FIFTH EDITION

FOOD CHEMICALS CODEX

Effective January 1, 2004

COMMITTEE ON FOOD CHEMICALS CODEX

Food and Nutrition Board

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Willing is not enough; we must do."
—Goethe

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Preface

The Fifth Edition of the *Food Chemicals Codex* (FCC) is a result of the collective efforts of the many members, past and present, of the Committee on Food Chemicals Codex over the past 42 years. The current committee, whose members have brought all these efforts to fruition with this edition, was appointed following a request from the U.S. Food and Drug Administration (FDA) to continue this activity. The charge to the committee states that ''the committee shall (1) provide information on matters related to the purity of food ingredients used in the United States and shall be knowledgeable of the purity of food ingredients used in other countries; (2) provide information on food-grade specifications for food additives, GRAS [generally recognized as safe] substances, and any other food substances used as ingredients; and (3) publish specification monographs in a Fifth Edition of the *Food Chemicals Codex*. To provide such information, the committee shall review proposals from industry, government, and any other source.''

The FCC project, currently under the Food and Nutrition Board of the Institute of Medicine of the National Academies, began in 1961, soon after the passage of the 1958 Food Additives Amendment to the federal Food, Drug, and Cosmetic Act. Although the FDA had, by regulations and informal statements, defined in general terms the quality requirements for GRAS and other food chemicals, these requirements were not sufficiently specific to serve as release, procurement, and acceptance specifications for manufacturers and users of food chemicals. Therefore, regulators and other interested parties believed that the publication of a book of standards designed especially for food chemicals would promote uniformity of quality and added assurance of safety for such chemicals. For these reasons, the Food Protection Committee of the National Academy of Sciences/National Research Council received requests in 1958 from its Industry Liaison Panel and other sources to undertake a project to produce a *Food Chemicals Codex* comparable in many respects to the *United States Pharmacopeia* and the *National Formulary* for drugs. As a result of these requests, representatives of industry and government agencies agreed that there was a definite need for such a *Codex* and that the Food Protection Committee was a suitable body to undertake the project.

The first edition, published in 1966, was supported by a Public Health Service grant and more than 100 supplementary grants from industry, associations, and foundations. Its role, which is still that of the *Food Chemicals Codex*, was to define the quality of food-grade chemicals in terms of identity, strength, and purity based on the elements of safety and good manufacturing practice. Later editions were supported by direct contracts with the FDA. Such sponsorship has been sufficient to support the publication of 4 earlier editions and 14 supplements in a 42-year span.

SCOPE

The scope of the *Food Chemicals Codex* has expanded with each new edition. Substances included in the first edition were limited to chemicals added directly to foods to achieve a desired function. Succeeding editions included these substances as well as such processing aids as enzymes, extraction solvents, filter media, and boiler water additives; those that are regarded as foods, such as fructose and dextrose, rather than as additives; and those that exhibit a functional effect, not on the foods to which they are added, but to the human body when the food is consumed. This Fifth Edition includes 961 monographs from the Fourth Edition; 49 monographs, along with those for 15 flavor chemicals, added in the three supplements to the Fourth Edition; and 19 new monographs, along with 33 for flavor chemicals, new to this Fifth Edition, bringing the total to 1077. Because of its regulatory status in countries other than the United States, and its worldwide use, the *Food Chemicals Codex* contains some monographs for chemicals not currently allowed in foods in the United States. This circumstance is clearly indicated in such monographs.

UPDATING AND DEVELOPING SPECIFICATIONS

The committee has invariably sought to define, using physicochemical and microbiological parameters, ingredients prepared under good manufacturing practices as safe for human consumption. Special emphasis has been placed on reducing contaminants, including trace elements, particularly lead. The committee removed Arsenic and Microbiological Criteria specifications from monographs that were unnecessarily burdened with them. More importantly, the committee revised the Lead and Heavy Metals Limits Policy by removing the Heavy Metals (as Pb) specifications and replacing them with specifications for relevant heavy metals. The committee also decided, based on research of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, the National Academies, that the intake of fluoride as a constituent of substances described in FCC monographs is not expected to significantly add to the human daily fluoride intake. However, because high levels of fluoride have been amply demonstrated to cause toxicological problems, as described in the report, the maintenance of fluoride limits in selected food additives appears consistent with sound public health policy. Because of the difficulties in analyzing for fluoride in food chemicals, the committee has adopted a new analytical method for fluoride and will continue to add more when adequate validation of new methods is submitted.

Limits on contaminants, specifically lead and other heavy metals, have been reduced in most monographs in this edition. This trend is expected to continue. Manufacturers and suppliers of food ingredients are encouraged to inform the committee of their ability to supply food ingredients with lead and other heavy metals limits lower than those specified in this edition. The arsenic specification remains in relatively few monographs in this edition where (1) the ingredient or additive is a high-volume consumption item (greater than 25 million pounds a year), (2) the ingredient or additive is derived from a natural (mineral) source where arsenic may be an intrinsic contaminant, or (3) there is reason to believe that arsenic constitutes a significant part of the total heavy metals content.

The committee is cognizant of the need for international harmonization of specifications in today's world. Efforts were made, where feasible, to harmonize the specifications in this edition with those of other standards-setting organizations, in particular with those in the *Compendium of Food Additive Specifications*, prepared by the Food and Agricultural Organization of the United Nations (FAO)/World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) and published by the FAO.

FORMAT

Generally the presentation follows that of the Fourth Edition, but a number of significant changes and additions have been made. As expected, the passage of 7 years since the appearance of the Fourth Edition has been accompanied by changes:

- Additional information in terms of FEMA (Flavor and Extract Manufacturers Association) numbers has been added to essential oil and other flavor monographs not in the Flavors Table, Chapter 3.
- Infrared Spectra for most substances requiring them for identification purposes have been rerun and thus are more accurate.
- New headers on each page of this book tell readers where they are by chapter, monograph, appendix, or test.
- The language in the monograph section has been revised to be more clear, consistent, and concise.
- All tests that occurred identically in three or more monographs were moved to the appendices.

FUTURE REVISIONS

The introduction of new food additives as well as constant changes and advances in manufacturing processes and analytical sciences lead to a need for continued revision of this compendium.

The committee recognizes the need to initiate an extensive update of the analytical methods described in this edition, in such a way that advanced new technologies are incorporated in the Sixth Edition, while maintaining a balance with other, less technology-intensive methods for use by laboratories and firms that may not have

access to such advanced technology. The committee specially recognizes the urgency of updating current chromatographic methods throughout the present edition and intends to complete this goal during the next 5 years. Users of this edition are requested and encouraged to submit suggestions for updating the specifications as well as the general analytical methods. Constructive criticism and notification of errors should also be brought to the attention of the Food Chemicals Codex, Institute of Medicine, 500 Fifth Street, N.W., Washington, D.C. 20001 or <fcc@nas.edu>.

LEGAL STATUS

The *Food Chemicals Codex* has earned international recognition by manufacturers, vendors, and users of food chemicals. The specifications herein serve as the basis for many buyer and seller contractual agreements.

In the United States, the first edition was given quasi-legal recognition in July 1966 by means of a letter of endorsement from FDA Commissioner James L. Goddard, which was reprinted in the book. The letter stated that ''the FDA will regard the specifications in the *Food Chemicals Codex* as defining an 'appropriate food grade' within the meaning of Sec. 121.101(b)(3) and Sec. 121.1000(a)(2) of the food additive regulations, subject to the following qualification: this endorsement is not construed to exempt any food chemical appearing in the *Food Chemicals Codex* from compliance with requirements of Acts of Congress or with regulations and rulings issued by the Food and Drug Administration under authority of such Acts.''

Subsequently, the specifications in the Second Edition, followed by those in the Third Edition, were cited, by reference, in the U.S. *Code of Federal Regulations* to define specific safe ingredients under title 21, in various parts of sections 172, 173, and 184.

In Canada, the current edition of the *Food Chemicals Codex*, including its supplements, is officially recognized in the *Canadian Food and Drug Regulations* under Section B.01.045(b) as the reference for specifications for food additives. The new Australia New Zealand Food Authority recognizes the *Food Chemicals Codex* as a primary source of identity and purity specifications in its Food Standards Code, Chapter 1 General Food Standards, Part 1.3 Substances Added to Food, Standard 1.3.4 Identity and Purity.

REVIEWERS

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Barbara P. Klein, University of Illinois. Appointed by the National Research Council and Institute of Medicine, she was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

ACKNOWLEDGMENTS

A compendium of this breadth can only result from the cooperation of many individuals and organizations. Underlying this, the support provided by FDA contract number 223-99-2321, monitored by project officers Paul M. Kuznesof and Daniel Folmer, is gratefully acknowledged.

Several monographs and various sections in this edition have portions based on other publications, and are used with permission granted by the parent organizations: the American Chemical Society; the American Oil Chemists Society; the American Society for Testing and Materials; AOAC International; and the United States Pharmacopeial Convention, Inc. This edition of the *Food Chemicals Codex* directly references the procedures in the Eighth Edition of the *FDA Bacteriological Analytical Manual*

(BAM) for its microbial limit tests. Where the sample size is not defined in the limit, the results are based on the sampling procedures described in BAM.

While participating individuals have been listed on pages vii–ix, the following organizations have also been active participants:

American Dairy Products Institute
Corn Refiners Association
Enzyme Technical Association
Flavor and Extract Manufacturers Association
Gelatin Manufacturers of Europe
Gelatin Manufacturers Institute of America
International Association of Color Manufacturers
International Dairy Federation
International Food Additives Council
International Pectin Producers Association
International Pharmaceutical Excipients Council
International Technical Caramel Association
National Association of Chewing Gum Manufacturers
Salt Institute
Soy Protein Council

Members of the National Academies Press—Sally S. Stanfield, James M. Gormley, Estelle H. Miller, Dan Parham, and William B. Mason—and staff of the Institute of Medicine Office of Reports and Communication—Jennifer Bitticks, Jennifer Otten, Bronwyn Schrecker, and Leah Covington—provided valuable support to the FCC staff toward the publication of this edition.

Success in the complex task of completing the Fifth Edition is due to the dedication and determination of the members of the Committee on Food Chemicals Codex under the focused leadership of its successive chairs, Steve L. Taylor and S. Suzanne Nielsen, during the past 58 months, and to those of the Food Chemicals Codex staff, Maria Oria and Marcia Lewis.

Washington, D.C.
September 2003

Ricardo A. Molins
Study Director

General Information

OPERATING PROCEDURES OF THE *FOOD CHEMICALS CODEX*

Organization

The Food Chemicals Codex (FCC) project is an activity of the Food and Nutrition Board, a unit of the Institute of Medicine of the National Academies. The immediate responsibility for developing the *Food Chemicals Codex* lies with the Board's Committee on Food Chemicals Codex. The committee consists of 12 to 15 members, chosen for their expertise in the various aspects of the committee's work, who are appointed, upon recommendation of the Food and Nutrition Board and the President of the Institute of Medicine, by the Chairman of the National Research Council. Committee members are paid no consulting fees or honoraria and are reimbursed only for expenses incurred while attending meetings and other activities of the committee.[1]

Functions of the Committee on Food Chemicals Codex

The committee's principal functions are as follows:

- To establish the general policies and guidelines by which FCC specifications are prepared.
- To evaluate comments submitted by interested parties on any aspect of the specifications and test procedures.
- To propose means by which the specifications may be kept current in reflecting food-grade quality on the basis of product safety and good manufacturing practices.
- To provide information on issues dealing with specifications for particular substances and analytical test procedures.

[1]The project scope, a committee roster, and meeting information are accessible on the National Academies' web site. Access <www.nationalacademies.org/cp.nsf> and search by name for ''Food Chemicals Codex.''

- To seek the advice of specialists when additional expert opinion is needed in making decisions regarding the appropriateness of specifications.
- To establish working groups consisting of committee members and other experts to address specific issues relevant to monograph development and to report their findings and recommendations to the full committee.
- To consider and act on any other issues concerning the development and publication of specifications and test procedures for food-grade ingredients.
- To approve the final manuscript for review before the publication of any edition of the FCC or its supplements.

Committee business is conducted through a central office at the National Academies in Washington, D.C. The appointed responsible study director at the Food and Nutrition Board, Institute of Medicine, coordinates all committee activities. The committee meets in regular session, usually once a year, to discuss the project's progress, including technical and policy issues relevant to the FCC. One or more members of the committee as well as the study director conduct ad hoc meetings on short-term projects as needed. The committee and study director also organize workshops and symposia as appropriate to exchange information with interested parties on key issues, whether of broad or limited scope.

Requirements for Listing Substances in the *Food Chemicals Codex*

The requirements are as follows: (1) the substance is permitted for use in food or in food processing in the United States (or, in certain cases, in other countries in which FCC specifications are recognized), (2) it is commercially available, and (3) suitable specifications and analytical test procedures are available to determine its identity and purity.

Criteria for *Food Chemicals Codex* Grade

The specifications published in the FCC are based primarily on the criteria of safety and good manufacturing practices (GMP). An FCC-grade substance is one that is prepared under GMP (discussed in detail later in this section) and that is of such purity as to ensure that potentially harmful or objectionable contaminants are not present at levels that would represent a hazard to the consumer of the foods in which the substance is intended to be used. Thus, FCC specifications define substances of sufficiently high quality to represent a reasonable certainty of safety when they are used under customary conditions of intentional use in food or in food processing. The specifications generally represent acceptable levels of quality and purity of food-grade substances available in the United States and in other countries in which FCC specifications are recognized. Because the different types of ingredients are diverse and complex, few general criteria can be established that will apply to all substances for which FCC specifications are prepared. The committee recognizes that limits and tests cannot be provided to cover all possible unusual or unexpected impurities, the

presence of which would be inconsistent with GMP. This matter is discussed further under *Trace Impurities*, in the *General Provisions*, and under *General Good Manufacturing Practices Guidelines for Food Chemicals*.

In addition to impurity limits, specifications, where applicable, must include the following: empirical formula, structural formula, and formula weight; description of the substance, including physical form, odor (flavoring agents only), and solubility (see the descriptive terms for solubility in the *General Provisions*); identification; assay (or a quantitative test to serve as an assay); physicochemical characteristics such as specific rotation, melting range or solidification point, viscosity, specific gravity, refractive index, and pH; loss on drying or water content; residual solvents; limits for mycotoxins and microbiological contaminants; and limits for byproducts and other adventitious constituents usually occurring in, or arising from the manufacture of, the substance. For safety, the committee deleted taste, as a characteristic of any substance, from all monographs, and odor from all but flavor monographs. The data provided, taken together, represent a complete compositional understanding of the substance. Additional information items include how the substance is to be packaged and stored to maintain its integrity and its functional use(s) in foods. If the substance contains an ''added substance,'' mentioning this fact enables the committee to judge whether the specifications should include it (see *Added Substances* under *General Provisions*).

Important Changes That May Affect How Information Is Submitted

Before submitting information to the Committee on Food Chemicals Codex, please read carefully the following paragraphs about the Federal Advisory Committee Act (FACA) Amendments of 1997, section 15, public law number 105-153. This act creates certain new requirements regarding studies performed for federal government agencies by the National Academies: the National Academy of Sciences (NAS), the National Academy of Engineering (NAE), the Institute of Medicine (IOM), and the National Research Council (NRC) (collectively referred to as ''the National Academies'').

The National Academies' policy applies to any committee (board, panel, etc.) appointed by the National Academies to develop a report (study reports, letter reports, workshop proceedings, summaries of symposia, and other manuscripts derived from institutional activities) that is intended for distribution outside the National Academies.

Documents Available to the Public at Committee Meetings Any meeting of a committee at which anyone other than committee members or officials, agents, or employees of the institution is present, whether in person or by telephone or audio or video teleconferences, is a ''data-gathering committee meeting.'' Except as provided by exemptions, all data-gathering committee meetings are open to the public.

Within the capacity of the meeting room, attendance at data-gathering committee meetings that are open to the public would not be limited. Any person, including members of the news media, may attend as observers (not participants), whether explicitly invited or not, provided that the individual is not disruptive. The chair of the meeting, assisted by officials and staff of the National Academies, is responsible for the conduct of the meeting.

Documents Unavailable to the Public at Committee Meetings Any committee meeting at which only committee members and officials, agents, and employees of the National Academies are present is a ''closed committee meeting.'' Closed committee meetings are not open to the public or to any person who is not a committee member or an official, agent, or employee of the National Academies. Deliberations by a committee in discussing, preparing, and finalizing a draft written report, including deliberations relating to review comments received in connection with review of the draft report under the National Academies' report review process, must be conducted in closed meetings.

After each closed committee meeting, the study director for the committee shall prepare a brief summary of the closed committee meeting and post the summary immediately on the National Academies' web site <www.nas.edu>. Except as provided by the exemptions, the brief summary of a closed committee meeting will identify the committee members present, the topics discussed, materials made available to the committee, and such other matters as the study director determines should be included, except that the brief summary will not disclose the substantive content or conclusions or recommendations of any draft report or discussions thereof or disclose any report review comments.

Public Access File A public access file for a committee project is established as soon as the study director creates a project record in the National Academies' current projects system.[2]

Materials provided at a data-gathering meeting or received by mail or fax from an organization or lay persons who are not officials, agents, or employees of the National Academies are placed in the public access file. Video tapes, audio tapes, or other alternative media such as diskettes or slides or viewgraphs presented to the committee by an organization or by individuals who are not officials, agents, or employees of the National Academies are considered by the National Academies to be subject to public disclosure as well.

Materials Exempt from the Public Access File The study director must request and receive advance written approval from the National Academies' Office of General Counsel (OGC) for withholding from the public any material presented to a committee by an organization or by a person other than an official, agent, or employee of the National Academies. This request must include adequate documentation to support such withholding. *For example, in the case of classified or statutorily protected information, the National Academies must receive a written statement addressed to the National Academies setting forth sufficient information to enable the National Academies' Office of General Counsel and the National Research Council's Executive Office to confirm that the information in question would be exempt from public disclosure under one or more of the Freedom of Information Act (FOIA)*[3] exemptions. The study director may not distribute to committee members any such materials (containing restrictive legends or markings limiting disclosure) without first consulting

[2]Access <www.nationalacademies.org/cp.nsf> and search by name for ''Food Chemicals Codex.''

[3]Access the Freedom of Information Act at <http://www.oalj.dol.gov/public/apa/refrnc/FOIA.HTM>.

the OGC. Only written materials that the OGC, in consultation with the National Research Council Executive Officer, determines to be exempt from disclosure under the exemptions to the disclosure requirements of the FOIA will be withheld from the Public Access File.

Procedures for Submission and Development of Specifications

The committee will consider suggested specifications, such as previously elaborated, submitted with supporting data by any interested party, including food ingredient manufacturers and suppliers, food processors, and industry associations. Suggested specifications should be submitted, in duplicate, to Food Chemicals Codex, Food and Nutrition Board, Institute of Medicine, 500 Fifth Street, N.W., Washington, D.C. 20001. The committee and/or the project staff examine suggested specifications and often expand them to meet the general criteria the committee requires. Because committee discussions involving quality characteristics of substances used in food or food processing might result in sharing privileged or proprietary information, contributors may request that such discussions be held in closed sessions. The final outcome of such discussions must be openly shared with all manufacturers, users, and parties interested in the substance discussed; therefore, open discussions are the norm, except during unusual circumstances. Where privileged or proprietary information is concerned, the project staff can put such information in a format so that the end results are not associated with particular manufacturers or users. The committee and/or the staff draft a new monograph and send it to the originator (and to any other manufacturers of that substance that can be identified) for comment. After the draft has gone through this process and all necessary revisions have been made, the committee votes by mail ballot whether to propose these specifications for public comment. If the committee finds deficiencies, or if any questions are raised, the draft is returned to the originator and other interested parties with the committee's comments and recommendations for improvement. Once a draft has gained committee acceptance, availability of the proposed specification for comment is announced in the *Federal Register* or online at <www.cfsan.fda.gov> or through notices in trade journals. This notification allows the public and other interested parties as well as manufacturers and users that may be inadvertently overlooked to provide their comments to the committee. Once the public comments are considered and any necessary changes made, the committee votes to determine whether the monograph is suitable for publication. Monographs as well as supporting materials such as general tests and infrared spectra are then reviewed through the National Research Council's report review process and, if approved, are published in the next edition of the FCC or a supplement.

Procedure for Revising Specifications

FCC specifications are subject to revision at any time, and suggested revisions may be initiated by regulatory bodies, manufacturers, suppliers, or users of the ingredients; by the committee itself; or by any other interested parties. All suggestions for revision must be accompanied by supporting data. In the case of revisions of test procedures

and analytical methods, comparative data for both the existing and suggested procedures must be submitted. Where changes in limits or other tolerances are suggested, supporting data should be presented on representative production batches. Suggestions for changing the limits of certain impurities (e.g., arsenic, cadmium, lead, fluoride, and mercury) may require the submission of safety data and information concerning the daily intake of the substance. All suggestions for revision, together with the supporting data, are reviewed by the Committee on Food Chemicals Codex and/or by the FCC staff. If other manufacturers are involved (and can be identified), they are also asked to comment. If the committee finds deficiencies, or if any questions arise, the suggested revised specifications are returned to the originator (and other manufacturers, where appropriate) with the committee's comments or questions. If agreement cannot be reached at this point between the committee and the originator, or among manufacturers and other interested parties, a special meeting may be held to discuss the matter, or the parties involved may be invited to one of the committee's regular meetings to examine the question in depth. Approved revisions are published either in the next edition of the FCC or in a supplement by the same procedure described under *Procedures for Submission and Development of Specifications*.

Additional Information

FCC users should become thoroughly familiar with the *General Provisions* pertaining to this edition. Inquiries regarding any aspect of the operation of the FCC project may be directed to Food Chemicals Codex, Food and Nutrition Board, Institute of Medicine, 500 Fifth Street, N.W., Washington, D.C. 20001 (telephone 202-334-2580; facsimile 202-334-2316; email fcc@nas.edu). Additionally, all interested parties may view new and revised materials as presented by the Committee on Food Chemicals Codex at <www.iom.edu/fcc>.

VALIDATION OF *FOOD CHEMICALS CODEX* METHODS

Submissions to the *Food Chemicals Codex*

Submissions for new or revised specifications and analytical methods must contain sufficient information to enable committee members to evaluate the proposals. In most cases, evaluations involve assessing the clarity and completeness of the analytical methods description, determining the need for the methods, and reviewing documentation that the methods have been appropriately validated. Information may vary depending on the type of test method involved. However, in most cases a submission will consist of the following sections:

Rationale Use this section to identify the need for the analytical method and describe the capability of the specific method proposed and why it is preferred over other types of determinations. For revised analytical methods, provide a comparison of limitations of the existing FCC analytical method and advantages offered by the suggested method.

Suggested Analytical Method Use this section to present a complete description of the analytical method sufficiently detailed to enable persons ''skilled in the art'' to replicate it. Include all important operational parameters and specific instructions such as reagent preparation, systems suitability tests performance, description of blanks used, precautions, and explicit formulas for calculating test results.

Data Elements Use this section to provide thorough and complete documentation of the validation of the analytical method. Include summaries of experimental data and calculations substantiating each of the applicable analytical performance parameters. These parameters are described in the following section.

Validation

Validation of an analytical method is the process of establishing, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Express performance characteristics in terms of analytical parameters. Each of the recommended parameters is defined in the next section of this chapter, along with a delineation of a typical method by which it may be measured.

