time: 17

Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: beta carotene (UV 445), vitamin A (UV 350)

KEY WORDS
rat; protect from light; liver; plasma; lung

REFERENCE
Van Vliet,T.; Van Schaik,F.; Van Schoonhoven,J.; Schrijver,J. Determination of several retinoids, carotenoids and E vitamers by high-performance liquid chromatography. Application to plasma and tissues of rats fed a diet rich in either β-carotene or canthaxanthin, *J.Chromatogr.*, **1991**, *553*, 179–186.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, discard the effluent, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 3.5 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Spherisorb RP-18
Mobile phase: MeCN:MeOH 70:30
Flow rate: 1.5
Injection volume: 10
Detector: UV 290

CHROMATOGRAM
Retention time: 7.78 (vitamin E), 8.79 (vitamin E acetate)
Internal standard: anthraquinone (1.89)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: vitamin A

KEY WORDS
plasma; SPE

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: cheese
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70° for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10. Combine the organic layers and evaporate them to dryness, dissolve the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 280 em 475 (vitamin E), UV 450 (β-carotene), and F ex 325 em 475 for 3.5 min, ex 280 em 475 for 10.5 min, ex 325 em 475 for 9 min (others)

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.90 ng

OTHER SUBSTANCES
Extracted: β-carotene, vitamin A (all-trans-retinol), β-tocopherol, gamma-tocopherol, δ-tocopherol, 13-cis-retinol

KEY WORDS
normal phase; cheese

REFERENCE
Panfili,G.; Manzi,P.; Pizzoferrato,L. High-performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses, *Analyst*, **1994**, *119*, 1161–1165.

SAMPLE
Matrix: food
Sample preparation: Melt 5 g margarine at 40° and homogenize it. Mix 20 g infant formula with 20 g water at 50°. Cut 5 g broccoli, mix in a homogenizer, freeze dry for 48 h at -40°, grind to a fine powder, mix 5 g in 30 mL water. Add 25 mL 600 g/L KOH to 5 g sample. Add solid KOH to the suspension until the KOH concentration is 60% (w/v). Add 15 mL EtOH to each 5 mL portion, shake. Add 25 mg ascorbic acid per gram, mix, flush with nitrogen, heat at 80° for 40 min. Immediately cool to room temperature. Add water to make EtOH:water ratio 0.3. Add 100 mL n-hexane:ethyl acetate 90:10, shake, let layers separate. Extract the aqueous phase with two 100 mL portions of n-hexane:ethyl acetate 90:10. Combine organic phases, wash with 100 ml water until the washes are neutral. Filter the organic phase through phase separating filter paper (S&S 597 HY, Schliecher and Schuell, Germany). Evaporate the filtrate under reduced pressure, dissolve the residue in hexane containing 20 mg/L 2,6-di-tert-butyl-4-methylphenol, inject a 30 mL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb Diol
Mobile phase: n-Hexane:MTBE 94:6
Column temperature: 23
Flow rate: 1
Injection volume: 30
Detector: F ex 295 em 330

CHROMATOGRAM
Retention time: 11.5 (α), 18.5 (β), 21.0 (γ), 30.5 (δ)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: 2-tert-butyl-4-hydroxyanisole, di-tert-butyl-4-methylphenol, plastochromanol-8, tocodienol, tocomoneols, tocotrienols

KEY WORDS
broccoli; formula; margarine

REFERENCE
Konings,E.J.M.; Roomans,H.H.S.; Beljaars,P.R. Liquid chromatographic determination of tocopherols and tocotrienols in margarine, infant foods, and vegetables, *J.AOAC Int.*, **1996**, *79*, 902–906.

SAMPLE
Matrix: food
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70, for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10 (Analyst 1994, 119, 1161). Combine the organic layers and evaporate them to dryness, dissolve the residue in MeOH:dichloromethane 90:10, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Supelco C18
Mobile phase: MeOH
Flow rate: 2
Detector: F ex 280 em 325

CHROMATOGRAM
Retention time: 4.2 (δ-tocopherol), 4.7 (β and γ-tocopherol), 5.3 (α-tocopherol)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: vitamin A (F ex 325 em 425), sterols (UV 208), carotenes (UV 450)

KEY WORDS
cheese

REFERENCE
Manzi,P.; Panfili,G.; Pizzoferrato,L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products, *Chromatographia*, **1996**, *43*, 89–91.

SAMPLE
Matrix: food
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70, for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10 (Analyst 1994, 119, 1161). Combine the organic layers and evaporate them to dryness, dissolve the residue in n-hexane:2-propanol 99:1, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si (Beckman)
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Detector: F ex 325 em 475

CHROMATOGRAM
Retention time: 4.5 (α-tocopherol), 8.0 (β-tocopherol), 8.7 (γ-tocopherol), 13.5 (δ-tocopherol)
Limit of detection: 900 pg (α), 800 pg (β), 500 pg (γ), 700 pg (δ)

OTHER SUBSTANCES
Extracted: vitamin A (13-cis-retinol, all trans-retinols) (F ex 325 em 425), sterols (UV 208), carotenes (UV 450)

KEY WORDS
cheese; normal phase

REFERENCE
Manzi,P.; Panfili,G.; Pizzoferrato,L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products, *Chromatographia*, **1996**, *43*, 89–91.

SAMPLE
Matrix: formula
Sample preparation: Reconstitute 28 g milk based infant formula with ca. 145 g 78-80° water, mix thoroughly. Add 15 mL boiling isopropanol to 6.5 g reconstituted infant formula, mix thoroughly. Add 7.5 g magnesium sulfate, 30 mL hexane:ethyl acetate 85:15 and 1 mL BHT. Homogenize mixture for 1 min, filter through a coarse porosity glass filter by vacuum, wash the magnesium sulfate cake with two 15 mL portions of hexane:ethyl acetate 85:15. Repeat the extraction with 5 mL isopropanol and 15 mL hexane:ethyl acetate 85:15. Add 1 g magnesium sulfate to the combined filtrate and evaporate it to dryness under nitrogen. Dissolve the residue

in 10 mL hexane, filter (0.45 μm), evaporate to a volume of less than 5 mL at 45°. Dilute to 10 mL with mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: Hexane:isopropanol 99.5:0.5
Flow rate: 1
Injection volume: 50
Detector: F ex 285 em 310

CHROMATOGRAM
Retention time: 2.7 (α-tocopheryl acetate), 10.1 (γ tocopherol), 17.3 (δ tocopherol)
Limit of detection: 3.14 ng/mL

KEY WORDS
infant formula; normal phase

REFERENCE
Chase,Jr.,G.W.; Eitenmiller,R.R.; Long,A.R. Liquid chromatographic analysis of all-RAC-α-tocopheryl acetate, tocopherols, and retinyl palmitate in SRM 1846, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3317–3327.

SAMPLE
Matrix: formula, reference material
Sample preparation: 10 g Zero-control reference material (ZRM) powder + 50 g hot water, mix. 6.5 g Reconstituted ZRM or 3.5 g concentrated commercial formula + 10 mL boiling isopropanol, mix, add 7.5 g anhydrous magnesium sulfate to the ZRM and 4 g to the concentrated commercial formula, mix thoroughly, add 25 mL hexane:ethyl acetate 85:15, add 1 mL 360 μg/mL BHT, mix, homogenize (Polytron) for 1 min, filter through 60 mL coarse-porosity fritted glass filter using vacuum, wash with two 15 mL portions of hexane:ethyl acetate 85:15. Re-extract with 20 mL hexane:ethyl acetate 85:15 and 5 mL isopropyl alcohol, homogenize for 1 min, filter through 60 mL coarse-porosity fritted glass filter using vacuum, wash with two 15 mL portions of hexane:ethyl acetate 85:15. Mix the combined filtrate with 500 mg anhydrous magnesium sulfate, evaporate to dryness, add 15 mL hexane to the residue, filter (0.45 μm nylon) using vacuum, wash with three 7 mL portions of hexane, evaporate to 1 mL with nitrogen at 45°, dilute to 10 mL with hexane, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: Hexane:isopropanol 99.5:0.5
Flow rate: 1
Injection volume: 50
Detector: F ex 285 em 310

CHROMATOGRAM
Retention time: 3.4 (vitamin E acetate)
Limit of quantitation: 2.0 μg/mL

OTHER SUBSTANCES
Simultaneous: (R,R,R)-α.-tocopherol, δ-tocopherol, γ -tocopherol
Also analyzed: vitamin A

KEY WORDS
normal phase; soy-based infant formula

REFERENCE
Chase,G.W.,Jr.; Long,A.R.; Eitenmiller,R.R. Liquid chromatographic method for analysis of all-rac-α-tocopheryl acetate and retinyl palmitate in soy-based infant formula using a zero-control reference material (ZRM) as a method development tool, *J.AOAC Int.*, **1998**, *81*, 577–581.

SAMPLE
Matrix: formulations

Sample preparation: Mix 100-150 mg of the formulation with celite and extract with supercritical carbon dioxide at 250 atmospheres at 40° at 190-220 mL/min with the restrictor at 100° (Dionex SFE-703), collect in 4 mL THF:MeOH 80:20 at 0°, make up to 5 mL, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH 25:75
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: 6 (vitamin E acetate)

OTHER SUBSTANCES
Simultaneous: vitamin A palmitate (UV 325)

KEY WORDS
SFE; cream; lotion; protect from light

REFERENCE
Scalia,S.; Renda,A.; Ruberto,G.; Bonina,F.; Menegatti,E. Assay of vitamin A palmitate and vitamin E acetate in cosmetic creams and lotions by supercritical fluid extraction and HPLC, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 273–277.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder, weigh out 100-150 mg, mix with sea sand. Extract with supercritical carbon dioxide (Dionex) in the dynamic mode at 250 atmospheres and 40° for 15 min (restrictor 60°, gaseous flow rate 190-220 mL/min), collect in 6 mL THF at 0°, make up to 10 mL with THF, inject an aliquot. [Alternatively, add 5 crushed tablets or the contents from 5 capsules to 10 mL DMSO, add 15 mL hexane, shake at 60° for 45 min, centrifuge at 3000 rpm for 10 min, remove hexane layer, add 15 mL hexane, vortex for 5 min at room temperature, remove hexane layer, repeat hexane extraction three more times, combine all hexane layers, dilute with THF, inject a 25 μL aliquot. J.Assoc.Off.Anal.Chem. 1989, 72, 247.]

HPLC VARIABLES
Guard column: 4 × 4 5 μm (Merck)
Column: 250 × 4 5 μm Lichrospher CH-8
Mobile phase: MeOH:MeCN 75:25
Flow rate: 1.3
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 6 (vitamin E acetate)

OTHER SUBSTANCES
Simultaneous: vitamin A palmitate (UV 325)

KEY WORDS
tablets; SFE

REFERENCE
Scalia,S.; Ruberto,G.; Bonina,F. Determination of vitamin A, vitamin E, and their esters in, *J.Pharm.Sci.*, **1995**, *84*, 433–436.

SAMPLE
Matrix: fat spread products, margarine

Sample preparation: Melt two sticks of margarine at 45°. Homogenize spreads without warming. Weigh 5.0 g sample. Add 40 mL hexane-BHT and sonicate with intermittent mixing. Rinse side with 10 mL hexane-BHT, add 3 drops of Tween 80, 3 g anhydrous magnesium sulfate (1g for each mL of water content/1g magnesium sulfate) and vortex. Stand for ≥ 2 h. Filter by medium porosity fritted glass filter. Wash with hexane-BHT. Filtrate dilute to 100 mL with hexane-BHT. Pipet 1.0 mL and dilute to 50 mL with hexane-BHT. Inject an 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrosorb Si 60
Column: 250 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: n-Hexane:isopropyl alcohol 99.1:0.9
Flow rate: 1.0
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM
Retention time: 10.6
Limit of detection: 1.98 μg/100g
Limit of quantitation: 3.02 μg/100g

OTHER SUBSTANCES
Extracted: α-tocopherol, γ-tocopherol

KEY WORDS
δ-tocopherol

REFERENCE
Ye,L.; Landen,W.O.,Jr.; Lee,J.; Eitenmiller,R.R. Vitamin E content of margarine and reduced fat products using a simplified extraction procedure and HPLC determination, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1227–1238.

SAMPLE
Matrix: fat spread products, margarine
Sample preparation: Melt two sticks of margarine at 45°. Homogenize spreads without warming. Weigh 5.0 g sample. Add 40 mL hexane-BHT and sonicate with intermittent mixing. Rinse side with 10 mL hexane-BHT, add 3 drops of Tween 80, 3 g anhydrous magnesium sulfate (1g for each mL of water content/1g magnesium sulfate) and vortex. Stand for ≥ 2 h. Filter by medium porosity fritted glass filter. Wash with hexane-BHT. Filtrate dilute to 100 mL with hexane-BHT. Pipet 1.0 mL and dilute to 50 mL with hexane-BHT. Inject an 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrosorb Si 60
Column: 250 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: n-Hexane:isopropyl alcohol 99.1:0.9
Flow rate: 1.0
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 2.96 μg/100g
Limit of quantitation: 5.00 μg/100g

OTHER SUBSTANCES
Extracted: α-tocopherol, δ-tocopherol

KEY WORDS
γ-tocopherol

REFERENCE

Ye,L.; Landen,W.O.,Jr.; Lee,J.; Eitenmiller,R.R. Vitamin E content of margarine and reduced fat products using a simplified extraction procedure and HPLC determination, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1227–1238.

SAMPLE

Matrix: margarine, spreads

Sample preparation: Sonicate 5.0 g homogenized margarine or spread with 40 mL 0.1% BHT in hexane with intermittent mixing until sample has dissolved. Rinse sides of flask with 10 mL 0.1% BHT in hexane, add 3 drops Tween 80, add 3 g anhydrous magnesium sulfate (1 g for each 1 mL of water content plus 1 g extra), mix, let stand for ≥ 2 h. Filter (medium porosity fritted glass filter) and wash the filter with 0.1% BHT in hexane, dilute the filtrate to 100 mL with 0.1% BHT in hexane. Dilute a 1.0 mL aliquot to 50 mL with 0.1% BHT in hexane, filter (0.45 μ m). Inject an 20 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm LiChrosorb Si 60
Column: 250 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: n-Hexane:isopropyl alcohol 99.1:0.9
Flow rate: 1.0
Injection volume: 20
Detector: F ex 290 em 330

CHROMATOGRAM

Retention time: 4.8
Limit of detection: 232 ng/g
Limit of quantitation: 398.4 ng/g

OTHER SUBSTANCES

Extracted: γ-tocopherol, δ-tocopherol

KEY WORDS

normal phase

REFERENCE

Ye,L.; Landen,W.O.,Jr.; Lee,J.; Eitenmiller,R.R. Vitamin E content of margarine and reduced fat products using a simplified extraction procedure and HPLC determination, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1227–1238.

SAMPLE

Matrix: milk

Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20min. Extract twice with 10 mL n-hexane. Evaporate to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM

Retention time: 8.9 (A), 11 (B)

OTHER SUBSTANCES

Extracted: isotretinoin, retinal, tretinoin, vitamin A, vitamin D2, vitamin D3, vitamin K1, vitamin K2

REFERENCE

Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: milk
Sample preparation: Add 500 mg ascorbic acid, 50 mL EtOH, and 10 mL 60% KOH to 25 g liquid or reconstituted powdered milk under a nitrogen stream, stir overnight at room temperature. Extract with three 50 mL portions of n-hexane and two 25 mL portions of n-hexane by shaking for 2 min each. Combine the n-hexane extracts, wash with 50 mL portions of water containing a few drops of phenolphthalein until the aqueous phase appears colorless, add 1 g butylated hydroxytoluene, filter through a Whatman No.1 filter containing 20 g anhydrous sodium sulfate, concentrate the filtrate under reduced pressure at 40°, reconstitute with 10 mL MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: Tracer Spherisorb ODS 2 C18
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeCN:MeOH:water 1:95:4
Injection volume: 20
Detector: UV 323 for 14 min then UV 292

CHROMATOGRAM
Retention time: 20
Limit of detection: 300 ng/mL
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: vitamin A

REFERENCE
Albalá-Hurtado,S.; Novella-Rodríguez,S.; Veciana-Nogués,M.; Mariné-Font,A. Determination of vitamins A and E in infant milk formulae by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 243–246.

SAMPLE
Matrix: milk
Sample preparation: 1 g Powdered milk or 25 mL liquid milk + alcoholic KOH, let stand overnight, extract with hexane. Remove the organic layer and evaporate it to dryness, reconstitute the residue in MeOH, filter, inject an aliquot. (Alcoholic KOH was prepared from 50 mL EtOH and 15 mL 60% KOH in water.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid/sodium acetate
Flow rate: 1.25
Injection volume: 10
Detector: E, EG & G PAR Model 400, MP 1304 glassy carbon series dual electrode, E1 (upstream) -1100 mV, E2 (downstream) +700 mV (Condition electrodes for 30 min at E1 -1200 mV and E2 +1500 mV at the start of each day.)

CHROMATOGRAM
Retention time: 9
Limit of detection: 0.19 ng

OTHER SUBSTANCES
Extracted: vitamin A

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez Perez,A.; Gomez Perez,M.C.; Fernandez Moro,M.A.; Hernandez Mendez,J. Determination of vitamins A, E and K1 in milk by high-performance liquid chromatography with dual amperometric detection, *Analyst*, **1995**, *120*, 2489–2492.

SAMPLE
Matrix: milk

Sample preparation: Dilute milk to 30% with water, mix with reagent and pass through a 5 m × 0.5 mm i.d. PTFE tube knotted reactor at 1.25 mL/min, mix with 2.5 M acetic acid, pass onto column a Sep-Pak Plus C18 SPE cartridge for 5 min, wash column A with MeOH:water 40:60 for 4 min, elute column A with MeOH for 4 min, inject the last 100 μL aliquot of the eluate. (Reagent was 50 mL EtOH + 15 mL 60% aqueous NaOH + 5 mL 10% ascorbic acid.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid-sodium acetate
Flow rate: 1
Injection volume: 100
Detector: E, glassy carbon working electrode +1300 mV

CHROMATOGRAM
Retention time: 12
Limit of detection: 311 nM

OTHER SUBSTANCES
Extracted: vitamin A, vitamin D3

KEY WORDS
SPE

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez-Perez,A.; Gomez-Perez,M.C.; Hernandez-Mendez,J. Directly coupled sample treatment-high-performance liquid chromatography for on-line automatic determination of liposoluble vitamins in milk, *J.Chromatogr.A*, **1995**, *694*, 399–406.

SAMPLE
Matrix: silicone oils
Sample preparation: Condition a 1 g Si Bond-Elut SPE cartridge with 5 mL n-hexane. Mix 1 g silicone oil with 2 mL dichloromethane, vortex for 2 min, centrifuge at 3000 g. Withdrawn the supernatant, repeat this procedure twice, filter (0.45 μm), heat the filtrate at 50°, expose to a stream of helium for 30 min. Add 2.5 μg retinol acetate, 2.5 μg α-tocopherol acetate, and 25 μg BHT. Add the mixture to the SPE cartridge, elute with 500 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. A was MeCN:200 mM ammonium acetate 72:25. B was MeOH:water 95:5. A:B 100:0 for 10 min, to 0:100 over 1 min, maintain at 0:100 for 14 min
Flow rate: 2 for 10 min then 1.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 16
Internal standard: α-tocopherol acetate (18)
Limit of detection: 604.9 ng/mL
Limit of quantitation: 2.016 μg/mL

OTHER SUBSTANCES
Extracted: cholesterol (UV 210), retinal (UV 350), retinoic acid (UV 350), retinol acetate (UV 350), vitamin A (UV 350)

KEY WORDS
ophthalmic silicone oils; SPE

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Marinero,P. Simultaneous HPLC determination of cholesterol, α-tocopherol, retinol, retinal and retinoic acid in silicone oils used as vitreous substitutes in eye surgery, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1151–1167.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 8 μm Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)
Mobile phase: MeOH:water 92:8
Flow rate: 1
Detector: UV 330

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: retinoic acid, retinol acetate, vitamin A

REFERENCE
Jedrejewski,P.T.; Taylor,L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1995**, *33*, 438–445.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 3 μm Adsorbosphere-HS C18
Mobile phase: MeCN:isopropanol:MeOH 60:30:10 containing 0.1% ammonium acetate
Flow rate: 1
Detector: UV 234, UV 295, UV 450

OTHER SUBSTANCES
Simultaneous: β-carotene

REFERENCE
Maitra,I.; Marcocci,L.; Droy-Lefaix,M.T.; Packer,L. Peroxyl radical scavenging activity of *Gingko biloba* extract EGb 761, *Biochem.Pharmacol.*, **1995**, *49*, 1649–1655.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in hexane.

HPLC VARIABLES
Guard column: 15 × 3.2 silica (Applied Biosystems)
Column: 300 × 3.9 μBondapak NH2 aminopropylmethylsilyl bonded silica
Mobile phase: Cyclohexane:MTBE 90:10
Flow rate: 1
Injection volume: 10
Detector: F ex 298 em 345

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Simultaneous: β-tocopherol, gamma-tocopherol, δ-tocopherol

KEY WORDS
normal phase; discussion of other columns and mobile phases

REFERENCE
Abidi,S.L.; Mounts,T.L. Normal phase high-performance liquid chromatography of tocopherols on polar phases, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 509–520.

SAMPLE
Matrix: tissue
Sample preparation: Add aliquots of 1.6 nM IS to samples, resuspend them by sonication in 300 μL 1% ascorbate in 100 mM sodium dodecyl sulphate and 450 μL absolute EtOH, extract once with 800 μL hexane, mix for 30 s. Evaporate hexane extracts to dryness with nitrogen, resuspend in 1 mL MeOH containing 2.5% ascorbate. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS II
Mobile phase: MeOH:water 96:4
Flow rate: 1.8
Injection volume: 100
Detector: F ex 210 em 300

CHROMATOGRAM
Retention time: 8.0
Internal standard: δ-tocopherol

OTHER SUBSTANCES
Extracted: α-tocopherol, α-tocopheryloxybutyric acid

KEY WORDS
rat; liver; human; leukemia cell; δ-tocopherol is IS

REFERENCE
Tirmenstein,M.A.; Watson,B.W.; Haar,N.C.; Fariss,M.W. Sensitive method for measuring tissue α-tocopherol and α-tocopheryloxybutyric acid by high-performance liquid chromatography with fluorometric detection, *J.Chromatogr.B*, **1998**, *707*, 308–311.

SAMPLE
Matrix: tissue
Sample preparation: Add aliquots of 1.6 nmoles IS to tissue samples, resuspend by sonication in 300 μL 1% ascorbate in 100 mM sodium dodecyl sulfate and 450 μL absolute EtOH, extract once with 800 μL hexane, mix for 30 s. Evaporate hexane extracts to dryness with nitrogen, resuspend in 1 mL MeOH containing 2.5% ascorbate. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS II
Mobile phase: MeOH:water 96:4
Flow rate: 1.8
Injection volume: 100
Detector: F ex 210 em 300

CHROMATOGRAM
Retention time: 11.5
Internal standard: d-δ-tocopherol (Henkel) (8.0)
Limit of detection: 3 pmoles
Limit of quantitation: 6 pmoles

OTHER SUBSTANCES
Extracted: α-tocopheryloxybutyric acid

KEY WORDS
rat; liver; human; leukemia cell

REFERENCE
Tirmenstein,M.A.; Watson,B.W.; Haar,N.C.; Fariss,M.W. Sensitive method for measuring tissue α-tocopherol and α-tocopheryloxybutyric acid by high-performance liquid chromatography with fluorometric detection, *J.Chromatogr.B*, **1998**, *707*, 308–311.

SAMPLE
Matrix: tissue
Sample preparation: 50 mg Tissue + 50 μL 560 μg/mL vitamin K in EtOH, extract twice with 1 mL n-hexane using a sonicator, centrifuge at 2000 g for 5 min. Evaporate the supernatant to dryness, reconstitute it in 200 μL chloroform:MeOH 25:75, inject an aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeOH:water 96.5:3.5
Column temperature: 40
Flow rate: 2
Detector: F ex 295 em 350

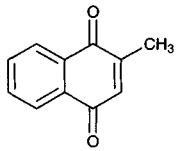
CHROMATOGRAM
Retention time: ca. 5
Internal standard: vitamin K (9)

OTHER SUBSTANCES
Extracted: vitamin A (UV 325), vitamin A_p (UV 325)

KEY WORDS
rat; liver; placenta; brain

REFERENCE
Barbas,C.; Castro,M.; Bonet,B.; Viana,M.; Herrera,E. Simultaneous determination of vitamins A and E in rat tissues by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 415–420.

Vitamin K



CAS Registry No.: 83-70-5, 523-68-2 (N-acetyl analog), 130-24-5 (K_5 HCl), 84-80-0 (K_1), 25486-55-9 (K_1 oxide), 84-81-1 ($K_{2(30)}$), 58-27-5 (K_3)
Merck Index: 10161

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20min. Extract twice with 10 mL n-hexane. Evaporate the extracts to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 10.8 (K1 (?), A), 16.7 (K2 (?), A), 12 (K1 (?), B), 20 (K2 (?), B)

OTHER SUBSTANCES
Extracted: isotretinoin, retinal, tretinoin, vitamin A, vitamin D2, vitamin D3, vitamin E

REFERENCE
Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in chloroform:MeOH 25:75. (Caution! Chloroform is a carcinogen!)

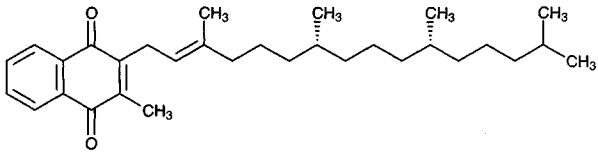
HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeOH:water 96.5:3.5
Column temperature: 40
Flow rate: 2
Detector: UV 325, F ex 295 em 350

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: vitamin A, vitamin A_p, vitamin E

REFERENCE
Barbas,C.; Castro,M.; Bonet,B.; Viana,M.; Herrera,E. Simultaneous determination of vitamins A and E in rat tissues by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 415–420.

Vitamin K1



Molecular formula: $C_{31}H_{46}O_2$
Molecular weight: 450.71
CAS Registry No.: 84-80-0
Merck Index: 7536

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 ng IS + 1 mL 0.9% NaCl + 3 mL isopropanol + 10 mL n-hexane, mix by inversion at 20 rpm for 1 h, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 1 mL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Hypersil MOS
Mobile phase: MeOH:water 92.5:7.5 containing 30 mM sodium chlorate (Continuously bubble oxygen-free nitrogen (presaturated with mobile phase) through the mobile phase reservoir.)
Flow rate: 0.9
Injection volume: 50
Detector: F ex 320 em 420 following post-column reaction. The mobile phase flowed through an electrochemical cell with coulometric reduction at -400 mV to the detector.

CHROMATOGRAM
Retention time: 10
Internal standard: vitamin $K_{2(30)}$ (17)
Limit of quantitation: 1 ng/mL

KEY WORDS
post-column reaction; plasma; protect from light

REFERENCE

Langenberg,J.P.; Tjaden,U.R. Determination of (endogenous) vitamin K_1 in human plasma by reversed-phase high-performance liquid chromatography using fluorometric detection after post-column electrochemical reduction. Comparison with ultraviolet, single and dual electrochemical detection, *J.Chromatogr.*, **1984**, *305*, 61–72.

SAMPLE

Matrix: blood

Sample preparation: 0.5-1 mL Plasma + 20 μL 87.5 ng/mL IS in EtOH + 2 mL EtOH + 6 mL hexane, mix, centrifuge at 3500 g for 5 min. Remove the upper hexane layer and evaporate it to dryness at 30° in a vortex-type evaporator, reconstitute the residue in hexane, add to a Sep-Pak silica SPE cartridge, wash with 8 mL hexane, elute with 8 mL hexane:diethyl ether 97:3, evaporate the eluate to dryness in a vortex-type evaporator, reconstitute with 1 mL hexane, add 4 mL reagent, add 5-10 mg zinc metal, vortex for 2 min, centrifuge, discard the hexane layer. Remove the lower (MeCN) layer and evaporate it to dryness, reconstitute the residue in 6 mL hexane and 2 mL water, vortex, centrifuge. Remove the upper hexane layer and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. (Reagent was 70 mM zinc chloride in MeCN:acetic 97:3.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil-ODS

Mobile phase: MeOH:dichloromethane:buffer 80:20:0.5 (Buffer was 2 M zinc chloride containing 1 M sodium acetate and 1 M acetic acid.)

Flow rate: 1

Injection volume: 100

Detector: F ex 248 em 418 (longpass cut-off filter) following post-column reaction. The column effluent flowed through a 20 × 3.9 column packed with 200 mesh zinc particles (to remove oxygen and reduce the vitamin K) to the detector.

CHROMATOGRAM

Retention time: 9.5

Internal standard: dihydro-vitamin K_1 (10.5)

Limit of detection: 50 pg/mL

OTHER SUBSTANCES

Extracted: vitamin K_1 epoxide

KEY WORDS

plasma; SPE; post-column reaction; pharmacokinetics

REFERENCE

Haroon,Y.; Bacon,D.S.; Sadowski,J.A. Liquid-chromatographic determination of vitamin K_1 in plasma, with fluorimetric detection, *Clin.Chem.*, **1986**, *32*, 1925–1929.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 2 mL EtOH, vortex for 3 min, add 3 mL hexane, vortex for 5 min, centrifuge at 1500 g for 10 min, repeat extraction with 3 mL hexane. Combine the hexane layers and wash with 2 mL MeOH:water 9:1. Remove the upper organic layer and filter (0.45 μm) it, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 33.4 μL EtOH, inject a 5 μL aliquot.

HPLC VARIABLES

Guard column: ODS

Column: 125 × 4 5 μm LiChrospher 100 RP-18 or 100 × 2.1 3 μm Spherisorb ODS-2

Mobile phase: Gradient. A was MeOH:water 99:1. B was MeOH:THF 70:30. A:B 100:0 for 2 min, 5:95 for 3.5 min, 0:100 for 4.5 min, re-equilibrate for 2 min (step gradients).

Flow rate: 1.5 (125 × 4 column) or 0.2 (100 × 2.1 column)

Injection volume: 5

Detector: UV 250

CHROMATOGRAM
Retention time: 6.5 (125 × 4 column), 7.5 (100 × 2.1 column)
Limit of detection: 0.42 ng (100 × 2.1 column), 6.4 ng (125 × 4 column)

OTHER SUBSTANCES
Extracted: retinyl palmitate (UV 328), vitamin E (α-tocopherol) (UV 284), vitamin D3 (cholecalciferol) (UV 265)

KEY WORDS
cow; plasma; protect from light; degas stock solutions with helium; narrow bore

REFERENCE
Gomis,D.B.; Escotet Arias,V.J.; Fidalgo Alvarez,L.E.; Gutiérrez Alvarez,M.D. Simultaneous determination of vitamins D3, E and K1 and retinyl palmitate in cattle plasma by liquid chromatography with a narrow-bore column, *J.Chromatogr.B*, **1994**, *660*, 49–55.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Enviroprep Inert silica SPE cartridge (Baxter) with 5 mL hexane. 500 μL Plasma + 1 mL 1 ng/mL IS in isopropanol, vortex for 5 s, add 2 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, remove the hexane layer, repeat extraction twice more. Combine the organic layers and evaporate them to dryness under a stream of nitrogen (make sure all alcohol is removed), reconstitute the residue in 500 μL hexane, vortex thoroughly, add to the SPE cartridge, wash with 10 mL hexane, elute with 5 mL hexane: ether 97:3, discard the first 1 mL, evaporate the next 4 mL eluate to dryness under a stream of nitrogen, reconstitute with 100-500 μL EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 201TP 54 (Vydac)
Mobile phase: EtOH:MeOH 40:60
Flow rate: 1
Injection volume: 50
Detector: F ex 242 em 430 (or 280 cut-off filter) following post-column reaction. The column effluent flowed through a 50 × 4 column packed with 10% Pt on alumina catalyst (Alfa) to the detector. (Caution! Catalyst/flammable solvent mixtures may ignite!)

CHROMATOGRAM
Retention time: 11
Internal standard: vitamin K_2 (menaquinone-4) (8)
Limit of detection: 20 pg/mL

KEY WORDS
serum; post-column reaction; SPE

REFERENCE
MacCrehan,W.A.; Schönberger,E. Determination of vitamin K1 in serum using catalytic-reduction liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1995**, *670*, 209–217.

SAMPLE
Matrix: formula
Sample preparation: 20 mL Infant formula + 4 mL concentrated ammonium hydroxide, swirl for 1 min, add 60 mL MeOH, swirl for 30 s, add 100 mL dichloromethane, add 50 mL isooctane, shake well, repeat extraction. Combine the organic layers and evaporate them to dryness under reduced pressure at <75°, reconstitute the residue in 20 mL acetone, evaporate, repeat acetone evaporation twice, flush flask with nitrogen, reconstitute with 10 mL isooctane:isopropanol 99.99:0.01, add to silica column, rinse flask with two 5 mL portions of isooctane:isopropanol 99.99:0.01, add rinses to column, elute column until solvent is 5 mm below top of sodium sulfate layer, rinse column top with 10 mL isooctane:isopropanol 99.99:0.01, discard eluate, elute with 100 mL isooctane:dichloromethane:isopropanol 84.98:15:0.02 (volume may need to be determined experimentally), evaporate eluate to dryness under reduced pressure at <75°, reconstitute with 5 mL isooctane, inject a 40 μL aliquot. (Prepare silica column by adding 5 g 60-200

mesh silica (Mallinckrodt SiliCAR Grade 62 Special, dry at 100° overnight) and 2 g anhydrous sodium, sulfate (dried at 100° overnight) to a 300 × 10 glass column, add 20 mL isooctane: isopropanol 99.99:0.01, elute until top of solvent layer is 5 mm below top of sodium sulfate layer.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Apex I silica (Jones Chromatography)
Mobile phase: Isooctane:dichloromethane:isopropanol 69.98:30:0.02
Flow rate: 1
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 6.7 (cis), 7.7 (trans)

KEY WORDS
protect from light; normal phase; SPE

REFERENCE
Tanner,J.T.; Barnett,S.A.; Mountford,M.K. Analysis of milk-based infant formula. Phase IV. Iodide, linoleic acid, and vitamins D and K: U.S. Food and Drug Administration-Infant Formula Council: Collaborative study, *J.AOAC Int.*, **1993**, *76*, 1042–1056.

SAMPLE
Matrix: formula, milk
Sample preparation: Dissolve 3 g infant formula or milk powder in 15 mL warm water with thorough mixing. 15 mL Ready-to-use formula or prepared solution + 5 mL buffer + 1 g lipase (Type VII from Candida cylindracea, 700-1500 U/mg, Sigma), shake mechanically for 5 min, heat at 37° with sonication for 2 h (shake vigorously every 20 min), cool, add 10 mL EtOH: MeOH 95:5, add 1 g potassium carbonate, add 1 mL 100 μg/mL cholesteryl phenylacetate in hexane, add 15 mL hexane, shake mechanically for 7 min, centrifuge for 5 min, repeat extraction with 15 mL hexane. Combine the organic layers and evaporate a 25 mL aliquot to near dryness under reduced pressure at 40°, reconstitute the residue in 100 μL hexane inject the whole amount into a 100 × 8 5 μm Resolve silica radial compression column (Waters) with a Guard-Pak silica precolumn (Waters) eluted with hexane:isopropanol 99.9:0.1 at 2 mL/min, monitor effluent at UV 269, collect the fraction between 2 and 4.5 min (then purge column at 8 mL/min for 30 min). Evaporate the fraction to dryness under a stream of nitrogen, reconstitute the residue in 200-500 μL isopropanol, inject a 20-50 μL aliquot. (Buffer was 800 mM KH_2PO_4 adjusted to pH 8.0 with NaOH.)

HPLC VARIABLES
Guard column: Guard-Pak C18
Column: 100 × 8 5 μm Resolve C18 radial compression (Waters)
Mobile phase: MeOH:isopropanol:ethyl acetate:water 45:35:14.5:13.5 (After IS elutes purge column with MeOH:ethyl acetate 50:50 for 10 min, re-equilibrate for 5 min.)
Flow rate: 2
Injection volume: 20-50
Detector: UV 269, UV 277

CHROMATOGRAM
Retention time: 26
Internal standard: cholesteryl phenylacetate (43)
Limit of detection: 5 ng/g
Limit of quantitation: 50 ng/mL

REFERENCE
Indyk,H.E.; Littlejohn,V.C.; Lawrence,R.J.; Woollard,D.C. Liquid chromatographic determination of vitamin K1 in infant formulas and milk, *J.AOAC Int.*, **1995**, *78*, 719–723.

SAMPLE
Matrix: milk

Sample preparation: 500 μL Milk + 50 μL 54 ng/mL IS in EtOH + 10 μL 200 mg/mL albumin + 200 μL water containing 50 mM sodium taurocholate, 100 mM calcium chloride, and 150 mM NaCl, sonicate (titanium probe MSE Scientific Instruments) for 2 min (30 s on, 30 s wait) in an ice bath, add 1.2 mL 3.4 mg/mL crude lipase (porcine pancreas Type II (EC 3.1.1.3), Sigma), in 200 mM pH 7.7 Tris buffer, shake at 37° at 100 strokes/min for 45 min, add 4 mL EtOH, add 2 mL water, add 200 μL 50 g/L ammonium hydroxide, add 7.5 mL n-hexane, vortex for 2 min, inject an aliquot of the hexane layer on to a 200 × 4.6 5 μm RSIL column (RSL, Eke, Belgium) and elute with n-hexane:diisopropyl ether 98.5:1.5 at 0.85 mL/min (Caution! Diisopropyl ether readily forms explosive peroxides!), collect the fraction containing vitamin K and the IS, evaporate to dryness, reconstitute with 75 μL MeOH:ethyl acetate 96:4, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.2 5 μm RoSIL HL (RSL, Eke, Belgium)
Mobile phase: MeOH:ethyl acetate 96:4 containing 2.25 g/L tetramethylammonium octahydridotriborate (Alfa)
Flow rate: 0.7
Injection volume: 50
Detector: F ex 325 em 430 following post-column reaction. The column effluent flowed through a 5 m × 0.5 mm i.d. knitted PTFE coil at 80° to the detector (the phylloquinone is reduced).

CHROMATOGRAM

Retention time: 7
Internal standard: a structural analog of phylloquinone with one more isoprene unit (Hoffman-La Roche) (11)
Limit of detection: 0.035 ng/mL
Limit of quantitation: 0.08 ng/mL

KEY WORDS

protect from light; post-column reaction

REFERENCE

Lambert,W.E.; Vanneste,L.; De Leenheer,A.P. Enzymatic sample hydrolysis and HPLC in a study of phylloquinone concentration in human milk, *Clin.Chem.*, **1992**, *38*, 1743–1748.

SAMPLE

Matrix: milk
Sample preparation: Condition a Sep-Pak silica SPE cartridge with hexane. Treat 25 (?) mL milk with 2.5 g lipase at 37° for 90 min, treat with alcoholic NaOH for 15 s, extract twice with 50 mL portions of hexane. Evaporate the hexane extract, reconstitute with 5 mL hexane, add to the SPE cartridge, wash with 4 mL hexane, elute with 4 mL hexane:ether 96:4, inject an aliquot of the eluate.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid/sodium acetate
Flow rate: 1.25
Injection volume: 10
Detector: E, EG & G PAR Model 400, MP 1304 glassy carbon series dual electrode, E1 (upstream) -1100 mV, E2 (downstream) +700 mV (Condition electrodes for 30 min at E1 -1200 mV and E2 +1500 mV at the start of each day.)

CHROMATOGRAM

Retention time: 19
Limit of detection: 3.1 ng

OTHER SUBSTANCES

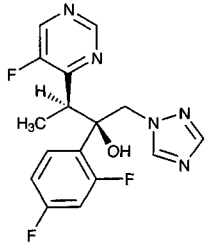
Noninterfering: vitamin A, vitamin E

KEY WORDS

SPE

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez Perez,A.; Gomez Perez,M.C.; Fernandez Moro,M.A.; Hernandez Mendez,J. Determination of vitamins A, E and K1 in milk by high-performance liquid chromatography with dual amperometric detection, *Analyst*, **1995**, *120*, 2489–2492.

Voriconazole



Molecular formula: $C_{16}H_{14}F_3N_5O$
Molecular weight: 349.32
CAS Registry No.: 137234-62-9

SAMPLE

Matrix: blood
Sample preparation: Mix 700 μL plasma with 300 μL IS in 20 mM pH 6.5 ammonium phosphate buffer, inject an 800 μL aliquot of the mixture onto column A and elute to waste with mobile phase A, after 4 min divert the effluent from column A onto column B. After 8 min elute the contents of column B onto column C with mobile phase B, after 1 min remove column B from the circuit, elute column C with mobile phase B, monitor the effluent from column C. (Flush column B with MeOH at 0.8 mL/min when not in line with column A.)

HPLC VARIABLES

Column: A 100 × 10 Sephadex G-25 superfine Pharmacia HR 10/10 column (Pharmacia Biotech, UK); B 10 × 2.1 37.5 μm Whatman pellicular ODS; C 10 × 3.2 5 μm Spherisorb ODS2 + 250 × 4.6 5 μm Spherisorb ODS2
Mobile phase: A 20 mM pH 6.5 ammonium phosphate buffer; B MeCN:buffer 42:58 (Buffer was 100 mM tetramethylethylenediamine solution adjusted to pH 7.0 with 85% phosphoric acid.)
Flow rate: A 1; B 0.8
Detector: UV 255

CHROMATOGRAM

Retention time: 11.51
Internal standard: (αR,βS)-α-(2-chlorophenyl)-5-fluoro-β-methyl-α-(1H-1,2,4-triazol-1-ylmethyl)-4-pyrimidineethanol (UK-115 794) (13.55)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

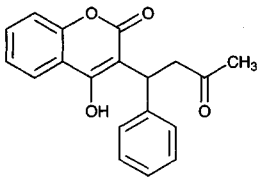
Noninterfering: acetaminophen, didanosine, prednisolone, rifampicin, zidovudine

KEY WORDS

column-switching; plasma; SEC

REFERENCE
Stopher,D.A.; Gage,R. Determination of a new antifungal agent, voriconazole, by multidimensional high-performance liquid chromatography with direct plasma injection onto a size-exclusion column, *J.Chromatogr.B*, **1997**, *691*, 441–448.