Typical analytical parameters used in assay validation are accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range, and ruggedness.

Accuracy

Definition The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amounts of analyte.

Determination Determine the accuracy of an analytical method by applying that method to samples to which known amounts of analyte have been added both above and below the normal levels expected in the samples. Calculate the accuracy from the test results as the percentage of analyte recovered by the assay.

Precision

Definition The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation). Precision may be a measure of the degree of either reproducibility or repeatability of the analytical method under normal operating conditions. In this context, reproducibility refers to the use of the analytical procedure in different laboratories. Intermediate precision expresses within-laboratory variation, as on different days, or with different analysts or equipment within the same laboratory. Repeatability refers to the use of the analytical procedure within a laboratory over a short time, using the same analyst with the same equipment.

Determination Determine the precision of an analytical method by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation (coefficient of variation). Assays in this context are independent analyses of samples that have

been carried through the complete analytical procedure from sample preparation to final test result.

Specificity

Definition The specificity of an analytical method is its ability to measure, both accurately and specifically, the analyte in the presence of components that may be expected to be present in the sample matrix. Specificity may often be expressed as the degree of bias of test results obtained by analysis of samples containing added impurities, degradation products, or related chemical compounds when compared with test results from samples without added substances. The bias may be expressed as the difference in assay results between the two groups of samples. Specificity is a measure of the degree of interference (or absence thereof) in the analysis of complex sample mixtures.

Determination Determine the specificity of an analytical method by comparing test results obtained from the analysis of samples containing impurities, degradation products, or related chemical compounds with those obtained from the analysis of samples without these elements. The bias of the assay, if any, is the difference in test results between the two groups of samples.

When impurities or degradation products are unidentified, demonstrate specificity by analyzing samples (with the method in question) containing impurities or degradation products and by comparing the results to those from additional purity assays (e.g., chromatographic assay). The degree of agreement of test results is a measure of the specificity.

Limit of Detection

Definition The limit of detection is a parameter of limit tests. It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Thus, limit tests merely substantiate that the analyte concentration is above or below a certain level. The limit of detection is usually expressed as the concentration of analyte (e.g., percentage, milligrams per gram, parts per billion) in the sample.

Determination Determining the limit of detection of an analytical method will vary depending on whether it is an instrumental or noninstrumental procedure. For instrumental procedures, different techniques may be used. Some investigators determine the signal-to-noise ratio by comparing test results from samples containing known concentrations of analyte with those of blank samples and establish the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. Other investigators measure the magnitude of analytical background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation, multiplied by a factor, usually 2 or 3, provides an estimate of the limit of detection. This limit is subsequently validated by the analysis of a suitable number of samples known to be close to or at the limit of detection.

For noninstrumental methods, determine the limit of detection by analyzing samples with known concentrations of analyte and by establishing the minimum level at which the analyte can reliably be detected.

Limit of Quantitation

Definition Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices, such as impurities and degradation products in food additives and processing aids. It is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The limit of quantitation is expressed as the concentration of analyte (e.g., percentage, milligram per kilogram, parts per billion) in the sample.

Determination Determining the limit of quantitation of an analytical method may vary depending on whether it is an instrumental or a noninstrumental procedure. For instrumental procedures, a common approach is to measure the magnitude of analytical background response by analyzing a number of blank samples and calculating the standard deviation of this response Multiplying the standard deviation by a factor, usually 10, provides an estimate of the limit of quantitation. This limit is subsequently validated by the analysis of a suitable number of samples known to be close to or at the limit of quantitation.

For noninstrumental methods, determine the limit of quantitation by analyzing samples having known concentrations of analyte and by establishing the minimum level at which the analyte can be detected with acceptable accuracy and precision.

Linearity and Range

Definition of Linearity The linearity of an analytical method is its ability (within a given range) to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line (correlation coefficient), calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

Definition of Range The range of an analytical method is the interval between and including the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the method as written. The range is normally expressed in the same units as test results (e.g., percent, milligrams per kilogram, parts per million) obtained by the analytical method.

Determination of Linearity and Range Determine the linearity of an analytical method by mathematically treating test results obtained from analysis of samples with analyte concentrations across the claimed range of the method. The treatment is normally a calculation of a regression line by the method of least squares of test results versus analyte concentrations. In some cases, to obtain proportionality between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation before the regression analysis. The slope of the regression line and its variance (correlation coefficient) provide a mathematical measure of linearity; the y-intercept is a measure of the potential assay bias.

Validate the range of the method by verifying that the analytical method provides acceptable precision, accuracy, and linearity when applied to samples containing analyte at the extremes of the range as well as within the range.

Ruggedness

Definition The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions, such as different laboratories, analysts, instruments, lots of reagents, elapsed assay times, assay temperatures, and days. Ruggedness is normally expressed as the lack of influence on test results of operational and environmental variables of the analytical method. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst.

Determination Determine the ruggedness of an analytical method by analyzing aliquots from homogeneous lots in different laboratories, by different analysts, using operational and environmental conditions that may differ but still are within the specified parameters of the assay. Determine the degree of reproducibility of test results as a function of the assay variables. This reproducibility may be compared to the precision of the assay under normal conditions to obtain a measure of the ruggedness of the analytical method.

Robustness

The robustness of an analytical method is a measure of the method's capacity to remain unaffected by small, but deliberate, variations in method parameters, and it provides an indication of the method's reliability during normal use.

Data Elements Required for Assay Validation

FCC assay procedures vary from highly exacting analytical determinations to subjective evaluation of attributes. Considering this variety of assays, it is only logical that different test methods require different validation schemes. This section covers only the most common categories of assays for which validation data should be required. These categories are as follows:

Category I Analytical methods for quantitation of major components of food additives or processing aids (including preservatives).

Category II Analytical methods for determination of impurities in food additives or processing aids. These methods include quantitative assays and limit tests.

Category III Analytical methods for determination of performance characteristics (e.g., solubility, melting point).

For each assay category, different analytical information is needed. In the following table, data elements that are normally required for each assay category are listed.

Already-established general assays and tests (e.g., titrimetric method of water determination, identification test) should also be validated to verify their accuracy (and absence of possible interference) when used for a new product or raw material.

The validity of an analytical method can be verified only by laboratory studies. Therefore, documentation of the successful completion of such studies is a basic requirement for determining whether a method is suitable for its intended applications. Appropriate documentation should accompany any proposal for new or revised compendial analytical procedures.

Data Elements Required for Assay Validation

Analytical Performance Parameter	Assay Category I	Assay Category II		Assay Category III
		Quantitative	Limit Tests	
Accuracy	Yes	Yes	*	*
Precision	Yes	Yes	No	Yes
Specificity	No	Yes	No	*
Limit of Detection	Yes	Yes	Yes	*
Limit of Quantitation	No	No	Yes	*
Linearity	Yes	Yes	No	*
Range	Yes	Yes	*	*
Ruggedness	Yes	Yes	Yes	Yes

*May be required, depending on the nature of the specific test.

GENERAL GOOD MANUFACTURING PRACTICES GUIDELINES FOR FOOD CHEMICALS[4]

Food chemicals and other substances employed as adjuncts in foods and as aids in food processing must meet recognized standards of performance and quality for their intended uses and applications. The requirements contained in the monographs of the FCC pertain to the characteristics of food chemicals at the time of their use.

It is not sufficient, however, for an end product merely to meet the FCC requirements. Production of food-quality chemicals is best achieved by implementing procedures that place primary emphasis on preventing defects and deficiencies. Thus, a product must be made and handled in a sanitary manner, in a way designed either to preclude the formation of undesirable by-products, or to ensure their adequate removal, as well as to prevent contamination, deterioration, mix-up and mislabeling, and the introduction of unusual or unexpected impurities.

Food chemicals are subject to applicable regulations promulgated by the responsible government agencies in countries in which FCC specifications are recognized. In the United States, for example, the pertinent regulations that deal primarily with sanitation are the ''Current Good Manufacturing Practices in Manufacturing, Packing, or Holding Human Food.''[5]

Beyond requirements related to sanitation, however, manufacturers, processors, packers, and distributors should establish and exercise other appropriate systems of controls throughout their operations, including food safety assurance systems such as Hazard Analysis and Critical Control Points (HACCP), where applicable, to ensure that FCC substances are safe and otherwise suitable for their intended use. These controls, together with the regulations cited above, constitute ''good manufacturing practices.'' While the

[4]These guidelines are presented for information only and are not intended to be mandatory in any sense as regards compliance with FCC specifications.

[5]*Code of Federal Regulations,* Title 21, Part 110, which may be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Also, Parts 113 and 114 are of interest, particularly with regard to record keeping.

details of the application of the principles of good manufacturing practices to the manufacturing, processing, packing, and distribution of food chemical substances will vary, the fundamental relevance of such principles at all stages of an operation should be recognized.

The principles of good manufacturing practices encompass such considerations as

- Systems of quality control and assurance, including self-auditing procedures.
- Clearly defined responsibilities of supervisory and other personnel, all of whom must be qualified and adequately trained.
- Design, operation and maintenance of buildings and equipment, with attention to housekeeping, sanitation, pest control, prevention of contamination of product, cleaning of equipment, a calibration program for all instruments and gauges, and environmentally satisfactory methods of waste disposal.
- Documentation of validation studies pertaining to the manufacturing process, laboratory test methods, and equipment and computer applications, when any such studies are appropriate.
- Written operational instructions that should include such items as

  —General instructions and hazards.
  —Master manufacturing instructions.
  —Master packaging instructions.
  —Master specifications for raw materials, in-process materials, packaging materials, labels, and finished products.
  —Laboratory test methods.
  —Control instrumentation and computer applications.
  —Labeling, holding, and distribution instructions.

- Handling and control, including the testing and approval, of raw materials, process aids, intermediates, and finished products.
- Product containers, closures, and labeling (including the control of labels and labeling).
- Laboratory and inspection controls and records (including the effect of process changes).
- Reserve samples of raw materials and products.
- Written records that contain essential operational data for each individual lot of food chemical and that permit tracing the lot history from the raw materials through manufacturing, packaging, holding, and distributing the product.
- Product stability and lifespan.
- Systems for holding, evaluating, and disposing of rejected products and returned materials.
- Procedures for investigating complaints and taking appropriate corrective action.

Some food chemicals have uses other than as food chemicals—in fact, the food-grade material may be only a small part of production for industrial or other uses. In such situations, the principles of good manufacturing practices must apply, and particular attention must be paid to the suitability of the raw materials used; the prevention of cross-contamination; and the segregation of food chemicals from nonfood chemicals, including material in process, final product, and product in storage. The necessary controls to ensure the above must be developed and implemented.

Note: Depending on the processes used, it frequently is possible to divert the food-grade product from the main product stream as the final steps in producing a food-grade product are approached, and to complete the processing under conditions suitable for food-grade substances. In such cases, if the diverted material can be adequately characterized by a knowledge of its history, and/or by appropriate analytical testing, it may be considered to be the raw material for the food-grade product.

Biotechnology (processes involving the use of biological systems) is an important source of chemicals, enzymes, and other substances used in foods and in food processing. Some food ingredients have long been made by fermentation and by enzymatic processes, but now processes involving genetically modified organisms have become a prominent emerging source of such substances.

The manufacture of food chemicals, whether it involves chemical or biological synthesis and purification, or recovery from natural materials, has a number of characteristics that must be taken into account in establishing a system of good manufacturing practice. For example, in the production of many chemicals, recycling of process liquors and recovery from waste streams are necessary for reasons of quality, economics, and environmental protection. In addition, the production of some food chemicals involves processes in which chemical and biochemical mechanisms have not been fully elucidated, and thus the methods and procedures for materials accountability usually will differ from those applicable to the manufacture of other classes of materials.

Another aspect of good manufacturing practices for food chemicals relates to the possible presence of objectionable impurities. While the limits and tests provided in the FCC are consistent with the information available to the committee regarding current methods of manufacture and common impurities that may be present, it obviously is impossible to provide limits and tests in each FCC monograph for the detection of all possible impurities because these may vary with the raw materials and the method of processing used in making the chemical. Thus, to evaluate whether other undesirable impurities may be present, the manufacturer should understand, to the best degree possible for the process at hand, the factors that contribute to the presence of impurities. Solvents as well as impurities in the raw materials and processing aids, all of which might carry into the final product, must be considered. In synthetic processes, it is necessary similarly to consider intermediates and the products of side reactions, as well as the possible formation of isomeric compounds, including epimers and enantiomorphs.

The same general considerations apply to biological processes, be they traditional or based on newer biotechnology. For products of biotechnology, a review that includes adequate characterization and documentation of the genetic origins of the starting materials and the characteristics of the process provides the necessary guidance for identifying and setting levels of undesirable impurities, which need to be controlled to suitable levels or to be absent altogether. Therefore, the active genetic components in the process (e.g., culture, recombinant DNA) should be known, well characterized, and free from any potential for introducing biologically significant levels of undesirable constituents (e.g., toxins, antibiotics, antinutrients, allergens) that cannot be kept out of the final product by preliminary processing. As in the production of all food chemicals, testing is appropriate when possible and particularly to demonstrate the absence of certain toxins and certain specific DNA sequences.

Because of the necessity to maintain the purity and integrity of the genetic materials associated with the biotechnological process, containment[6] is a particularly important consideration in preventing cross-contamination as well as the inadvertent release of biologically active materials.

Exposure of all products used in foods and food processing to foreign material contamination must be prevented. If objectionable impurities from any source, other than those covered by FCC requirements, are suspected to be present, good manufacturing practice requires the manufacturer to ensure that the substance is suitable for its intended applications as a food chemical by applying additional tests and limits. Current analytical technology should be applied wherever possible.

[6]See *NIH Guidelines for Research Involving Recombinant DNA Molecules, Federal Register,* Vol. 51, No. 88, pages 16957–16985, May 7, 1986. Copies, which include later revisions of the Guidelines, may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, MD 20892.

MONOGRAPHS ADDED TO THE *FOOD CHEMICALS CODEX*, FIFTH EDITION, FROM SUPPLEMENTS 1 THROUGH 3

Supplement 1
Calcium Lignosulfonate
beta-Cyclodextrin
Dimethyl Dicarbonate
Glyceryl Palmitostearate
4-Hexylresorcinol
Magnesium Phosphate, Dibasic, Mixed Hydrates
Manganese Citrate
Olestra
Sodium Lignosulfonate
Sucrose Fatty Acid Esters
Sugar Beet Fiber
Vitamin K
Whey Protein Concentrate
Whey, Reduced Lactose
Whey, Reduced Minerals
Yeast, Autolyzed

Flavors
ω-Pentadecalactone
1,3,5-Undecatriene
Veratraldehyde

Supplement 2
L-Carnitine
Erythritol
Ferric Citrate
Ferrous Citrate
Maltitol
Menhaden Oil, Hydrogenated
Menhaden Oil, Refined
Sheanut Oil, Refined

Supplement 3
Acidified Sodium Chlorite Solutions
Aspartame-Acesulfame Salt
Curdlan
gamma-Cyclodextrin
Polyglycerol Polyricinoleic Acid
Pork Collagen
Salatrim
Solin Oil
Soy Protein Concentrate
Sucrose Acetate Isobutyrate

Flavors
Acetaldehyde Diethyl Acetal
2-Acetyl Thiazole
Allyl Phenoxy Acetate
Allyl Propionate
Borneol
2-*sec*-Butyl Cyclohexanone
Butyl 2-Methyl Butyrate
Diphenyl Ether
d-Fenchone
Fenchyl Alcohol
Furfuryl Alcohol
2-Furyl Methyl Ketone

NEW MONOGRAPHS IN THE *FOOD CHEMICALS CODEX*, FIFTH EDITION

Allura Red
Arabinogalactan
Bohenin
Brilliant Blue
Butadiene-Styrene Rubber
Curdlan
Erythrosine
Fast Green
Ferrous Glycinate
Hydrogenated Starch Hydrolysates
Indigotine
Sunset Yellow
Tartrazine
Transglutaminase
Trehalose
Vegetable Oil Phytosterol Esters
Wheat Protein Isolate
Whey Mineral Concentrate
Whey Protein Isolate

Flavors
2,6-Dimethoxy Phenol
3,4-Dimethyl 1,2-Cyclopentandione
5-Ethyl 3-Hydroxy 4-Methyl 2(5H)-Furanone
3-Ethyl Pyridine
Furfuryl Mercaptan
Geranyl Isovalerate
2,3-Heptandione
(*Z*)-3-Hexenyl Butyrate
(*Z*)-3-Hexenyl Formate
Hexyl Butyrate
Hexyl Hexanoate
Isoamyl Isobutyrate
Isobutyl Formate
Isobutyl Hexanoate
Linalool Oxide
Maltol Isobutyrate
2-Methoxy 3-(or 5- or 6-) Isopropyl Pyrazine
5H-5-Methyl-6,7-dihydrocyclopenta[b]pyrazine
5-Methyl Furfural
Methyl Furoate
Methyl Hexanoate
Methyl Isovalerate
5-Methyl 2-Phenyl 2-Hexenal
Methyl Thiobutyrate
Methyl Valerate
β-Naphthyl Ethyl Ether
Phenyl Ethyl Cinnamate
Phenyl Ethyl Propionate
Propyl Formate
Propyl Mercaptan
Salicylaldehyde
δ-Tetradecalactone
2-Tridecanone

FORMER AND CURRENT TITLES OF *FOOD CHEMICALS CODEX* MONOGRAPHS

Fourth Edition Title	*Fifth Edition Title*
Acacia	Gum Arabic
Ammonium Hydroxide	Ammonia Solution
DL-α-Tocopherol	All-rac-α-Tocopherol
D-α-Tocopherol Concentrate	RRR-α-Tocopherol
Tocopherols Concentrate, Mixed	RRR-Tocopherols Concentrate, Mixed
D-α-Tocopheryl Acetate	RRR-α-Tocopheryl Acetate
DL-α-Tocopheryl Acetate	All-rac-α-Tocopheryl Acetate
D-α-Tocopheryl Acetate Concentrate	RRR-α-Tocopheryl Acetate Concentrate
D-α-Tocopheryl Acid Succinate	RRR-α-Tocopheryl Acid Succinate

MONOGRAPHS COMBINED

Fourth Edition Title	*Fifth Edition Title*
Butadiene-Styrene 50/50 Rubber Butadiene-Styrene 75/25 Rubber	Butadiene-Styrene Rubber
Sodium Acetate Sodium Acetate, Anhydrous	Sodium Acetate

Contents

1 / *General Provisions and Requirements Applying to Specifications, Tests, and Assays of the* **Food Chemicals Codex**

The General Provisions provide, in summary form, the basic policies and guidelines for the interpretation and application of the standards, tests, assays, and other specifications of the *Food Chemicals Codex* and make it unnecessary to repeat throughout the book those requirements that are pertinent in numerous instances.

Where exceptions to the General Provisions are made, the wording in the individual monograph or general test chapter takes precedence and specifically indicates the directions or the intent.

TITLE OF BOOK

The title of this book, including supplements thereto issued separately, is the *Food Chemicals Codex*, Fifth Edition. It may be abbreviated to FCC V.

Where the term ''Codex'' is used without further qualification in the text of this book, it applies to the *Food Chemicals Codex*, Fifth Edition.

INQUIRIES

Inquiries regarding any aspect of the operation of the Food Chemicals Codex may be directed to the Food Chemicals Codex, Institute of Medicine, 500 Fifth Street, N.W., Washington, D.C. 20001.

CODEX SPECIFICATIONS

Food Chemicals Codex specifications, comprising the *Description*, *Requirements*, and *Tests*, are presented in monograph form (*Section 2*) or tabular form (*Section 3*) for each substance or group of related substances. They are designed to ensure that food additives have a sufficiently high level of quality to be safe under usual conditions of intentional use in foods, both directly or indirectly, or in food processing. Thus, FCC specifications generally represent acceptable levels of quality and purity of food-grade ingredients available in the United States (or in other countries in which FCC specifications are recognized).

The titles of FCC monographs are in most instances the common or usual names. The FCC specifications apply equally to substances bearing the main titles, synonyms listed under the main titles, and names derived by transposition of definitive words in main titles. The Committee on Food Chemicals Codex recognizes that the nomenclature used for flavor chemicals may not be consistent with other authoritative sources.

Although the assays and tests described constitute methods upon which the specifications of the *Food Chemicals Codex* depend, analysts are not prevented from applying alternative methods if they are satisfied that the procedures used will produce results of equal or greater accuracy. In the event of doubt or disagreement concerning a substance purported to comply with the requirements of this Codex, only the methods described herein are applicable and authoritative.

POLICIES AND GUIDELINES

General Policy It is the policy of the Codex to set maximum limits for trace impurities wherever they are deemed to be important for a particular food chemical, and they shall be set at levels consistent with food safety and good manufacturing practice.

Maximum limits for inorganic trace impurities (e.g., arsenic, cadmium, fluoride, lead, mercury, selenium) will be included in any monographs for which consumer safety or manufacturing experience indicates their desirability. No limits for

arsenic, lead, and other heavy metals are required for flavor chemicals because of the very low levels at which these substances are added to foods.

All requests to increase limits shall be considered on the basis of the toxicological risk involved, the principles of good manufacturing practice, and the availability of the same substances from other sources that meet the FCC limits in question.

Added Substances (Policy) FCC specifications are intended for application to individual substances (single entities) and not to proprietary blends or other mixtures. Some specifications, however, allow ''added substances'' (i.e., functional secondary ingredients such as anticaking agents, antioxidants, diluents, emulsifiers, and preservatives) intentionally added when necessary to ensure the integrity, stability, utility, or functionality of the primary substance in commercial use.

If an FCC monograph allows such additions, each added substance must meet the following requirements: (1) it is approved for use in foods by the U.S. Food and Drug Administration or by the responsible government agency in other countries in which FCC specifications are recognized; (2) it is of appropriate food-grade quality and meets the requirements of the *Food Chemicals Codex*, if listed therein; (3) it is used in an amount not to exceed the minimum required to impart its intended technical effect or function in the primary substance; (4) its use will not result in concentrations of contaminants exceeding permitted levels in any food as a consequence of the affected FCC primary substance's being used in food; and (5) it does not interfere with the assay and tests prescribed for determining compliance with the FCC requirements for the primary substance, unless the monograph for the primary substance has provided for such interferences.

Where added substances are specifically permitted in an FCC substance, the label shall state the name(s) and amount(s) of any added substance(s).

Adding substances not specifically provided for and mentioned by name of function in the monograph of an FCC substance will cause the substance to no longer be designated as an FCC substance. Such a combination is a mixture to be described by disclosure of its ingredients, including any that are not FCC substances.

Allergens The Committee on Food Chemicals Codex recognizes the issue of food allergens, but current limitations regarding (1) the threshold levels and (2) the analytical methods to detect allergens at very low levels have thus far prevented the inclusion in FCC monographs of specifications related to allergens.

Arsenic Specifications (Policy) Arsenic specifications will be included in monographs only when there is specific reason for the committee to believe that arsenic constitutes a likely contaminant in the substance in question.

Fluoride Limits (Guideline) The Committee on Food Chemicals Codex has established limits for fluoride in numerous monographs. For phosphates, this reflects the natural occurrence of fluoride in the inorganic phosphate starting material. Fluoride limits in other monographs may reflect the natural occurrence of fluoride in the article or in reagents used in food additive manufacture.

Following issuance of the *Fluoride Limits (Guideline)* in earlier editions of the *Food Chemicals Codex*, considerable research has been completed demonstrating the cariostatic—caries preventing—properties of fluoride. Fluoride is now added to many municipal water supplies to provide a level of 1 mg/L, and many dental products are formulated with it. Fluoride is in the formulation of many over-the-counter dietary supplements as well.

During and after the time the *Food Chemicals Codex*, Fourth Edition, was in preparation, the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, The National Academies, completed a comprehensive review of fluoride (IOM, 1997).[1] The committee reviewed the literature, noted the toxicological manifestations of fluoride in animals and humans, and established a general No Observed Adverse Effect Level (NOAEL) of 10 mg/day. As fluoride has the ability to induce fluorosis—mottling of the primary teeth of young children—an upper limit (UL) of 0.7 mg/day was established for infants, with increasing ULs assigned to older children. In addition, an Adequate Intake (AI) of 3 mg/day was assigned for adult females and of 4 mg/day for adult males, with the UL for both being 10 mg/day.

The intake of fluoride as a constituent of substances described in FCC monographs, even at the maximum limits established for fluoride, is not expected to significantly add to the human daily fluoride intake from other sources and is well within the various limits described in the Institute of Medicine's committee report. Nonetheless, given that toxicological manifestations have been amply demonstrated for fluoride, as described in the report, the maintenance of fluoride limits in drinking water and food, and thus food additives, appears consistent with sound public health policy. Therefore, the Committee on Food Chemicals Codex considers that maintaining fluoride limits for relevant food additives and ingredients is justified.

Because of the difficulties in analyzing for fluoride in food chemicals, the committee intends to adopt new analytical methods for fluoride as soon as adequate validation is submitted. Furthermore, in view of the considerable variation in fluoride limits for additives and ingredients in various national and international compendia, the committee deems harmonization of fluoride limits between the FCC and other compendia to be desirable.

FCC Substances Containing Sulfiting Agents (Policy) If an FCC substance contains 10 mg/kg or more of any sulfiting agent, the presence of such sulfiting agent shall be indicated on the labeling.

Labeling (Policy) For purposes of compliance with *Food Chemicals Codex* monographs, ''labeling'' means all labels and other written, printed, or graphic matter (1) on any article or any of its containers or wrappers or (2) accompanying such article, or otherwise provided by vendors to purchasers for

[1]IOM (Institute of Medicine). 1997. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. Washington, DC: National Academy Press.

purposes of product identification.

In addition to FCC labeling requirements, substances included in the *Food Chemicals Codex* are subject to compliance with such labeling requirements as may be promulgated by government bodies. Such substances are intended for use in foods, either directly or indirectly, and in food processing.

The name of the substance on a container label, plus the designation ''Food Chemicals Codex Grade,'' ''FCC Grade,'' or simply ''FCC,'' is a representation by the manufacturer, vendor, or user of the substance that at the time of shipment, it conforms to the specifications in FCC V, including its supplements that are current at that time.

When an FCC substance is available commercially in solution form or as a component of a mixture, and there is no provision in the *Food Chemicals Codex* for such solution or mixture, the manufacturer, vendor, or user may indicate on the label that the product contains substances meeting FCC specifications by use of the initials ''FCC'' after the name of those components that meet the FCC requirements.

For the labeling of FCC substances in which added substances are permitted, see *Added Substances (Policy)*, above.

For the labeling of FCC substances that contain 10 mg/kg or more of a sulfiting agent, see *FCC Substances Containing Sulfiting Agents (Policy)*, above.

Heavy Metals Limits (Policy) The Committee on Food Chemicals Codex notes the importance of providing limits for individual heavy metals as required by the source and composition of individual food additives. Thus, it has decided to remove from most monographs the general heavy metals (as lead) limits and tests and, based on the current level and availability of scientific information and on the policy stated below, to replace them with limits and tests for specific heavy metals such as lead, cadmium, and mercury as may be relevant to each substance.

The committee recognizes the desirability of lowering exposure to lead and other heavy metals, especially in the case of infants and children. Overall exposure to heavy metals in general, and to lead in particular, is a public health concern. Although diet is not the largest source of lead exposure, it is a significant one. While ingestion of FCC substances does not represent the major source of dietary lead, it is desirable to lower the lead limits for all FCC substances, particularly for those substances consumed in high amounts. Therefore, the committee's policy is to reduce lead and other heavy metals limits to the lowest extent feasible, especially given that more recent evidence shows deleterious neurobehavioral effects occurring in children exposed to lead at levels below those previously considered acceptable.

In setting limits for lead and other heavy metals, the committee considers the amount of a food chemical consumed, the feasibility of manufacturing a product within these limits, and the availability of analytical methods to ensure compliance. The constraints of good manufacturing practice and the availability of reliable analytical methods are often limiting factors in setting lower limits for lead and other heavy metals.

The committee regards as one of its goals the assurance of the safety of properly used food chemicals. This means that FCC specifications will respond to advances in knowledge about new manufacturing methods, analytical techniques, or toxicology and safety issues.

Microbiological Attributes (Policy) Manufacturers, vendors, and users of FCC substances are expected to exercise good manufacturing practices (GMPs) and to establish food safety assurance systems such as Hazard Analysis and Critical Control Points (HACCP) to ensure that FCC substances are safe and otherwise suitable for their intended use. FCC substances are expected to meet applicable regulatory requirements, including microbiological criteria, for safety and quality. According to *Codex Alimentarius* recommendations for establishing and applying microbiological criteria for foods, ''Mandatory *(regulatory)* microbiological criteria shall apply to those products and/or points of the food chain where no other more effective tools are available, and where they are expected to improve the degree of protection to the consumer. Where these are appropriate they shall be product-type specific and only applied at the point of the food chain specified in the regulation.'' In addition, businesses may develop microbiological criteria for specific food additives or ingredients, processes, and products. The General Policy for microbiological safety and quality of FCC substances is that such substances be produced, handled, and used in food processing following GMPs and applicable food safety systems. Therefore, the FCC does not list specific microbiological criteria for FCC substances other than those for which scientifically valid data are available to the committee that support the need for such criteria. In such cases, the *Codex Alimentarius* principles for establishing and applying microbiological criteria have been followed.[2] These principles include the following:

1. Microbiological criteria should be established and applied only where there is a definite need and their application is practical.
2. Consideration is given to
   i. the evidence of actual or potential hazards to health;
   ii. the microbiological status of raw material(s);
   iii. the effect of processing on the microbiological status of the ingredient or food additive;
   iv. the likelihood and consequences of microbial contamination and/or growth during subsequent handling, storage, and use;
   v. the category(ies) of consumers concerned; and
   vi. the intended use of the ingredient or food additive.
3. The sampling plan, method, and handling are stated.
4. The microorganism(s) included in the criteria is (are) widely accepted as relevant to the particular ingredient or food additive—as pathogen(s), as indicator organism(s), or as spoilage organism(s).
5. Limits used in the criteria are based on microbiological data appropriate to the ingredient or food additive and other similar substances.

[2]Codex Alimentarius Commission. 1997. Joint FAO/WHO Food Standards Programme, Codex Committee on Food Hygiene, Supplement to Volume 1B-1997. *Principles for the Establishment and Application of Microbiological Criteria for Foods*. CAC/GL 21-997. Pp. 47–54.

Mg/Kg and Percent (Policy) Beginning with the Second Supplement to FCC III, to bring the Codex into concordance with current nomenclature as used in analytical chemistry, the term ''ppm'' [parts per million (by weight)] was replaced by ''mg/kg'' (milligrams per kilogram).

The term ''mg/kg'' is used for expressing the concentrations of trace amounts of substances, such as impurities, up to 10 mg/kg. Above 10 mg/kg, percent (by weight) is used. For example, a monograph requirement equivalent to 20 mg/kg is expressed as 0.002%, or 0.0020%, depending on the number of significant figures justified by the test specified for use in conjunction with the requirement.

ATOMIC WEIGHTS AND CHEMICAL FORMULAS

The atomic weights used in computing formula weights and volumetric and gravimetric factors stated in tests and assays are those recommended in 1991 by the IUPAC Commission on Isotopic Abundances and Atomic Weights.

Molecular and structural formulas and formula weights immediately following titles are included for the purpose of information and are not to be considered an indication of the purity of the substance. Molecular formulas given in specifications, tests, and assays, however, denote the pure chemical entity.

ASSAYS AND TESTS

Every FCC substance in commerce, when tested in accord with these assays and tests, meets all of the requirements in the monograph defining it.

Many materials in concentrated forms, whether to be used in food or as test reagents, are a skin or respiratory irritant or are otherwise toxic. Use caution when handling these materials.

Analytical Samples In the description of assays and tests, the approximate quantity of the analytical sample to be used is usually indicated. The quantity actually used, however, should not deviate by more than 10% from that stated.

Some substances must be dried before a sample is taken for an assay or test. When a *Loss on Drying* or *Water* test is specified, the undried substance may be used and the results calculated on the dried basis, provided that any moisture or other volatile matter in the undried sample does not interfere with the specified assay and test procedures.

The word ''accurately,'' used in connection with gravimetric or volumetric measurements, means that the operation should be carried out within the limits of error prescribed under *Volumetric Apparatus* or *Weights and Balances*, Appendix I. The same significance also applies to the term ''exactly'' or quantitative expressions such as ''100.0 mL'' or ''50.0 mg.''

The word ''transfer,'' when used in describing assays and tests, means that the procedure should be carried out quantitatively.

Apparatus With the exception of volumetric flasks and other exact measuring or weighing devices, directions to use a definite size or type of container or other laboratory apparatus are intended only as recommendations, unless otherwise specified.

Where an instrument for physical measurement, such as a thermometer, spectrophotometer, or gas chromatograph, is designated by its distinctive name or tradename in a test or assay, a similar instrument of equivalent or greater sensitivity or accuracy may be employed.

Where low-actinic or light-resistant containers are specified, clear glass containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

Blank Tests Where a blank determination is specified in a test or assay, it is to be conducted using the same quantities of the same reagents and by the same procedure repeated in every detail except that the substance being tested is omitted.

A *residual blank titration* may be stipulated in assays and tests involving a back titration in which a volume of a volumetric solution larger than is required to react with the sample is added, and the excess of this solution is then titrated with a second volumetric solution. Where a residual blank titration is specified or where the procedure involves such a titration, a blank is run as directed in the preceding paragraph. The volume of the titrant consumed in the back titration is then subtracted from the volume required for the blank. The difference between the two, equivalent to the actual volume consumed by the sample, is the corrected volume of the volumetric solution to be used in calculating the quantity of the substance being determined.

Centrifuge Where the use of a centrifuge is indicated, unless otherwise specified, the directions are predicated upon the use of apparatus having an effective radius of about 20 cm (8 in.) and driven at a speed sufficient to clarify the supernatant layer within 15 min. If necessary, determine the gravity by using the equation

$$g = \{[(\text{rpm} \times 2 \times \pi)/60] \times r_m\}/980,$$

in which rpm is the rotor speed and r_m is the mean radius, in centimeters, of the tube holding the sample in the rotor.

Desiccators and Desiccants The expression ''in a desiccator'' means using a tightly closed container of appropriate design in which a low moisture content can be maintained by means of a suitable desiccant. Preferred desiccants include anhydrous calcium chloride, magnesium perchlorate, phosphorus pentoxide, and silica gel.

Filtration Where it is directed to ''filter,'' without further qualification, the intent is that the liquid be filtered through suitable filter paper or an equivalent device until the filtrate is clear.

Identification The tests described under this heading in monographs are designed for application to substances taken from labeled containers and are provided only as an aid to substantiate identification. These tests, regardless of their specificity, are not necessarily sufficient to establish proof of identity, but failure of a substance taken from a labeled container to meet the requirements of a prescribed identification test means that it does not conform to the requirements of the monograph.

Indicators The quantity of an indicator solution used should be 0.2 mL (approximately 3 drops) unless otherwise directed in an assay or test.

Negligible The term ''negligible,'' as used in some *Residue on Ignition* specifications, indicates a quantity not exceeding 0.5 mg.

Odorless This term, when used in describing a flavoring material, applies to the examination, after exposure to air for 15 min, of about 25 g of the material that has been transferred quickly from the original container to an open evaporating dish of about 100-mL capacity. If the package contains 25 g or less, the entire contents should be examined.

Pressure Measurements The term ''mm Hg'' used with respect to pressure within an apparatus, or atmospheric pressure, refers to the use of a suitable manometer or barometer calibrated in terms of the pressure exerted by a column of mercury of the stated height.

Reagents Specifications for reagents are not included in the *Food Chemicals Codex*. Unless otherwise specified, reagents required in tests and assays should conform to the specifications of the current editions of *Reagent Chemicals—American Chemical Society Specifications* or in the section on Reagent Specifications in the *United States Pharmacopeia*. Reagents not covered by any of these specifications should be of a grade suitable to the proper performance of the method of assay or test involved.

Acids and Ammonium Hydroxide When ammonium hydroxide, glacial acetic acid, hydrochloric acid, hydrofluoric acid, nitric acid, phosphoric acid, or sulfuric acid is called for in tests and assays, reagents of ACS grade and strengths are to be used. (These reagents sometimes are called ''concentrated,'' but this term is not used in the *Food Chemicals Codex*.)

Alcohol, Ethyl Alcohol, Ethanol When one of these substances is called for in tests and assays, use ACS-grade Ethyl Alcohol (95%).

Alcohol Absolute, Anhydrous Alcohol, Dehydrated Alcohol When one of these substances is called for in tests and assays, use ACS-grade Ethyl Alcohol, Absolute.

Water When water is called for in tests and assays and in the preparation of solutions, it shall have been prepared by distillation, ion-exchange treatment, or reverse osmosis.

Water, Carbon Dioxide-Free When this type of water is called for, it shall have been boiled vigorously for 5 min or more, and allowed to cool while protected from absorption of carbon dioxide from the atmosphere. ''Deaerated water'' is water that has been treated to reduce the content of dissolved air by suitable means, such as by boiling vigorously for 5 min and cooling or by the application of ultrasonic vibration.

> **Note**: Certain chemical reagents specified in FCC test procedures may be considered to be hazardous or toxic by the Occupational Safety and Health Administration, by the Environmental Protection Agency (under provisions of the Toxic Substances Control Act), or by health authorities in other countries in which the *Food Chemicals Codex* is recognized. In preparing this edition, the Committee on Food Chemicals Codex has attempted to specify use of different reagents where suitable substitutes are known. For some procedures, however, the original chemicals have been retained due to the lack of information on suitable substitutes. In such cases, the analyst is encouraged to investigate the use of suitable substitute reagents, as appropriate, and to inform the committee of the results so obtained.

The methods and analytical procedures described in the Codex are designed for use by properly trained personnel in a suitably equipped laboratory. In common with many laboratory procedures, the methods quoted frequently involve hazardous materials.

In performing the assay or test procedures in the Codex, safe laboratory practices must be followed. This includes the use of precautionary measures, protective equipment, and work practices consistent with the chemicals and procedures used. Before undertaking any assay or procedures described in this compendium, the individual should be aware of the hazards associated with the chemicals and of the procedures and means of protecting against them. Material Safety Data Sheets, which contain precautionary information related to safety and health concerns, are available from manufacturers and distributors of many chemicals and should provide helpful information about the safe use of such chemicals.

Reference Standards Some instrumental and chromatographic tests and assays specify the use of a reference standard. Where a reference standard is designated as USP, it may be obtained from the United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 <http://www.usp.org>. Where a reference standard is designated as a NIST (National Institute of Standards and Technology) Standard Reference Material, it may be obtained from the Standard Reference Materials Program, NIST, 100 Bureau Drive, Stop 2322, Gaithersburg, MD 20899-2322 <http://ts.nist.gov/ts/htdocs/230/232/232.htm>.

To serve its intended purpose, each reference standard must be properly stored, handled, and used. Generally, reference standards should be stored in their original containers away from heat and protected from light. Follow any special instructions accompanying the containers.

Assay and test results are determined on the basis of comparison of the test sample with the reference standard that has been freed from or corrected for volatile residues or water content as instructed on the reference standard label. If a reference standard is required to be dried before use, transfer a sufficient amount to a clean, dry vessel. Do not use the original container as the drying vessel, and do not dry a reference standard repeatedly at temperatures above 25°. Where the titrimetric determination of water is required at the time a reference standard is to be used, proceed as directed in the *Karl Fischer Titrimetric Method* under *Water Determination,* Appendix IIB.

Unless a reference standard label bears a specific potency or content, assume the reference standard is 100.0% pure.

Significant Figures When tolerance limits are expressed numerically, the values are significant to the number of digits

indicated. Record the observed or calculated analytical result with only one digit included in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, eliminate it and leave the preceding digit unchanged. If this digit is greater than 5, eliminate it and increase the preceding digit by one. If this digit equals 5, eliminate it and increase the preceding digit by one. For example, a requirement of not less than 96.0% would not be met by a result of 95.94%, but would be met by results of 95.96% or 95.95%, both of which would be rounded to 96.0%. When a range is stated, the upper and lower limits are inclusive so that the range consists of the two values themselves, properly rounded, and all intermediate values between them.

Solutions Prepare all solutions, unless otherwise specified, with water prepared by distillation, ion-exchange treatment, reverse osmosis, or as otherwise indicated in the monograph.

Such expressions as ''1:10'' or ''10%'' mean that *1 part by volume* of a liquid or *1 part by weight* of a solid is to be dissolved in a volume of the diluent or solvent sufficient to make the finished solution 10 parts by volume. Directions for the preparation of colorimetric solutions (CS), test solutions (TS), and volumetric solutions (VS), are provided in the section on *Solutions and Indicators* under *General Tests and Assays*, following Appendix X.

Prepare a volumetric solution to have a normality (molarity) within 10% of the stated value and to be standardized to four significant figures. When volumetric equivalence factors are provided in tests and assays, the term ''0.X *N* (*M*)'' is understood to mean a VS having a normality (molarity) of exactly 0.X000 *N* (*M*). If the normality (molarity) of the VS employed in a particular procedure differs from 0.X000, apply an appropriate correction factor.

Specific Gravity Numerical values for specific gravity, unless otherwise noted, refer to the ratio of the weight of a substance in air at 25° to that of an equal volume of water at the same temperature. Determine specific gravity by any reliable method, unless otherwise specified.

Temperatures Unless otherwise specified, temperatures are expressed in centigrade (Celsius) degrees, and all measurements are to be made at 25°, unless otherwise directed.

Test Solutions See *Solutions and Indicators* under *General Tests and Assays*, following Appendix X.

Time Limits Unless otherwise specified, allow 5 min for a reaction to take place when conducting limit tests for trace impurities such as chloride or iron.

Expressions such as ''exactly 5 min'' mean that the stated period should be accurately timed.

Tolerances The minimum purity tolerances specified for FCC items have been established with the expectation that the substances to which they apply will be used as direct or indirect food additives, ingredients, or food-processing aids. These tolerance limits should neither bar the use of lots of articles that more nearly approach 100% purity nor constitute a basis for a claim that such lots exceed the quality prescribed by the *Food Chemicals Codex*.

When a maximum assay tolerance is not given, the assay should show the equivalent of not more than 100.5%.

Trace Impurities Tests for inherent trace impurities are provided to limit such substances to levels that are consistent with good manufacturing practice and that are safe and otherwise unobjectionable under conditions in which the food additive or ingredient is customarily employed.

It obviously is impossible to provide limits and tests in each monograph for the detection of all possible unusual or unexpected impurities, the presence of which would be inconsistent with good manufacturing practice. The limits and tests provided are those considered to be necessary according to currently recognized methods of manufacture and are based on information available to or provided to the Committee on Food Chemicals Codex. If other methods of manufacture or other than the usual raw materials are used, or if other possible impurities may be present, additional tests may be required and should be applied, as necessary, by the manufacturer, vendor, or user to demonstrate that the substance is suitable for its intended application.

Vacuum The unqualified use of the term ''in vacuum'' means a pressure at least as low as that obtainable by an efficient aspirating water pump (not higher than 20 mm Hg).

Water and Loss on Drying In general, for compounds containing water of crystallization or adsorbed water, a limit test, to be determined by the *Karl Fischer Titrimetric Method*, is provided under the heading *Water*. For compounds in which the loss on drying may not necessarily be attributable to water, a limit test, to be determined by other methods, is provided under the heading *Loss on Drying*.

Weighing Practices

Constant Weight A direction that a substance is to be ''dried to constant weight'' means that the drying should continue until two consecutive weighings differ by not more than 0.5 mg/g of sample taken, the second weighing to follow an additional hour of drying.

The direction ''ignite to constant weight'' means that the ignition should be continued at 800° ± 25°, unless otherwise specified, until two consecutive weighings do not differ by more than 0.5 mg/g of sample taken, the second weighing to follow an additional 15 min of ignition.

Tared Container When a tared container, such as a glass filtering crucible, a porcelain crucible, or a platinum dish, is called for in an analytical procedure, it shall be treated as is specified in the procedure. For example, dried or ignited for a specified time, or to constant weight, cooled in a desiccator as necessary, and weighed accurately.

Weights and Measures, Symbols and Abbreviations The International System of Units (SI), to the extent possible, is used in most specifications, assays, and tests in this *Food Chemicals Codex*. The SI metric units, and other units and abbreviations commonly employed, are as follows:

°	= degrees centigrade
kg	= kilogram
g	= gram

mg = milligram
μg = microgram
ng = nanogram
pg = picogram
L = liter
mL = milliliter
μL = microliter
m = meter
cm = centimeter
dm = decimeter
mm = millimeter
μm = micrometer (0.001 mm)
nm = nanometer
C = coulomb
A = ampere
V = volt
mV = millivolt
W = watt
dc = direct current
ft = foot
in. = inch
$in.^3$ = cubic inch
gal = gallon
lb = pound
oz = ounce
ppm = parts per million (10^6) parts
mg/kg = parts per million (by weight)
ppb = parts per billion (10^9) parts
ng/kg = parts per billion (by weight)
psi = pounds per square inch
sp. gr. = specific gravity
b.p. = boiling point
m.p. = melting point
id = inside diameter
od = outside diameter
h = hour
min = minute
s = second
% = percent
N = normality
M = molarity
μ*M* = micromolar
μmol = micromole
ACS = American Chemical Society
AOAC = AOAC International
AOCS = American Oil Chemists Society
ASTM = American Society for Testing and Materials
CAS = Chemical Abstracts Service
CFU = colony-forming unit(s)
FDA = United States Food and Drug Administration
FEMA = Flavor and Extract Manufacturers Association
INS = International Numbering System
NIST = National Institute of Standards and Technology
USP = United States Pharmacopeia

GENERAL SPECIFICATIONS AND STATEMENTS

Certain specifications and statements in the monographs of the *Food Chemicals Codex* are not amenable to precise description and accurate determination within narrow limiting ranges. Because of the subjective or general nature of these specifications, good judgment, based on experience, must be used in interpreting and attaching significance to them. Specifications or statements that are most likely to cause doubt are discussed in the subsequent paragraphs.

Description The material given under this heading in monographs is provided for general information. It includes a description of physical characteristics such as color and form and information on stability under certain conditions of exposure to air and light. Statements in this section may also cover approximate indications of properties such as solubility (see below) in various solvents, pH, melting point, and boiling point, with numerical values modified by ''about,'' ''approximately,'' ''usually,'' and other comparable nonspecific terms. These characteristics and statements are not requirements, but are provided as information that may assist with the overall evaluation of a food chemical. As the committee revises existing monographs and develops new ones, it will, as appropriate, include information about why certain specifications and limits are given.