Warfarin



Molecular formula: $C_{19}H_{16}O_4$
Molecular weight: 308.33
CAS Registry No.: 81-81-2, 129-06-6 (sodium salt)
Merck Index: 10174
Lednicer No.: 1 131

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL 2 M HCl, 100 μL 10 μg/mL diclofenac in 10:90 isopropanol: hexane, and 5 mL ether to 0.5 mL human plasma. Shake vigorously for 10 min and centrifuge at 4° at 1200 g for 10 min. Remove a 4 mL aliquot of the organic layer and evaporate it to dryness using a centrifugal vacuum evaporator at 50° for 30 min. Reconstitute residue with 200 μL isopropanol:hexane 10:90 and inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 Chiralcel OD
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Isopropanol:acetic acid:hexane 18:0.5:81.5
Column temperature: 25
Flow rate: 1
Injection volume: 40
Detector: UV 312; circular dichroism detector (Model J-720, Jasco, Tokyo)

CHROMATOGRAM

Retention time: 13.1 (R), 25.3 (S)
Internal standard: diclofenac (9.6)
Limit of detection: 20 ng/mL (R), 40 ng/mL (S)

OTHER SUBSTANCES

Simultaneous: hydroxywarfarin
Noninterfering: acetaminophenon, aspirin, baraprost, captopril, cilostazol, diltiazem, dipyridamole, disopyramide, furosemide, ibuprofen, ketoprofen, metoprolol, ticlopidine
Interfering: indomethacin

KEY WORDS

plasma; chiral; pharmacokinetics

REFERENCE

Takahashi,H.; Kashima,T.; Kimura,S.; Muramoto,N.; Nakahata,H.; Kubo,S.; Shimoyama,Y.; Kajiwara,M.; Echizen,H. Determination of unbound warfarin enantiomers in human plasma and 7-hydroxywarfarin in human urine by chiral stationary-phase liquid chromatography with ultraviolet or fluorescence and on-line circular dichroism detection, *J.Chromatogr.B*, **1997**, *701*, 71–80.

SAMPLE

Matrix: blood
Sample preparation: Condition a Bakerbond C18 SPE cartridge with 2 mL MeOH and 2 mL 1 M HCl. Mix 300 μL plasma with 2 mL 1 M HCl. Add to the SPE cartridge, wash with 2 mL HCl, elute with 2 mL MeOH. Concentrate the eluate to 25 μL under a stream of nitrogen, dilute with 85 μL mobile phase, centrifuge at 16000 g for 15 min. Inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Pirkle covalent (R, R) Whelk-O 1 (Regis)
Mobile phase: MeCN:0.5% glacial acetic acid 40:60
Flow rate: 1
Injection volume: 100
Detector: UV 313

CHROMATOGRAM
Retention time: 13.6 (S), 15.8 (R)
Internal standard: ethylwarfarin (16.5 (S), 18.5 (R))
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Simultaneous: amiodarone, aspirin, diclofenac, furosemide, ibuprofen, isosorbide dinitrate, lisinopril, phenytoin, tolbutamide

KEY WORDS
plasma; chiral; SPE

REFERENCE
Henne,K.R.; Gaedigk,A.; Gupta,G.; Leeder,J.S.; Rettie.A.E., Chiral phase analysis of warfarin enantiomers in patient plasma in relation to CYP2C9 genotype, *J.Chromatogr.B*, **1998**, *710*, 143–148.

SAMPLE
Matrix: blood, tissue
Sample preparation: Condition a silica Sep-Pak SPE cartridge also containing 2 g sodium sulfate (?) with 5 mL MeOH and 5 mL cyclohexane. Mix 3 mL blood or crushed tissue with 1 mL 20 μg/mL IS, adjust to pH 3-4 with 0.5 M sulfuric acid, extract three times with 10 mL MeOH: chloroform 10:90 (Caution! Chloroform is a carcinogen!). Evaporate at 40°, re-dissolve the residue in 5 mL cyclohexane, sonicate and centrifuge three times. Remove a 5 mL aliquot of the top layer, evaporate at 40°. Reconstitute the residue in 5 mL cyclohexane. Add to the SPE cartridge, elute with 5 mL MeOH, evaporate at 40°, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 200 mm long μBondapak C18
Mobile phase: MeOH:0.8% acetic acid 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5
Internal standard: N,N-diphenylbenzidine (9.3)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: bromadiolone, brodifacoum, coumarin, coumatetralyl

KEY WORDS
SPE; plasma; heart; lung; liver; kidney; spleen

REFERENCE
Park,S.W.; Seo,B.S.; Kim,E.H.; Kim,D.H.; Paeng,K.-J. Purification and determination procedure of coumarin derivatives, *J.Forensic Sci.*, **1996**, *41*, 685–688.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Mix 500 μL plasma with 30 μL MeOH, 50 μL 6 M hydrochloric acid and 3 mL diethyl ether, vortex for 30 s, centrifuge at 2000 g for 10 min, evaporate ether layer in a 45° water bath under a stream of nitrogen. Reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot. Urine. Mix 200 μL urine with 20 μL 6 M hydrochloric acid, 40 μL MeOH and 8 mL diethyl ether, vortex for 30 s, evaporate to dryness in a 45° water bath under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak C18 (plasma), 150 × 3.9 5 μm Resolve Spherical C18 (urine)

Mobile phase: MeCN:buffer 38:62 (Buffer was 10 mM potassium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 229 em 389

CHROMATOGRAM
Retention time: 9
Internal standard: warfarin

OTHER SUBSTANCES
Extracted: furosemide
Simultaneous: quinidine, sulfamethoxazole
Noninterfering: carbamazepine, cimetidine, diazepam, disopyramide, fluvoxamine, furosemide metabolite, meclofenamate, metoclopramide, phenobarbital, phenylbutazone, phenytoin, ranitidine, theophylline, trimethoprim

KEY WORDS
plasma; warfarin is IS

REFERENCE
Abou-Auda,H.S.; Al-Yamani,M.J.; Morad,A.M.; Bawazir,S.A.; Khan,S.Z.; al-Khamis,K.I. High-performance liquid chromatographic determination of furosemide in plasma and urine and its use in bioavailability studies, *J.Chromatogr.B*, **1998**, *710*, 121–128.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 20.358

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk

Sample preparation: Mix 100 μmol warfarin, 1 mg 4-(N,N-dimethylamino)pyridine, 200 μL triethylamine, and 1 mL dichloromethane, completely flush the vessel with nitrogen and cap it. Add dropwise 45 μL (S)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (ACROS, Fairlawn NJ) via a syringe. Stir at room temperature for 3 h. Add 20 mL dichloromethane and 30 mL 100 mM pH 6.0 phosphate buffer. wash the organic layer twice with 30 mL portions of phosphate buffer, extract aqueous layer twice with 30 mL portions of dichloromethane. Dry the combined organic extracts over sodium sulfate, filter through a 2 g zirconia. Evaporate solvent in vacuum, re-suspend residue in THF. Inject a 0.1 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 2.5 μm Hp-C/ZrO2 (The carbon-coated zirconium particles were prepared by chemical vapor deposition. Pass heptane through a zirconium dioxide particles in a tube furnace at ca. 700° and at reduced pressure (15-10 Torr) for 1.5 hour. Rinse particles with THF, extract particles with toluene in Soxhlet extractor.)
Mobile phase: THF:water 45:55
Column temperature: 30
Flow rate: 1.0
Injection volume: 0.1
Detector: UV 254

CHROMATOGRAM

Retention time: 6.2, 6.9 (enantiomers)

OTHER SUBSTANCES

Also analyzed: 4-chloroamphetamine, phenylalanine

KEY WORDS

chiral; derivatization; details of column preparation

REFERENCE

Jackson,P.T.; Kim,T.-Y.; Carr,P.W. Diastereomeric selectivity of carbon-coated zirconia reversed-phase liquid chromatographic media, *Anal.Chem.*, **1997**, *69*, 5011–5017.

SAMPLE

Matrix: eggs
Sample preparation: Add 2 g anhydrous sodium sulfate to 5 g egg white or yolk. Extract twice with 15 mL acetone:diethyl ether 90:10. Homogenize for 5 min, centrifuge at 10000 g at -8° for 5 min, evaporate combined supernatants to dryness at 40°. Reconstitute dried extract with 3 mL MeCN. Wash twice with 3 mL hexane and centrifuge at 10000 g at -8° for 5 min. Discard hexane phase, evaporate MeCN phase to dryness under a stream of nitrogen in a 40° dry bath. Reconstitute with 1 mL mobile phase and filter it through a 0.45 μm filter. Inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 4.6 × 4.0 5 μm LiChroSpher 100 RP-18E
Column: 125 × 4.6 5 μm LiChroSpher 100 RP-18E
Mobile phase: MeOH:ammonium acetate triethylamine buffer 62:38 (Prepare buffer as follows. Mix 3.85 g ammonium acetate, 2 mL glacial acetic acid and 2 mL triethylamine in water, adjust to pH 5.2 with glacial acetic acid and make up to 1 L with water.)
Flow rate: 1.0
Injection volume: 50
Detector: UV 281

CHROMATOGRAM

Retention time: 3.1
Limit of detection: 6 ng/g (white); 5 ng/g (yolk)
Limit of quantitation: 20 ng/g (white); 15 ng/g (yolk)

KEY WORDS

eggs; yolk; white

REFERENCE
Pouliquen,H.; Fauconnet,V.; Morvan,M.-L.; Pinault,L. Determination of warfarin in the yolk and the white of hens' eggs by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 143–148.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 250 μL 1 M HCl to 500 μL microsomal incubation, extract with 3 mL MTBE, evaporate the organic layer under nitrogen, reconstitute the residue in 100 μL MeCN: water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 Novapak C18
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM acetic acid adjusted to pH 4.8 with ammonium hydroxide.)
Flow rate: 1
Injection volume: 120
Detector: Radioactivity, Inus β-Ram using Inus Tru-Count scintillation fluid at a flow rate of 5 mL/min

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

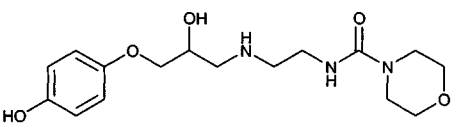
REFERENCE
Obach,R.S. Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol, *Drug Metab.Dispos.*, **1997**, *25*, 1359–1369.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm), dilute the filtrate with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:10 mM pH 4.7 acetate buffer 50:50
Detector: UV 214

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

Xamoterol



Molecular formula: $C_{16}H_{25}N_3O_5$
Molecular weight: 339.39
CAS Registry No.: 81801-12-9, 73210-73-8 (hemifumarate)
Merck Index: 10189

SAMPLE
Matrix: blood

REFERENCE
Pouliquen,H.; Fauconnet,V.; Morvan,M.-L.; Pinault,L. Determination of warfarin in the yolk and the white of hens' eggs by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 143–148.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 250 μL 1 M HCl to 500 μL microsomal incubation, extract with 3 mL MTBE, evaporate the organic layer under nitrogen, reconstitute the residue in 100 μL MeCN: water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 Novapak C18
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM acetic acid adjusted to pH 4.8 with ammonium hydroxide.)
Flow rate: 1
Injection volume: 120
Detector: Radioactivity, Inus β-Ram using Inus Tru-Count scintillation fluid at a flow rate of 5 mL/min

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

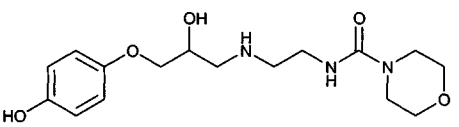
REFERENCE
Obach,R.S. Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol, *Drug Metab.Dispos.*, **1997**, *25*, 1359–1369.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm), dilute the filtrate with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:10 mM pH 4.7 acetate buffer 50:50
Detector: UV 214

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

Xamoterol



Molecular formula: $C_{16}H_{25}N_3O_5$
Molecular weight: 339.39
CAS Registry No.: 81801-12-9, 73210-73-8 (hemifumarate)
Merck Index: 10189

SAMPLE
Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with MeOH and water. 1 mL Plasma + 2 mL pH 7.0 phosphate-citrate buffer, add to the SPE cartridge, wash with 10 mL water, wash with 5 mL MeOH:water 10:90, elute with 2 mL MeOH. Evaporate the eluate to dryness at 60°, reconstitute with 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 5 μm Hypersil-ODS
Mobile phase: MeOH:THF:30 mM perchloric acid 8:0.6:91.4
Flow rate: 1.5
Injection volume: 100
Detector: E, Bioanalytical Systems Model LC-4A, TL-5A thin-layer glassy carbon electrode + 0.85 V, RE-1 Ag/AgCl reference electrode

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CHROMATOGRAM
Retention time: 8
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, acetazolamide, caffeine, chlordiazepoxide, cimetidine, codeine, diazepam, digoxin, docusate sodium, flurazepam, furosemide, heparin, hydrochlorothiazide, meclofenamate, methyldopa, nitroglycerin, quinidine, simethicone, spironolactone, thioridazine, triamterene, warfarin
Interfering: aspirin, benzyl alcohol, hydralazine

KEY WORDS
plasma; SPE

REFERENCE
Davis,P.C. Determination of xamoterol in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1987**, *417*, 233–235.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or 100 μL urine + 40 ng prenalterol + 5 mL 0.1% disodium EDTA + 100 μL 1 M pH 6.5 ammonium acetate, add to the ion-exchange column, wash with two 10 mL portions of water, elute with 3 mL 1 M ammonium hydroxide. Evaporate the eluate to dryness under reduced pressure at 50°, reconstitute with 250 μL ice-cold 100 mM perchloric acid, centrifuge at 4° at 10000 g for 10 min, inject a 100 μL aliquot of the supernatant. (Prepare an ion-exchange column by packing 50-100 mesh Bio-Rex 70 Na+ cation-exchange resin (Bio-Rad) into a 40 × 10 column, wash with 3 M HCl, 3 M NaOH, 3 M acetic acid, 1 M pH 6.5 ammonium acetate, and water.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:10 mM perchloric acid 15:85
Flow rate: 2
Injection volume: 100
Detector: F ex 190 em 320-400 (filter)

CHROMATOGRAM
Retention time: 6.9
Internal standard: prenalterol (5.8)
Limit of detection: 10 ng/mL (urine), 1 ng/mL (plasma)

OTHER SUBSTANCES
Simultaneous: albuterol, fenoterol
Noninterfering: atropine, clonidine, diazepam, nitrazepam, prazosin, quinidine

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KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Oddie,C.J.; Jackman,G.P.; Bobik,A. Measurement of xamoterol in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *308*, 370–375.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

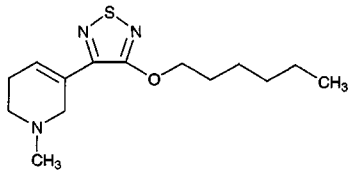
CHROMATOGRAM
Retention time: k' 1.13, k' 2.27 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

Xanomeline



Molecular formula: $C_{14}H_{23}N_3OS$
Molecular weight: 281.42
CAS Registry No.: 131986-45-3, 141064-23-5 (oxalate), 152854-19-8 (tartrate)
Merck Index: 10190

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 500 mg C18 Bond-Elut SPE cartridge with MeOH and water. Mix 2 mL microsomal incubation with 4.5 mL ice-cold MeOH, centrifuge at 4000 g for 10 min, dilute the supernatant with 15 mL water. Add a 2 mL aliquot to the SPE cartridge, wash with

2 mL water, elute with 2 mL MeOH:25% aqueous ammonia 96:4, evaporate the eluate to dryness under reduced pressure, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm ChromSpher Si (Chrompack)
Mobile phase: n-Heptane:2-propanol:water:25% ammonia 90:10:0.075:0.075 (A) or n-heptane:2-propanol:MeOH:25% ammonia 50:50:10:1 (B)
Detector: UV 295; MS, VG TRIO 1000, particle beam interface at 50°, helium at 25-30 psi, ion source 200°, positive ionization mode, electron current 150 μA, electron energy 70 eV

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
normal phase; rat; liver; SPE

REFERENCE
Andersen,J.V.; Hansen,K.T. Normal-phase liquid chromatography-particle-beam mass spectrometry in drug metabolism studies of the dopamine receptor antagonist Odapipam and the muscarine M1 receptor agonist Xanomeline, *Xenobiotica*, **1997**, *27*, 901–912.

SAMPLE
Matrix: perfusate
Sample preparation: Inject an aliquot of perfusate directly onto the column.

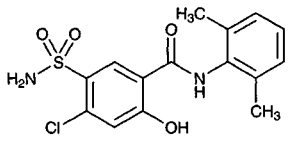
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb Phenyl
Mobile phase: MeCN:buffer 40:60 (Buffer was 30 mM tetramethylammonium hydroxide adjusted to pH 3.0 with perchloric acid.)
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: UV 295

CHROMATOGRAM
Limit of detection: 100 nM

KEY WORDS
liver

REFERENCE
Andersen,J.V.; Hansen,K.T. Normal-phase liquid chromatography-particle-beam mass spectrometry in drug metabolism studies of the dopamine receptor antagonist Odapipam and the muscarine M1 receptor agonist Xanomeline, *Xenobiotica*, **1997**, *27*, 901–912.

Xipamide



Molecular formula: $C_{15}H_{15}ClN_2O_4S$
Molecular weight: 354.81
CAS Registry No.: 14293-44-8
Merck Index: 10212
Lednicer No.: 2 93

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL dichloromethane:2-propanol 75:25, shake for 10 min. Centrifuge at 2000 g for 10 min at 4°. Remove the organic phase and evaporate it to

dryness under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL mobile phase, mix for 10 s. Centrifuge at 6500 g for 10 min. Inject a 40 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm Supelcosil LC-18 (Supelco)
Mobile phase: n-Propanol:buffer 5:95 (Buffer was 50 mM sodium dodecyl sulfate in 10 mM pH 5.8 sodium phosphate buffer.)
Flow rate: 1.3
Injection volume: 40
Detector: UV 225

CHROMATOGRAM
Retention time: 8.58
Internal standard: xipamide

OTHER SUBSTANCES
Extracted: albuterol, atenolol, chlorthalidone

KEY WORDS
plasma; xipamide is IS

REFERENCE
Giachetti,C.; Tenconi,A.; Canali,S.; Zanolo,G. Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography. Application to pharmacokinetic studies in man, *J.Chromatogr.B*, **1997**, *698*, 187–194.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

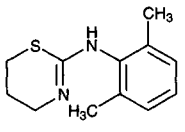
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 218.1

CHROMATOGRAM
Retention time: 18.823

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Xylazine



Molecular formula: $C_{12}H_{16}N_2S$
Molecular weight: 220.34
CAS Registry No.: 7361-61-7, 23076-35-9 (HCl)
Merck Index: 10213
Lednicer No.: 2 307

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 10 µL 100 µg/mL doxapram in MeOH + 1 mL 50 mM pH 11 borax buffer, vortex for 5 s, add 10 mL chloroform, shake for 10 min, centrifuge at 11400 g for 10 min, filter (Whatman No. 1 PS phase-separating paper). Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 100 µL mobile phase, inject the whole sample.

HPLC VARIABLES

Column: 300 × 4 10 µm µBondapak C18
Mobile phase: MeOH:water:heptanesulfonic acid (Pic B7) 45:55:0.2 containing 2% glacial acetic acid
Flow rate: 2
Injection volume: 100
Detector: UV 225

CHROMATOGRAM

Retention time: 4
Internal standard: doxapram (5.5)
Limit of detection: 20 ng/mL

KEY WORDS

plasma; sheep

REFERENCE

Alvinerie,M.; Toutain,P.L. Determination of xylazine in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *222*, 308–310.

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. Add 200 µL plasma to the SPE cartridge, wash with 100 mM ammonium acetate, elute with MeOH:100 mM ammonium acetate 3:1. Evaporate the eluate to dryness under reduced pressure, dissolve the residue in 200 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Hitachi gel 3056 octadecylsilica
Mobile phase: MeOH:100 mM ammonium acetate 60:40
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M1000, APCI, nebulizer 260°, vaporizer 399

CHROMATOGRAM

Retention time: 7.0
Limit of detection: 0.5-2.5 ng/mL

OTHER SUBSTANCES

Simultaneous: atipamezole, atropine, butorphanol, flumazenil, ketamine, medetomidine, midazolam

KEY WORDS
plasma; SPE; dog

REFERENCE
Kanazawa,H.; Nagata,Y.; Matsushima,Y.; Takai,N.; Uchiyama,H.; Nishimura,R.; Takeuchi,A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, *631*, 215–220.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.53

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 μL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 μL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES
Guard column: 10 × 2.1 37-50 μm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 4 ng/g

OTHER SUBSTANCES
Extracted: azaperol, carazolol, acepromazine, azaperone, haloperidol, propiomazine, chlorpromazine

KEY WORDS
SPE; pig; kidney

REFERENCE
Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Cut pig kidney or liver into small pieces and homogenize. 5 g Homogenate + 10 mL MeCN, shake, vortex for 30 s, sonicate for 3 min, vortex for 30 s, sonicate for 3 min, centrifuge at 10000 g for 20 min. Add 7.5 mL supernatant + 40 mL 10% NaCl to the SPE cartridge at about 1 mL/min, do not allow cartridge to dry out, wash with 850 μL 10 mM sulfuric acid, dry with air, elute with 3.5 mL acidic MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL 10 mM sulfuric acid, vortex briefly, add 1 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, inject an aliquot of the aqueous layer. (Acidic MeCN was 1 mL 50 mM sulfuric acid in 100 mL MeCN.)