Solubility Statements included in the *Requirements* section of a monograph under a heading such as *Solubility in Alcohol* express exact requirements and constitute quality specifications.

Statements relating to solubility given in the *Description*, however, are intended as information regarding approximate solubilities only and are not to be considered as Codex-quality requirements. Such statements are considered to be of minor significance as a means of identification or determination of purity. For those purposes, dependence must be placed upon other specifications.

Approximate solubilities are indicated by the following descriptive terms:

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very Soluble	less than 1
Freely Soluble	from 1 to 10
Soluble	from 10 to 30
Sparingly Soluble	from 30 to 100
Slightly Soluble	from 100 to 1000
Very Slightly Soluble	from 1000 to 10,000
Practically Insoluble or Insoluble	more than 10,000

Soluble substances, when brought into solution, may show slight physical impurities, such as fragments of filter paper, fibers, and dust particles. Unless excluded by definite tests or other requirements; however, significant amounts of black specks, metallic chips, glass fragments, or other insoluble matter are not permitted.

Function A statement of function is provided in each monograph to indicate the principal applications or technical effects of the substance in foods or in food processing. The statement is not intended to limit in any way the choice or use of the substance or to indicate that it has no other utility.

In preparing the Fifth Edition, the committee considered a number of new monographs describing substances that may, following ingestion, provide certain health benefits. The committee noted that these substances are intended to exhibit a functional effect, not on the foods to which they are added, but on the human body, in a manner similar to that of some nutrients, when that food is consumed. They also noted that these substances are products of an emerging science and that a comprehensive understanding of their beneficial effects had yet to be developed at the time their respective monographs were reviewed. The committee, nevertheless, believes that including monographs for these substances in the *Food Chemicals Codex* is consistent with sound public health policy to standardize the material and minimize possible contaminants. Accordingly, the committee selected the term, ''Source of . . . ,'' in describing the function of these materials. Including these monographs was approved, taking into account the emerging science, but extensive monograph revisions in future editions might be necessary. By including these monographs in this Fifth Edition, the committee neither judges nor endorses any potential health benefits.

Additionally, because of the current usage of the term ''dietary supplement,'' the committee replaced this term with ''nutrient.''

Packaging and Storage Statements in monographs relating to packaging and storage are advisory in character and are intended only as general information to emphasize instances where deterioration may be accelerated under adverse packaging and storage conditions, such as exposure to air, light, or temperature extremes, or where safety hazards are involved. Additionally, to reduce the risk of intentional or accidental introduction of undesirable materials into food substances, containers should be equipped with tamper-resistant closures.

Cool Place A cool place is one where the temperature is between 8° and 15° (46° and 59°F). Alternatively, it may be a refrigerator, unless otherwise specified in the monograph.

Excessive Heat Any temperature above 40° (104°F).

Storage Under Nonspecific Conditions Where no specific storage directions or limitations are provided in the individual monograph, the conditions of storage and distribution include protection from moisture, freezing, and excessive heat. Containers should be stored in secure areas when not in use to reduce the possibility of tampering.

Containers The container is the device that holds the substance and that is or may be in direct contact with it. The immediate container is in direct contact with the substance at all times. The closure is a part of the container. Closures should be tamper resistant and tamper evident.

The container should not interact physically or chemically with the material that it holds so as to alter its strength, quality, or purity, and the food additive contact surface of the container should comply with the food additive regulations promulgated under the Food, Drug, and Cosmetic Act (or with applicable laws and regulations in other countries in which FCC specifications are recognized).

Polyunsaturated fats and oils are susceptible to oxidation when stored in metal containers, at elevated temperatures, and/or in open containers. Oxidation can be minimized by storing them in closed, nonmetal containers with minimal headspace or flushed with nitrogen gas.

Light-Resistant Container A light-resistant container is designed to prevent deterioration of the contents beyond the prescribed limits of strength, quality, or purity under the ordinary or customary conditions of handling, shipment, storage, and sale. A colorless container may be made light resistant by enclosing it in an opaque carton or wrapper (see also *Apparatus*, above).

Well-Closed Container A well-closed container protects the contents from extraneous solids and from loss of the chemical under the ordinary or customary conditions of handling, shipment, storage, and sale.

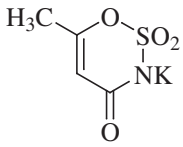
Tight Container A tight container protects the contents from contamination of extraneous liquids, solids, or vapors; from loss of the chemical; and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, and sale, and is capable of tight reclosure.

Product Security Tamper-evident packaging closures and security tags should be used. Containers that appear to have been opened or otherwise altered by unauthorized persons should not be used until the purity of the substance has been confirmed.

2 / *Monographs*

Acesulfame Potassium

Acesulfame K; 6-Methyl-1,2,3-oxathiazine-4(3H)-one-2,2 Dioxide Potassium Salt



$C_4H_4KNO_4S$ Formula wt 201.24

INS: 950 CAS: [55589-62-3]

DESCRIPTION

Acesulfame Potassium occurs as a white, free-flowing crystalline powder. It is freely soluble in water and very slightly soluble in ethanol.

Function Nonnutritive sweetener; flavor enhancer.

REQUIREMENTS

Identification

A. Add a few drops of sodium cobaltinitrite TS to a solution of 0.3 g of sample in 1 mL of glacial acetic acid and 5 mL of water. A yellow precipitate forms.

B. Dissolve 10 mg of sample in 1000 mL of water. The solution shows an absorption maximum at 227 ± 2 nm.

Assay Not less than 99.0% and not more than 101.0% of $C_4H_4KNO_4S$ after drying.

Fluoride Not more than 3 mg/kg.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 1.0%.

Organic Impurities Not more than 20 μg/g of UV-active compounds.

pH of a 1:100 Solution Between 5.5 and 7.5.

TESTS

Assay Dissolve between 200 and 300 mg of sample, previously dried at 105° for 2 h and accurately weighed, in 50 mL of glacial acetic acid contained in a 250-mL flask.

Note: Dissolution may be slow.

Add 2 or 3 drops of crystal violet TS to the flask, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint that persists for at least 30 s.

Caution: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 20.12 mg of $C_4H_4KNO_4S$.

Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB, using a 4-g sample.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 2 g of sample in 20 mL of water, and 2 μg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Organic Impurities

Reference Standard 4-Hydroxybenzoic acid ethyl ester.

Sample Solution A solution of 10 g of sample per liter of deionized water.

Procedure (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph capable of separating acesulfame potassium and 4-hydroxybenzoic acid ethyl ester with a resolution of 2. Use a chromatograph equipped with a UV or diode array (227 nm) detector and a 25-cm × 4.6-mm (id) stainless steel column, or equivalent, packed with 3 to 5 μm of reversed phase C18 silica gel, or equivalent. The elution is isocratic. Use a 40:60 (v/v) solution of acetonitrile:0.01 *M*/L tetrabutyl ammonium hydrogen sulfate (TBAHS) in water as the mobile phase, with a flow rate of about 1 mL/min.

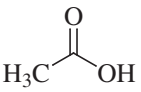
Inject 20 μL of *Sample Solution* into the chromatograph, and obtain the chromatogram. If peaks other than that caused by acesulfame potassium appear within three times the elution time of acesulfame potassium, carry out a second run using 20 μL of *Sample Solution* diluted to 0.2 mg/L.

The sum of the areas of all peaks eluted in the first run within three times the elution time of acesulfame potassium, except for the acesulfame potassium peak, does not exceed the peak area of acesulfame potassium in the second run.

pH of a 1:100 Solution Determine as directed under *pH Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers in a cool, dry place.

Acetic Acid, Glacial



$C_2H_4O_2$ Formula wt 60.05

INS: 260 CAS: [64-19-7]

FEMA: 2006

DESCRIPTION

Acetic Acid, Glacial, occurs as a clear, colorless liquid. It boils at about 118°. When well diluted with water (e.g., 1:100), it has a vinegar odor and taste. It is miscible with water, with alcohol, and with glycerin.

Function Acidifier; flavoring agent.

REQUIREMENTS

Identification A 1:3 aqueous solution gives positive tests for *Acetate*, Appendix IIIA.
Assay Not less than 99.5% and not more than 100.5%, by weight, of $C_2H_4O_2$.
Lead Not more than 0.5 mg/kg.
Nonvolatile Residue Not more than 0.005%.
Readily Oxidizable Substances Passes test.
Solidification Point Not cooler than 15.6°.

TESTS

Assay Transfer about 2 mL of sample into a tared, glass-stoppered flask, and accurately weigh. Add 40 mL of water, then add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sodium hydroxide is equivalent to 60.05 mg of $C_2H_4O_2$.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Nonvolatile Residue Evaporate 19 mL (20 g) of sample, accurately measured, in a tared dish on a steam bath, and dry at 105° for 1 h.
Readily Oxidizable Substances Dilute 2 mL of sample in a glass-stoppered container with 10 mL of water, and add 0.1 mL of 0.1 *N* potassium permanganate. The pink color does not change to brown within 2 h.
Solidification Point Determine as directed under *Solidification Point*, Appendix IIB.

Packaging and Storage Store in tightly closed containers.

Acetone

2-Propanone; Dimethyl Ketone

CH_3COCH_3

C_3H_6O Formula wt 58.08

CAS: [67-64-1]

DESCRIPTION

Acetone occurs as a clear, colorless, volatile liquid. It is miscible with water, with alcohol, with ether, with chloroform, and with most volatile oils.

Caution: Acetone is highly flammable.

Function Extraction solvent.

REQUIREMENTS

Identification Mix 0.1 mL of sample with 10 mL of water, add 5 mL of 1 *N* sodium hydroxide, warm, and add 5 mL of iodine TS. A yellow precipitate of iodoform forms.
Assay Not less than 99.5% and not more than 100.5% of C_3H_6O, by weight.
Acidity (as acetic acid) Not more than 0.002%.
Aldehydes (as formaldehyde) Not more than 0.002%.
Alkalinity (as ammonia) Not more than 10 mg/kg.
Distillation Range Within a range of 1°, including 56.1°.

Lead Not more than 1 mg/kg.
Methanol Not more than 0.05%.
Nonvolatile Residue Not more than 10 mg/kg.
Phenols Passes test.
Refractive Index Between 1.358 and 1.360.
Solubility in Water Passes test.
Specific Gravity Not greater than 0.7880 at 25°/25° (equivalent to 0.7930 at 20°/20°).
Substances Reducing Permanganate Passes test.
Water Not more than 0.5%.

TESTS

Assay Transfer about 1 g of sample, accurately weighed, into a 1000-mL flask containing 20 mL of water, and dilute to volume with water. Place 10 mL of this solution into a glass-stoppered flask, add 25 mL of sodium hydroxide TS, and allow the mixture to stand for 5 min. Add 25 mL of 0.1 *N* iodine, stopper the flask, allow the contents to stand in a cold, dark place for 10 min, and add 30 mL of 1 *N* sulfuric acid. Titrate the excess iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* iodine is equivalent to 0.9675 mg of C_3H_6O.
Acidity (as acetic acid) Mix 38 mL of sample with an equal volume of carbon dioxide-free water, add 0.1 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Not more than 0.1 mL is required to produce a pink color.
Aldehydes (as formaldehyde) Prepare a *Sample Solution* by diluting 2.5 mL of sample with 7.5 mL of water. Prepare a *Standard Solution* containing 40 µg of formaldehyde in 10 mL of water. Add 0.15 mL of a 5% solution of 5,5-dimethyl-1,3-cyclohexanedione in alcohol to each solution, and evaporate on a steam bath until the Acetone is volatilized. Dilute to 10 mL with water, and cool quickly in an ice bath while stirring vigorously. Any turbidity produced in the *Sample Solution* does not exceed that produced in the *Standard Solution*.
Alkalinity (as ammonia) Add 1 drop of methyl red TS to 25 mL of water, add 0.1 *N* sulfuric acid until a red color just appears, then add 23 mL of sample, and mix. Not more than 0.1 mL of 0.1 *N* sulfuric acid is required to restore the red color.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Methanol Prepare a *Sample Solution* by diluting 10 mL of sample to 100 mL with water. Prepare a *Standard Solution* in water containing 40 µg of methanol in each milliliter. Add 0.2 mL of 10% phosphoric acid and 0.25 mL of 1:20 potassium permanganate solution to 1 mL of each solution. Allow the mixtures to stand for 15 min, then add 0.3 mL of 1:10 sodium bisulfite solution to each, and shake until colorless. Slowly add 5 mL of ice-cold 80% sulfuric acid, keeping the mixtures cold during the addition. Add 0.1 mL of 1:100 chromotropic acid solution, mix, and digest on a steam bath for 20 min. Any violet color produced in the *Sample Solution* does not exceed that produced in the *Standard Solution*.
Nonvolatile Residue Evaporate 125 mL (about 100 g) of sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.
Phenols Evaporate 3 mL of sample to dryness at 60°. Add 3 drops of a solution of 100 mg of sodium nitrite in 5 mL of sulfuric acid to the residue, allow the mixture to stand for about 3 min, and then carefully add 3 mL of 2 *N* sodium hydroxide. No color appears.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Water Mix 38 mL of sample with an equal volume of carbon dioxide-free water. The solution remains clear for at least 30 min.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Substances Reducing Permanganate Transfer 10 mL of sample into a glass-stoppered cylinder, add 0.05 mL of 0.1 *N* potassium permanganate, mix, and allow to stand for 15 min. The pink color does not entirely disappear.
Water Determine as directed under *Water Determination*, Appendix IIB, using freshly distilled pyridine instead of methanol as the solvent.

Packaging and Storage Store in tight containers remote from fire.

Acetone Peroxides

INS: 929 CAS: [1336-17-0]

DESCRIPTION

Acetone Peroxides, usually mixed with an edible carrier such as cornstarch, occur as a fine, white, free-flowing powder. They are a mixture of monomeric and linear dimeric acetone peroxides (mainly 2,2-hydroperoxypropane), with minor proportions of higher polymers.

> **Caution**: Acetone Peroxides are strong oxidizing agents. Avoid exposure to the skin and eyes.

Function Bleaching agent; maturing agent; dough conditioner.

REQUIREMENTS

Identification Dissolve about 20 mg of sample in 5 mL of 1:10 sulfuric acid, allow to stand for a few minutes, and add a drop of potassium permanganate TS. The pink color disappears.
Assay A sample yields an amount of hydrogen peroxide equivalent to not less than 16.0% of Acetone Peroxides.
Lead Not more than 4 mg/kg.

TESTS

Assay Transfer about 200 mg of sample, accurately weighed, into a 250-mL beaker, add 50 mL of 1:10 sulfuric acid, allow to stand for at least 3 min, stirring occasionally, and titrate with 0.1 *N* potassium permanganate to a light pink color that persists for at least 20 s. Calculate the total peroxides, *P*, as grams of hydrogen peroxide equivalents per 100 g of the sample, by the equation

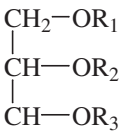
$$P = V \times N \times 0.017 \times 100/W,$$

in which *V* and *N* are the volume and exact normality, respectively, of the potassium permanganate; 0.017 is the milliequivalent weight of hydrogen peroxide; and *W* is the weight, in grams, of the sample taken. Multiply the value *P* so obtained by 1.6 to convert to percent Acetone Peroxides.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 4 µg of lead (Pb) ion in the control.

Packaging and Storage Store in tightly closed containers in a cool, dry place, preferably below 24°.

Acetylated Monoglycerides

Acetylated Mono- and Diglycerides; Acetic and Fatty Acid Esters of Glycerol; Acetoglycerides



in which R_1, R_2, and R_3 each may be a fatty acid moiety, $COCH_3$, or H. At least one R must be a fatty acid, and at least one must be $COCH_3$.

INS: 472a

DESCRIPTION

Acetylated Monoglycerides occur as clear, thin liquids or solids, ranging in color from white to pale yellow. They consist of partial or complete esters of glycerin with a mixture of acetic acid and edible fat-forming fatty acids. They may be manufactured by the interesterification of edible fats with triacetin and glycerin in the presence of catalytic agents, followed by molecular distillation, or by the direct acetylation of edible monoglycerides with acetic anhydride and without the use of a catalyst or molecular distillation. They are insoluble in water, but are soluble in alcohol, in acetone, and in other organic solvents, the extent of solubility depending on the degree of esterification and the melting range.

Function Emulsifier; coating agent; texture-modifying agent; solvent; lubricant.

REQUIREMENTS

Acid Value Not more than 6.
Lead Not more than 2 mg/kg.
Reichert-Meissl Value Between 75 and 200.

The following specifications should conform to the representations of the vendor: *Free Glycerin*, *Iodine Value*, and *Saponification Value*.

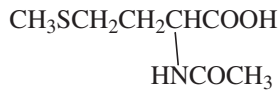
TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
Free Glycerin Determine as directed under *Free Glycerin or Propylene Glycol*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Reichert-Meissl Value Determine as directed under *Reichert-Meissl Value*, Appendix VII.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Packaging and Storage Store in well-closed containers.

N-Acetyl-L-Methionine

N-Acetyl-L-2-amino-4-(methylthio)butyric Acid



$C_7H_{13}NO_3S$ Formula wt 191.25

CAS: [65-82-7]

View IR

DESCRIPTION

N-Acetyl-L-Methionine occurs as a colorless or lustrous, white, crystalline solid or a white powder. It is soluble in water, in alcohol, in alkali solutions, and in dilute mineral acids, but practically insoluble in ether.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those

of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_7H_{13}NO_3S$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.5%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between –18.0° and –22.0° after drying.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 250 mg of the sample, accurately weighed, into a glass-stoppered flask, and add 100 mL of water, 5 g of dibasic potassium phosphate, 2 g of monobasic potassium phosphate, and 2 g of potassium iodide. Mix well to dissolve, add 50.0 mL of 0.1 *N* iodine, stopper the flask, and mix. Allow to stand for 30 min, add starch TS indicator, and then titrate the excess iodine with 0.1 *N* sodium thiosulfate. Perform a residual blank titration. Each milliliter of 0.1 *N* iodine is equivalent to 9.563 mg of $C_7H_{13}NO_3S$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Dry at 105° for 2 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 2 g of the previously dried sample in sufficient water to make 100 mL.
Residue on Ignition Ignite 1 g as directed in *Method I* under *Residue on Ignition (Sulfated Ash)*, Appendix IIC.

Packaging and Storage Store in tightly closed, light-resistant containers.

Acid Hydrolysates of Proteins

Acid-Hydrolyzed Proteins; Hydrolyzed Vegetable Protein (HVP); Hydrolyzed Plant Protein (HPP); Hydrolyzed (Source) Protein Extract; Acid-Hydrolyzed Milk Protein

DESCRIPTION

Acid Hydrolysates of Proteins occur as liquids, pastes, powders, or granules. They are composed primarily of amino acids, small peptides (peptide chains of five or fewer amino acids), and salts resulting from the essentially complete hydrolysis of peptide bonds in edible proteinaceous materials, catalyzed by food-grade acids and/or heat. Cleavage of peptide bonds typically ranges from a low of 85% to essentially 100%. In processing, the protein hydrolysates may be treated with safe and suitable alkaline materials. The edible proteinaceous materials used as raw materials are derived from corn, soy, wheat, yeast, peanuts, rice, or other safe and suitable vegetable or plant sources, or from milk.

Function Flavoring agent; flavor enhancer.

REQUIREMENTS

Note: Perform all calculations on the dried basis. Evaporate liquid and paste samples to dryness in a suitable tared container; then, as for the powdered and granular forms, dry to constant weight at 105° (see *General Provisions*).

Assay (Total Nitrogen; TN) Not less than 4.0%.
α-Amino Nitrogen (AN) Not less than 3.0%.
α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Not less than 62.0% and not more than 85.0% when calculated on an ammonia nitrogen-free basis.
Ammonia Nitrogen (NH_3-N) Not more than 1.5%.
3-Chloropropane-1,2-diol (3-CPD) Not more than 1 mg/kg.
1,3-Dichloro-2-propanol (DCP) Not more than 0.05 mg/kg.
Glutamic Acid Not more than 20.0% as glutamic acid ($C_5H_9NO_4$) and not more than 35.0% of the total amino acids.
Insoluble Matter Not more than 0.5%.
Lead Not more than 3 mg/kg.
Potassium Not more than 30.0%.
Sodium Not more than 20.0%.

TESTS

Assay (Total Nitrogen; TN) Determine as directed under *Nitrogen Determination*, Appendix IIIC.
α-Amino Nitrogen (AN) Determine as directed under α-*Amino Nitrogen Determination*, Appendix IIIC.
α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Calculate by dividing the percent of α-amino nitrogen (*AN*) by the percent of total nitrogen (*TN*) as corrected for ammonia nitrogen (NH_3-*N*) according to the formula

$$100[(AN - NH_3\text{-}N)/(TN - NH_3\text{-}N)].$$

Ammonia Nitrogen (NH_3-N) Determine as directed under *Ammonia Nitrogen*, Appendix IIIC.
3-Chloropropane-1,2-diol (3-CPD)

3-CPD Stock Solution Transfer 12.5 mg of reagent-grade 3-chloropropane-1,2-diol (3-CPD), accurately weighed, into a 100-mL volumetric flask, dilute to volume with ethyl acetate, and mix.

Dilute 3-CPD Solution Dilute 5 mL of *3-CPD Stock Solution* to 100 mL with ethyl acetate to yield a solution containing 6.25 µg/mL of 3-CPD.

Internal Standard Solution Transfer 50 mg of 1-chlorotetradecane into a 50-mL volumetric flask, and dilute to volume with ethyl acetate. Dilute 1 mL of this solution to 100 mL with ethyl acetate to yield a solution containing 10 µg/mL of 1-chlorotetradecane.

Standard Solutions

A. Pipet 2 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask,

dilute to volume with ethyl acetate, and mix. The resulting *Standard Solution A* contains 0.5 μg/mL of 3-CPD.

B. Pipet 8 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask, dilute to volume with ethyl acetate, and mix. The resulting *Standard Solution B* contains 2.0 μg/mL of 3-CPD.

C. Pipet 16 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask, dilute to volume with ethyl acetate, and mix. The resulting *Standard Solution C* contains 4.0 μg/mL of 3-CPD.

Procedure (See *Chromatography*, Appendix IIA) Use a gas chromatograph equipped with an electrolytic conductivity detector operated in the halogen mode and fitted either with a capillary injector operated in the splitless mode or with a purged, packed injector with a glass insert. Use a 30-m × 0.53-mm (id), fused-silica column, or equivalent, coated with 1-μm Supelcowax 10 or an equivalent bonded carbowax column fitted with a 50-cm retention gap of 0.53-mm, deactivated, fused silica, or equivalent. Set the column temperature to 170° for 5 min, raise the temperature at a rate of 5°/min to 250°, and hold it at that temperature for 10 min. Maintain the injector temperature at 225°. Use helium as the carrier gas at a flow rate of 8 mL/min.

Use hydrogen as the reactant gas at a flow rate of 30 mL/min, and use 1-propanol as the solvent at a flow rate through the cell of 0.5 mL/min or at the manufacturer's specified flow rate for the optimum operation of the electrolytic conductivity detector. The reactor temperature should be 900°, with a base temperature of 275°. Minimize contamination of the reaction tube by venting flow from the column at all times, except for the time during which compounds of interest elute.