HPLC VARIABLES
Guard column: Hypersil 5 μm SAS C1
Column: 250 mm long 5 μm Hypersil SAS C1
Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate
Flow rate: 2
Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

CHROMATOGRAM
Retention time: 10
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: azaperol, acepromazine, carazolol, azaperone, haloperidol, propriomazine, chlorpromazine

KEY WORDS
SPE; pig; kidney; liver

REFERENCE
Rose,M.D.; Shearer,G. Determination of tranquilisers and carazolol residues in animal tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *624*, 471–477.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Tissuemizer) 20 g kidney and 70 mL chloroform at medium-high speed for 1-1.5 min, rinse blade with 5 mL chloroform, centrifuge at 1800 rpm for 10 min, filter (glass-fiber) supernatant, repeat extraction, rinse filter with 30 mL chloroform. Combine filtrates, add 1 mL 1 M HCl, evaporate to dryness under reduced pressure at 55°, add 25 mL MeOH, evaporate to dryness, reconstitute with 25 mL petroleum ether, add to Celite column, rinse flask with three 10 mL portions of petroleum ether, add rinses to column, rinse flask with two 50 mL portions of petroleum ether:MeOH 98.5:1.5, add to column, discard all effluent, elute with two 50 mL portions of MeOH. Evaporate eluate to dryness under reduced pressure at 55°, reconstitute with 25 mL MeOH, evaporate to dryness, reconstitute with 4-10 mL mobile phase, filter (0.45 μm), inject a 50 μL aliquot. (Petroleum ether was water saturated. Prepare Celite column as follows. Slurry 250 g Celite 545 and 800 mL HCl:water 50:50, heat on a steam bath with occasional stirring for several hours, allow to settle, pour off liquid, add 800 mL HCl: water 50:50, slurry, heat on a steam bath for several hours, allow to settle, decant liquid, wash with water until pH is neutral, wash with 250 mL MeOH, wash with 250 mL n-hexane, wash with 250 mL petroleum ether, heat on a steam bath to remove residual solvent, dry at 105°. Blend 2 g acid-washed Celite and 0.5 mL water, add to a 300 × 22 column, blend 3 g Celite and 1 mL 1 M HCl, add to the column.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:water:2 M sodium acetate:1 M acetic acid 32:64:2:2
Flow rate: 1
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
cow; pig; kidney; SPE

REFERENCE
Holland,D.C.; Munns,R.K.; Roybal,J.E.; Hurlbut,J.A.; Long,A.R. Simultaneous determination of xylazine and its major metabolite, 2,6-dimethylaniline, in bovine and swine kidney by liquid chromatography, *J.AOAC Int.*, **1993**, *76*, 720–724.

SAMPLE
Matrix: urine
Sample preparation: Condition a 1 mL 100 mg Bond-Elut cyanopropyl SPE cartridge with two 1 mL portions of MeOH and 1 mL water, do not allow to dry. 1 mL Urine + 500 μL water + 100 μL 15 μg/mL diazepam in MeOH, add to the SPE cartridge, dry under vacuum for 3 min, elute with two 250 μL portions of MeCN:methanolic HCl 50:50, inject an aliquot of the eluate. (Prepare methanolic HCl by adding 3 mL concentrated HCl to 50 mL MeOH.)

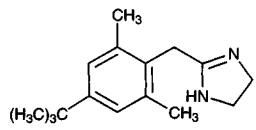
HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeCN:MeOH:buffer 30:2:50 (Buffer was 4 g/L tetramethylammonium hydroxide.)
Flow rate: 2
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 6.5
Internal standard: diazepam (9.4)
Limit of detection: 10 ng/mL

KEY WORDS
dog; SPE; pharmacokinetics

REFERENCE
Moore,C.M.; Oliver,J.S. Rapid extraction and determination of xylazine in greyhound urine using high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *491*, 519–524.

Xylometazoline



Molecular formula: $C_{16}H_{24}N_2$
Molecular weight: 244.38
CAS Registry No.: 526-36-3, 1218-35-5 (HCl)
Merck Index: 10219
Lednicer No.: 1 242

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 6.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: benzalkonium chloride, domiphen bromide, thimerosal

KEY WORDS
nasal drops

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: formulations
Sample preparation: Dilute nasal solution 10-fold with water, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. MeCN:1 mM NaOH from 10:90 to 80:20 over 25 min.
Column temperature: 25
Flow rate: 1.2
Injection volume: 10
Detector: UV 224

CHROMATOGRAM
Retention time: 14.5
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: ephedrine, naphazoline, oxymetazoline

KEY WORDS
nasal solutions

REFERENCE
De Orsi,D.; Gagliardi,L.; Cavazzutti,G.; Mediati,M.G.; Tonelli,D. Simultaneous determination of ephedrine and 2-imidazolines in pharmaceutical formulations by reversed-phase HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 3233–3242.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 5.9
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: atropine, codeine, dansylamide, dansylcadaverine, doxorubicin, methylatropine, naphazoline, noscapine

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 RSIL C18 (RSL, Eke, Belgium)
Mobile phase: MeOH:water 40:60 containing 20 mM sodium 1-octanesulfonate and 10 mM N,N-dimethyloctylamine, pH adjusted to 3.0 with orthophosphoric acid
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 40

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline, coumazoline, lidocaine, naphazoline, oxymetazoline, prednisolone, sulfadimidine, sulfanilamide, sulfathiazole, tenaphtoxaline, tetrahydrozoline, tolazoline, tramazoline

REFERENCE
De Schutter,J.A.; Van den Bossche,W.; De Moerloose,P. Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography, *J.Chromatogr.*, **1987**, *391*, 303–308.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 12.68 (A), 7.16 (B)

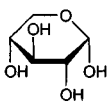
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene,

desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Xylose



Molecular formula: $C_5H_{10}O_5$
Molecular weight: 150.13
CAS Registry No.: 58-86-6
Merck Index: 10220

SAMPLE
Matrix: beverages, juice, milk
Sample preparation: Orange juice. Dilute orange juice 100-fold with water, filter (Millipore HV, 0.45 μm), dilute filtrate 10-fold, inject an aliquot. Beverages. Dilute soft drinks 1000-fold with water, inject an aliquot. Milk. Dilute 5 mL milk to 100 mL with mobile phase, filter (Millipore HV, 0.45 μm), dilute filtrate 50-fold, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Cation H (Bio-Rad)
Column: 300 × 3.8 9 μm HPX 87-H Aminex (Bio-Rad)
Mobile phase: 10 mM Sulfuric acid
Column temperature: 50
Flow rate: 0.5
Injection volume: 40
Detector: E following post-column reaction, Hewlett-Packard 1049A programmable electrochemical detector, Metrohm detector cell, cuprous oxide working electrode +550 mV, glassy carbon auxiliary electrode, Ag/AgCl (3 M KCl) reference electrode. The column effluent mixed with 200 mM NaOH pumped at 0.4 mL/min, the mixture flowed through a 220 × 0.8 single-bead string reactor packed with 0.6 mm glass beads to the detector. (Prepare cuprous oxide electrode as follows. Stir 300 mg conductive carbon cement (Gerhard Neubauer, Munster), 60 mg cuprous oxide (Fluka), and 300 μL acetone until a thick paste forms as the acetone evaporates. Pack

conductive carbon cement into the base of a 3 mm diameter cavity carbon paste electrode base (Metrohm), allow to dry, polish with dry emery paper (grade 2/0, Oakey), remove surface layer with an acetone-soaked tissue, pack the paste into the cavity, allow to dry overnight, polish with dry emery paper (grade 2/0), 3 μm imperial micro finishing film sheet (3M), 0.3 μm imperial micro finishing film sheet (3M), and 0.05 μm alumina particles on a Buehler pad, sonicate for 2 min in water (Anal. Chim. Acta 1995, 300, 5).)

CHROMATOGRAM
Retention time: 11.25
Limit of detection: 0.9 μM

OTHER SUBSTANCES
Also analyzed: arabinose, cellobiose, dextrose, fructose, fucose, galactitol, galactose, galacturonic acid, lactose, lactulose, lyxose, maltose, mannitol, mannose, myo-inositol, raffinose, rhamnose, ribose, sorbose, sucrose

KEY WORDS
orange juice; soft drinks; post-column reaction; fruit

REFERENCE
Huang,X.; Pot,J.J.; Kok,W.T. Determination of sugars by liquid chromatography and amperometric detection with a cuprous oxide modified electrode, *Chromatographia*, **1995**, *40*, 684–689.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 500 μL MeOH, shake for 1 min, centrifuge at 10000 rpm for 1 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 4 Aminex A-27
Mobile phase: 500 mM Boric acid adjusted to pH 8.7 with KOH
Flow rate: 2
Injection volume: 10
Detector: F ex 357 (low-pressure mercury lamp) em 436 (420 nm cutoff filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.8 mm ID stainless steel coil at 150° to the detector. (Reagent was 20 g boric acid and 20 g ethanolamine in 1 L water.)

CHROMATOGRAM
Retention time: 20
Limit of quantitation: 5 nmoles

OTHER SUBSTANCES
Extracted: dextrose, fructose, galactose, maltose, ribose

KEY WORDS
post-column reaction; serum

REFERENCE
Kato,T.; Kinoshita,T. Fluorometric detection and determination of carbohydrates by high-performance liquid chromatography using ethanolamine, *Anal.Biochem.*, **1980**, *106*, 238–243.

SAMPLE
Matrix: bulk
Sample preparation: Evaporate hydrolysates of glycosaminoglycans to dryness, reconstitute in 500 μL 10% benzoic anhydride in pyridine containing 5% 4-dimethylaminopyridine, heat at 37° for 1.5 h, add 4.5 mL water, shake vigorously, pass through a Sep-Pak C18 SPE cartridge three times, wash with 10 mL pyridine:water 10:90, wash with 5 mL water, reverse the direction of flow and elute with 2.5 mL MeCN, evaporate the eluate to dryness, reconstitute with MeCN, centrifuge at 11000 g for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 RP-18
Column: 250 × 4.6 Supelcosil LC-18
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 9.4

OTHER SUBSTANCES
Simultaneous: N-acetylgalactosamine, N-acetylglucosamine, 1,6-anhydroidose, dextrose, fucose, galactosamine, galactose, glucosamine, mannose, 1-methylfucose, 1-methylgalactose, 1-methylmannose, 1-methylxylose
Interfering: 1-methylglucose

KEY WORDS
derivatization; SPE

REFERENCE
Karamanos,N.K.; Hjerpe,A.; Tsegenidis,T.; Engfeldt,B.; Antonopoulos,C.A. Determination of iduronic acid and glucuronic acid in glycosaminoglycans after stoichiometric reduction and depolymerization using high-performance liquid chromatography and ultraviolet detection, *Anal.Biochem.*, **1988**, *172*, 410–419.

SAMPLE
Matrix: carbohydrates
Sample preparation: Mix 10 nmoles total monosaccharides with 200 μL 2 M trifluoroacetic acid, flush with nitrogen for a few min, seal, heat at 100° for 6 h, evaporate to dryness under reduced pressure in a desiccator over NaOH pellets, reconstitute with water, inject an aliquot.

HPLC VARIABLES
Column: 80 × 8 11 μm Hitachi No. 2633 resin (quaternary ammonium)
Mobile phase: Gradient. A was 250 mM pH 8.2 borate buffer. B was 400 mM pH 7.4 borate buffer. C was 600 mM pH 9.3 borate buffer. A:B:C from 100:0:0 to 0:100:0 over 15 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 10 min, maintain at 0:0:100.
Column temperature: 65
Flow rate: 1
Injection volume: 20
Detector: F ex 331 em 383 following post-column reaction. The column effluent mixed with 10% 2-cyanoacetamide in water pumped at 0.25 mL/min and 600 mM pH 9.3 borate buffer pumped at 0.25 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100 ± 0.5° to the detector.

CHROMATOGRAM
Retention time: 58
Limit of detection: 0.1-1 nmole

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fucose, galactose, lyxose, mannose, rhamnose, ribose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Takahashi,M.; Kakehi,K.; Ganno,S. Rapid, automated analysis of monosaccharides by high-performance anion-exchange chromatography of borate complexes with fluorimetric detection using 2-cyanoacetamide, *Anal.Biochem.*, **1981**, *113*, 130–138.

SAMPLE
Matrix: glycoconjugates

Sample preparation: Mix 0.1-1.5 mg glycoconjugate with 200 μL 2 M trifluoroacetic acid, flush with nitrogen for a few min, seal, heat at 100° for 6 h, evaporate to dryness under reduced pressure in a desiccator over NaOH pellets, reconstitute with 200 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Hitachi No. 2633 resin
Mobile phase: Gradient. A was 250 mM pH 8.2 borate buffer. B was 400 mM pH 7.4 borate buffer. C was 600 mM pH 9.3 borate buffer. A:B:C from 100:0:0 to 0:100:0 over 15 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 11 min, maintain at 0:0:100.
Column temperature: 65 ± 1
Flow rate: 1
Injection volume: 20
Detector: UV 276 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide pumped at 0.5 mL/min and 600 mM pH 10.5 borate buffer pumped at 0.5 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100 ± 0.2° and a 1 m × 0.5 mm ID PTFE cooling coil to the detector.

CHROMATOGRAM
Retention time: 57
Limit of detection: 1 nmole

OTHER SUBSTANCES
Extracted: arabinose, dextrose, fucose, galactose, lyxose, mannose, rhamnose, ribose

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KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Takahashi,M.; Nishimura,Y.; Kakehi,K.; Ganno,S. Sensitive ultraviolet monitoring of aldoses in automated borate complex anion-exchange chromatography with 2-cyanoacetamide, *Anal.Biochem.*, **1981**, *118*, 162–167.

SAMPLE
Matrix: glycoproteins
Sample preparation: 200 μg Glycoprotein + 100 μL water + 100 μL 4 M trifluoroacetic acid, heat at 100° for 6 h, cool to room temperature, evaporate to dryness under reduced pressure at 35°, add 40 μL reagent, heat at 80° for 1 h, cool to room temperature, add 200 μL water, add 200 μL chloroform, vortex vigorously, centrifuge for 1 min, inject an aliquot of the upper aqueous layer. (Prepare the reagent by mixing 165 mg ethyl p-aminobenzoate, 35 mg sodium cyanoborohydride, 41 μL glacial acetic acid, and 350 μL glacial acetic acid.)

HPLC VARIABLES
Column: 150 × 3.9 Pico.Tag (Waters)
Mobile phase: MeCN:MeOH:50 mM pH 4.5 sodium acetate 10:5:85
Column temperature: 45
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: N-acetylgalactosamine, N-acetylglucosamine, 2-deoxyglucose, fucose, galactosamine, galactose, glucosamine, glucose, lactose, maltose, mannose

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KEY WORDS
derivatization

REFERENCE
Kwon,H.; Kim,J. Determination of monosaccharides in glycoproteins by reverse-phase high-performance liquid chromatography, *Anal.Biochem.*, **1993**, *215*, 243–252.

SAMPLE
Matrix: plants
Sample preparation: Condition trimethylaminopropylsilica SAX and cyclohexylsilica SPE cartridges (Analytichem) with 4 mL MeOH and 4 mL water. Heat 1 g plant material with 10 mL EtOH:water 80:20 in a sealed tube at 100° for 15-30 min, evaporate the extract to dryness, reconstitute with water, pass through the SPE cartridges, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 6.5 Sugar Pak-1 microparticulate gel, calcium form (Waters)
Mobile phase: 100 μM Calcium EDTA
Column temperature: 70
Flow rate: 0.4
Injection volume: 50
Detector: F ex 360 em 470 following post-column reaction. The column effluent mixed with 30 mM benzamidine in 1 M KOH pumped at 1 mL/min and the mixture flowed through a 530 μL reaction coil (Varian PCR1) at 100° to the detector.

CHROMATOGRAM
Retention time: 10.92
Limit of detection: 15.8-62.5

OTHER SUBSTANCES
Extracted: arabinose, dextrose, fructose, fucose, galactose, lactose, mannose
Interfering: rhamnose

KEY WORDS
post-column reaction; SPE

REFERENCE
Coquet,A.; Veuthey,J.-L.; Haerdi,W. Selective post-column fluorigenic reaction with benzamidine for trace level detection of reducing saccharides in liquid chromatography, *J.Chromatogr.*, **1991**, *553*, 255–263.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 160 × 4 8 μm DAX8 anion-exchange resin, sulfate form (Durrum Chemical Co.) (Regenerate resin outside the column by washing 10 g resin with 400 mL water, 400 mL 500 mM NaCl at 50°, water, 0.5 N sodium sulfate at 50° (until a negative chloride test is obtained), water, and EtOH:water 95:5. Slurry pack below 70° with mobile phase at 1.14 mL/min.)
Mobile phase: EtOH:water 87.6:12.4
Column temperature: 88
Flow rate: 0.57
Injection volume: 5-100
Detector: UV 562 following post-column reaction. The column effluent mixed with the reagent pumped at 0.3 mL/min and the mixture flowed for 5 min through a coil of 0.3 mm ID PTFE at 100° to the detector. (Prepare reagent by mixing equal volumes of solution A and solution B, the mixture is stable for at least 1 month. Solution A is 1 g of copper sulfate pentahydrate and 3.7 g aspartic acid in 1 L water. Solution B is 38 g sodium carbonate decahydrate and 2 g sodium bicinchoninate (Pierce Chemical Co.) in 1 L water.)

CHROMATOGRAM
Retention time: 83
Limit of detection: <500 pmole

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, digitose, fructose, fucose, galactose, 2-d-galactose, 6-d-glucose, gulose, lyxose, mannose, 3-O-methylglucose, rhamnose, 2-d-ribose, ribose, sorbose, tagatose

KEY WORDS
post-column reaction

REFERENCE
Mopper,K. Improved chromatographic separations on anion-exchange resins. I. Partition chromatography of sugars in ethanol, *Anal.Biochem.*, **1978**, *85*, 528–532.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: 150 × 6 6 μm Shodex RSPak DC-613 sulfonated polystyrene 55% cross-linked with divinylbenzene calcium form (Showa Denko)
Mobile phase: MeCN:water 80:20
Column temperature: 4
Flow rate: 0.5
Injection volume: 20
Detector: UV 280 following post-column reaction. The column effluent mixed with 500 mM pH 8.5 borate buffer pumped at 0.5 mL/min and 1% 2-cyanoacetamide in water pumped at 0.5 mL/min and the mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 100 ± 1° and a 1 m × 0.5 mm PTFE cooling coil to the detector.

CHROMATOGRAM
Retention time: k' 2.68 (α-D-xylose), k' 2.20 (β-D-xylose)

OTHER SUBSTANCES
Also analyzed: allose, altrose, arabinose, dextrose, fucose, galactose, gulose, idose, lyxose, mannose, rhamnose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Suzuki,S.; Kakehi,K. Improved analysis of aldose anomers by high-performance liquid chromatography on cation-exchange columns, *J.Chromatogr.*, **1984**, *291*, 317–325.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 6 μm Shodex RSPak DC-613 sulfonated polystyrene 55% cross-linked with divinylbenzene (H^+) (Showa Denko)
Mobile phase: MeCN:water 90:10
Flow rate: 0.6
Injection volume: 20
Detector: E, Irika E-502, glassy carbon working electrode 0.30 V, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 200 mM pH 9.5 borate buffer pumped at 0.25 mL/min and 1.5% 2-cyanoacetamide in water pumped at 0.25 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 100° and a 1 m × 0.5 mm PTFE cooling coil to the detector.

CHROMATOGRAM
Retention time: 13
Limit of detection: 20 pmole

OTHER SUBSTANCES
Simultaneous: fucose, galactose, rhamnose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Konishi,T.; Suzuki,S. Electrochemical detection of reducing carbohydrates in high-performance liquid chromatography after post-column derivatization with 2-cyanoacetamide, *J.Chromatogr.*, **1984**, *299*, 245–251.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 3 mL MeCN and 5 mL water. Mix 250 μL of an aqueous solution with 225 μL 1% dansylhydrazine in EtOH and 45 μL 10%trichloroacetic acid in water, heat at 65° for 20 min, dilute with water to an organic solvent concentration of ≤5%, add a 5 mL aliquot to the SPE cartridge, wash with 5 mL MeCN:water 5:95 at ≤2 mL/min, elute with 6 mL MeCN:water 20:80 at ≤2 mL/min (J. Chromatogr. 1983, 256, 27), lyophilize the eluate, reconstitute with MeCN:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm 600 RPB C18 (Alltech)
Mobile phase: MeCN:water 20:80 containing 10 mM formic acid, 40 mM acetic acid, and 1 mM triethylamine. (After each run flush column with MeCN:MeOH 20:80 for 5 min.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Limit of detection: 200-300 pmole

OTHER SUBSTANCES
Simultaneous: dextrose, fucose, galactose, lyxose, mannose

KEY WORDS
derivatization; SPE

REFERENCE
Eggert,F.M.; Jones,M. Measurement of neutral sugars in glycoproteins as dansyl derivatives by automated high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *333*, 123–131.

SAMPLE
Matrix: solutions
Sample preparation: Heat 100-200 pmole sample with 20 μL 4 M trifluoroacetic acid and 20 μL 4 M HCl in a tube sealed under vacuum at 100° for 6 h, add 500 pmole L-rhamnose, evaporate to dryness under reduced pressure at 50°, add 50 μL 9.8% sodium bicarbonate solution (freshly prepared), add 2 μL acetic anhydride, let stand at room temperature with occasional stirring for 30 min, add 200 μL 100-200 mesh Dowex 50W-X2 (H^+), check that pH is about 3. Add the mixture to a 100 × 5 column and wash it with 5 bed volumes of water, evaporate to dryness under reduced pressure, add 5 μL reagent, seal tube, heat at 100° for 13-15 min, add 2 μL 20 mg/mL sodium cyanoborohydride in water (freshly prepared), reseal the tube, heat at 90° for 8 h, dilute with 20 μL water, inject the whole amount on to a 600 × 7.5 10 μm TSK-GEL G2000PW column (Toyo Soda) and elute with 20 mM pH 7.5 ammonium acetate buffer at 0.5 mL/min, collect the sugar fraction at 40-55 min. Evaporate the eluate to dryness and reconstitute it with 250 μL water, inject a 5 μL aliquot. (Prepare reagent by mixing 500 mg 2-aminopyridine, 400 μL concentrated HCl, and 11 mL water.)

HPLC VARIABLES
Column: two 250 × 4.6 5 μm Ultrasphere-ODS column in series
Mobile phase: MeCN:250 mM pH 4.0 sodium citrate buffer 1:99
Flow rate: 0.5
Injection volume: 5
Detector: F ex 320 em 400

CHROMATOGRAM
Retention time: 39

Internal standard: L-rhamnose
Limit of quantitation: 10 pmoles

OTHER SUBSTANCES
Simultaneous: N-acetyl-D-mannosamine, N-acetylgalactosamine, N-acetylglucosamine, 2-deoxy-D-ribose, dextrose, fucose, galactose, mannnose, ribose

KEY WORDS
derivatization; SPE

REFERENCE
Takemoto,H.; Hase,S.; Ikenaka,T. Microquantitative analysis of neutral and amino sugars as fluorescent pyridylamino derivatives by high-performance liquid chromatography, *Anal.Biochem.*, **1985**, *145*, 245–250.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 80 × 3 Hitachi 2633
Mobile phase: 700 mM pH 8.5 Borate buffer containing 0.01% EDTA
Column temperature: 60
Flow rate: 0.7
Injection volume: 20
Detector: E, Irika E-502, glassy carbon working electrode 350 mV, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 100 mM ethylenediamine sulfate pumped at 0.25 mL/min and 700 mM pH 9.0 borate buffer pumped at 0.25 mL/min and the mixture flowed through a 30 m × 0.5 mm ID PTFE coil at 140° and a 10 m × 0.2 mm ID cooling coil to the detector.

CHROMATOGRAM
Retention time: 30
Limit of detection: 1 pmole

OTHER SUBSTANCES
Simultaneous: dextrose, galactose, mannose, rhamnose

KEY WORDS
post-column reaction

REFERENCE
Honda,S.; Enami,K.; Konishi,T.; Suzuki,S.; Kakehi,K. Use of ethylenediamine sulphate for post-column derivatization of reducing carbohydrates to electrochemically oxidizable compounds in high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *361*, 321–329.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: HPIC-AS6 anion-exchange (Dionex)
Mobile phase: 150 mM NaOH
Column temperature: 36
Flow rate: 0.5
Injection volume: 20
Detector: UV 500 following post-column reaction. The column effluent mixed with the reagent pumped at 0.2 mL/min (?) and the mixture flowed through a knitted 10 m × 0.3 mm ID PTFE coil at 90° and then a short knitted PTFE coil at 22° to the detector. (Reagent was 2 mg/mL thymol in concentrated sulfuric acid, let stand for 30 min after preparation, discard after 48 h. (The reagent was displaced from a pressure vessel into the post-column reaction system by pumping n-heptane into the vessel.)

CHROMATOGRAM
Retention time: 9.3
Limit of detection: 100 ng

OTHER SUBSTANCES
Simultaneous: arabinose, desoxyribose, dextrose, fructose, galactose, lactose, maltose, mannose, raffinose, ribose, saccharose
Noninterfering: methyl arabinose, methyl glycose, rhamnose, rutinose, trehalose

KEY WORDS
post-column reaction

REFERENCE
Engelhardt,H.; Ohs,P. Trace analysis of sugars by HPLC and post-column derivatization, *Chromatographia*, **1987**, *23*, 657–662.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve 50 mg sugars in 700 μL pyridine, add 700 μL 720 mM hydroxylamine hydrochloride in pyridine, heat at 60° for 10 min, add 250 μL acetic anhydride, heat at 75° for 10 min, evaporate to dryness under reduced pressure, reconstitute with 3 mL chloroform. Wash the organic layer three times with 6 mL portions of water and dry it over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, take up in chloroform, pass through silica gel using chloroform, evaporate the eluate to dryness, reconstitute, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm μBondapak C18
Mobile phase: Gradient. MeCN:water from 35:75 to 50:50 over 15 min.
Flow rate: 1
Injection volume: 5
Detector: UV 207

CHROMATOGRAM
Retention time: k' 3.1
Limit of detection: 3 μg

OTHER SUBSTANCES
Also analyzed: allose, altrose, arabinose, dextrose, fructose, galactose, gulose, idose, lyxose, mannose, ribose, talose

KEY WORDS
derivatization

REFERENCE
Velasco,D.; Castells,J.; Lopez-Calahorra,F. High-performance liquid chromatographic separation of monosaccharides as their peracetylated ketoximes and aldononitriles, *J.Chromatogr.*, **1990**, *519*, 228–236.

SAMPLE
Matrix: solutions
Sample preparation: 10 μL EtOH containing sugars + 110 μL EtOH:acetic acid 99.9:0.1 + 100 μL Fmoc-hydrazine in MeCN, mix, heat at 65° for 3 h, cool to room temperature, dilute with EtOH or EtOH:acetic acid 99.9:0.1, inject an aliquot. (Prepare Fmoc-hydrazine as follows. Dissolve 100 mg 9-fluorenylmethylchloroformate in 25 mL MeCN, add this solution dropwise with stirring to 1 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!), stir for 30 min, evaporate under reduced pressure, use the crude product or recrystallize from EtOH or MeCN (mp 173-5°). Prepare a solution of Fmoc-hydrazine in MeCN so that the hydrazine:sugar ratio is 10:1.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax ODS

Mobile phase: Gradient. A was MeCN:water 27:73 containing 80 mM acetic acid. B was MeCN:water 30:70 containing 80 mM acetic acid. A:B from 100:0 to 0:100 over 30 min.
Flow rate: 1
Detector: F ex 270 em 320

CHROMATOGRAM
Retention time: 15
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Simultaneous: fructose, galactose, lactose, maltose, mannose, ribose

KEY WORDS
derivatization

REFERENCE
Zhang,R.-E.; Cao,Y.-L.; Hearn,M.W. Synthesis and application of Fmoc-hydrazine for the quantitative determination of saccharides by reversed-phase high-performance liquid chromatography in the low and subpicomole range, *Anal.Biochem.*, **1991**, *195*, 160–167.

SAMPLE
Matrix: solutions
Sample preparation: Add 55 μL phenylisocyanate to a 1 mg/mL solution in DMF, heat at 55° for 95 min, cool, add 500 μL MeOH, let stand for 5 min, make up to 6 mL with DMF, dilute an aliquot 10-fold with DMF, inject an aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm ODS 224 RP18 (Brownlee)
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 9.42
Limit of detection: 0.2 ng

OTHER SUBSTANCES
Simultaneous: allose, arabinose, deoxyglucose, deoxyribose, dextrose, fucose, galactose, lyxose, mannose, methylgalactoside, methylglucoside, methylmannoside, rhamnose, ribose

KEY WORDS
derivatization; more than one derivative was observed, retention time is for major derivative

REFERENCE
Rakotomanga,S.; Baillet,A.; Pellerin,F.; Baylocq-Ferrier,D. Liquid chromatographic analysis of monosaccharides with phenylisocyanate derivatization, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 587–591.

SAMPLE
Matrix: solutions
Sample preparation: Add 25 nmoles 3-O-methylglucose, evaporate the solution to dryness, add 50 μL 300 mM sodium cyanoborohydride in 2 M pH 7.0 ammonium acetate (freshly prepared), heat at 105° for 4 h, add 100 μL water, add 40 μL 6 M formic acid, evaporate to dryness under reduced pressure, add 500 μL MeOH, evaporate to dryness, repeat MeOH evaporation twice more, add 100 μL EtOH:water:triethylamine 40:40:20, evaporate to dryness, add 100 μL EtOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under reduced pressure, reconstitute with 20 μL MeCN:water 60:40, add 180 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C18

Mobile phase: Gradient. A was 50 mM pH 6.8 ammonium acetate. B was 100 mM pH 6.8 ammonium acetate in MeCN:MeOH:water 44:10:46. A:B 78:22 until the run is over, to 0:100 over 5 min, maintain at 0:100 for 6 min, return to initial conditions over 5 min
Column temperature: 30
Flow rate: 0.8
Detector: UV 254

CHROMATOGRAM
Retention time: 13.5
Internal standard: 3-O-methylglucose (20)
Limit of detection: 50 pmole

OTHER SUBSTANCES
Simultaneous: dextrose, fucose, galactose, mannose, ribose

KEY WORDS
derivatization

REFERENCE
Spiro,M.J.; Spiro,R.G. Monosaccharide determination of glycoconjugates by reverse-phase high-performance liquid chromatography of their phenylthiocarbamyl derivatives, *Anal.Biochem.*, **1992**, *204*, 152–157.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 7.8 Aminex HPX-87P cation-exchange (Bio-Rad)
Mobile phase: Water
Column temperature: 85
Flow rate: 0.8
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 20 m × 0.3 mm ID PTFE coil at 90° and a coil at 0° to the detector. (Reagent was prepared from 4000 ppm Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) in 2 M NaOH (A) and 40 mM hydrogen peroxide (B). A:B 70:30.)

CHROMATOGRAM
Retention time: 16.5
Limit of detection: 30 ng

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fructose, galactose, mannose, ribose

KEY WORDS
post-column reaction

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Hernandez,V.; Toribio,L.; Mendez,R. Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) as a reagent for post-column derivatization of neutral monosaccharides in high pressure liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 1105–1116.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a 500 μM saccharide solution in MeCN:water 30:70 with 50 μL 10 mM reagent in MeCN and 100 μL 0.5% trichloroacetic acid in MeCN, heat at 65° in the dark for 3 h. Remove a 50 μL aliquot of the reaction mixture, add 200 μL water, add 200 μL ethyl acetate, mix, centrifuge at 3000 rpm for 2 min, repeat the ethyl acetate wash twice more. Dry the aqueous layer under reduced pressure, reconstitute with 200 μL MeCN, inject an aliquot. (Synthesis of reagent, R-(+)-DBD-ProCZ, is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the

flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro- 2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Add 100 mg DBD-F in 10 mL MeCN to 47 mg R-(+)-proline in 20 mL 250 mM pH 11.5 sodium carbonate solution, stir at room temperature for 30 min, wash with ethyl acetate, adjust the pH of the aqueous layer to 1-2 with 2 M HCl, extract three times with 30 mL ethyl acetate. Combine the extracts and evaporate them under reduced pressure, recrystallize from benzene/ethyl acetate to give R-(+)-4-(N,N-dimethylaminosulfonyl)-7-(2-carboxypyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-Pro) as yellow needles (mp 187-9° d) (Analyst 1989, 114, 1233). Suspend 55 mg (R)-(+)-DBD-Pro in 55 mL anhydrous diethyl ether at 0°, add 110 mg phosphorus pentachloride, stir at 5° for 1 h, filter quickly, evaporate to dryness under reduced pressure, dry under vacuum over phosphorus pentoxide for 12 h to give R-(+)-4-(N,N-dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-Pro-Cl) as yellow crystals (mp 116-17°) (Analyst 1993, 118, 759). Add 130 mg DBD-Pro-Cl dissolved in 25 mL anhydrous benzene dropwise to 100 mL MeOH containing 70 mg hydrazine hydrate, stir for 30 min at room temperature, evaporate under reduced pressure, recrystallize from ethyl acetate:MeOH 90:10 to give R-(+)-4-(2-carbazolylpyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (R-(+)-DBD-ProCZ) as orange crystals (mp 107-109°) (Anal. Proc. 1994, 31, 265).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water 15:85
Column temperature: 40
Flow rate: 1
Detector: F ex 450 em 540

CHROMATOGRAM
Retention time: 15.81

OTHER SUBSTANCES
Simultaneous: N-acetyl-D-glucosamine, arabinose, dextrose, galactose, mannose

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KEY WORDS
derivatization

REFERENCE

Toyo'oka,T.; Kuze,A. Determination of saccharides labelled with a fluorescent reagent, DBD-ProCZ, by liquid chromatography, *Biomed.Chromatogr.*, **1997**, *11*, 132–136.

SAMPLE

Matrix: urine

Sample preparation: Mix acetone with urine so as to make a 63:47 acetone:urine mixture, centrifuge a 6 mL aliquot. Evaporate the acetone from the supernatant under a stream of helium at 35°, add 30 mg Dowex 50W-X8, add 30 mg Dowex 1-X8, agitate, centrifuge, inject a 1-10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.3 Hitachi 3013 N anion-exchange resin, phosphate form
Mobile phase: MeCN:water 83:17
Column temperature: 60
Flow rate: 1
Injection volume: 1-10
Detector: UV 530 following post-column reaction. The column effluent mixed with the reagent pumped at 1.5 mL/min, the mixture flowed through a 3 m × 0.5 mm i.d. coil of PTFE tubing at 85° and a 1 m × 0.5 mm i.d. coil of PTFE tubing at room temperature to the detector. (Reagent was 2 g/L blue tetrazolium in EtOH:water 50:50 containing 180 mM NaOH.)

CHROMATOGRAM

Retention time: 10
Limit of detection: 10 ng

OTHER SUBSTANCES

Extracted: arabinose, dextrose, fructose, fucose, galactose, lactose, ribose

KEY WORDS

post-column reaction

REFERENCE

D'Amboise,M.; Hanai,T.; Noël,D. Liquid-chromatographic measurement of urinary monosaccharides, *Clin. Chem.*, **1980**, *26*, 1348–1350.

SAMPLE

Matrix: urine

Sample preparation: 100 μL Urine + 10 μL 10% trichloroacetic acid in water + 50 μL 5% dansyl hydrazine in MeCN, heat at 65° for 20 min, cool in ice, add an equal volume of water, inject a 10-300 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeCN:80 mM acetic acid 21:79
Flow rate: 1
Injection volume: 10
Detector: F ex 360 em >470

CHROMATOGRAM

Retention time: 11.5
Limit of detection: 5-15 pmole

OTHER SUBSTANCES

Extracted: cellobiose, 2-deoxyglucose, 2-deoxyribose, dextrose, fucose, galactose, gentobiose, lactose, maltose, mannose, rhamnose, ribose
Interfering: arabinose, fructose

KEY WORDS

derivatization

REFERENCE
Mopper,K.; Johnson,L. Reversed-phase liquid chromatographic analysis of Dns-sugars. Optimization of derivatization and chromatographic procedures and applications to natural samples, *J.Chromatogr.*, **1983**, *256*, 27–38.

SAMPLE
Matrix: urine
Sample preparation: 10 μL Urine + 200 μL reagent, heat at 65° for 16 h, cool to room temperature, inject a 5 μL aliquot of the clear supernatant. (Prepare reagent by dissolving 5 mg Fmoc-hydrazine in 1 mL MeCN, add 10 μL buffer. Buffer was 1.44 M formic acid containing 600 mM NaOH. Prepare Fmoc-hydrazine as follows. Dissolve 1 g 9-fluorenylmethyl chloroformate in 100 mL EtOH, add this solution dropwise with stirring to 10 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!), stir for 30 min, filter off the precipitate, wash it twice with 20 mL portions of ice-cold EtOH, dry at room temperature.)

HPLC VARIABLES
Guard column: 10 × 4.6 3 μm Spherisorb ODS II
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. Isopropanol:isobutyl alcohol:water 6:6:88 for 13 min, to 80:0:20 (step gradient), maintain at 80:0:20 for 6 min, re-equilibrate at initial conditions.
Column temperature: 50
Injection volume: 5
Detector: F ex 270 em 315

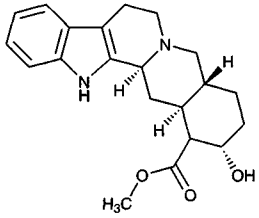
CHROMATOGRAM
Retention time: 9.8
Limit of detection: 20 nM

OTHER SUBSTANCES
Extracted: lactulose, 3-O-methyl-D-glucose, rhamnose

KEY WORDS
derivatization

REFERENCE
Rooyakkers,D.R.; van Eijk,H.M.H.; Deutz,N.E.P. Simple and sensitive multi-sugar-probe gut permeability test by high-performance liquid chromatography with fluorescence labelling, *J.Chromatogr.A*, **1996**, *730*, 99–105.

Yohimbine



Molecular formula: $C_{21}H_{26}N_2O_3$
Molecular weight: 354.45
CAS Registry No.: 146-48-5
Merck Index: 10236

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

REFERENCE
Mopper,K.; Johnson,L. Reversed-phase liquid chromatographic analysis of Dns-sugars. Optimization of derivatization and chromatographic procedures and applications to natural samples, *J.Chromatogr.*, **1983**, *256*, 27–38.

SAMPLE
Matrix: urine
Sample preparation: 10 μL Urine + 200 μL reagent, heat at 65° for 16 h, cool to room temperature, inject a 5 μL aliquot of the clear supernatant. (Prepare reagent by dissolving 5 mg Fmoc-hydrazine in 1 mL MeCN, add 10 μL buffer. Buffer was 1.44 M formic acid containing 600 mM NaOH. Prepare Fmoc-hydrazine as follows. Dissolve 1 g 9-fluorenylmethyl chloroformate in 100 mL EtOH, add this solution dropwise with stirring to 10 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!), stir for 30 min, filter off the precipitate, wash it twice with 20 mL portions of ice-cold EtOH, dry at room temperature.)

HPLC VARIABLES
Guard column: 10 × 4.6 3 μm Spherisorb ODS II
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. Isopropanol:isobutyl alcohol:water 6:6:88 for 13 min, to 80:0:20 (step gradient), maintain at 80:0:20 for 6 min, re-equilibrate at initial conditions.
Column temperature: 50
Injection volume: 5
Detector: F ex 270 em 315

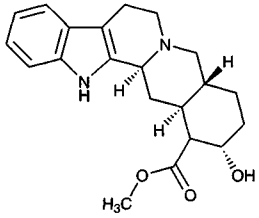
CHROMATOGRAM
Retention time: 9.8
Limit of detection: 20 nM

OTHER SUBSTANCES
Extracted: lactulose, 3-O-methyl-D-glucose, rhamnose

KEY WORDS
derivatization

REFERENCE
Rooyakkers,D.R.; van Eijk,H.M.H.; Deutz,N.E.P. Simple and sensitive multi-sugar-probe gut permeability test by high-performance liquid chromatography with fluorescence labelling, *J.Chromatogr.A*, **1996**, *730*, 99–105.

Yohimbine



Molecular formula: $C_{21}H_{26}N_2O_3$
Molecular weight: 354.45
CAS Registry No.: 146-48-5
Merck Index: 10236

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 272

CHROMATOGRAM

Retention time: 4.40
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue
Sample preparation: Serum. 200-450 μL Serum + 800 μL 1 M pH 9.2 Delory King carbonate buffer, vortex, add 5 mL diethyl ether, mix for 30 s, centrifuge at 1000 g for 2 min. Remove the organic phase and add it to 100 μL 100 mM HCl, mix for 30 s, centrifuge at 1000 g for 2 min, discard the ether, volatilize residual ether from the aqueous phase under a stream of nitrogen, inject a 10-70 μL of the aqueous phase. Liver. Homogenize 1 g of liver in 3 mL ice cold 1 M pH 9.2 Delory King carbonate buffer, add 5 mL diethyl ether, mix for 30 s, centrifuge at 1000 g for 2 min. Remove the organic phase and add it to 100 μL 100 mM HCl, mix for 30 s, centrifuge at 1000 g for 2 min, discard the ether, volatilize residual ether from the aqueous phase under a stream of nitrogen, inject a 10-70 μL of the aqueous phase.

HPLC VARIABLES
Column: 100 × 3.2 3 μm Phase-2 ODS
Mobile phase: MeCN:15 mM pH 3.0 monochloroacetate buffer 25:75 containing 350 mg/L EDTA
Flow rate: 0.6
Injection volume: 10-70
Detector: E, Bioanalytical Systems LC-4B, LC-17 oxidative flow cell, TL-5 glassy carbon electrode + 900 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.0
Internal standard: yohimbine

OTHER SUBSTANCES
Extracted: phentolamine

KEY WORDS
yohimbine is IS; serum; liver; mouse

REFERENCE
Kerger,B.D.; James,R.C.; Roberts,S.M. An assay for phentolamine using high performance liquid chromatography with electrochemical detection, *Anal.Biochem.*, **1988**, *170*, 145–151.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Plasma or 500 μL urine + 100 μL 5 μg/mL eserine chlorohydrate + 0.25 (urine) or 1 (plasma) mL 500 mM pH 11 Na_2HPO_4 + 2 mL chloroform, shake for 5 min, centrifuge at 5000 g for 5 min. Remove 1.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeOH:EtOH 85:15, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm LiChrosorb Si 60
Mobile phase: MeOH:20 mM pH 5 sodium acetate 95:5
Column temperature: 30
Flow rate: 1
Injection volume: 80
Detector: F ex 280 em 320 (cell temp 15°) or UV 280

CHROMATOGRAM
Retention time: 7
Internal standard: eserine chlorohydrate (17)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Le Verge,R.; Le Corre,P.; Chevanne,F.; Doe De Maindreville,M.; Royer,D.; Levy,J. Determination of yohimbine and its two hydroxylated metabolites in humans by high-performance liquid chromatography and mass spectral analysis, *J.Chromatogr.*, **1992**, *574*, 283–292.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of

maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM

Retention time: 11.51

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: cell cultures
Sample preparation: Extract 5 g cell culture with 20 mL MeOH with sonication for 10 min, repeat extraction twice. Evaporate extracts to dryness under reduced pressure, reconstitute in 100 mL 10 mM HCl, filter, adjust pH to 6 with 10 mM NaOH, inject a 5-100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm Armsorb-300-C8 (Armchrom, Yerevan, Armenia)
Mobile phase: Gradient. A was MeCN:water 10:90 containing 0.1% trifluoroacetic acid. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 50:50 over 50 min.
Flow rate: 0.8
Injection volume: 5-100
Detector: UV 280

CHROMATOGRAM

Retention time: 21

OTHER SUBSTANCES

Extracted: ajmaline, ajmalicine, reserpine, raucaffricine, serpentine

REFERENCE

Klyushnichenko,V.E.; Yakimov,S.A.; Tuzova,T.P.; Syagailo,Y.V.; Kuzovkina,I.N.; Wulfson,A.N.; Miroshnikov,A.I. Determination of indole alkaloids from *R. serpentina* and *R. vomitoria* by high-performance liquid chromatography and high-performance thin-layer chromatography, *J.Chromatogr.A*, **1995**, *704*, 357–362.