Inject 1 μL of each *Standard Solution A*, *B*, and *C*, into the gas chromatograph. Calculate the response area ratios of 3-CPD to the *Internal Standard Solution* for each *Standard Solution*. Plot the response area ratios versus the micrograms of 3-CPD in each *Standard Solution* to obtain the standard curve.

Adjust an accurately weighed sample, as needed, with 20% aqueous sodium chloride to obtain a *Sample Solution* with a solids content of 36%. Transfer a 20-g aliquot of the *Sample Solution* into a 20-mL Extrelut NT column (EM Science, Gibbstown, NJ), or equivalent, and allow it to equilibrate for 15 min. Elute the column with 150 mL of ethyl acetate, collecting the eluent in a 250-mL short-neck, round-bottom flask with a 24/40 joint. Using a rotary evaporator at 50°, concentrate the eluent to a volume of approximately 3 mL. Add 0.5 mL of *Internal Standard Solution* to the eluent, transfer this mixture to a 4-dram screw-cap vial, and dilute to a volume of 5.0 mL. Inject 1 μL into the gas chromatograph, measure its response area ratio of 3-CPD to the *Internal Standard Solution*, and determine from the standard curve the micrograms of 3-CPD in the 20-g aliquot taken.

1,3-Dichloro-2-propanol (DCP)

Eluent Transfer 850 mL of chromatographic-grade pentane and 150 mL of chromatographic-grade diethyl ether into a suitable container, and mix well.

DCP Stock Solution Transfer 50 mg of reagent-grade 1,3-dichloro-2-propanol (DCP), accurately weighed, into a 50-mL volumetric flask, dilute to volume with *Eluent*, and mix.

Dilute DCP Solution Stepwise and quantitatively dilute the *DCP Stock Solution* with *Eluent* to obtain a final solution containing 1 μg/mL of DCP.

Internal Standard Solution Transfer 50 mg of trichlorobenzene into a 50-mL volumetric flask, dilute to volume with *Eluent*, and mix. Use a 1:1000 dilution of this solution as the *Internal Standard Solution*.

Standard Solutions Pipet 1-, 2-, 3-, and 4-mL portions of *Dilute DCP Solution* into separate 50-mL volumetric flasks. Add 1.0 mL of *Internal Standard Solution* to each, dilute to volume with *Eluent*, and mix.

Sample Preparation Dissolve 5.0 g of sample, accurately weighed, in a minimal volume of 20% aqueous sodium chloride solution. Quantitatively transfer this solution to an Extrelut NT column (EM Science, Gibbstown, NJ), or equivalent. After 15 min, elute the column with three 20-mL portions of *Eluent*, and collect all the eluate. Carefully evaporate the eluate to less than 4 mL. Add 1.0 mL of *Internal Standard Solution*, and dilute with *Eluent*, as necessary, to bring the final volume to 5.0 mL.

Procedure (See *Chromatography*, Appendix IIA) Use a gas chromatograph equipped with a split injector and an electron-capture detector and fitted with a 50-m × 0.2-mm (id), fused-silica column (Carbowax 20M, or equivalent) coated with dimethylpolysiloxane, or equivalent. Use nitrogen as the carrier gas at a flow rate of 8 mL/min. Before use, precondition the column by heating it at 200° and the detector at 300° for 24 h. Set the injector temperature at 250° and the electron-capture detector at 300°, and program the column temperature as follows: Maintain for 10 min at 115°, raise rapidly at 30°/min to 200°, and maintain at 200° for 12 min.

Inject 1.0 μL of each of the four *Standard Solutions* into the gas chromatograph. For each *Standard Solution*, calculate the response area ratios of DCP to the *Internal Standard Solution*. Plot the response area ratios versus the micrograms of DCP in each *Standard Solution* to obtain the standard curve.

Similarly, inject 1.0 μL of *Sample Preparation*. Measure its response ratio, and determine from the standard curve the micrograms of DCP in the sample taken.

Glutamic Acid Determine as directed under *Glutamic Acid*, Appendix IIIC.

Insoluble Matter Transfer about 5 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, add 75 mL of water, cover the flask with a watch glass, and boil gently for 2 min. Filter the solution through a tared filtering crucible, dry at 105° for 1 h, cool, and weigh.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB using a *Sample Solution* prepared as directed for organic compounds, and 3 μg of lead (Pb) ion in the control.

Potassium

Standard Solution Transfer 38.20 mg of reagent-grade potassium chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with deionized water, and mix. Transfer 5.0 mL of this solution into a 1000-

mL volumetric flask, dilute to volume with deionized water, and mix. Each milliliter contains 1.0 μg of potassium (K).

Sample Solution Transfer 1.00 ± 0.05 g of previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 1000-mL volumetric flask. Wash the filter paper with hot water, dilute to volume, and mix. Use a 1:300 aqueous dilution as the *Sample Solution*.

Spectrophotometer Use any suitable atomic absorption spectrophotometer.

Procedure Determine the absorbance of each solution at 766.5 nm, following the manufacturer's instructions for optimum operation of the spectrophotometer. The absorbance of the *Sample Solution* does not exceed that of the *Standard Solution*.

Sodium

Standard Solution Transfer 25.42 mg of reagent-grade sodium chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with deionized water, and mix. Transfer 5.0 mL of this solution into a 1000-mL volumetric flask, dilute to volume with deionized water, and mix. Each milliliter of the final *Standard Solution* contains 0.5 μg of sodium (Na). Prepare a 1:100 aqueous dilution of this solution to obtain the final working *Standard Solution*.

Sample Solution Transfer 1.00 ± 0.05 g of previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 100-mL volumetric flask. Wash the filter paper with hot water, dilute to volume, and mix. Prepare a 1:4000 aqueous dilution of this solution to obtain the final *Sample Solution*.

Spectrophotometer Use any suitable atomic absorption spectrophotometer.

Procedure Determine the absorbance of each solution at 589.0 nm, following the manufacturer's instructions for optimum operation of the spectrophotometer. The absorbance of the *Sample Solution* does not exceed that of the *Standard Solution*.

Packaging and Storage Store in well-closed containers.

Acidified Sodium Chlorite Solutions

DESCRIPTION

Acidified Sodium Chlorite (ASC) Solutions occur as clear, colorless to pale-yellow liquids. The ASC Solutions are equilibrium mixtures of sodium chlorite ($NaClO_2$) and chlorous acid ($HClO_2$). ASC Solutions are produced by lowering the pH of a sodium chlorite solution with a safe and suitable acid to achieve a pH within the range 2.3 to 3.9 depending on the intended use.

Function Antimicrobial agent in processing water used to spray, dip, rinse, or store food before processing, to be followed by rinsing in potable water or by blanching, cooking, or canning; sanitizer for hard surfaces; broad-spectrum bactericide, virucide, fungicide, and sporicide.

REQUIREMENTS

Lead Not more than 1 mg/kg.
Mercury Not more than 1 mg/kg.
pH Between 2.3 and 3.9.

> **Note**: The pH is chosen depending on the application; it controls the concentration of metastable chlorous acid, which rapidly breaks down into chlorine dioxide, chloride, and in some applications, chlorate.
>
> **Caution**: To minimize the evolution of hazardous chlorine dioxide gas, do not adjust the pH below 2.3.

Sodium Chlorite Between 40 and 1200 ppm, depending on the application.

TESTS

Lead Determine as directed under *Lead Limit* Test, Appendix IIIB, using a 1.0-mL portion of sample mixed with 5 mL of water and 11 mL of 2.7 *N* hydrochloric acid, and 10 μg of lead (Pb) ion in the control.

Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB, using the following as the *Sample Preparation*: Transfer 2.0 mL of sample into a 50-mL beaker; add 10 mL of water, 1 mL of 1:5 sulfuric acid, and 1 mL of a 1:25 potassium permanganate solution; cover with a watch glass; boil for a few seconds; and cool.

pH Determine as directed under *pH Determination*, Appendix IIB.

Sodium Chlorite (**21** *CFR* 173.325; ''Determination of Sodium Chlorite: 50 ppm to 1500 ppm,'' Alcide Corporation)

Sample For solutions containing 40 to 250 ppm, use a 100-g sample; for those containing 250 to 500 ppm, use a 50-g sample; for those containing 500 to 1100 ppm, use a 20-g sample; for those containing 1100 to 1500 ppm, use a 15-g sample.

Procedure Transfer the sample into a tared 250-mL Erlenmeyer flask, and record the weight to the nearest 0.1 mg. Add a magnetic stirring bar. Add approximately 2 g of potassium iodide, place the flask over a magnetic stirrer, and stir until the potassium iodide crystals dissolve (about 1 min). Add 1 mL of 6 *N* hydrochloric acid, and stir for 30 s. While continuously stirring, titrate the liberated iodine with standardized 0.025 *N* sodium thiosulfate ($Na_2S_2O_3$). When most of the brown iodine color has faded, add 2 mL of starch indicator solution, and titrate to a clear endpoint, allowing adequate mixing time between additions of titrant near the endpoint. Record the volume of titrant, *V*, in milliliters.

Calculation Calculate the amount of Sodium Chlorite, in parts per million, by the equation

ppm of Sodium Chlorite = $(V \times N \times 90.44 \times 1000)/(W \times 4)$,

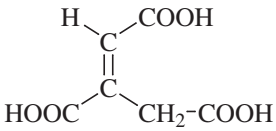
in which V is the volume, in milliliters, of titrant; N is the normality of the sodium thiosulfate titrant; 90.44 is the molecular weight of Sodium Chlorite; 1000 is a conversion factor from milligrams per gram to parts per million; W is the weight, in grams, of the sample; and 4 is the milliequivalents of sodium thiosulfate per milliequivalent of Sodium Chlorite.

Alternative Methods The concentration of Sodium Chlorite also can be determined using ion chromatography by following U.S. Environmental Protection Agency Method 300.1[1] or amperometrically by following American Public Health Association Method 4500-ClO_2.[2]

Packaging and Storage Store in closed, opaque containers. Avoid exposure to sun or ultraviolet light because chlorine dioxide gas will generate in the solution.

Aconitic Acid

Equisetic Acid; Citridic Acid; Achilleic Acid; 1-Propene-1,2,3-tricarboxylic Acid



$C_6H_6O_6$ Formula wt 174.11

CAS: [499-12-7]

FEMA: 2010

DESCRIPTION

Aconitic Acid occurs in the leaves and tubers of *Aconitum napellus* L. (Fam. Ranunculaceae) and various species of *Achillea* and *Equisetum*, in beet root, and in sugar cane. It may be synthesized by the dehydration of citric acid by sulfuric or methanesulfonic acid. Aconitic Acid from the above sources has the ''*trans*'' configuration. It has a melting point of 195° to 200° with decomposition. It is practically odorless and has a winy taste. It is soluble in water and in alcohol and is slightly soluble in ether.

Function Flavoring substance and adjuvant.

REQUIREMENTS

Identification The infrared spectrum of the sample, determined neat as a potassium bromide dispersion, exhibits infrared absorption bands at 3030, 2630, and 1720 cm^{-1}. An aqueous solution of the substance exhibits major absorption peaks at 411 and 432 nm, with little or no absorption at 389 nm.
Assay Not less than 98.0% and not more than 100.5% of $C_6H_6O_6$, calculated on the anhydrous basis.
Lead Not more than 0.5 mg/kg.
Oxalate Passes test.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.1%.
Water Not more than 0.5%.

TESTS

Assay Dissolve about 3 g of sample, accurately weighed, in 40 mL of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sodium hydroxide is equivalent to 58.04 mg of $C_6H_6O_6$.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Oxalate Neutralize 10 mL of a 1:10 aqueous solution with 6 *N* ammonium hydroxide, add 5 drops of 2.7 *N* hydrochloric acid, cool, and add 2 mL of calcium chloride TS. No turbidity develops.
Readily Carbonizable Substances Transfer 1.0 g of sample, finely powdered, into a 22- × 175-mm test tube previously rinsed with 10 mL of 95% sulfuric acid and allowed to drain for 10 min. Add 10 mL of 95% sulfuric acid, agitate the tube until solution is complete, and immerse the tube in a water bath at 90° ± 1° for 60 ± 0.5 min, keeping the level of the acid below the level of the water during the heating period. Cool the tube in a stream of water, and transfer the acid solution into a color comparison tube. The color of the acid solution is not darker than that of the same volume of *Matching Fluid K* (see *Readily Carbonizable Substances*, Appendix IIB) in a similar matching tube, viewing the tubes vertically against a white background.
Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 4-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tightly closed containers.

[1]Hautman, Daniel P. and Munch, David J. ''Method 300.1: Determination of inorganic anions in drinking water by ion chromatography, Revision 1.0.'' U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water. 1997. Online. Available: <http://www.epa.gov/OGWDW/methods/sourcalt.html> [accessed November 18, 2002].

[2]Franson, MA, ed. 1998. Standard methods 4500-ClO_2, amperometric method II. In: *Standard Methods for the Examination of Water and Wastewater*, 20th Ed. Baltimore, MD: APHA/AWWA/WEF. Pp. 4-73–4-79.

Adipic Acid

Hexanedioic Acid; 1,4-Butanedicarboxylic Acid

$HOOC(CH_2)_4COOH$

$C_6H_{10}O_4$ Formula wt 146.14

INS: 355 CAS: [124-04-9]

DESCRIPTION

Adipic Acid occurs as white crystals or a crystalline powder. It is not hygroscopic. It is freely soluble in alcohol, soluble in acetone, and slightly soluble in water.

Function Buffer; neutralizing agent.

REQUIREMENTS

Assay Not less than 99.6% and not more than 101.0% of $C_6H_{10}O_4$, calculated on the anhydrous basis.
Lead Not more than 2 mg/kg.
Melting Range Between 151.5° and 154°.
Residue on Ignition Not more than 0.002%.
Water Not more than 0.2%.

TESTS

Assay Mix about 1.5 g of sample, accurately weighed, with 75 mL of recently boiled and cooled water contained in a 250-mL glass-stoppered Erlenmeyer flask, add phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a faint pink endpoint that persists for at least 30 s, shaking the flask as the endpoint is approached. Each milliliter of 0.5 *N* sodium hydroxide is equivalent to 36.54 mg of $C_6H_{10}O_4$.
Lead Determine as directed in *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Transfer 100.0 g of sample into a tared 125-mL platinum dish that has been previously cleaned by fusing with 5 g of potassium pyrosulfate or bisulfate, followed by boiling in 2 *N* sulfuric acid and rinsing with water. Melt the sample completely over a gas burner, then ignite the melt with the burner. After ignition starts, lower or remove the flame to prevent the sample from boiling and to keep it burning slowly until it is completely carbonized. Ignite at 850° in a muffle furnace for 30 min or until the carbon is completely removed, then cool and weigh.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Agar

INS: 406 CAS: [9002-18-0]

DESCRIPTION

Agar is commercially available as white to pale yellow bundles consisting of thin, membranous agglutinated strips, or in cut, flaked, granulated, or powdered forms. Agar is a generic name given to a group of related molecules with a repeating unit of agarobiose formed basically by D- and L-galactose units interlinked with α-1,3 and β-1,4 linkages. Approximately every tenth D-galactopyranose unit contains a sulfate ester group. It is extracted from the cellular walls of agarophyte seaweed, considering as such the red seaweed from phylum Rodophyta, which belong to the Gelidiceae, Gracilariaceae, and Ahnpheltiaceae families. It is insoluble in cold water, but it is soluble in boiling water.

Function Stabilizer; emulsifier; thickener.

REQUIREMENTS

Identification

A. Place a few fragments of unground sample or a small amount of the powder on a slide, add a few drops of water, and examine microscopically. The sample appears granular and somewhat filamentous. A few fragments of the spicules of sponges and a few frustules of diatoms may be present.

B. While stirring continuously, boil 1 g of sample with 65 mL of water for 10 min, and adjust to a concentration of 1.5%, by weight, with hot water. A clear liquid results that congeals between 32° and 39° to form a firm, resilient gel that does not liquefy below 85°.
Arsenic Not more than 3 mg/kg.
Ash (Acid-Insoluble) Not more than 0.5%, calculated on the dried basis.
Ash (Total) Not more than 6.5%, calculated on the dried basis.
Gelatin Passes test.
Insoluble Matter Not more than 1.0%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 20.0%.
Starch Passes test.
Water Absorption Passes test.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Ash (Acid-Insoluble) Determine as directed under *Ash (Acid-Insoluble)*, Appendix IIC.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Gelatin Dissolve about 1 g of sample in 100 mL of boiling water, and allow the solution to cool to about 50°. Add 5 mL

of trinitrophenol TS to 5 mL of the solution. No turbidity forms within 10 min.
Insoluble Matter Add sufficient water to 7.5 g of sample to make 500 g, boil for 15 min, and readjust to the original weight. Add hot water to 100 g of the mixture to make 200 mL, heat almost to boiling, filter while hot through a tared filtering crucible, rinse the container with several portions of hot water, and pass the rinsings through the crucible. Dry the crucible and its contents at 105° to constant weight, cool, and weigh. The weight of the residue does not exceed 15 mg.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 5 h. Cut unground sample into 2- to 5-mm square pieces before drying.
Starch Boil 100 mg of sample in 100 mL of water, cool, and add a few drops of iodine TS. No blue color appears.
Water Absorption Place 5 g of sample into a 100-mL graduated cylinder, fill to volume with water, mix, and allow to stand at about 25° for 24 h. Pour the contents of the cylinder through moistened glass wool, allowing the water to drain into another 100-mL graduated cylinder. Not more than 75 mL of water is obtained.

Packaging and Storage Store in well-closed containers.

DL-Alanine

DL-2-Aminopropanoic Acid

$CH_3CH(NH_2)COOH$

$C_3H_7NO_2$ Formula wt 89.09

CAS: [302-72-7]

View IR

DESCRIPTION

DL-Alanine occurs as a white crystalline powder. It is freely soluble in water, but sparingly soluble in alcohol. The pH of a 1:20 aqueous solution is between 5.5 and 7.0. It melts with decomposition at about 198°. It is optically inactive.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_3H_7NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint.

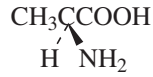
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 8.909 mg of $C_3H_7NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

L-Alanine

L-2-Aminopropanoic Acid



$C_3H_7NO_2$ Formula wt 89.09

CAS: [56-41-7]

View IR

DESCRIPTION

L-Alanine occurs as a white crystalline powder. It is freely soluble in water, sparingly soluble in alcohol, and insoluble in ether. The pH of a 1:20 aqueous solution is between 5.5 and 7.0.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_3H_7NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.

Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +13.5° and +15.5°, calculated on the dried basis; or $[\alpha]_D^{25°}$: between +13.2° and +15.2°, calculated on the dried basis.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 8.909 mg of $C_3H_7NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Alginic Acid

$(C_6H_8O_6)_n$ Formula wt, calculated 176.13
Formula wt, actual (avg.) 200.00

INS: 400 CAS: [9005-32-7]

DESCRIPTION

Alginic Acid occurs as a white to yellow-white, fibrous powder. It is a hydrophilic colloidal carbohydrate extracted from various species of brown seaweeds (*phaeophyceae*) with dilute alkali. It may be described chemically as a linear glycuronoglycan consisting mainly of β-1,4 linked D-mannuronic and L-guluronic acid units in the pyranose ring form. Alginic Acid is insoluble in water, readily soluble in alkaline solutions, and insoluble in organic solvents. The pH of a 3:100 suspension in water is between 2.0 and 3.4.

Function Stabilizer; thickener; emulsifier.

REQUIREMENTS

Identification

A. Add 1 mL of calcium chloride TS to 5 mL of a 1:150 solution of sample in 0.1 *N* sodium hydroxide. A voluminous gelatinous precipitate forms.

B. Add 1 mL of 2 *N* sulfuric acid to 5 mL of the solution prepared for *Identification Test A*. A heavy gelatinous precipitate forms.

C. Place about 5 mg of sample into a test tube, add 5 mL of water, 1 mL of a freshly prepared 1:100 naphtholresorcinol:ethanol solution, and 5 mL of hydrochloric acid. Heat the mixture to boiling, boil gently for about 3 min, and then cool to about 15°. Transfer the contents of the test tube to a 30-mL separator with the aid of 5 mL of water, and extract with 15 mL of isopropyl ether. Perform a blank determination (see *General Provisions*), and make any necessary correction. The isopropyl ether extract from the sample exhibits a deeper purple hue than that from the blank.
Assay A sample yields not less than 20% and not more than 23% of carbon dioxide (CO_2), corresponding to between 91.0% and 104.5% of $(C_6H_8O_6)_n$ (equiv wt 200.00), calculated on the dried basis.
Arsenic Not more than 3 mg/kg.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 15.0%.
Residue on Ignition (Sulfated Ash) Not more than 8.0%, calculated on the dried basis.

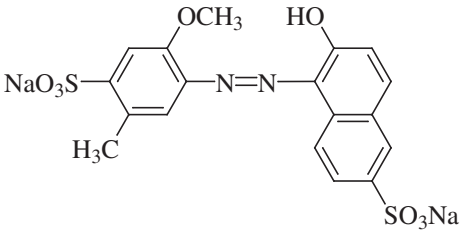
TESTS

Assay Determine as directed under *Alginates Assay*, Appendix IIIC. Each milliliter of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 25 mg of $(C_6H_8O_6)_n$ (equiv wt 200.00).
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead ion (Pb) in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.
Residue on Ignition (Sulfated Ash) Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 3-g sample.

Packaging and Storage Store in well-closed containers.

Allura Red[1]

Allura Red AC; CI 16035; Class: Monoazo



$C_{18}H_{14}N_2O_8S_2Na_2$ Formula wt 496.43

INS: 129 CAS: [25956-17-6]

DESCRIPTION

Allura Red occurs as a red-brown powder or granule. It is principally the disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid. It dissolves in water to give a solution red at neutrality and in acid and dark red in base. It is insoluble in ethanol.

Function Color.

REQUIREMENTS

Identification An aqueous solution containing 16.4 mg/L exhibits absorbance intensities (*A*) and wavelength maxima as follows: at pH 7, *A* = 0.87 at 500 nm; at pH 1, *A* = 0.83 at 490 nm (both neutral and acid solutions exhibit a shoulder at about 410 nm); and at pH 13, *A* = 0.37 at 500 nm, and *A* = 0.41 at 450 nm.

Assay Not less than 85.0% total coloring matters.

Arsenic Not more than 3 mg/kg.

Ether Extracts Not more than 0.2%.

Lead Not more than 10 mg/kg.

Loss on Drying (Volatile Matter) at 135°, Chlorides, and **Sulfates** (as sodium salts) Not more than 15.0% in combination.

Subsidiary Colors

Disodium 6-Hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-8-(2-methoxy-5-methyl-4-sulfophenoxy)-2-naphthalenesulfonic Acid Not more than 1.0%.

Higher and Lower Sulfonated Subsidiary Colors (as sodium salts) Not more than 1.0% each.

Uncombined Intermediates and Products of Side Reactions

*4-Amino-5-methoxy-*o*-toluenesulfonic Acid* Not more than 0.2%.

Disodium 6,6′-Oxybis(2-naphthalenesulfonic Acid) Not more than 1.0%.

Sodium 6-Hydroxy-2-naphthalenesulfonic Acid Not more than 0.3%.