SAMPLE

Matrix: plants
Sample preparation: Freeze leaves with liquid nitrogen, air dry, grind to a fine powder. Mix 0.5 g powder and 2 mL MeOH, sonicate for 30 min, allow to settle, decant the liquid, repeat extraction. Combine the extracts and filter (0.45 μm) them, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Microsorb C18

Mobile phase: Gradient. MeCN:buffer 15:85 for 2 min, to 40:60 over 58 min, maintain at 40:60 for 5 min, to 95:5 over 5 min, maintain at 95:5 over 5 min. (Prepare buffer by mixing 2 mL trifluoroacetic acid and 1 mL triethylamine in water, make up to 1 L with water, adjust pH to 2.4 with ammonium hydroxide.)
Injection volume: 10
Detector: UV 274

CHROMATOGRAM
Retention time: 16.25

OTHER SUBSTANCES
Extracted: tetrahydroalstonine, tryptamine, vinblastine, vincamine, vincristine
Interfering: ajmalacine

KEY WORDS
leaves

REFERENCE
Bowman,R.N.; Gerber,R.E.; Terry,M.E. Analysis of anti-cancer alkaloids vincristine & vinblastine, *Rainin Chromatography Update (TB-13)*, **1996**, 1–2.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 10 μm PRP-1 (Hamilton)
Mobile phase: Gradient. MeCN:20 mM ammonium hydroxide from 15:85 to 100:0 over 17 min
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: cocaine, codeine, methadone, reserpine, thebaine

REFERENCE
Keystone Scientific Catalog, 1993-4, p. 22.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 1-25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 Zorbax cyanopropyl
Mobile phase: MeCN:500 mM acetic acid:t-butylamine 30:70:0.01
Flow rate: 2.5
Injection volume: 1-25
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: aspidospermine, quebrachamine

REFERENCE
Deutsch,H.F.; Evenson,M.A.; Drescher,P.; Sparwasser,C.; Madsen.P.O., Isolation and biological activity of aspidospermine and quebrachamine from an Aspidosperma tree source, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1283–1287.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.15 (A), 4.37 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

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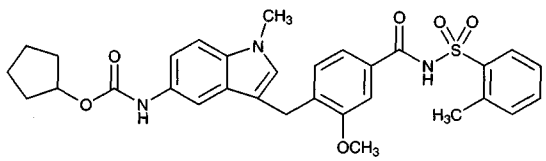

Molecular formula: $C_{31}H_{33}N_3O_6S$
Molecular weight: 575.69
CAS Registry No.: 107753-78-6
Merck Index: 10241

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
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Detector: UV 229

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Retention time: 8.15 (A), 4.37 (B)

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KEY WORDS
details of plasma extraction

REFERENCE
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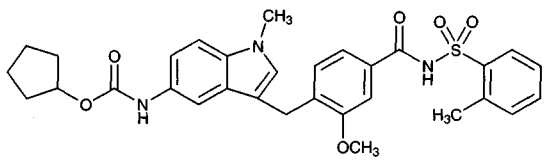

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SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut (LRBE) C18 SPE cartridge (Varian) with two 1 mL portions of MeOH and two 1 mL portions of water. Add 1 mL MeCN to 1 mL plasma, mix, centrifuge at 1000 g for 5 min, decant the supernatant, add it to 5 mL 10 mM pH 7.0 triethylammonium phosphate buffer. Add the diluted supernatant to the SPE cartridge, let the whole supernatant volume pass through the cartridge, elute with three separate aliquots of 500 μL MeCN:THF:triethylamine 90:10:0.2, evaporate the solvent under nitrogen at 40°, reconstitute the sample in 250 μL mobile phase, filter (0.45 μm syringe filter), inject an aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4.0 5 μm Zorbax CN
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: THF:hexane:90% glacial acetic acid 30:70:0.1
Flow rate: 0.9
Injection volume: 150
Detector: F ex 250 em 452

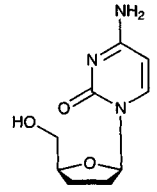
CHROMATOGRAM
Retention time: 9-10
Internal standard: 4-5(-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-a-benzylsulfonylbenzamide (ICI 198 707) (10-11)
Limit of quantitation: 750 pg/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, albuterol, aspirin, benzyl alcohol, caffeine, ibuprofen

KEY WORDS
plasma; SPE

REFERENCE
Bui,K.H.; Kennedy,C.M.; Azumaya,C.T.; Birmingham,B.K. Determination of zafirlukast, a selective leukotriene antagonist, human plasma by normal-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *696*, 131–136.

Zalcitabine



Molecular formula: $C_9H_{13}N_3O_3$
Molecular weight: 211.22
CAS Registry No.: 7481-89-2
Merck Index: 10242
Lednicer No.: 5 98

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 6 mL MeOH and 12 mL MeOH. Add 1.25 mL plasma to the SPE cartridge at 0.5 mL/min, wash with 1.5 mL water, elute with 2 mL MeOH at 0.5 mL/min. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, filter (Ultrafree-MC 0.45 μm) while centrifuging at 11900 g for 10 min, inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:water:heptafluorobutyric acid 6.5:93.4:0.1
Flow rate: 2
Injection volume: 100
Detector: UV 288

CHROMATOGRAM
Retention time: 5.8
Internal standard: 5-methyldeoxycytidine (UV 306) (3.8)
Limit of detection: 30 nM

KEY WORDS
SPE; pharmacokinetics; plasma

REFERENCE
Hawkins,M.E.; Poplack,D.G.; Pizzo,P.A.; Balis,F.M. High-performance liquid chromatographic method for the analysis of 2',3'-dideoxycytidine in human plasma, *J.Chromatogr.*, **1990**, *532*, 442–444.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Supelclean LC-18 SPE cartridge (Supelco) with 2 mL MeOH and 2 mL water. 1 mL Plasma + 5 ng IS, vortex, add to the SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH:water 20:80. Evaporate the eluate to dryness, reconstitute the residue in 50 μL MeOH:water 10:90, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:50 mM ammonium acetate 10:90
Flow rate: 1
Injection volume: 25
Detector: MS, VG Trio-3 triple quadrupole, thermospray interface with heated capillary at 280°, source 200°, repeller electrode 200 V, SIM, m/z 212 for zalcitabine, m/z 216 for IS

CHROMATOGRAM
Retention time: 8.9
Internal standard: [$^{15}N_2$,$^{2}H_2$]zalcitabine
Limit of quantitation: 0.25 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Jajoo,H.K.; Bennett,S.M.; Kornhauser,D.M. Thermospray liquid chromatographic-mass spectrometric analysis of anti-AIDS nucleosides: quantification of 2',3'-dideoxycytidine in plasma samples, *J.Chromatogr.*, **1992**, *577*, 299–304.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 400 μL ethyl acetate:MeCN 50:50, vortex for 30 s. Remove a 200 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL mobile phase, vortex for 10 s, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 60 RP-select B
Column: 125 × 4 5 μm Lichrospher 60 RP-select B
Mobile phase: MeOH:pH 7.0 phosphate buffer 5:95
Flow rate: 1
Injection volume: 10
Detector: UV 270

OTHER SUBSTANCES
Also analyzed: didanosine (UV 250)

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Mirchandani,H.L.; Chien,Y.W. Intestinal absorption of dideoxynucleosides: Characterization using a multiloop in situ technique, *J.Pharm.Sci.*, **1995**, *84*, 44–48.

SAMPLE
Matrix: blood
Sample preparation: Filter (Millipore Ultrafree-MC, 10000 molecular mass limit) 250 μL serum while centrifuging at 17000 g for 1.5 h, inject a 50 μL aliquot of the clear ultrafiltrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: Isopropanol:20 mM pH 5 sodium citrate 2.5:97.5
Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 3.0

OTHER SUBSTANCES
Extracted: didanosine, zidovudine

KEY WORDS
serum; ultrafiltrate

REFERENCE
Rosell-Rovira,M.L.; Pou-Clavé,L.; Lopez-Galera,R.; Pascual-Mostaza,C. Determination of free serum didanosine by ultrafiltration and high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 89–92.

SAMPLE
Matrix: blood, cell suspensions, perfusate
Sample preparation: Centrifuge cellular suspensions at 17000 g for 5 min, inject a 25 μL aliquot. Centrifuge perfusion fluid at 17000 g for 5 min, inject a 50 μL aliquot. Dilute 1 mL plasma with 1 mL saturated ammonium sulfate, vortex for 30 s, centrifuge at 3000 g for 2 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm Phenyl Hypersil NC-04
Mobile phase: MeOH:1.4 g/L sodium acetate 3:97, adjusted to pH 6.55
Flow rate: 1
Injection volume: 25-50
Detector: UV 271

CHROMATOGRAM
Retention time: 10
Limit of detection: 50 ng/mL

KEY WORDS
plasma

REFERENCE
Frijus-Plessen,N.; Michaelis,H.C.; Foth,H.; Kahl,G.F. Determination of 3'-azido-3'-deoxythymidine, 2',3'-dideoxycytidine, 3'-fluoro-3'-deoxythymidine and 2',3'-dideoxyinosine in biological samples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 101–107.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 100 μg/mL aqueous solution, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cyclobond I RSP (Advanced Separation Technologies)
Mobile phase: 2.5 mL/L Triethylamine in water adjusted to pH 6.5 with glacial acetic acid (Pass mobile phase through a silica column to saturate it with silica.)
Flow rate: 0.25
Injection volume: 5
Detector: UV 270

CHROMATOGRAM
Retention time: 29
Limit of detection: 0.05-0.1% (of zalcitabine)

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Scypinski,S.; Ross,A.J. Liquid chromatographic separation of zalcitabine and its stereoisomers, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1271–1276.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 5:95
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 3.89

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type anti-HIV drugs. 1. Stability studies for hairless rat skin permeation, *J.Pharm.Sci.*, **1995**, *84*, 1061–1066.

SAMPLE
Matrix: solutions
Sample preparation: Briefly vortex 100 μL of an aqueous solution with 300 μL MeCN:water:acetic acid 80:19:1, centrifuge at 15000 g for 5 min. Remove the supernatant and add it to 100 μL 1.25 mM phenacyl bromide in MeCN, heat at 80° for 45 min, evaporate to dryness under reduced pressure at room temperature, reconstitute with 60 μL water, vortex briefly, centrifuge at 15000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Prodigy ODS-2 (Phenomenex)
Mobile phase: MeCN:water:trifluoroacetic acid 16:84:0.1
Column temperature: 45
Flow rate: 2.2
Injection volume: 20
Detector: F ex 305 em 370

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: (-)-cis-5-(4-amino-1,2-dihydro-2-oxo-1-pyrimidinyl)-1,3-oxothiolane-2-methanol (3-TC)), cytidine, cytosine monophosphate, deoxycytidine
Noninterfering: guanidine, hypoxanthine, thymine, uracil, xanthine
Interfering: cytosine

KEY WORDS
derivatization

REFERENCE
Eisenberg,E.J.; Cundy,K.C. High-performance liquid chromatographic determination of cytosine-containing compounds by precolumn fluorescence derivatization with phenacyl bromide: application to antiviral nucleosides and nucleotides, *J.Chromatogr.B*, **1996**, *679*, 119–127.

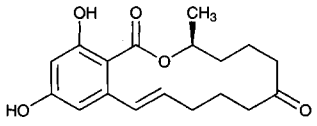
SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 5:95
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 3.89

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type Anti-HIV drugs. 2. The effect of vehicle and enhancer on skin permeation, *J.Pharm.Sci.*, **1996**, *85*, 214–219.

Zearalenone



Molecular formula: $C_{18}H_{22}O_5$
Molecular weight: 318.37
CAS Registry No.: 17924-92-4
Merck Index: 10246

SAMPLE
Matrix: solutions

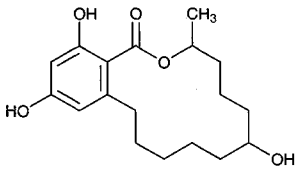
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeOH:water 80:20
Flow rate: 1
Detector: F ex 285 em 440 following post-column reaction. The column effluent mixed with 250 mM aluminum chloride hexahydrate in MeOH:water 75:25 pumped at 0.5 mL/min and the mixture flowed through a 5 m × 0.3 mm ID PTFE coil at 50° to the detector. (At the end of each day flush system with MeOH:water 75:25.)

CHROMATOGRAM
Retention time: 6.5

KEY WORDS
post-column reaction

REFERENCE
Hetmanski,M.T.; Scudamore,K.A. Detection of zearalenone in cereal extracts using high-performance liquid chromatography with post-column derivatization, *J.Chromatogr.*, **1991**, *588*, 47–52.

Zeranol



Molecular formula: $C_{18}H_{26}O_5$
Molecular weight: 322.40
CAS Registry No.: 26538-44-3, 55331-29-8
Merck Index: 10251

SAMPLE
Matrix: blood
Sample preparation: Hydrolyse serum or plasma with β-glucuronidase and sulfatase, extract with diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 40 μL buffer, add 100 μL 1.5 mg/mL dansyl chloride in acetone, shake vigorously for 30 s, heat at 100° for 5 min, inject a 20 μL aliquot. (Prepare buffer by adjusting the pH of 4 g/L sodium bicarbonate in water to 10.5 with 5 M NaOH.)

HPLC VARIABLES
Column: 250 × 2.6 PAH-10 C18 (Perkin-Elmer)
Mobile phase: Gradient. MeCN:water from 60:40 to 95:5 over 15 min (Perkin-Elmer curve 1), maintain at 95:5 for 10 min. (Flush column with MeCN at 0.1 mL/min overnight.)
Flow rate: 1
Injection volume: 20
Detector: F ex 335 em 540

CHROMATOGRAM
Retention time: 11.5
Limit of detection: 80 ng

OTHER SUBSTANCES
Extracted: diethylstilbestrol, estriol, estrone, hexestrol, zanone, zenone

KEY WORDS
derivatization; cow; sheep; plasma; serum; LOD is too high for practical detection of compounds in serum and plasma.

REFERENCE
Rhys Williams,A.T.; Winfield,S.A.; Belloli,R.C. Dns derivatization of anabolic agents with high-performance liquid chromatographic separation and fluorescence detection, *J.Chromatogr.*, **1982**, *240*, 224–229.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL dichloromethane, shake for 20 min, centrifuge at 4000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot. To measure conjugates add 1 mL residual plasma from above procedure to 1 mL pH 5.5 phosphate buffer, add 1 mL 1000 U/mL helicase (type H-2 from Helix pomatia, Sigma), heat at 37° overnight, add 10 mL dichloromethane, shake for 20 min, centrifuge at 4000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS-1 C18
Mobile phase: MeCN:MeOH:0.2% acetic acid 30:20:50

Flow rate: 1.2
Detector: radioactivity

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: taleranol, zeralanone

KEY WORDS
pig; plasma; tritium-labeled

REFERENCE
Bories,G.; Suarez,A.F. Profiling of free and conjugated [^{3}H]zeranol metabolites in pig plasma, *J.Chromatogr.*, **1989**, *489*, 191–197.

SAMPLE
Matrix: filters
Sample preparation: Sonicate filter with 2 mL MeOH for 1 h, add 1 mL water, filter (0.5 μm PTFE), inject an aliquot.

HPLC VARIABLES
Column: C18 Radial Compression (Waters)
Mobile phase: MeOH:water 60:40
Flow rate: 2
Detector: UV 236

CHROMATOGRAM
Retention time: 10.0
Limit of detection: 7 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: taleranol, zearalanone, zearalenol, zearalenone

REFERENCE
Neumeister,C.E. Environmental sampling and analysis for zeranol, *Am.Ind.Hyg.Assoc.J.*, **1987**, *48*, 919–921.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: diethylstilbestrol, trenbolone, nandrolone, dienestrol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE
Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE
Matrix: tissue

Sample preparation: Homogenize 2.5 g tissue with 10 mL acetone for 20 s, sonicate for 5 min, centrifuge at 3200 rpm. Decant the supernatant into a silanized tube. Add 8 mL acetone to the pellet and repeat the extraction. Combine the supernatants. Add to a 5 mL pipette tip containing 1.5 g alumina (80-200 mesh, Brockman activity 1) followed by an Econo-Column filled with 1.0 g AGMP-1 resin (Bio-Rad), allow to pass through by gravity. Wash with four 1 mL portions of acetone:water 95:5. Remove the alumina column, wash the ion-exchange column with 1 mL acetone:water 95:5, elute with four 1 mL portions of 10% acetic acid in acetone. Evaporate the combined eluates to dryness with nitrogen at 40°. Add 500 μL water to the residue, extract twice with 2 mL potions of ether. Combine the ether layers and evaporate them to dryness. Reconstitute the residue in mobile phase B. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelco silica
Mobile phase: Gradient. A was hexane. B was MeOH:hexane:2-propanol 45:40:15. A:B from 100:0 to 60:40 over 15 min.
Flow rate: 2.0
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 11.54
Limit of detection: 10 ng

OTHER SUBSTANCES

Extracted: diethylstilbestrol, estradiol
Simultaneous: estrone, zeralenol, zeralanone, zeralenone

KEY WORDS

chicken; muscle; normal phase; SPE

REFERENCE

Medina,M.B.; Sherman,J.T. High performance liquid chromatographic separation of anabolic oestrogens and ultraviolet detection of 17β-oestradiol, zeranol, diethylstilboestrol or zearalenone in avian muscle tissue extracts, *Food Addit.Contam.*, **1986**, *3*, 263–272.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Tekmar Model 3412-A20) 80 g ground tissue and 160 mL MeOH at medium-high speed for two 1 min intervals, centrifuge at 1500 rpm for 10 min, remove the supernatant, rinse apparatus with two 10 mL portions of MeOH. Combine the organic layers and evaporate them to 25 mL under a stream of nitrogen, adjust pH to 5.0 ± 0.2 with 20% acetic acid, add 4 mL freshly prepared β-glucuronidase solution in water (1 mg/mL, Type B-1, 500 000 U/g, Sigma), add 5 mL chloroform, mix by swirling, heat at 37° for 12-16 h, cool, add 45 mL chloroform, shake for 10 s, pass organic layer through 30-35 g anhydrous sodium sulfate (prewashed with 30 mL chloroform), extract aqueous layer with 50 mL chloroform, pass organic layer through sodium sulfate, wash sodium sulfate with two 5 mL portions of chloroform. Combine the chloroform layers and add 10 mL 2 M NaOH, shake vigorously for 30 s, discard organic layer, wash aqueous layer with two 50 mL portions of chloroform. Adjust the pH of the aqueous layer to 10.6-10.8 with 19-20 mL 1 M sodium bicarbonate solution, extract three times with 25 mL portions of chloroform, pass extracts through 30-35 g anhydrous sodium sulfate (prewashed with chloroform), wash sodium sulfate with 10 mL chloroform, evaporate chloroform layer to dryness under reduced pressure at 40°, reconstitute with 2 mL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Nova-Pak RP-C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 90 mM sodium acetate containing 10 mg/L EDTA adjusted to pH 6.9 with acetic acid.)
Flow rate: 1
Injection volume: 25
Detector: E, Bioanalytical Systems LC4B-17D, glassy carbon electrode +0.90 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 20
Limit of detection: 100 pg

OTHER SUBSTANCES
Extracted: zearalanone, β-zearalenol, zearalenone

KEY WORDS
chicken; cow; muscle; liver

REFERENCE
Roybal,J.E.; Munns,R.K.; Morris,W.J.; Hurlbut,J.A.; Shimoda,W. Determination of zeranol/zearalenone and their metabolites in edible animal tissue by liquid chromatography with electrochemical detection and confirmation by gas chromatography/mass spectrometry, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 263–271.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH: water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, wash with 1 mL MeOH, 1 mL 1 M HCl, elute with 2 mL 30 mM HCl in MeCN:MeOH 20:80. Evaporate eluate to dryness with nitrogen at 40°, take up in 100 μL MeCN:MeOH:THF:10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 22:8:13:57, inject 50 μL aliquot

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: MeCN:MeOH:THF:10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 21: 7:12:60
Flow rate: 1.5
Injection volume: 50
Detector: E, Coulochem 5100A, detector 1 0.05 V, detector 2 0.60 V

CHROMATOGRAM
Retention time: 12
Limit of detection: 1 ng/g

OTHER SUBSTANCES
Simultaneous: taleranol, zearalenol, zearalenone

KEY WORDS
muscle; liver; chicken; ox

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM

ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM
Retention time: 11.3
Limit of detection: 100 ppb

OTHER SUBSTANCES
Extracted: dexamethasone, diethylstilbestrol, medroxyprogesterone, melengestrol acetate, trenbolone, triamcinolone acetonide

KEY WORDS
cow; muscle; liver; SFE

REFERENCE
Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

SAMPLE
Matrix: urine
Sample preparation: 10 mL Urine + glucuronidase/sulfatase (Helix pomatia), incubate at 37° for 1 h, extract twice with 5 mL diethyl ether, add 225 μL water and evaporate ether under nitrogen, add 400 μL MeOH, inject a 250 μL aliquot of this mixture.

HPLC VARIABLES
Guard column: 75 × 2.1 Corasil C18
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 250
Detector: UV 240

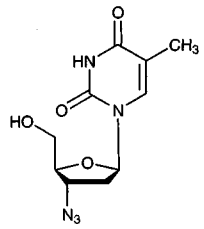
CHROMATOGRAM
Retention time: 6.5
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES
Simultaneous: 17α-methyltestosterone, 17β-trenbolone, trans-diethylstilbestrol, medroxyprogesterone, nandrolone

KEY WORDS
cow

REFERENCE
Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

Zidovudine



Molecular formula: $C_{10}H_{13}N_5O_4$
Molecular weight: 267.24
CAS Registry No.: 30516-87-1
Merck Index: 10252
Lednicer No.: 4 118

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 3 mL MeOH and 2 mL buffer, do not allow to go dry. 1 mL Plasma + 25 μL 40 μg/mL 7-ethyltheophylline in water, vortex, add to the SPE cartridge at ≤0.5 mL/min, wash with 1 mL buffer, air dry for 3 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeCN:5% acetic acid 10:90, vortex vigorously, centrifuge at 10000 g for 5 min, inject a 20 μL aliquot of the supernatant. (Prepare buffer by diluting 1.44 mL concentrated phosphoric acid to 1 L with water and adjusting the pH to 6.55 with concentrated ammonia.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. A was 1.44 mL concentrated phosphoric acid and 4 mL n-octylamine in 1 L water, pH adjusted to 6.55 with concentrated ammonia. B was MeCN. A:B from 95:5 to 70:30 over 7 min, to 20:80 over 1.5 min, return to initial conditions over 1 min, re-equilibrate for 2.5 min.
Flow rate: 1
Injection volume: 20
Detector: UV 266

CHROMATOGRAM

Retention time: 3.6
Internal standard: 7-ethyltheophylline (4.2)
Limit of detection: 7 ng/mL
Limit of quantitation: 22 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, caffeine

KEY WORDS

plasma; SPE

REFERENCE

Nadal,T.; Ortuño,J.; Pascual,J.A. Rapid and sensitive determination of zidovudine and zidovudine glucuronide in human plasma by ion-pair high-performance liquid chromatography, *J.Chromatogr.A*, **1996**, *721*, 127–137.

SAMPLE

Matrix: blood
Sample preparation: Filter (Millipore Ultrafree-MC, 10000 molecular mass limit) 250 μL serum while centrifuging at 17000 g for 1.5 h, inject a 50 μL aliquot of the clear ultrafiltrate.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: Isopropanol:20 mM pH 5 sodium citrate 2.5:97.5
Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 22.4

OTHER SUBSTANCES
Extracted: didanosine, zalcitabine

KEY WORDS
serum; ultrafiltrate

REFERENCE
Rosell-Rovira,M.L.; Pou-Clavé,L.; Lopez-Galera,R.; Pascual-Mostaza,C. Determination of free serum didanosine by ultrafiltration and high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 89–92.

SAMPLE
Matrix: intestinal mucosal homogenate
Sample preparation: Homogenate mixture + 100 μL 250 mM NaCN, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:100 mM potassium phosphate 25:75
Flow rate: 1
Detector: UV 254

KEY WORDS
rat

REFERENCE
Sinko,P.J.; Hu,P. Determining intestinal metabolism and permeability for several compounds in rats. Implications on regional bioavailability in humans, *Pharm.Res.*, **1996**, *13*, 108–113.

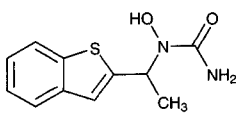
SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 20:80
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 4.18

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type Anti-HIV drugs. 2. The effect of vehicle and enhancer on skin permeation, *J.Pharm.Sci.*, **1996**, *85*, 214–219.

Zileuton



Molecular formula: $C_{11}H_{12}N_2O_2S$
Molecular weight: 236.29
CAS Registry No.: 111406-87-2
Merck Index: 10253

SAMPLE
Matrix: blood
Sample preparation: Condition a 300 μL 200 mg Analytichem Bond Elut C8 SPE cartridge with two 2 mL portions of MeOH and two 2 mL portions of water. 1 mL Plasma + 0.1-1 μg/mL IS in MeOH, mix, add to the SPE cartridge, wash with 1 mL water, dry for 3 min. Elute with two 1 ml portions of diethyl ether, evaporate under a gentle stream of air or nitrogen at 40°. Reconstitute the residue in 200 μL mobile phase or water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:MeOH:THF:water 15:5:10:70 (or 10:2:14:74 for rat blood) containing 40 mM NaH_2PO_4, 7.5 mM H_3PO_4, and 5 mM acetohydroxamic acid, pH 4.0
Flow rate: 1.0–1.5
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 15-19
Internal standard: A-66649 (Abbott Laboratories, USA) (19-25)
Limit of quantitation: 10-20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; human; monkey; rat; pharmacokinetics

REFERENCE
Granneman; G.R.; Breackman; R.A.; Erdman; K.A. Determination of a new 5-lipoxygenase inhibitor, zileuton, and its inactive *N*-dehydroxylated metabolite in plasma by high performance liquid chromatography, *Clin.Pharmacokinet.*, **1995**, *29*, 1–8.

SAMPLE
Matrix: blood
Sample preparation: Condition Bond Elut C8 cartridge with MeOH and water. Add 1 mL plasma or diluted plasma to the SPE cartridge, aspirate, wash with water, elute with ether, evaporate the eluate, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm AGP 100.4
Mobile phase: 50 mM Sodium perchlorate containing 17 mM acetic acid, adjusted to pH 2.0
Detector: F ex 260 em 321

CHROMATOGRAM
Internal standard: Abbott-66649 (Abbott Laboratories, USA)
Limit of quantitation: 10 ng/mL (R(+)), 10 ng/mL (S(-))

KEY WORDS
SPE; plasma; chiral; pharmacokinetics

REFERENCE
Wong; S.L.; Awni; W.M.; Cavanaugh; J.H.; El-Shourbagy; T.; Locke; Ch.S.; Dubé; L.M. The pharmacokinetics of zileuton 200 to 800 mg, its enantiomers, and its metabolites in normal healthy volunteers, *Clin.Pharmacokinet.*, **1995**, *29*, 9–21.

SAMPLE
Matrix: urine
Sample preparation: Directly inject a urine sample.

HPLC VARIABLES
Column: 250 × 4.6 5 μm IB-sil CN-bonded silica (Phenomenex)

Mobile phase: Isopropanol:25 mM sodium dodecyl sulfate containing 10 mM phosphate buffer 3:97, pH 3.0
Column temperature: 50
Flow rate: 1
Injection volume: 50
Detector: UV 262

CHROMATOGRAM
Retention time: 26.19
Limit of quantitation: 0.08-0.10 ppm

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Thomas,S.B.; Albazi,S.J. Simultaneous determination of the 5-lipoxygenase inhibitor "Zileuton" and its N-dehydroxylated metabolite in untreated rat urine by micellar liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 977–991.

Zinostatin

Molecular weight: ca. 16000
CAS Registry No.: 9014-02-2, 123760-07-6
Merck Index: 10302

SAMPLE
Matrix: bulk
Sample preparation: Inject 200 μL of a 660 μg/mL solution in 15 mM pH 5 sodium acetate buffer.

HPLC VARIABLES
Column: Mono Q HR 5/5 anion exchange (Pharmacia)
Mobile phase: Gradient. A was 20 mM pH 5 ammonium acetate. B was 20 mM pH 5 ammonium acetate containing 1 M NaCl. A:B 100:0 to 3:97 over 2.5 min, to 75:25 over 11 min, to 0:100 over 2 min.
Flow rate: 2
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
protect from daylight

REFERENCE
Denklau,D.; Kohnlein,W.; Luders,G.; Stellmach,J. Isolation and fast purification of neocarzinostatin by FPLC -ion exchange chromatography, *Z.Naturforsch.[C].*, **1983**, *38*, 939–942.

SAMPLE
Matrix: solutions

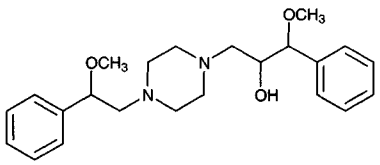
HPLC VARIABLES
Column: TSK G-3000SW (Toyo Soda)

Mobile phase: 10 mM pH 7.9 ammonium bicarbonate containing 30 mM NaCl
Flow rate: 1
Detector: UV 254, UV 280

REFERENCE

Maeda,H.; Ueda,M.; Morinaga,T.; Matsumoto,T. Conjugation of poly(styrene-co-maleic acid) derivatives to the antitumor protein neocarzinostatin: pronounced improvements in pharmacological properties, *J.Med.Chem.*, **1985**, *28*, 455–461.

Zipeprol



Molecular formula: $C_{23}H_{32}N_2O_3$
Molecular weight: 384.52
CAS Registry No.: 34758-83-3, 34758-84-4 (2.HCl)
Merck Index: 10303

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM

Retention time: 13.45

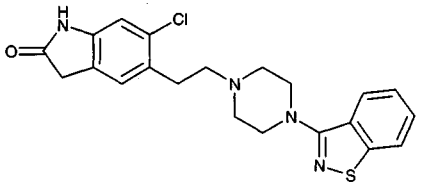
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ziprasidone



Molecular formula: $C_{21}H_{21}ClN_4OS$
Molecular weight: 412.94
CAS Registry No.: 146939-27-7, 138982-67-9 ($H_2O.HCl$)
Merck Index: 10304

SAMPLE

Matrix: blood, feces, urine
Sample preparation: Serum. 5 mL Serum + 10 mL MeCN, mix, centrifuge, wash the pellet with 2 mL MeCN, combine the supernatants, concentrate, reconstitute the residue with 200 μL mobile phase, inject an 80 μL aliquot. Urine. Condition a Sep-Pak C18 cartridge. 10 mL Urine + pH 5.0 acetate buffer, add to the SPE cartridge, wash with water, elute with MeOH, evaporate to dryness. Reconstitute the residue with 200 μL MeOH:ammonium acetate 20:80, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC basic
Mobile phase: Gradient. MeOH:20 mM pH 5.0 ammonium acetate 10:90 for 10 min, to 80:20 over 50 min, maintain at 80:20 for 7 min, return to initial conditions over 8 min, re-equilibrate for 10 min.
Injection volume: 80
Detector: UV; Radioactivity, β-RAM; MS, Perkin-Elmer Sciex API III, ion spray interface at 6000 V, collision gas argon, collision energy 25 eV

CHROMATOGRAM

Retention time: 48
Limit of quantitation: 0.5-50 ng/mL (serum)

OTHER SUBSTANCES

Extracted: metabolites

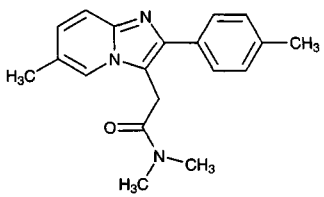
KEY WORDS

SPE; serum; pharmacokinetics; radiolabeled

REFERENCE

Prakash,C.; Kamel,A.; Gummerus,J.; Wilner,K. Metabolism and excretion of a new antipsychotic drug, ziprasidone, in humans, *Drug Metab.Dispos.*, **1997**, *25*, 863–872.

Zolpidem



Molecular formula: $C_{19}H_{21}N_3O$
Molecular weight: 307.40
CAS Registry No.: 82626-48-0, 99294-93-6 ((+)-tartrate (2:1))
Merck Index: 10321
Lednicer No.: 4 162

SAMPLE

Matrix: blood
Sample preparation: Mix 2 mL plasma, 2 mL pH 9.2 carbonate buffer, and 4 mL hexane:dichloromethane 4:3, centrifuge at 3000 rpm for 10 min. Remove the upper organic phase and evaporate it to dryness under a stream of air. Reconstitute the residue with mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 5 μm C18 Spherisorb ODS-2
Mobile phase: MeOH:THF:buffer 30:65:5 (Buffer was 10 mM potassium dihydrogen phosphate containing 0.1% triethylamine, adjusted to pH 2.6 with orthophosphoric acid.)
Flow rate: 0.8
Injection volume: 100
Detector: UV 305

CHROMATOGRAM

Retention time: 5.5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: zopiclone
Simultaneous: acepromazine, alprazolam, amisulpiride, amitriptyline, clobazam, clotiazepam, cyamemazine, desipramine, diazepam, flunitrazepam, haloperidol, imipramine, levomepromazine, lormetazepam, midazolam, nitrazepam, nortriptyline, prazepam
Interfering: bromazepam, lorazepam, niaprazine

KEY WORDS

plasma

REFERENCE

Stanke,F.; Jourdil,N.; Lauby,V.; Bessard,G. Zopiclone and zolpidem quantification in human plasma by high performance liquid chromatography with photodiode-array detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2623–2633.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM

Retention time: 11.882

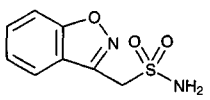
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Zonisamide



Molecular formula: $C_8H_8N_2O_3S$
Molecular weight: 212.23
CAS Registry No.: 68291-97-4
Merck Index: 10323
Lednicer No.: 4 130

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 250 μL 500 mM pH 4.5 NaH_2PO_4 + 50 μL 40 mg/mL IS in MeOH + 7 mL dichloroethane, vortex for 1 min, let stand for a few min. Remove the organic layer and filter (Whatman No. 1 paper) it, evaporate the filtrate to dryness under a stream of air in a warm water bath, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.4 30-38 μm CO:Pell ODS
Column: 250 × 4.4 Hypersil 5 MOS
Mobile phase: MeCN:buffer 58:100 (Buffer was 50 mL 1 M NaOH and 58 mL 1 M acetic acid made up to 1 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 4
Internal standard: 3-sulfamoylmethyl-6-fluoro-1,2-benzisoxazole (5)
Limit of quantitation: 900 ng/mL

OTHER SUBSTANCES

Simultaneous: carbamazepine, carbamazepine 10,11-epoxide, carbamazepine 10,11-diol
Noninterfering: acetaminophen, acetazolamide, N-acetylprocainamide, ampicillin, caffeine, cefuroxime, cimetidine, chloramphenicol, clobazam, desmethylclobazam, desmethylsuximide, ethosuximide, mefenamic acid, metronidazole, naproxen, paraxanthine, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, salicylic acid, sulfamethazine, sulfamethoxazole, sulthiame, theophylline, thiopental

KEY WORDS

plasma

REFERENCE

Berry,D.J. Determination of zonisamide (3-sulphamoylmethyl-1,2-benzisoxazole) in plasma at therapeutic concentrations by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 173–181.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 600 μL allobarbital in 75 mM pH 6.8 buffer, add 200 units β-glucuronidase (Type VII-A from E. coli), incubate at 37° for 30 min, add 1 mL of the sample to an Extrelut-1 SPE cartridge, after 10 min elute with 2.5 mL MTBE, dry the eluate under a stream of nitrogen, dissolve the residue in 50 μL MeOH:water 1:1, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 25 × 4 4 μm Superspher RP-18e (Merck)
Mobile phase: MeOH:11.2 mM β-cyclodextrin in 20 mM KH_2PO_4 5:95
Flow rate: 0.8
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 7
Internal standard: allobarbital (16)
Limit of detection: 2.1 ng/mL

OTHER SUBSTANCES
Simultaneous: phenobarbital, mephobarbital

KEY WORDS
serum; SPE

REFERENCE
Eto,S.; Noda,H.; Noda,A. Simultaneous determination of antiepileptic drugs and their metabolites, including chiral compounds, via β-cyclodextrin inclusion complexes by a column-switching chromatographic technique, *J.Chromatogr.B*, **1994**, *658*, 385–390.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut SPE cartridge (cat. no. 607101) with two 1 mL portions of MeOH and two 1 mL portions of water. Add 20 μL serum, 20 μL 100 μg/mL IS in MeOH:water 50:50, and 800 μL water to the SPE cartridge, wash with 1 mL water, elute with 250 μL MeOH, inject a 40 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 17:20:63
Flow rate: 1.4
Injection volume: 40
Detector: UV 246

CHROMATOGRAM
Retention time: 3.9
Internal standard: N,N-dimethylzonisamide (8.4)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: carbamazepine, carbamazepine epoxide, phenobarbital, phenytoin
Noninterfering: valproic acid

KEY WORDS
serum; SPE

REFERENCE
Furuno,K.; Oishi,R.; Gomita,Y.; Eto,K. Simple and sensitive assay of zonisamide in human serum by high-performance liquid chromatography using a solid-phase extraction technique, *J.Chromatogr.B*, **1994**, *656*, 456–459.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 1 mL MeCN, vortex for 30 s, centrifuge at 13400 g for 2 min, inject a 20 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 75 × 4.6 TSK gel ODS-80Tm (Tosoh)
Mobile phase: MeCN:5 mM pH 4.7 KH_2PO_4 31:69
Flow rate: 1
Injection volume: 20
Detector: UV 210

KEY WORDS
serum; comparison with capillary electrophoresis

REFERENCE

Makino,K.; Goto,Y.; Sueyasu,M.; Futagami,K.; Kataoka,Y.; Oishi,R. Micellar electrokinetic capillary chromatography for therapeutic drug monitoring of zonisamide, *J.Chromatogr.B*, **1997**, *695*, 417–425.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. 200 μL Serum, plasma, or whole blood + 300 μL water + 1 mL 100 mM pH 6.0 phosphate buffer + 50 μL 100 μg/mL dimethylzonisamide in chloroform:EtOH 10:1 + 6 mL chloroform:EtOH 10:1, shake for 10 min, centrifuge at 3500 rpm for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL EtOH, centrifuge at 10000 rpm for 1 min, inject a 20 μL aliquot. Tissue. Homogenize (glass homogenizer) rat brain with 100 mM pH 6.0 phosphate buffer. 500 μL Homogenate + 50 μL 100 μg/mL dimethylzonisamide in chloroform:EtOH 10:1 + 1 mL MeCN, let stand for 30 min at room temperature, centrifuge at 14000 rpm for 1 min. Remove the supernatant and evaporate it to 200 μL, centrifuge at 14000 rpm, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Develosil ODS-7 (Chemco, Osaka)
Mobile phase: MeCN:isopropanol:1% acetic acid 11:10:70
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 285

CHROMATOGRAM

Internal standard: dimethylzonisamide
Limit of detection: 50 ng/g (brain), 100 ng/mL (blood)

KEY WORDS

rat; serum; whole blood; brain; human; plasma; pharmacokinetics

REFERENCE

Nishiguchi,K.; Ohnishi,N.; Iwakawa,S.; Yagi,J.; Nakayama,S.; Takada,S.; Nakamura,H.; Yokoyama,T.; Okumura,K. Pharmacokinetics of zonisamide; saturable distribution into human and rat erythrocytes and into rat brain, *J.Pharmacobiodyn.*, **1992**, *15*, 409–415.

SAMPLE

Matrix: urine

Sample preparation: Inject a 100 μL aliquot of urine.

HPLC VARIABLES

Column: 250 × 10 10 μm Econosil C18
Mobile phase: Gradient. MeCN:MeOH:50 mM ammonium acetate from 1.6:2.4:96 to 28:42:30 over 30 min.
Flow rate: 4
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Retention time: 21

OTHER SUBSTANCES

Extracted: metabolites

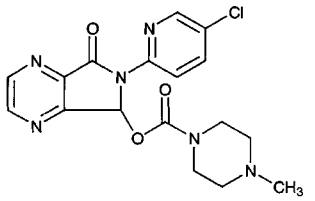
KEY WORDS

rat; preparative

REFERENCE

Stiff,D.D.; Zemaitis,M.A. Metabolism of the anticonvulsant agent zonisamide in the rat, *Drug Metab.Dispos.*, **1990**, *18*, 888–894.