Water-Insoluble Matter Not more than 0.2%.

TESTS

Assay Determine the total color strength as the weight percent of the sample taken using *Methods I* and *II* in *Total Color* under *Colors*, Appendix IIIC. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 175 to 225 mg of sample, accurately weighed, into a 1-L volumetric flask, dissolve in and dilute to volume with water. The absorptivity (*a*) for Allura Red is 0.052 mg/L/cm at 502 nm.

Method II (Sample Preparation) Transfer approximately 0.2 g of sample, accurately weighed, into the titration flask. The stoichiometric factor (F_s) for Allura Red is 8.06.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Chloride Determine as directed in *Sodium Chloride* under *Colors*, Appendix IIIC.

Ether Extracts Determine as directed in *Ether Extracts* under *Colors*, Appendix IIIC.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 μg of lead (Pb) ion in the control.

Loss on Drying (Volatile Matter) at 135° Determine as directed in *Loss on Drying (Volatile Matter)* under *Colors*, Appendix IIIC.

Subsidiary Colors

Solvent System Use a solvent system composed of 20 mL each of acetonitrile, dioxane, ethyl acetate, isoamyl alcohol, and water and 4 mL of ammonium hydroxide.

Standard Solution Transfer approximately 1 g of purified Allura Red (free of subsidiary colors), accurately weighed, into a 50-mL volumetric flask. Add 50 mg each of lower and higher sulfonated subsidiary colors, accurately weighed, dissolve in and dilute to volume with water. Store in the dark.

Sample Solution Transfer approximately 2 g of sample, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with water.

Procedure Spot 3-μL aliquots of *Sample Solution* and *Standard Solution* side by side 3 cm from the bottom of a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G. Up to seven samples and standards may be run simultaneously.

When the plate has air dried for 15 min, develop it in an unlined tank equilibrated with the *Solvent System* for at least 20 min. Allow the solvent front to reach to within 3 cm from the top of the plate.

Allow the plate to dry in a fume hood, and by visual inspection, compare the intensities of the lower and higher

[1]To be used or sold for use to color food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Red No. 40 can be applied only to FDA-certified batches of this color additive. Allura Red is a common name given to the uncertified colorant. See the monograph entitled FD&C Red No. 40 for directions for producing an FDA-certified batch.

sulfonated subsidiary colors with those in the *Standard Solution*. If the subsidiary colors in the *Sample Solution* appear more concentrated than those in the *Standard Solution*, determine the quantity of each, using a densitometer set to monitor the absorbance maximum of each. Calculate the concentrations of the subsidiary colors in percent (P), if present above 0.1%, by the equation

$$P = (A \times p)/A_S,$$

in which A is the area of the densitometer curve, p is the percent of subsidiary color in the *Standard Solution*, and A_S is the area of the densitometer curve for the subsidiary color in the *Standard Solution*.

Sulfate Determine as directed in *Sodium Sulfate* under *Colors*, Appendix IIIC.

Uncombined Intermediates and Products of Side Reactions Determine as directed for *Method II* in *Uncombined Intermediates and Products of Side Reactions* under *Colors*, Appendix IIIC, injecting 20 µL of the following *Sample Preparation*: Transfer 0.25 g of sample, accurately weighed, into a 100-mL volumetric flask. Dissolve in and dilute to volume with 0.1 *M* disodium borate ($Na_2B_4O_7$).

Water-Insoluble Matter Determine as directed in *Water-Insoluble Matter* under *Colors*, Appendix IIIC.

Packaging and Storage Store in well-closed containers.

Almond Oil, Bitter, FFPA

Bitter Almond Oil Free from Prussic Acid

CAS: [8013-76-1]

View IR

DESCRIPTION

Almond Oil, Bitter, FFPA, occurs as a colorless to slightly yellow liquid with a strong almond aroma and a slightly astringent, mild taste. It is a volatile oil obtained from the nuts of the bitter almond tree, *Prunus amygdalus* Batsch var. *amara* (De Candolle) Focke (Fam. Rosaceae), apricot kernel (*Prunus armeniaca* L.), and other fruit kernels containing amygdalin. It is prepared by steam distillation of a water-macerated, powdered, and pressed cake that has been specially treated and redistilled to remove hydrocyanic acid. It is soluble in most fixed oils and in propylene glycol, slightly soluble in mineral oil, and insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 95.0% of aldehydes, calculated as benzaldehyde (C_7H_6O).

Acid Value Not more than 8.0.

Chlorinated Compounds Passes test.

Hydrocyanic Acid Passes test (about 0.015%).

Optical (Specific) Rotation Optically inactive, or not more than ±0.15°.

Refractive Index Between 1.541 and 1.546 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 1.040 and 1.050.

TESTS

Assay Determine as directed under *Aldehydes*, Appendix VI, using about 1 mL of sample, accurately weighed, and 53.05 as the equivalence factor (e) in the calculation.

Acid Value Determine as directed under *Acid Value*, Appendix VI.

Chlorinated Compounds Determine as directed under *Chlorinated Compounds*, Appendix VI.

Hydrocyanic Acid Transfer 1 mL of sample into a test tube. Add 1 mL of water, 5 drops of a 1:10 solution of sodium hydroxide, and 5 drops of a 1:10 solution of ferrous sulfate. Shake thoroughly, and acidify with 0.5 *N* hydrochloric acid. No blue precipitate or color appears.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves to form a clear solution in 2 mL of 70% alcohol.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Aluminum Ammonium Sulfate

Ammonium Alum

$AlNH_4(SO_4)_2 \cdot 12H_2O$ Formula wt 453.32

INS: 523 CAS: [7784-25-0]

DESCRIPTION

Aluminum Ammonium Sulfate occurs as large, colorless crystals, white granules, or a powder. One gram dissolves in 7 mL of water at 25° and in about 0.3 mL of boiling water. Its

solutions are acid to litmus. It is insoluble in alcohol, and is freely, but slowly, soluble in glycerin.

Function Buffer; neutralizing agent.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Aluminum*, for *Ammonium*, and for *Sulfate*, Appendix IIIA.
Assay Not less than 99.5% and not more than 100.5% of $AlNH_4(SO_4)_2 \cdot 12H_2O$.
Alkalies and Alkaline Earths Passes test.
Fluoride Not more than 0.003%.
Lead Not more than 3 mg/kg.
Selenium Not more than 0.003%.

TESTS

Assay Dissolve about 1 g of sample, accurately weighed, in 50 mL of water, add 50.0 mL of 0.05 *M* disodium EDTA and 20 mL of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 mL of glacial acetic acid in 1000 mL of solution), and boil gently for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Back titrate with 0.05 *M* zinc sulfate to a bright rose-pink color. Perform a blank determination (see *General Provisions*), and make any necessary correction. The milliliters of 0.05 *M* disodium EDTA consumed is equivalent to 50 minus the milliliters of 0.05 *M* zinc sulfate used. Each milliliter of 0.05 *M* disodium EDTA consumed is equivalent to 22.67 mg of $AlNH_4(SO_4)_2 \cdot 12H_2O$.
Alkalies and Alkaline Earths Completely precipitate the aluminum from a boiling solution of 1 g of sample in 100 mL of water by adding enough 6 *N* ammonium hydroxide to render the solution distinctly alkaline to methyl red TS, and filter. Evaporate the filtrate to dryness, and ignite. The weight of the residue does not exceed 5 mg.
Fluoride Determine as directed in *Method V* under *Fluoride Limit Test*, Appendix IIIB.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Packaging and Storage Store in well-closed containers.

Aluminum Potassium Sulfate

Potassium Alum

$AlK(SO_4)_2 \cdot 12H_2O$ Formula wt 474.38

INS: 522 CAS: [7784-24-9]

DESCRIPTION

Aluminum Potassium Sulfate occurs as large, transparent crystals or crystalline fragments, or as a white crystalline powder. One gram dissolves in 7.5 mL of water at 25° and in about 0.3 mL of boiling water. Its solutions are acid to litmus. It is insoluble in alcohol, but is freely soluble in glycerin.

Function Buffer; neutralizing agent; firming agent.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Aluminum*, for *Potassium*, and for *Sulfate*, Appendix IIIA.
Assay Not less than 99.5% and not more than 100.5% of $AlK(SO_4)_2 \cdot 12H_2O$.
Ammonium Salts Passes test.
Fluoride Not more than 0.003%.
Lead Not more than 3 mg/kg.
Selenium Not more than 0.003%.

TESTS

Assay Dissolve about 1 g of sample, accurately weighed, in 50 mL of water, add 50.0 mL of 0.05 *M* disodium EDTA and 20 mL of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 mL of glacial acetic acid in 1000 mL of aqueous solution), and boil gently for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Back titrate with 0.05 *M* zinc sulfate to a bright rose-pink color. Perform a blank determination (see *General Provisions*), and make any necessary correction. The milliliters of 0.05 *M* disodium EDTA consumed is equivalent to 50 minus the milliliters of 0.05 *M* zinc sulfate used. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 23.72 mg of $AlK(SO_4)_2 \cdot 12H_2O$.
Ammonium Salts Add 1 g of sample to 10 mL of 1 *N* sodium hydroxide in a small beaker, and heat on a steam bath for 1 min. The odor of ammonia is not perceptible.
Fluoride Determine as directed in *Method V* under *Fluoride Limit Test*, Appendix IIIB.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Packaging and Storage Store in well-closed containers.

Aluminum Sodium Sulfate

Soda Alum; Sodium Alum

$AlNa(SO_4)_2$ Formula wt, anhydrous 242.09
$AlNa(SO_4)_2 \cdot 12H_2O$ Formula wt, dodecahydrate 458.29

INS: 521 CAS: anhydrous [10102-71-3]
CAS: dodecahydrate [7784-28-3]

DESCRIPTION

Aluminum Sodium Sulfate occurs as colorless crystals, white granules, or a powder. It is anhydrous or may contain up to

12 molecules of water of hydration. The anhydrous form is slowly soluble in water. The dodecahydrate is freely soluble in water, and it effloresces in air. Both forms are insoluble in alcohol.

Function Buffer; neutralizing agent; firming agent.

REQUIREMENTS

Identification A sample responds to the flame test for *Sodium*, Appendix IIIA, and gives positive tests for *Aluminum* and for *Sulfate*, Appendix IIIA.
Assay *Anhydrous*: Not less than 99.0% and not more than 104.0% of $AlNa(SO_4)_2$ after drying; *Dodecahydrate*: Not less than 99.5% of $AlNa(SO_4)_2$ after drying.
Ammonium Salts Passes test.
Fluoride Not more than 0.003%.
Lead Not more than 3 mg/kg.
Loss on Drying *Anhydrous*: Not more than 10%; *Dodecahydrate*: Not more than 47.2%.
Neutralizing Value *Anhydrous*: Between 104 and 108.
Selenium Not more than 0.003%.

TESTS

Assay Accurately weigh about 500 mg of sample, previously dried as directed in the test for *Loss on Drying* (below), moisten with 1 mL of glacial acetic acid, and dissolve in 50 mL of water, warming gently on a steam bath until solution is complete. Cool, neutralize with 6 *N* ammonium hydroxide, add 50.0 mL of 0.05 *M* disodium EDTA and 20 mL of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 mL of glacial acetic acid in 1000 mL of aqueous solution), and boil gently for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Back titrate with 0.05 *M* zinc sulfate to a bright pink color. Perform a blank determination (see *General Provisions*), and make any necessary correction. The milliliters of 0.05 *M* disodium EDTA consumed is equivalent to 50 minus the milliliters of 0.05 *M* zinc sulfate used. Each milliliter of 0.05 *M* disodium EDTA consumed is equivalent to 12.10 mg of $AlNa(SO_4)_2$.
Ammonium Salts Heat 1 g of sample with 10 mL of 1 *N* sodium hydroxide on a steam bath for 1 min. The odor of ammonia is not perceptible.
Fluoride Determine as directed in *Method V* under the *Fluoride Limit Test*, Appendix IIIB, using a 1.76-g sample.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying *Anhydrous*: Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 200° for 16 h; *Dodecahydrate*: Determine as directed for the anhydrous sample, but dry the sample first at 50° to 55° for 1 h, then at 200° for 16 h.
Neutralizing Value Transfer 500 mg of anhydrous sample, accurately weighed, into a 200-mL Erlenmeyer flask, add 30 mL of water and 4 drops of phenolphthalein TS, and boil until the sample dissolves. Add 13.0 mL of 0.5 *N* sodium hydroxide, boil for a few seconds, and titrate with 0.5 *N* hydrochloric acid to the disappearance of the pink color, adding the acid dropwise and agitating vigorously after each addition. Calculate the neutralizing value, as parts of $NaHCO_3$ equivalent to 100 parts of the sample, by the formula

$$8.4V,$$

in which V is the volume, in milliliters, of 0.5 *N* sodium hydroxide consumed by the sample.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Packaging and Storage Store in tight containers.

Aluminum Sulfate

$Al_2(SO_4)_3$ Formula wt, anhydrous 342.14
$Al_2(SO_4)_3 \cdot 18H_2O$ Formula wt, octadecahydrate 666.41

INS: 520 CAS: anhydrous [10043-01-3]
CAS: octadecahydrate [7784-31-8]

DESCRIPTION

Aluminum Sulfate occurs as a white powder, as shining plates, or as crystalline fragments. It is anhydrous or contains 18 molecules of water of crystallization. Because of efflorescence, the hydrate may have a composition approximating the formula $Al_2(SO_4)_3 \cdot 14H_2O$. One gram of the hydrate dissolves in about 2 mL of water. The anhydrous approaches the same solubility, but the rate of solution is so slow that it initially appears to be relatively insoluble. The pH of a 1:20 aqueous solution is 2.9 or above.

Function Firming agent.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Aluminum* and for *Sulfate*, Appendix IIIA.
Assay *Anhydrous*: Not less than 99.5% of $Al_2(SO_4)_3$, calculated on the ignited basis; *Octadecahydrate*: Not less than 99.5% and not more than 114.0% of $Al_2(SO_4)_3 \cdot 18H_2O$, corresponding to not more than approximately 101.7% of $Al_2(SO_4)_3 \cdot 14H_2O$.
Alkalies and Alkaline Earths Passes test (about 0.4%).
Ammonium Salts Passes test.
Fluoride Not more than 0.003%.
Lead Not more than 3 mg/kg.
Loss on Ignition *Anhydrous*: Not more than 5%.

> **Note**: This *Requirement* does not apply to $Al_2(SO_4)_3 \cdot 18H_2O$.

Selenium Not more than 0.003%.

TESTS

Assay Accurately weigh an amount of sample equivalent to about 4 g of $Al_2(SO_4)_3$, transfer into a 250-mL volumetric

flask, dissolve in and dilute to volume with water, and mix. Pipet 10 mL of this solution into a 250-mL beaker, add 25.0 mL of 0.05 *M* disodium EDTA and 20 mL of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 mL of glacial acetic acid in 1000 mL of aqueous solution), and boil gently for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Back titrate with 0.05 *M* zinc sulfate to a bright pink color. Perform a blank determination (see *General Provisions*), and make any necessary correction. The milliliters of 0.05 *M* disodium EDTA consumed is equivalent to 50 minus the milliliters of 0.05 *M* zinc sulfate used. Each milliliter of 0.05 *M* disodium EDTA consumed is equivalent to 8.554 mg of $Al_2(SO_4)_3$ or to 16.66 mg of $Al_2(SO_4)_3 \cdot 18H_2O$.

Alkalies and Alkaline Earths Add a few drops of methyl red TS to a boiling solution of 2 g of sample in 150 mL of water, and then add 6 *N* ammonium hydroxide until the color of the solution just changes to a distinct yellow. Add hot water to restore the original volume, and filter while hot. Evaporate 75 mL of the filtrate to dryness, and ignite to constant weight. Not more than 4 mg of residue remains.

Ammonium Salts Heat 1 g of sample with 10 mL of 1 *N* sodium hydroxide on a steam bath for 1 min. The odor of ammonia is not perceptible.

Fluoride Determine as directed in *Method V* under the *Fluoride Test*, Appendix IIIB, using 1.67 g of sample.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Ignition *Anhydrous*: Accurately weigh about 2 g of sample, and ignite, preferably in a muffle furnace, at about 500° for 3 h.

Note: This *Test* does not apply to $Al_2(SO_4)_3 \cdot 18H_2O$.

Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Packaging and Storage Store in well-closed containers.

Ambrette Seed Oil

Ambrette Seed Liquid CAS: [8015-62-1]

View IR

DESCRIPTION

Ambrette Seed Oil occurs as a clear yellow to amber liquid with the strong, musky odor of ambrettolide. It is a volatile oil obtained by steam distillation from the partially dried and crushed seeds of the plant *Abelmoschus moschatus* Moench, syn. *Hibiscus abelmoschus* L. (Fam. Malvaceae). It is refined by solvent extraction to remove fatty acids or by precipitation of the fatty acid salts. It is soluble in most fixed oils and in mineral oil, often with cloudiness, but relatively insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 3.0.
Optical (Specific) Rotation Between –2.5° and +3°.
Refractive Index Between 1.468 and 1.485 at 20°.
Saponification Value Between 140 and 200.
Specific Gravity Between 0.898 and 0.920.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 1 g of sample, accurately weighed.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Ammonia Solution

Ammonium Hydroxide; Stronger Ammonia Water

NH_3 Formula wt 17.03

INS: 527 CAS: [7664-41-7]

DESCRIPTION

Ammonia Solution occurs as a clear, colorless liquid. Upon exposure to air it loses ammonia rapidly. Its specific gravity is about 0.90.

> **Caution**: Ammonia Solution is irritating to the oral mucosa and respiratory tract. Perform tests in a well-ventilated fume hood.

Function pH control agent; surface finishing agent; boiler water additive.

REQUIREMENTS

Identification Hold a glass rod wet with hydrochloric acid near the surface of the sample liquid. Dense, white fumes evolve.

Assay Not less than 27.0% and not more than 30.0%, by weight, of NH_3.
Lead Not more than 0.5 mg/kg.
Nonvolatile Residue Not more than 0.02%.
Readily Oxidizable Substances Passes test.

TESTS

Assay Accurately tare a 125-mL glass-stoppered Erlenmeyer flask containing 35.0 mL of 1 *N* sulfuric acid. Cool a sample, contained in its original bottle, to 10° or cooler. Partially fill a 10-mL graduated pipet from near the bottom of this sample. (Do not use vacuum to draw up the sample.) Wipe off any liquid adhering to the outside of the pipet, and discard the first milliliter of sample. Hold the pipet just above the surface of the acid, and transfer 2 mL into the flask, leaving at least 1 mL in the pipet. Stopper the flask, mix, and weigh again to obtain the weight of the sample. Add methyl red TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sulfuric acid is equivalent to 17.03 mg of NH_3.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Nonvolatile Residue Evaporate 11 mL (10 g) of sample in a tared platinum or porcelain dish to dryness, dry at 105° for 1 h, cool, and weigh.
Readily Oxidizable Substances Dilute 4 mL of sample with 6 mL of water, and add a slight excess of 2 *N* sulfuric acid and 0.1 mL of 0.1 *N* potassium permanganate. The pink color does not completely disappear within 10 min.

Packaging and Storage Store in tight containers at a temperature not exceeding 25°.

Ammoniated Glycyrrhizin

CAS: [1407-03-0]

DESCRIPTION

Ammoniated Glycyrrhizin occurs as a brown powder. It is precipitated by acid from the water extract of dried and ground rhizomes and roots of *Glycyrrhiza glabra* or related *Glycyrrhiza* (licorice root) (Fam. Leguminosae) and neutralized with dilute ammonia. Suitable diluents may be added.

Function Flavoring agent; flavor enhancer.

REQUIREMENTS

Identification A sample gives positive tests for *Ammonium*, Appendix IIIA.
Assay Not less than 22.0% and not more than 32.0% of monoammonium glycyrrhizinate ($C_{42}H_{65}NO_{16}$), calculated on the dried basis.
Ash (Total) Not more than 2.5%.
Loss on Drying Not more than 6.0%.

TESTS

Assay (Based on AOAC method 982.19.)

Apparatus (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph operated at room temperature with a 10-μm particle size, 30-cm × 4-mm (id), C18 reverse-phase column (μBondapak C18 column, Waters Corp., 34-T Maple Street, Milford, MA 01757, or equivalent). Maintain the *Mobile Phase* at a pressure and flow rate (typically 2.0 mL/min) capable of giving the required elution time (see *System Suitability* in *High-Performance Liquid Chromatography*). Use an ultraviolet detector that monitors absorption at 254 nm (0.2 to 0.1 AUFS range).

Mobile Phase Add 380 mL of acetonitrile and 10 mL of glacial acetic acid to 610 mL of glass-distilled water that has been filtered through a 0.45-μm filter (Millipore, or equivalent). Mix, and de-gas thoroughly.

Standard Solution Dissolve about 10 mg of Monoammonium Glycyrrhizinate Standard for analytical use (Sigma, or equivalent), accurately weighed, in 20 mL of a 1:1 (v/v) solution of acetonitrile:water. Filter the solution through a 0.45-μm filter (Millipore, or equivalent). Prepare fresh daily.

> **Note**: Correct the weight of the Monoammonium Glycyrrhizinate Standard taken for the percent loss on drying shown on its label.

Assay Solution Dissolve about 40 g of sample, accurately weighed, in 20 mL of water. Filter the solution through a 0.45-μm filter (Millipore, or equivalent).

System Suitability Inject duplicate 10-μL portions of the *Standard Solution* into the chromatograph. The retention time of monoammonium glycyrrhizinate is approximately 6 min. Adjust the operating conditions if necessary. The mean standard deviation for replicate injections is not more than 2.0%.

Procedure Separately inject, in duplicate, 10-μL volumes of the *Standard Solution* and the *Assay Solution* into the chromatograph, and determine the mean peak area for each solution. Calculate the percent monoammonium glycyrrhizinate, equivalent to $C_{42}H_{65}NO_{16}$, in the portion of sample taken by the formula

$$100 \times (20C_S/W_U) \times (A_U/A_S),$$

in which C_S is the concentration, in milligrams per milliliter, of the *Standard Solution*; W_U is the weight, in milligrams, of the sample taken; and A_U and A_S are the peak areas of the *Assay Solution* and the *Standard Solution*, respectively.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 1-g sample at 105° for 1 h.

Packaging and Storage Store in a tightly closed container in a cool, dry place.

Ammonium Alginate

Algin

$(C_6H_7O_6NH_4)_n$ Formula wt, calculated 193.16
Formula wt, actual (avg.) 217.00

INS: 403 CAS: [9005-34-9]

DESCRIPTION

Ammonium Alginate occurs as a white to yellow, fibrous or granular powder. It is the ammonium salt of alginic acid (see the monograph for *Alginic Acid*). It dissolves in water to form a viscous, colloidal solution. It is insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater than about 30% by weight. It is insoluble in chloroform, in ether, and in acids having a pH lower than about 3.

Function Stabilizer; thickener; emulsifier.

REQUIREMENTS

Identification

A. Add 1 mL of calcium chloride TS to 5 mL of a 1:100 aqueous solution. A voluminous, gelatinous precipitate forms.

B. Add 1 mL of 2.7 *N* sulfuric acid to 10 mL of a 1:100 aqueous solution. A heavy, gelatinous precipitate forms.

C. Place about 5 mg of sample into a test tube, add 5 mL of water, 1 mL of a freshly prepared 1:100 solution naphtholresorcinol:ethanol, and 5 mL of hydrochloric acid. Heat the mixture to boiling, boil gently for about 3 min, and then cool to about 15°. Transfer the contents of the test tube to a 30-mL separator with the aid of 5 mL of water, and extract with 15 mL of isopropyl ether. Perform a blank determination (see *General Provisions*), and make any necessary correction. The isopropyl ether extract from the sample exhibits a deeper purple hue than that from the blank.

D. Add 5 mL of 1 *N* sodium hydroxide to about 1 g of sample contained in a test tube, and shake the mixture briefly. The odor of ammonia is evident.

Assay A sample yields not less than 18% and not more than 21% of carbon dioxide (CO_2), corresponding to between 88.7% and 103.6% of Ammonium Alginate (equiv wt 217.00), calculated on the dried basis.

Arsenic Not more than 3 mg/kg.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 15.0%.

Residue on Ignition (Sulfated Ash) Not more than 7.0%, calculated on the dried basis.

TESTS

Assay Determine as directed under *Alginates Assay*, Appendix IIIC. Each milliliter of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 27.12 mg of Ammonium Alginate (equiv wt 217.00).

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead ion (Pb) in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Residue on Ignition (Sulfated Ash) Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, using a 3-g sample.

Packaging and Storage Store in well-closed containers.

Ammonium Bicarbonate

NH_4HCO_3 Formula wt 79.06

INS: 503(ii) CAS: [1066-33-7]

DESCRIPTION

Ammonium Bicarbonate occurs as white crystals or as a crystalline powder. It volatilizes rapidly at 60°, dissociating into ammonia, carbon dioxide, and water, but it is quite stable at room temperature. One gram dissolves in about 6 mL of water. It is insoluble in alcohol.

Function Alkali; leavening agent.

REQUIREMENTS

Identification A sample gives positive tests for *Ammonium* and for *Bicarbonate*, Appendix IIIA.

Assay Not less than 99.0% and not more than 100.5% of NH_4HCO_3.

Chloride Not more than 0.003%.

Lead Not more than 3 mg/kg.

Nonvolatile Residue Not more than 0.05% (0.55% for products containing a suitable anticaking agent).

Sulfur Compounds (as SO_4) Not more than 0.007%.

TESTS

Assay Dissolve about 3 g of sample, accurately weighed, in 40 mL of water. Add 2 drops of methyl red TS, and while constantly stirring, titrate with 1 *N* hydrochloric acid, adding the acid slowly, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the faint pink color no longer fades after boiling. Each milliliter of 1 *N* hydrochloric acid is equivalent to 79.06 mg of NH_4HCO_3.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, using a 500-mg sample. Any turbidity produced by the sample does not exceed that produced by a control containing 15 µg of chloride ion (Cl).

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Nonvolatile Residue Transfer 4 g of sample into a tared dish, add 10 mL of water, and evaporate to dryness on a steam bath. Heat the dish at 105° for 1 h, cool in a desiccator, and weigh.
Sulfur Compounds (as SO_4) Dissolve 4 g of sample in 40 mL of water, add about 10 mg of sodium carbonate and 1 mL of 30% hydrogen peroxide, and evaporate the solution to dryness on a steam bath. Treat the residue as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by the sample does not exceed that produced by a control containing 280 μg of sulfate ion (SO_4).

Packaging and Storage Store in well-closed containers.

Ammonium Carbonate

INS: 503(i) CAS: [10361-29-2]

DESCRIPTION

Ammonium Carbonate occurs as a white powder or as hard, white or translucent masses. It consists of ammonium bicarbonate (NH_4HCO_3) and ammonium carbamate ($NH_2{\cdot}COONH_4$) in varying proportions. On exposure to air it becomes opaque and is finally converted into porous lumps or a white powder of ammonium bicarbonate because of the loss of ammonia and carbon dioxide. One gram dissolves slowly in about 4 mL of water. Its solutions are alkaline to litmus.

Function Buffer; leavening agent; neutralizing agent.

REQUIREMENTS

Identification When heated, a sample volatilizes without charring, and the vapor is alkaline to moistened litmus paper. A 1:20 aqueous solution effervesces when an acid is added.
Assay Not less than 30.0% and not more than 34.0% of NH_3.
Chloride Not more than 0.003%.
Lead Not more than 3 mg/kg.
Nonvolatile Residue Not more than 0.05%.
Sulfur Compounds (as SO_4) Not more than 0.005%.

TESTS

Assay Place about 10 mL of water in a weighing bottle, tare the bottle and its contents, add about 2 g of sample, and accurately weigh. Transfer the contents of the bottle to a 250-mL flask, and while mixing, slowly add 50.0 mL of 1 *N* sulfuric acid, allowing for the release of carbon dioxide. When solution has been effected, wash down the sides of the flask with a few milliliters of water, add a few drops of methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sulfuric acid is equivalent to 17.03 mg of NH_3.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, using the residue of the following: Dissolve 500 mg of sample in 10 mL of hot water, add about 5 mg of sodium carbonate, and evaporate to dryness on a steam bath. Any turbidity produced does not exceed that shown in a control containing 15 μg of chloride ion (Cl).
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Nonvolatile Residue Transfer 4 g of sample into a tared dish, add 10 mL of water, and evaporate on a steam bath. Heat the dish at 105° for 1 h, cool in a desiccator, and weigh.
Sulfur Compounds Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, using the residue of the following: Dissolve 4 g of sample in 40 mL of water, add about 10 mg of sodium carbonate and 1 mL of 30% hydrogen peroxide, and evaporate the solution to dryness on a steam bath. Any turbidity produced does not exceed that shown in a control containing 200 μg of sulfate (SO_4) ion.

Packaging and Storage Store in tight, light-resistant containers, preferably at a temperature not exceeding 30°.

Ammonium Chloride

NH_4Cl Formula wt 53.49

INS: 510 CAS: [12125-02-9]

DESCRIPTION

Ammonium Chloride occurs as colorless crystals or as a white, fine or coarse, crystalline powder. It is somewhat hygroscopic. One gram dissolves in 2.6 mL of water at 25°, in 1.4 mL of boiling water, in about 100 mL of alcohol, and in about 8 mL of glycerin. The pH of a 1:20 solution is between 4.5 and 6.0.

Function Yeast food; dough conditioner.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Ammonium* and for *Chloride*, Appendix IIIA.
Assay Not less than 99.0% of NH_4Cl after drying.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 0.5%.

TESTS

Assay Dissolve about 200 mg of sample, previously dried over silica gel for 4 h and accurately weighed, in about 40 mL of water contained in a glass-stoppered flask. While agitating the mixture, add 3 mL of nitric acid, 5 mL of nitrobenzene,

and 50.0 mL of 0.1 *N* silver nitrate; shake vigorously; add 2 mL of ferric ammonium sulfate TS; and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Each milliliter of 0.1 *N* silver nitrate is equivalent to 5.349 mg of NH_4Cl.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 4 μg of lead (Pb) ion in the control
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample over silica gel for 4 h.

Packaging and Storage Store in tight containers.

Ammonium Phosphate, Dibasic

Diammonium Hydrogen Phosphate; Diammonium Phosphate

$(NH_4)_2HPO_4$ Formula wt 132.06

INS: 342(ii) CAS: [7783-28-0]

DESCRIPTION

Ammonium Phosphate, Dibasic, occurs as white crystals, a crystalline powder, or granules. It is freely soluble in water. The pH of a 1:100 aqueous solution is between 7.6 and 8.2.

Function Buffer; dough conditioner; leavening agent; yeast food.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Ammonium* and for *Phosphate*, Appendix IIIA.
Assay Not less than 96.0% and not more than 102.0% of $(NH_4)_2HPO_4$.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Lead Not more than 4 mg/kg.

TESTS

Assay Dissolve about 600 mg of sample, accurately weighed, in 40 mL of water, and titrate to a pH of 4.6 with 0.1 *N* sulfuric acid. Each milliliter of 0.1 *N* sulfuric acid is equivalent to 13.21 mg of $(NH_4)_2HPO_4$.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.
Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample, *Buffer Solution A*, and 0.1 mL of *Fluoride Standard Solution*.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Packaging and Storage Store in tightly closed containers.

Ammonium Phosphate, Monobasic

Ammonium Dihydrogen Phosphate; Monoammonium Phosphate

$NH_4H_2PO_4$ Formula wt 115.03

INS: 342(i) CAS: [7722-76-1]

DESCRIPTION

Ammonium Phosphate, Monobasic, occurs as white crystals, a crystalline powder, or granules. It is freely soluble in water. The pH of a 1:100 aqueous solution is between 4.3 and 5.0.

Function Buffer; dough conditioner; leavening agent; yeast food.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Ammonium* and for *Phosphate*, Appendix IIIA.
Assay Not less than 96.0% and not more than 102.0% of $NH_4H_2PO_4$.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Lead Not more than 4 mg/kg.

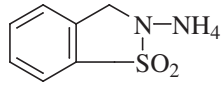
TESTS

Assay Dissolve about 500 mg of sample, accurately weighed, in 50 mL of water, and titrate to a pH of 8.0 with 0.1 *N* sodium hydroxide. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 11.50 mg of $NH_4H_2PO_4$.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.
Fluoride Determine as directed in *Method IV* under the *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample, *Buffer Solution B*, and 0.1 mL of *Fluoride Standard Solution*.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Packaging and Storage Store in tightly closed containers.

Ammonium Saccharin

1,2-Benzisothiazolin-3-one 1,1-Dioxide Ammonium Salt



$C_7H_8N_2O_3S$ Formula wt 200.21

DESCRIPTION

Ammonium Saccharin occurs as white crystals or as white,

crystalline powder. It is freely soluble in water. The pH of a 1:3 aqueous solution is between 5 and 6.

Function Nonnutritive sweetener.

REQUIREMENTS

Identification

A. Dissolve about 100 mg of sample in 5 mL of a 1:20 solution of sodium hydroxide, evaporate to dryness, and gently fuse the residue over a small flame until ammonia no longer evolves. After the residue has cooled, dissolve it in 20 mL of water, neutralize the solution with 2.7 *N* hydrochloric acid, and filter. Add 1 drop of ferric chloride TS to the filtrate. A violet color appears.

B. Mix 20 mg of sample with 40 mg of resorcinol, cautiously add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 mL of water and an excess of 1 *N* sodium hydroxide. A fluorescent green liquid results.

C. A 1:10 aqueous solution gives positive tests for *Ammonium*, Appendix IIIA.

D. Add 1 mL of hydrochloric acid to 10 mL of a 1:10 aqueous solution. A crystalline precipitate of saccharin forms. Wash the precipitate well with cold water and dry at 105° for 2 h. The saccharin thus obtained melts between 226° and 230° (see *Melting Range or Temperature*, Appendix IIB).

Assay Not less than 98.0% and not more than 101.0% of $C_7H_8N_2O_3S$, calculated on the anhydrous basis.

Benzoate and Salicylate Passes test.

Lead Not more than 2 mg/kg.

Readily Carbonizable Substances Passes test.

Selenium Not more than 0.003%.

Toluenesulfonamides Not more than 0.0025%.

Water Not more than 0.3%.

TESTS

Assay With the aid of 10 mL of water, quantitatively transfer about 500 mg of sample, accurately weighed, into a separator Add 2 mL of 2.7 *N* hydrochloric acid, and extract the precipitated saccharin, first with 30 mL, then with five 20-mL portions of a solvent comprising 9:1 (v/v) chloroform:alcohol. Filter each extract through a small filter paper moistened with the solvent mixture, and evaporate the combined filtrates to dryness on a steam bath with the aid of a current of air. Dissolve the residue in 75 mL of hot water, cool, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 20.02 mg of $C_7H_8N_2O_3S$.

Benzoate and Salicylate Add 3 drops of ferric chloride TS to 10 mL of a 1:20 aqueous solution previously acidified with 5 drops of glacial acetic acid. No precipitate or violet color appears.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Readily Carbonizable Substances Determine as directed under *Readily Carbonizable Substances*, Appendix IIB, using 200 mg of sample dissolved in 5 mL of 95% sulfuric acid and kept at 48° to 50° for 10 min. The color is no darker than that of *Matching Fluid A*.

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Toluenesulfonamides

Methylene Chloride Use a suitable grade (such as that obtainable from Burdick & Jackson Laboratories, Inc.), equivalent to the product obtained by distillation in an all-glass apparatus.

Internal Standard Stock Solution Transfer 100.0 mg of 95% *n*-tricosane (obtainable from Chemical Samples Co.) into a 10-mL volumetric flask, dissolve in and dilute to volume with *n*-heptane, and mix.

Stock Standard Preparation Transfer 20.0 mg each of reagent-grade *o*-toluenesulfonamide and *p*-toluenesulfonamide into a 10-mL volumetric flask, dissolve in and dilute to volume with methylene chloride, and mix.

Diluted Standard Preparations Pipet 0.1, 0.25, 1.0, 2.5, and 5.0 mL, respectively, of the *Stock Standard Preparation* into five 10-mL volumetric flasks. Pipet 0.25 mL of the *Internal Standard Stock Solution* into each flask, dilute each to volume with methylene chloride, and mix. These solutions contain, respectively, 20, 50, 200, 500, and 1000 μg/mL of each toluenesulfonamide, plus 250 μg of *n*-tricosane.

Test Preparation (See *Chromatography*, Appendix IIA.) Dissolve 2.00 g of sample in 8.0 mL of 5% sodium bicarbonate solution, and mix the solution thoroughly with 10.0 g of chromatographic siliceous earth (Celite 545, Johns-Manville, or equivalent). Transfer the mixture into a 250- × 25-mm chromatographic tube having a fritted-glass disk and a Teflon stopcock at the bottom and a reservoir at the top. Pack the contents of the tube by tapping the column on a padded surface, and then by tamping firmly from the top. Place 100 mL of methylene chloride in the reservoir, and adjust the stopcock so that 50 mL of eluate is collected in 20 to 30 min. Add 25 μL of *Internal Standard Stock Solution* to the eluate, mix, and then concentrate the solution to a volume of 1.0 mL in a suitable concentrator tube fitted with a modified Snyder column, using a Kontes tube heater maintained at 90°.

Procedure Inject 2.5 μL of the *Test Preparation* into a suitable gas chromatograph equipped with a flame-ionization detector and a 3-m × 2-mm (id) glass column, or equivalent, packed with 3% phenyl methyl silicone (OV-17, Applied Science Laboratories, Inc., or equivalent) on 100- to 120-mesh, silanized, calcined, diatomaceous silica (Gas-Chrom Q, Applied Science, or equivalent).

> **Caution**: The glass column should extend into the injector for on-column injection and into the detector base to avoid contact with metal.

Maintain the column at 108°. Set the injection port temperature to 225° and the detector to 250°. Use helium as the carrier gas, with a flow rate of 30 mL/min. Set the instrument attenuation setting so that 2.5 μL of the *Diluted Standard Preparation* containing 200 μg/mL of each toluenesulfonamide gives a response of 40% to 80% of full-scale deflection. Record the

chromatogram, note the peaks for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and the *n*-tricosane internal standard, and calculate the areas for each peak by suitable means. The retention times for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane are about 5, 6, and 15 min, respectively.

In a similar manner, obtain the chromatograms for 2.5-µL portions of each of the five *Diluted Standard Preparations*, and for each solution, determine the areas of the *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane peaks. From the values thus obtained, prepare standard curves by plotting concentration of each toluenesulfonamide, in micrograms per milliliter, versus the ratio of the respective toluenesulfonamide peak area to that of *n*-tricosane. From the standard curve, determine the concentration, in micrograms per milliliter, of each toluenesulfonamide in the *Test Preparation*. Divide each value by 2 to convert the result to milligrams per kilogram of the toluenesulfonamide in the 2-g sample taken for analysis.

> **Note**: If the toluenesulfonamide content of the sample is greater than about 500 mg/kg, the impurity may crystallize out of the methylene chloride concentrate (see *Test Preparation*). Although this level of impurity exceeds that permitted by the specification, the analysis may be completed by diluting the concentrate with methylene chloride containing 250 µg of *n*-tricosane per milliliter, and by applying appropriate dilution factors in the calculation. Care must be taken to redissolve completely any crystalline toluenesulfonamide to give a homogeneous solution.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Ammonium Sulfate

$(NH_4)_2SO_4$ Formula wt 132.14

INS: 517 CAS: [7783-20-2]

DESCRIPTION

Ammonium Sulfate occurs as colorless or white crystals or granules that decompose at temperatures above 280°. One gram is soluble in about 1.5 mL of water. It is insoluble in alcohol. The pH of a 0.1 *M* solution is between 4.5 and 6.0.

Function Dough conditioner; yeast nutrient.

REQUIREMENTS

Identification A sample gives positive tests for *Ammonium* and for *Sulfate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of $(NH_4)_2SO_4$.
Lead Not more than 3 mg/kg.
Residue on Ignition Not more than 0.25%.
Selenium Not more than 0.003%.

TESTS

Assay Transfer about 2 g of sample, accurately weighed, into a 250-mL flask, and dissolve it in 100 mL of water. Add 40 mL of a mixture of equal volumes of formaldehyde and water, previously neutralized to phenolphthalein TS with 1 *N* sodium hydroxide. Mix, allow to stand for 30 min, and titrate the mixture with 1 *N* sodium hydroxide to a pink endpoint that persists for 5 min. Each milliliter of 1 *N* sodium hydroxide is equivalent to 66.06 mg of $(NH_4)_2SO_4$.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.

Amyris Oil, West Indian Type

Sandalwood Oil, West Indian Type

View IR

DESCRIPTION

Amyris Oil, West Indian Type, occurs as a clear, pale yellow, viscous liquid having a distinct odor suggestive of sandalwood. It is the volatile oil obtained by steam distillation from the wood of *Amyris balsamifera* L. (Fam. Rutaceae). It is soluble in most fixed oils and usually in mineral oil. It is soluble in an equal volume of propylene glycol, the solution often becoming opalescent on further dilution. It is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those shown in the respective spectrum in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 3.0.
Angular Rotation Between +10° and +53°.
Ester Value Not more than 7.
Ester Value after Acetylation Between 115 and 165.
Refractive Index Between 1.503 and 1.512 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.943 and 0.976.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed in *Ester Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Ester Value after Acetylation Determine as directed under *Total Alcohols*, Appendix VI, using about 2 g of the dried acetylated oil, accurately weighed. Reflux for a period of 2 h. Calculate the *Ester Value after Acetylation* by the formula

$$A \times 28.05/B,$$

in which A is the number of milliliters of 0.5 N alcoholic potassium hydroxide consumed in the saponification, and B is the weight, in grams, of the acetylated oil used in the test.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 80% alcohol, often with opalescence.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made of aluminum or glass or that are lined with tin.

Angelica Root Oil

CAS: [8015-64-3]

View IR

DESCRIPTION

Angelica Root Oil occurs as a pale yellow to deep amber liquid with a warm, pungent odor and bittersweet taste. It is obtained by steam distillation of the dried slender rootlets of *Angelica archangelica* L. (Fam. Umbelliferae). It is soluble in most fixed oils, slightly soluble in mineral oil, but relatively insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 7.0.
Angular Rotation Between 0° and +46°.
Ester Value Between 10 and 65.
Refractive Index Between 1.473 and 1.487 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.850 and 0.880.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed under *Ester Value*, Appendix VI, using about 5 g of sample, accurately weighed.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 90% alcohol, often with turbidity, and remains in solution on further addition of alcohol to a total of 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined. The oil increases in specific gravity and viscosity during storage.

Angelica Seed Oil

View IR

DESCRIPTION

Angelica Seed Oil occurs as a light yellow liquid having a sweeter and more delicate aroma than the root oil. It is obtained by steam distillation of the fresh seeds of *Angelica archangelica* L. (Fam. Umbelliferae). It is soluble in most fixed oils, slightly soluble in mineral oil, but relatively insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those shown in the respective spectrum in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 3.0.
Angular Rotation Between +4° and +16°.
Ester Value Between 14.0 and 32.0.
Refractive Index Between 1.480 and 1.488 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.853 and 0.876.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed under *Ester Determination*, Appendix VI, using about 5 g of sample, accurately weighed.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 4 mL of 90% alcohol, often with considerable turbidity, and it remains in solution on further addition of alcohol to a total of 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Anise Oil

CAS: [8007-70-3]

View IR

DESCRIPTION

Anise Oil occurs as a colorless to pale yellow, strongly refractive liquid with the characteristic odor and taste of anise. It is obtained by steam distillation of the dried ripe fruit of *Pimpinella anisum* L. (Fam. Umbelliferae) or *Illicium verum* Hooker filius (Fam. Magnoliaceae).

> **Note**: If solid material has separated, carefully warm the sample until it is completely liquefied, and mix before using it.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between +1° and –1°.
Phenols Passes test.
Refractive Index Between 1.553 and 1.560 at 20°.
Solidification Point Not lower than 15°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.978 and 0.988.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Phenols Prepare a 1:3 solution of recently distilled sample in 90% alcohol. It is neutral to moistened litmus paper, and adding 1 drop of ferric chloride TS to 5 mL of the solution produces no blue or brown color.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solidification Point Determine as directed under *Solidification Point*, Appendix IIB.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI, using 3 mL of 90% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Annatto Extracts

INS: 160b CAS: [1393-63-1]

DESCRIPTION

Annatto Extracts occur as dark red solutions, emulsions, or suspensions in water or oil or as dark red powders. The extract is prepared from annatto seed, *Bixa orellana* L. (Fam. Bixaceae), using a food-grade extraction solvent. Bixin is the principal pigment of oil-soluble Annatto Extracts. Norbixin is the principal pigment of alkaline water-soluble Annatto Extracts. Commercial preparations are usually mixtures of bixin, norbixin, and other carotenoids.

Function Color.

REQUIREMENTS

Identification

A. *Oil- and Water-Soluble Annatto Extracts* Oil-soluble Annatto Extracts diluted with acetone exhibit absorbance maxima at 439, 470, and 501 nm. Water-soluble Annatto Extracts diluted with water exhibit absorbance maxima at 451 to 455 nm and 480 to 484 nm.

B. *Carr-Price Reaction* (See *Chromatography*, Appendix IIA.) Prepare a small chromatography column by filling a 200- × 7-mm glass tube, stoppered with glass wool, with alumina (80- to 200-mesh) slurried in toluene so that the settled alumina fills about $^2/_3$ of the tube. Using a rubber outlet tube and clamp, adjust the flow rate to about 30 drops/min.

Oil-Soluble Annatto Extracts Add to the top of the alumina column 3 mL of a solution containing sufficient sample,

in toluene, to impart a color equivalent to a solution of 0.1% potassium dichromate. Elute with toluene until a pale yellow fraction is washed from the column. Wash the column with three 10-mL volumes of dry acetone, add 5 mL of *Carr-Price Reagent* (see *Solutions and Indicators*), and allow it to run onto the top of the column. The orange-red zone (bixin) at the top of the column immediately turns blue-green.