Zopiclone



Molecular formula: $C_{17}H_{17}ClN_6O_3$
Molecular weight: 388.81
CAS Registry No.: 43200-80-2
Merck Index: 10324

SAMPLE

Matrix: blood
Sample preparation: Condition a 500 mg Bond Elut LRC C2 SPE cartridge with 2 mL MeOH, 2 mL MeCN:water:trifluoroacetic acid 80:20:0.1, and 5 mL Tris buffer. Mix 100 µL 100 ng/mL IS with 2 mL 50 mM Tris buffer adjusted to pH 7.5 with concentrated HCl and 500 µL plasma. Add sample to the SPE cartridge and draw through by vacuum at 2 mL/min. Wash with 20 mL Tris buffer, dry with vacuum for 1 min. Elute with 2 mL MeCN:water:trifluoroacetic acid 80:20:0.1, evaporate the eluate to dryness under a stream of nitrogen at 40°. Reconstitute the residue in 1 mL MeOH, vortex for 40 s, centrifuge at 3000 rpm for 15 min, decant the supernatant, evaporate to dryness. Reconstitute the residue in 200 µL mobile phase, vortex for 20 s. Inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 4 µm Nova-Pak phenyl Guard-Pak
Column: 300 × 4.6 5 µm C18 Spherisorb ODS-2
Mobile phase: MeOH:THF:buffer 30:65:5 (Buffer was 10 mM potassium dihydrogen phosphate containing 0.1% triethylamine, adjusted to pH 2.6 with orthophosphoric acid.)
Flow rate: 0.8
Injection volume: 100
Detector: UV 305

CHROMATOGRAM

Retention time: 4.5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: zolpidem
Simultaneous: acepromazine, alprazolam, amisulpride, amitriptyline, clobazam, clotiazepam, cyamemazine, desipramine, diazepam, flunitrazepam, haloperidol, imipramine, levomepromazine, lormetazepam, midazolam, nitrazepam, nortriptyline, prazepam
Interfering: bromazepam, lorazepam, niaprazine

KEY WORDS

plasma; SPE

REFERENCE

Stanke,F.; Jourdil,N.; Lauby,V.; Bessard,G. Zopiclone and zolpidem quantification in human plasma by high performance liquid chromatography with photodiode-array detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2623–2633.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL 2 µg/mL IS in MeCN + 1 mL 10 mM pH 8 sodium phosphate buffer + 10 mL dichloromethane:isopropanol 90:10, shake gently for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 µL mobile phase, inject a 100 µL aliquot on to a 250 × 4.6 5 µm Nucleosil silica column and elute with MeCN:MeOH 95:5 at 1 mL min, collect the fraction containing zopiclone (about 7.5 min), evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: cellulose carbamate (Société Française Chromato Colonne)

Column: 250 × 4.6 cellulose carbamate (Société Francaise Chromato Colonne)
Mobile phase: Hexane:EtOH 40:60
Column temperature: 35
Flow rate: 1
Injection volume: 100
Detector: F ex 300 em 470

CHROMATOGRAM
Retention time: 8 (-), 10 (+)
Internal standard: 6-(7-chloro-2-quinolyl)-5-hydroxy-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-7-one 4-methyl-1-piperazine carboxylate (5.5 (on achiral system)

KEY WORDS
plasma; normal phase; pharmacokinetics; chiral

REFERENCE
Fernandez,C.; Baune,B.; Gimenez,F.; Thuillier,A.; Farinotti,R. Determination of zopiclone enantiomers in plasma by liquid chromatography using a chiral cellulose carbamate column, *J.Chromatogr.*, **1991**, *572*, 195–202.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 10 μL 8.5 μg/mL IS in MeCN, let stand for 5 min, add 300 μL saturated borate buffer (pH 9.2), add 3 mL n-butyl chloride, vortex for 2 min, centrifuge at 2140 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL MeCN, inject a 1-30 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Spheri 5 C18
Mobile phase: MeCN:buffer 60:40 (Buffer was 1.15 g $(NH_4)H_4PO_4$ and 1 mL triethylamine in 1 L water, pH 6.8.)
Flow rate: 1
Injection volume: 10-30
Detector: UV 305

CHROMATOGRAM
Retention time: 4
Internal standard: 6-(6-chloro-2-quinolyl)-5-hydroxy-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-7-one 4-methyl-1-piperazine carboxylate (29481 R.P.) (6)
Limit of quantitation: 4 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
whole blood

REFERENCE
Boniface,P.J.; Martin,I.C.; Nolan,S.L.; Tan,S.T. Development of a method for the determination of zopiclone in whole blood, *J.Chromatogr.*, **1992**, *584*, 199–206.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma + 250 ng hydroquinidine + 1 mL pH 8 phosphate buffer + 6 mL dichloromethane, mix, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS C18

Mobile phase: MeCN:MeOH:THF:buffer 15:5:2:78 (Buffer was 10 mM trimethylamine adjusted to pH 2.5 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 305

CHROMATOGRAM
Retention time: 4.6
Internal standard: hydroquinidine (3.3)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: quinidine, quinine
Noninterfering: benzodiazepines, phenothiazines, tricyclic antidepressants, zolpidem

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Royer-Morrot,M.J.; Rambourg,M.; Jacob,I.; Bauer,P.; Royer,R.J. Determination of zopiclone in plasma using column liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1992**, *581*, 297–299.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently on a horizontal agitator for 10 min, centrifuge at 2800 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot. (Buffer was saturated ammonium chloride, diluted 25% with water, adjusted to pH 9.5 with 25% diluted ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm Nova-Pak C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 10 mM KH_2PO_4 adjusted to pH 2.6 with orthophosphoric acid. At the end of the day wash column with water at 0.8 mL/min for 1 h and MeOH at 0.8 mL/min for 1 h.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 200
Detector: UV 305

CHROMATOGRAM
Retention time: 4.05
Limit of detection: 24.8 ng/mL

OTHER SUBSTANCES
Extracted: alpidem, zolpidem, suriclone
Simultaneous: p-nitrophenol, ketotifen, tiaprofenic acid, vincristine, sultopride, pyrimethamine, nimodipine

KEY WORDS
plasma

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. High-performance liquid chromatographic assay with diode-array detection for toxicological screening of zopiclone, zolpidem, suriclone and alpidem in human plasma, *J.Chromatogr.*, **1993**, *616*, 95–103.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 100 µg/mL chlordiazepoxide hydrochloride in water + 100 µL 70 mM pH 8 phosphate buffer + 5 mL MTBE:isooctane 75:25, vortex for 30 s, cen-

trifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 75 μL hexane:EtOH 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Chiralcel OD-H
Mobile phase: Hexane:EtOH 40:60
Flow rate: 0.6
Injection volume: 50
Detector: F ex 300 em 470

CHROMATOGRAM
Retention time: 19 (-), 28 (+)
Internal standard: chlordiazepoxide (9.5)
Limit of detection: 1 ng/mL
Limit of quantitation: 2.5 ng/mL

KEY WORDS
plasma; chiral; pharmacokinetics

REFERENCE
Foster,R.T.; Caillé,G.; Ngoc,A.H.; Lemko,C.H.; Kherani,R.; Pasutto,F.M. Stereospecific high-performance liquid chromatographic assay of zopiclone in human plasma, *J.Chromatogr.B*, **1994**, *658*, 161–166.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 305

CHROMATOGRAM
Retention time: 4.05
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; almino-

profen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents

Sample preparation: 1 mL Whole blood or gastric contents + 50 µL 400 µg/mL IS in MeOH + 1 mL EtOH + 5 drops 1 M pH 9 potassium carbonate + 2 mL water + 8 mL n-hexane:MTBE 25:75, rotate for 15 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Hypersil BDS C18

Mobile phase: Gradient. A was MeCN:MeOH:1.5 M ammonium acetate:water 10:10:3:77. B was MeCN:MeOH:1.5 M ammonium acetate:water 40:40:3:17. A:B from 95:5 to 50:50 over 20 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 15.6

Internal standard: N-allylnormetazocine (Sigma) (9)

OTHER SUBSTANCES

Extracted: pentazocine

KEY WORDS

whole blood

REFERENCE

Van Bocxlaer,J.; Meyer,E.; Clauwaert,K.; Lambert,W.; Piette,M.; De Leenheer,A. Analysis of zopiclone (Imovane) in postmortem specimens by GC-MS and HPLC with diode-array detection, *J.Anal.Toxicol.*, **1996**, *20*, 52–54.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. 1 mL Whole blood + 10 µL 8.5 µg/mL IS in MeCN + 300 µL saturated pH 9 borate buffer + 3 mL n-butyl chloride, vortex for 2 min, centrifuge, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL MeCN, inject a 10-30 µL aliquot. Tissue. Stir

10 g homogenized liver tissue, 40 mL Tris buffer, and 10 mg Subtilisin-Carlsberg protease at room temperature overnight, filter through glass wool. 1 mL Homogenate + 10 μL 8.5 μg/mL IS in MeCN + 300 μL saturated pH 9 borate buffer + 3 mL n-butyl chloride, vortex for 2 min, centrifuge, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL hexane, extract with two 1 mL portions of MeCN. Combine the MeCN layers and evaporate them to dryness, reconstitute the residue in 100 μL MeCN, inject a 10-30 μL aliquot. (Prepare Tris buffer by dissolving 121 g Tris in 1 L water, adjust pH to 7.0 with concentrated sulfuric acid.)

HPLC VARIABLES

Column: 220 × 4.6 5 μm Spheri 5 silica
Mobile phase: MeCN:buffer 60:40 (Prepare buffer by dissolving 1.15 g $(NH_4)H_2PO_4$ in 1 L water and adding 1 mL triethylamine, pH 6.8.)
Flow rate: 1
Injection volume: 10-30
Detector: UV 305

CHROMATOGRAM

Internal standard: 6-(6-chloro-2-quinolyl)-7-[(4-methyl-1-piperazinyl)carbonyloxy]-6,7-dihydro [5H]pyrrolo[3,4-b]pyrazine-5-one
Limit of detection: 50 ng/g

KEY WORDS

whole blood; liver

REFERENCE

Boniface,P.J.; Russell,S.G.G. Two cases of fatal zopiclone overdose, *J.Anal.Toxicol.*, **1996**, *20*, 131–133.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with one column volume of 1 M HCl, with 2 column volumes of MeOH, and with 1 column volume of water. Hemolyze 1 mL blood with 500 μL water, centrifuge. Add 250 μL 250 ng/mL harmane hydrochloride in 200 mM NaH_2PO_4 and 1 mL serum or hemolysate or 500 μL urine to the SPE cartridge at 1 mL/min, wash with 2 column volumes of water, wash with two 500 μL aliquots of MeCN (drain completely after each wash), elute with 250 μL MeOH:35% perchloric acid 99:1 (remove the final portion of eluate by centrifuging for 20 s), inject a 10 (urine) or 25 (serum) μL aliquot of the eluate.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:0.1% tetramethylammonium perchlorate 17:83, adjusted to pH 3.8 with 10% perchloric acid
Flow rate: 1.8
Injection volume: 10-25
Detector: F ex 320 em 520, UV 305

CHROMATOGRAM

Retention time: 12.2
Internal standard: harmane (8.3)
Limit of quantitation: 2 ng/mL (serum, F), 10 ng/mL (urine, F)

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: acetaminophen, atenolol, barbiturates, benzodiazepines, chlordiazepoxide, fluoxetine, imipramine, metoprolol, nadolol, paroxetine, salicylic acid

KEY WORDS

serum; SPE

REFERENCE

Gupta,R.N. Simultaneous determination of zopiclone and its two major metabolites (N-oxide and N-desmethyl) in human biological fluids by column liquid chromatography after solid-phase extraction, *J.Liq. Chromatogr.Rel.Technol.*, **1996**, *19*, 699–709.

SAMPLE

Matrix: formulations
Sample preparation: Grind tablets to a fine powder, weigh out amount equivalent to 5 mg zopiclone, add 40 mL 100 mM HCl, sonicate for 15 min, cool to room temperature, make up to 50 mL with 100 mM HCl, filter (1.6 μm glass fiber, Whatman GF/A), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher-60 RP Select B
Mobile phase: MeCN:THF:buffer 18:1:81 (Buffer was 3.4 g monosodium hexane sulfonate and 7.0 g $NaH_2PO_4.2H_2O$ in 1 L water, pH 4.55.)
Column temperature: 25
Flow rate: 1.5
Injection volume: 20
Detector: UV 303

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 0.05% (of zopiclone)

OTHER SUBSTANCES

Simultaneous: degradation products, impurities
Noninterfering: excipients

KEY WORDS

protect from light; tablets; rugged; stability-indicating

REFERENCE

Bounine,J.P.; Tardif,B.; Beltran,P.; Mazzo,D.J. High-performance liquid chromatographic stability-indicating determination of zopiclone in tablets, *J.Chromatogr.A*, **1994**, *677*, 87–93.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 212, 300

CHROMATOGRAM

Retention time: 1.4
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 7.50 (A), 3.79 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 5-fold with 50 mM pH 8.4 phosphate buffer. 1 mL Diluted urine + 100 μL 20 μg/mL suriclone in EtOH + 10 mL dichloromethane:isopropanol 95:5, shake gently for 5 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 μL EtOH, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 11.5 min elute the fraction containing zopiclone on to column B, after 1.5 min backflush the contents of column

B on to column C with mobile phase B, elute with mobile phase B, monitor the effluent from column C. (Chiral separation of metabolites can also be achieved by collecting the fractions containing the metabolites (20.5 to 25.5 min and 46.5 to 55.5 min) on column B then chromatographing them on column C.)

HPLC VARIABLES

Column: A 150 × 4.6 5 μm Nucleosil cyanopropyl; B 60 × 4 5 μm Kromasil silica (Informatiques & Technologies); C cellulose carbamate guard column + 250 × 4.6 5 μm cellulose carbamate (Société Française Chromato Colonne)
Mobile phase: A Hexane:EtOH:MeOH 80:5:15 containing 0.18% MeOH:diethylamine 99.9:0.1 and 0.05% water; B Hexane:EtOH:MeOH:diethylamine 55:30:15:1
Column temperature: 35 (column C only)
Flow rate: A 0.7; B 1.2
Injection volume: 100
Detector: F ex 305 em 470

CHROMATOGRAM

Retention time: 20 (-), 25 (+)
Internal standard: suriclone (18 (on column A only))
Limit of quantitation: 10 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

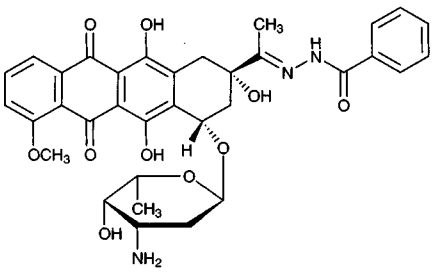
chiral; column-switching; pharmacokinetics; heart-cut

REFERENCE

Fernandez,C.; Gimenez,F.; Baune,B.; Maradeix,V.; Thuillier,A.; Farinotti,R. Determination of the enantiomers of zopiclone and its two chiral metabolites in urine using an automated coupled achiral-chiral chromatographic system, *J.Chromatogr.*, **1993**, *617*, 271–278.

Zorubicin

Molecular formula: $C_{34}H_{35}N_3O_{10}$
Molecular weight: 645.67
CAS Registry No.: 54083-22-6, 36508-71-1 (HCl)
Merck Index: 10326



SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8

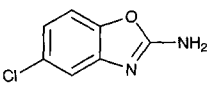
Injection volume: 50
Detector: UV 234

CHROMATOGRAM
Retention time: 8.10
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Zoxazolamine



Molecular formula: $C_7H_5ClN_2O$
Molecular weight: 168.58
CAS Registry No.: 61-80-3
Merck Index: 10328

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

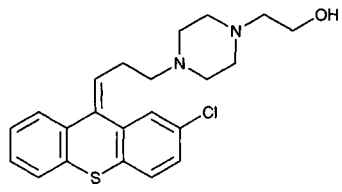
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Zuclopenthixol



Molecular formula: $C_{22}H_{25}ClN_2OS$
Molecular weight: 400.97
CAS Registry No.: 53772-83-1
Merck Index: 2455

SAMPLE

Matrix: blood
Sample preparation: 2 mL Serum + 25 ng IS + 300 μL EtOH + 100 μL 7 M NaOH + 8 mL hexane:isopropylamine 99.9:0.1, shake for 15 min, centrifuge at 2400 g for 5 min. Remove the organic layer and add it to 2 mL 100 mM HCl, shake for 15 min, centrifuge for 5 min. Discard the organic layer and add 200 μL 7 M NaOH and 4 mL hexane:isopropylamine 99.9:0.1 to the aqueous layer, shake for 15 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 1 mL hexane, evaporate, reconstitute with 100 μL hexane:isopropylamine 99.9:0.1, inject a 70 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb S5W silica
Mobile phase: n-Heptane:isopropanol:concentrated ammonia:water 85:15:0.4:0.2
Flow rate: 1
Injection volume: 70
Detector: UV 254

CHROMATOGRAM

Retention time: 7.5
Internal standard: 4-[3-(2-chloro-7-trifluoromethylthioxanthen-9-yl)propyl]-1-piperazineethanol (Lu 9-215) (6.5)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Extracted: trans(E)-isomer, metabolites
Simultaneous: estazolam
Noninterfering: amitriptyline, biperiden, imipramine, nortriptyline, orphenadrine, procyclidine
Interfering: benzodiazepines

KEY WORDS

serum; rat; dog; human; pharmacokinetics; normal phase

REFERENCE

Aaes-Jorgensen,T. Specific high-performance liquid chromatographic method for estimation of *cis(Z)* and *trans(E)*-isomers of clopenthixol and a N-dealkyl metabolite, *J.Chromatogr.*, **1980**, *183*, 239–245.

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Whole blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry under suction, wash with 2 mL acetone:chloroform 50:50, elute with 3 mL freshly prepared ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm Asahipak ODP-50
Mobile phase: MeCN:50 mM ammonium acetate 85:15
Flow rate: 0.6
Injection volume: 10

Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, Finnigan MAT TSP-2 interface, collision gas argon 3.0 mTorr, collision offset -17.5 V, repeller 70 V, vaporizer 130-5°, source 200°, filament off, multiplier 1500 V, dynode power 15 kV, scantime 1.20 s, MSMSC factor 0, monitor 316–271. (The effluent from the column was mixed with 50 mM ammonium acetate pumped at 0.6 mL/min. The mixture flowed to the detector.)

CHROMATOGRAM
Retention time: 3.40
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: chlorprothixene, flupenthixol, thiothixene

KEY WORDS
whole blood; SPE

REFERENCE
Verweij,A.M.A.; Hordijk,M.L.; Lipman,P.J.L. Quantitative liquid chromatography, thermospray/tandem mass spectrometric (LC/TSP/MS/MS) analysis of some tranquilizers of the thioxanthene group in whole-blood, *J.Liq.Chromatogr.*, **1994**, *17*, 4009–4110.

SAMPLE
Matrix: blood, gastric contents, tissue, urine, vitreous humor
Sample preparation: Homogenize tissue with 4 volumes water. Extract 3 mL Blood, gastric contents, urine, vitreous humor, or homogenized tissue with 1.5 mL saturated pH 9.5 ammonium chloride buffer and 5 mL chloroform:2-propanol:n-heptane 25:10:65 for 10 min. (Caution! Chloroform is a carcinogen!). Centrifuge at 3500 g for 10 min, evaporate the organic layer at 45°. Reconstitute with 30μL MeOH. Centrifuge at 10000 g for 5 min, remove 20 μL of the supernatant, inject an aliquot.

HPLC VARIABLES
Column: 150 × 2 4 μm NovaPak C18
Mobile phase: MeOH:2mM pH 3 ammonium acetate buffer 90:10
Flow rate: 0.2
Injection volume: 2
Detector: MS, PE Sciex API 100 double quadrupole, nebulizing gas nitrogen at 1.6 mL/min, curtain gas nitrogen at 1.08 mL/min, sprayer electrode +4.5 kV, electron multiplier +2.4 kV, m/z 100-450

CHROMATOGRAM
Retention time: 14.95 ((Z)-cis), ((E)-trans)

KEY WORDS
liver; kidney; lung; brain; skeletal muscle; comparison with HPLC/UV

REFERENCE
Tracqui,A.; Kintz,P.; Cirimele,V.; Berthault,F.; Mangin,P.; Ludes,B. HPLC-DAD and HPLC-MS findings in a fatality involving (Z)-cis-clopenthixol (zuclopenthixol), *J.Anal.Toxicol.*, **1997**, *21*, 314–318.

SAMPLE
Matrix: blood, gastric contents, tissue, urine, vitreous humor
Sample preparation: Homogenize tissue with 4 volumes water. 3 mL Blood, gastric contents, urine, vitreous humor, or homogenized tissue + 4 μg IS, extract with 1.5 mL saturated pH 9.5 ammonium chloride buffer and 5 mL chloroform:2-propanol:n-heptane 25:10:65 for 10 min. (Caution! Chloroform is a carcinogen!). Centrifuge at 3500 g for 10 min, evaporate the organic layer at 45°. Reconstitute with 30μL MeOH. Centrifuge at 10000 g for 5 min, remove 20 μL of the supernatant, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:THF:10 mM pH 2.6 KH_2PO_4 buffer 65:5:30

Flow rate: 0.8
Injection volume: 7
Detector: UV 228

CHROMATOGRAM
Retention time: 8.98 ((Z)-cis), 10.21 ((E)-trans)
Internal standard: prochlorperazine
Limit of detection: 7 ng/mL

KEY WORDS
liver; kidney; lung; brain; skeletal muscle; comparison with HPLC/MS

REFERENCE
Tracqui,A.; Kintz,P.; Cirimele,V.; Berthault,F.; Mangin,P.; Ludes,B. HPLC-DAD and HPLC-MS findings in a fatality involving (Z)-cis-clopenthixol (zuclopenthixol), *J.Anal.Toxicol.*, **1997**, *21*, 314–318.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 1 mL Bond Elut CN with 2 mL MeCN and 2 mL water. Add 2 mL plasma or urine to the SPE cartridge, wash with 2 mL water, elute with MeCN:n-butylamine 90:10. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb S5 CN
Mobile phase: MeCN:200 mM pH 6.5 potassium phosphate buffer:water 36:5:59 containing 6 mM dodecyl-N,N,N-trimethylammonium bromide
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: F ex 260 em 435 following post-column photolysis with a low-pressure 8 W mercury UV light in a 5 m × 0.5 mm i.d.coil of PTFE tubing

CHROMATOGRAM
Retention time: 9
Limit of detection: 0.05 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
protect from light; plasma; SPE; post-column photochemical derivatization

REFERENCE
Hansen,B.B.; Hansen,S.H. Determination of zuclopenthixol and its main N-dealkylated metabolite in biological fluids using high-performance liquid chromatography with post-column photochemical derivatization and fluorescence detection, *J.Chromatogr.B*, **1994**, *658*, 319–325.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 16.325

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Inject 1 mL onto column A. Elute column A onto column B with mobile phase for 30 s then remove it from the circuit. Elute column B with mobile phase and monitor the effluent.

HPLC VARIABLES
Column: A 10 × 6 packed with 40 μm material from a Bond Elut cartridge (cat. no. 620303); B 100 × 4 3 μm Spherisorb ODS Superpac
Mobile phase: MeCN:85% phosphoric acid:triethylamine:water 49.55:0.225:0.225:50
Flow rate: 0.65
Injection volume: 1000
Detector: UV 238

CHROMATOGRAM
Retention time: 3.30

OTHER SUBSTANCES
Simultaneous: alprazolam, amitriptyline, chlorpromazine, chlorprothixene, clomipramine, desclomipramine, desmethylimipramine, diazepam, flunitrazepam, imipramine, levomepromazine, maprotiline, nortriptyline, promethazine, thioridazine, thioridazine sulfone, thioridazine sulfoxide, trimipramine, zimeldine
Noninterfering: carbamazepine, clonazepam, lorazepam, nitrazepam, oxazepam, phenytoin
Interfering: fluphenazine, haloperidol, perphenazine, protriptyline

KEY WORDS
column-switching

REFERENCE
Svensson,C.; Nyberg,G.; Mårtensson,E. High-performance liquid chromatographic quantitation of amitriptyline and nortriptyline in dialysate from plasma or serum using on-line solid-phase extraction, *J.Chromatogr.*, **1988**, *432*, 363–369.