Water-Soluble Annatto Extracts Transfer 2 mL or 2 g of sample into a 50-mL separatory funnel, and add sufficient 2 *N* sulfuric acid to make the solution acidic to pH test paper (pH 1 to 2). Dissolve the red precipitate of norbixin by mixing the solution with 50 mL of toluene. Discard the water layer, and wash the toluene phase with water until it no longer gives an acid reaction. Remove any undissolved norbixin by centrifugation or filtration, and dry the solution over anhydrous sodium sulfate. Transfer 3 to 5 mL of the dry solution to the top of an alumina column prepared as described above. Elute the column with toluene, three 10-mL volumes of dry acetone, and 5 mL of *Carr-Price Reagent* (see *Solutions and Indicators*) added to the top of the column. The orange-red band of norbixin immediately turns blue-green.

Arsenic Not more than 3 mg/kg.

Color Intensity A sample meets the representations of the vendor.

Lead Not more than 10 mg/kg.

Residual Solvents *Acetone*: Not more than 0.003%; *Hexanes*: Not more than 0.0025%; *Isopropyl Alcohol*: Not more than 0.005%; *Methyl Alcohol*: Not more than 0.005%; *Trichloroethylene* and *Dichloromethane*: Not more than 0.003%, individually or in combination.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Color Intensity

Oil-Soluble Annatto Extracts Transfer a sample, accurately weighed, into a solution of 1% glacial acetic acid in acetone, and dilute to a suitable volume (absorbance of 0.5 to 1.0). Filter the sample to clarify if necessary. Measure the absorbance at 454 nm, and calculate the color intensity by the formula

$$A/(b \times c),$$

in which A is the absorbance of the *Sample Solution*; b is the length, in centimeters, of the cell; and c is the concentration, in grams per liter, of the *Sample Solution*.

Water-Soluble Annatto Extracts Determine as directed under *Oil-Soluble Annatto Extracts* (above), but dissolve the sample in 0.1 *M* sodium hydroxide, and measure the absorbance at 453 nm.

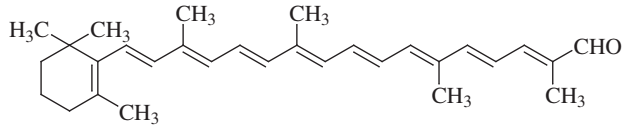
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 μg of lead (Pb) ion in the control.

Residual Solvents Determine as directed under *Residual Solvent*, Appendix VIII.

Packaging and Storage Store under refrigeration in full, well-closed containers that are made from steel or aluminum and that are suitably lined.

β-Apo-8′-Carotenal

Apocarotenal; APO



$C_{30}H_{40}O$ Formula wt 416.65

INS: 160e CAS: [1107-26-2]

DESCRIPTION

β-Apo-8′-Carotenal occurs as a fine, crystalline powder with a dark, metallic sheen. It is freely soluble in chloroform and sparingly soluble in acetone, but it is insoluble in water. It melts at 136° to 140° with decomposition.

Function Color.

REQUIREMENTS

Identification

A. Determine the absorbance of *Sample Solution B*, prepared as directed in the *Assay* (below), at 488 nm and at 460 nm. The ratio A_{488}/A_{460} is between 0.77 and 0.85.

B. Determine the absorbance of *Sample Solution B* at 460 nm, and that of *Sample Solution A*, prepared as directed in the *Assay* (below), at 332 nm. The ratio A_{332}/A_{460} is between 0.63 and 0.75.

Assay Not less than 96.0% and not more than 101.0% of $C_{30}H_{40}O$.

Arsenic Not more than 1 mg/kg.

Lead Not more than 10 mg/kg.

Residue on Ignition Not more than 0.2%.

TESTS

Assay (**Note**: Carry out all work in low-actinic glassware and in subdued light.)

Sample Solution A Transfer about 40 mg of sample, accurately weighed, into a 100-mL volumetric flask, dissolve in 10 mL of acid-free chloroform, dilute to volume with cyclohexane, and mix. Pipet 2 mL of this solution into a 50-mL volumetric flask, dilute to volume with cyclohexane, and mix.

Sample Solution B Pipet 5 mL of *Sample Solution A* into a 50-mL volumetric flask, dilute to volume with cyclohexane, and mix.

Procedure Determine the absorbance of *Sample Solution B* in a 1-cm cell at the wavelength of maximum absorption

at about 460 nm, with a suitable spectrophotometer, using cyclohexane as the blank. Calculate the quantity, in milligrams, of $C_{30}H_{40}O$ in the sample taken by the formula

$$25{,}000A/264,$$

in which A is the absorbance of the solution and 264 is the absorptivity of pure β-Apo-8′-Carotenal.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 µg of lead (Pb) ion in the control.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in tight, light-resistant containers under inert gas.

Arabinogalactan

Larch Fiber; Larch Gum

INS: 409 CAS: [9036-66-2]

DESCRIPTION

Arabinogalactan occurs as a white to yellow-white, coarse or fine powder. It is the dried water extract from the wood of the larch trees *Larix occidentalis* and *Larix laricina* (Fam. Pinaceae). It is a highly branched polysaccharide that has a molecular weight of 15,000 to 60,000 daltons and is composed of galactose units and arabinose units in the approximate ratio of 6:1. It is freely dispersible in hot or cold water. It is insoluble in alcohol.

Function Dietary fiber; humectant; stabilizer.

REQUIREMENTS

Assay (Total Carbohydrates) Not less than 80% of Arabinogalactan.

Identification Add 20 g of sample to 20 mL of water, and stir until completely dissolved. Pour the solution into a 500-mL beaker, and add 100 mL of water. Transfer 7 mL of the resulting solution into 250-mL beaker and add 0.2 mL of diluted lead subacetate TS. No precipitate forms. Add 280 mL of 95% ethyl alcohol to the remainder of the solution. A precipitate forms.

Ash (Total) Not more than 10.0%.

Lead Not more than 0.1 mg/kg.

Insoluble Matter Not more than 0.1%.

Loss on Drying Not more than 8.0%.

Protein Not more than 1.0%.

Starch Passes test.

Carbohydrates (Total) Not less than 80.0%.

TESTS

Assay (Total Carbohydrates) The remainder, after subtracting from 100% the sum of the percentages of *Ash, Loss on Drying*, and *Protein*, represents the percent of total carbohydrates (as arabinogalactan) in the sample.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test,* Appendix IIIB.

Insoluble Matter Dissolve 5 g of sample in about 100 mL of water contained in a 250-mL Erlenmeyer flask, add 10 mL of 2.7 *N* hydrochloric acid, and boil gently for 15 min. Use suction to filter the hot solution through a tared, filtered crucible; wash thoroughly with hot water; dry at 105° for 2 h; and weigh.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 5 h.

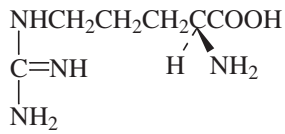
Protein Determine as directed under *Nitrogen Determination*, Appendix IIIC, transferring about 3.5 g of sample, accurately weighed, into a 500-mL Kjeldahl flask. The percent of protein equals the percent of N × 6.25.

Starch Add a few drops of iodine TS to a 1:10 aqueous solution. No blue or red color appears.

Packaging and Storage Store in well-closed containers.

L-Arginine

L-2-Amino-5-guanidinovaleric Acid



$C_6H_{14}N_4O_2$ Formula wt 174.20

CAS: [74-79-3]

View IR

DESCRIPTION

L-Arginine occurs as white crystals or as a white crystalline powder. It is soluble in water, insoluble in ether, and sparingly soluble in alcohol. It is strongly alkaline, and its water solutions absorb carbon dioxide from the air.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.5% and not more than 101.5% of $C_6H_{14}N_4O_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 1.0%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +26.0° and +27.9°, calculated on the dried basis; or $[\alpha]_D^{25^\circ}$: Between +25.8° and +27.7°, calculated on the dried basis.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely. Each milliliter of 0.1 *N* perchloric acid consumed in the assay is equivalent to 8.710 mg of $C_6H_{14}N_4O_2$.

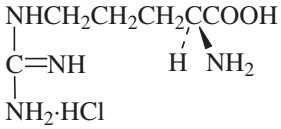
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying the sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

L-Arginine Monohydrochloride

L-2-Amino-5-guanidinovaleric Acid Monohydrochloride



$C_6H_{14}N_4O_2$·HCl Formula wt 210.66

CAS: [1119-34-2]

View IR

DESCRIPTION

L-Arginine Monohydrochloride occurs as a white or nearly white crystalline powder. It is soluble in water, slightly soluble in hot alcohol, and insoluble in ether. It is acidic and melts with decomposition at about 235°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_{14}N_4O_2$·HCl, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +21.3° and 23.5°, calculated on the dried basis; or $[\alpha]_D^{25^\circ}$: Between 21.3° and +23.4°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 100 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 2 mL of formic acid, add exactly 15.0 mL of 0.1 *N* perchloric acid, and heat on a water bath for 30 min.

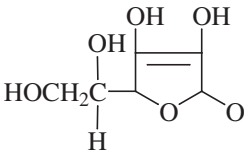
> **Caution**: Handle perchloric acid in an appropriate fume hood.

After cooling, add 45 mL of glacial acetic acid, and titrate the excess perchloric acid with 0.1 *N* sodium acetate, determining the endpoint potentiometrically. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 10.53 mg of $C_6H_{14}N_4O_2$·HCl.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying the sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Ascorbic Acid

Vitamin C; L-Ascorbic Acid



$C_6H_8O_6$ Formula wt 176.13

INS: 300 CAS: [50-81-7]

DESCRIPTION

Ascorbic Acid occurs as white or slightly yellow crystals or as powder. It melts at about 190°. It gradually darkens on exposure to light, is reasonably stable in air when dry, but rapidly deteriorates in solution in the presence of air. One gram is soluble in about 3 mL of water and in about 30 mL of alcohol. It is insoluble in chloroform and in ether.

Function Antioxidant; meat-curing aid; nutrient.

REQUIREMENTS

Identification

A. A 1:50 aqueous solution slowly reduces alkaline cupric tartrate TS at 25°, but more readily upon heating.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits maxima at the same wavelengths as those of a similar preparation of USP Ascorbic Acid Reference Standard.

Assay Not less than 99.0% and not more than 100.5% of $C_6H_8O_6$.

Lead Not more than 2 mg/kg.

Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Between +20.5° and +21.5°.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 400 mg of sample, accurately weighed, in a mixture of 100 mL of water, recently boiled and cooled, and 25 mL of 2 *N* sulfuric acid. Titrate the solution immediately with 0.1 *N* iodine, adding starch TS near the endpoint. Each milliliter of 0.1 *N* iodine is equivalent to 8.806 mg of $C_6H_8O_6$.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

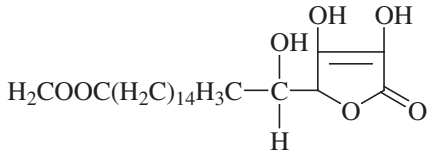
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 1 g of sample in 10 mL of carbon dioxide-free water.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in tight, light-resistant containers.

Ascorbyl Palmitate

Palmitoyl L-Ascorbic Acid



$C_{22}H_{38}O_7$ Formula wt 414.54

INS: 304 CAS: [137-66-6]

DESCRIPTION

Ascorbyl Palmitate occurs as a white or yellow-white powder. It is very slightly soluble in water and in vegetable oils. One gram dissolves in about 4.5 mL of alcohol.

Function Antioxidant.

REQUIREMENTS

Identification A 1:10 solution in alcohol decolorizes dichlorophenol–indophenol TS.

Assay Not less than 95.0% of $C_{22}H_{38}O_7$, calculated on the dried basis.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 2%.

Melting Range Between 107° and 117°.

Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Between +21° and +24°, calculated on the dried basis.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 300 mg of sample, accurately weighed, in 50 mL of alcohol contained in a 250-mL Erlenmeyer flask, add 30 mL of water, and immediately titrate with 0.1 *N* iodine to a yellow color that persists for at least 30 s. Each milliliter of 0.1 *N* iodine is equivalent to 20.73 mg of $C_{22}H_{38}O_7$.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in a vacuum oven at 560° to 600° for 1 h.

Melting Range Determine as directed in *Procedure for Class Ia*, under *Melting Range or Temperature*, Appendix IIB.

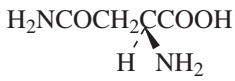
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 1 g of sample in 10 mL of methanol.

Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in tightly closed containers, preferably in a cool, dry place.

L-Asparagine

L-α-Aminosuccinamic Acid



$C_4H_8N_2O_3$ Formula wt, anhydrous 132.12
$C_4H_8N_2O_3 \cdot H_2O$ Formula wt, monohydrate 150.13

CAS: anhydrous [70-47-3]
CAS: monohydrate [5794-13-8]

View IR

DESCRIPTION

L-Asparagine occurs as white crystals or as a crystalline powder. It is soluble in water and practically insoluble in alcohol and in ether. Its solutions are acid to litmus. It melts at about 234°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.0% and not more than 101.5% of $C_4H_8N_2O_3$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Between 11.5% and 12.5%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +33.0° and +36.5°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 130 mg of sample, previously dried at 130° for 3 h and accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically.

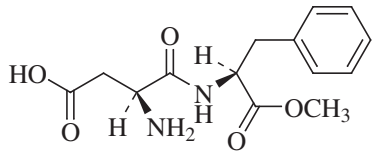
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 13.21 mg of $C_4H_8N_2O_3$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and using 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 130° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Aspartame

N-L-α-Aspartyl-L-phenylalanine 1-Methyl Ester; APM



$C_{14}H_{18}N_2O_5$ Formula wt 294.31
INS: 951 CAS: [22839-47-0]

DESCRIPTION

Aspartame occurs as a white, crystalline powder. It is sparingly soluble in water and slightly soluble in alcohol. The pH of a 0.8% solution is between about 4.5 and 6.0.

Function Sweetener; sugar substitute; flavor enhancer.

REQUIREMENTS

Identification The infrared absorption spectrum of a potassium bromide dispersion of sample exhibits maxima only at the same wavelengths as those of a similar preparation of USP Aspartame Reference Standard.
Assay Not less than 98.0% and not more than 102.0% of $C_{14}H_{18}N_2O_5$, calculated on the dried basis.
5-Benzyl-3,6-dioxo-2-piperazineacetic Acid Not more than 1.5%.
Lead Not more than 1 mg/kg.
Loss on Drying Not more than 4.5%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +14.5° and +16.5°, calculated on the dried basis.
Other Related Substances Not more than 2.0%.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Transfer about 300 mg of sample, accurately weighed, to a 150-mL beaker, dissolve in 1.5 mL of 96%

formic acid, and add 60 mL of glacial acetic acid. Add crystal violet TS, and titrate immediately with 0.1 *N* perchloric acid to a green endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

> **Note**: Use 0.1 *N* perchloric acid previously standardized to a green endpoint. A blank titration exceeding 0.1 mL may be due to excessive water content and may cause loss of visual endpoint sensitivity.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid

Mobile Phase Weigh and transfer 5.6 g of potassium phosphate monobasic into a 1-L flask, add 820 mL of water, and dissolve. Adjust the pH to 4.3 using phosphoric acid, add 180 mL of methanol, and mix. Filter through a 0.45-μm disk, and de-gas.

Diluting Solvent Add 200 mL of methanol to 1800 mL of water, and mix.

Impurity Standard Preparation Transfer about 25 mg of USP 5-Benzyl-3,6-dioxo-2-piperazineacetic Acid Reference Standard, accurately weighed, into a 100-mL volumetric flask. Add 10 mL of methanol, and dissolve. Dilute to volume with water, and mix. Pipet 15 mL of this solution into a 50-mL volumetric flask, dilute to volume with *Diluting Solvent*, and mix. Use a freshly prepared solution.

Sample Preparation Transfer about 50 mg of sample, accurately weighed, to a 10-mL volumetric flask. Dilute to volume with *Diluting Solvent*, and mix. Use a freshly prepared solution.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a suitable high-performance liquid chromatograph equipped with a detector measuring at 210 nm and a 250- × 4.6-mm (id) column packed with octadecyl silanized silica (10-μm Partisil ODS-3, or equivalent), and operated under isocratic conditions at 40°. The flow rate of the *Mobile Phase* is about 2 mL/min.

System Suitability The area responses of three replicate injections of *Impurity Standard Preparation* show a relative standard deviation of not more than 2.0%.

Procedure Separately inject equal 20-μL portions of *Impurity Standard Preparation* and *Sample Preparation* into the chromatograph, and record the chromatograms (the approximate retention time of 5-benzyl-3,6-dioxo-2-piperazineacetic acid is 4 min, and the approximate retention time of Aspartame is 11 min). Measure the peak area response of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in each chromatogram. Calculate the percentage of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the sample by the formula

$$1000(A_U C_S)/(A_S W_U),$$

in which A_U and A_S are the peak area responses of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the *Sample Preparation* and in the *Impurity Standard Preparation*, respectively; C_S is the concentration, in milligrams per milliliter, of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the *Impurity Standard Preparation*; and W_U is the weight, in milligrams, of Aspartame taken for the *Sample Preparation*.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 1-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 4 g of sample in sufficient 15 *N* formic acid to make 100 mL. Make the determination within 30 min of preparing the *Sample Solution*.

Other Related Impurities Proceed as directed in the test for *5-Benzyl-3,6-dioxo-2-piperazineacetic Acid* (above), except use the following in place of the *Standard Preparation*:

Other Related Substances Standard Preparation Pipet 2 mL of the *Sample Preparation* from the test for *5-Benzyl-3,6-dioxo-2-piperazineacetic Acid* into a 100-mL volumetric flask, dilute to volume with the *Diluting Solvent*, and mix.

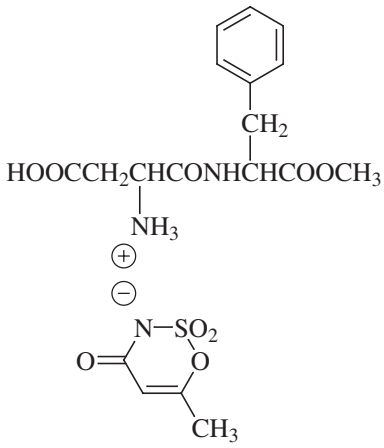
Procedure Inject about 20-μL portions of the *Other Related Substances Standard Preparation* and the *Sample Preparation* into the chromatograph, and record the chromatogram for a time equal to twice the retention time of Aspartame. In the chromatogram obtained from the *Sample Preparation*, the sum of the responses of all secondary peaks, other than that for 5-benzyl-3,6-dioxo-2-piperazineacetic acid, is not more than the response of the Aspartame peak obtained in the chromatogram from the *Other Related Substances Standard Preparation*.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers in a cool, dry place.

Aspartame-Acesulfame Salt

APM-Ace; [2-carboxy-β-(*N*-(*b*-methoxycarbonyl-2-phenyl)ethylcarbamoyl)]ethanaminium 6-methyl-4-oxo-1,2,3-oxathiazin-3-ide-2,2-dioxide; L-Phenylalanine, L-α-aspartyl-2-methyl ester compound with 6-methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (1:1)



$C_{18}H_{23}O_9N_3S$ Formula wt 457.45

CAS: [106372-55-8]

View IR

DESCRIPTION

Aspartame-Acesulfame Salt occurs as a white, crystalline powder. It is sparingly soluble in water and slightly soluble in alcohol.

Function Sweetener.

REQUIREMENTS

Identification An infrared absorption spectrum of a potassium bromide dispersion of Aspartame-Acesulfame Salt exhibits maxima only at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 63.0% and not more than 66.0% of aspartame, calculated on the dried basis. Not less than 34.0% and not more than 37.0% of acesulfame, calculated as acid formed on the dried basis.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid (DKP) Not more than 0.5%.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 0.5%.

Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +14.5° and +16.5°, calculated on the dried basis.

Other Related Impurities Not more than 1.0%.

Potassium Not more than 0.5%.

TESTS

Assay (**Note**: Use a combination pH-electrode for all titrations.) Dissolve 0.100 to 0.150 g of sample, accurately weighed, in 50 mL of ethanol. Under a flow of nitrogen, titrate the solution with standardized 0.1 *N* tetrabutylammonium hydroxide in methanol or 2-propanol. Determine the volume of titrant needed to reach the first equivalence point (V_1 mL) and the second equivalence point (V_2 mL).

Perform a blank titration with 50 mL of ethanol. Calculate the percent Acesulfame and Aspartame, respectively, taken by the formulas

$$[(V_1 - V_B) \times N \times 163]/(10 \times W),$$

$$[(V_2 - V_1) \times N \times 294]/(10 \times W),$$

in which V_1, V_2, and V_B are the number of milliliters of 0.1 *N* tetrabutylammonium hydroxide in methanol or 2-propanol used for the sample (first and second equivalence points) and the blank, respectively; *N* is the normalityof the tetrabutylammonium hydroxide; *W* is the weight, in grams, of sample taken; and 163 and 294 are the formula weights of acesulfame and aspartame, respectively.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid

Mobile Phase Dissolve 5.6 g of potassium phosphate, monobasic, accurately weighed, in 820 mL of water contained in a 1-L flask. Use phosphoric acid to adjust the pH to 4.3, add 180 mL of methanol, and mix. Filter through a 0.45-μm disk, and de-gas.

Diluting Solvent Add 200 mL of methanol to 1800 mL of water, and mix.

Standard Preparation Transfer about 25 mg of USP Reference Standard 5-Benzyl-3,6-dioxo-2-piperazineacetic Acid, accurately weighed, into a 100-mL volumetric flask. Add 10 mL of methanol, and dissolve. Dilute to volume with water, and mix. Pipet 15 mL of this solution into a 50-mL volumetric flask, dilute to volume with the *Diluting Solvent*, and mix. Use a freshly prepared solution on the day of use.

Sample Preparation Transfer about 50 mg of sample, accurately weighed, into a 10-mL volumetric flask. Dilute to volume with *Diluting Solvent*, and mix. Use a freshly prepared solution on the day of use.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a suitable high-performance liquid chromatograph equipped with a detector measuring at 210 nm and a 250- × 4.6-mm (id) column packed with octadecyl silanized silica (10-μm Partisil ODS-3, or equivalent), and operated under isocratic conditions at 40°. The flow rate of the *Mobile Phase* is about 2 mL/min.

System Suitability The area responses of three replicate injections of the *Standard Preparation* show a relative standard deviation of not more than 2.0%.

Procedure Separately inject equal 20-μL portions of the *Standard Preparation* and the *Sample Preparation* into the chromatograph, and record the chromatograms (the approximate retention time of 5-benzyl-3,6-dioxo-2-piperazineacetic acid is 4 min, and the approximate retention time of aspartame is 11 min). Measure the peak area response of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in each chromatogram.

Calculation Calculate the percent 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the sample taken by the formula

$$1000 \times (A_U C_S)/(A_S W_U),$$

in which A_U and A_S are the peak area responses of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the *Sample Preparation* and in the *Standard Preparation*, respectively; C_S is the concentration, in milligrams per milliliter, of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the *Standard Preparation*; and W_U is the weight, in milligrams, of sample taken for the *Sample Preparation*.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 6.2 g of sample in sufficient 15 *N* formic acid to make 100 mL. Make the determination within 30 min of preparation of the *Sample Solution*. Divide the calculated specific rotation by 0.646 to correct for the Aspartame content in Aspartame-Acesulfame Salt.

Other Related Impurities Determine as directed in the test for *5-Benzyl-3,6-dioxo-2-piperazineacetic Acid* (above), but use the following *Standard Preparation* and *Procedure*:

Standard Preparation Pipet 1.5 mL of the *Sample Preparation* from the test for *5-Benzyl-3,6-dioxo-2-piperazineacetic Acid* into a 100-mL volumetric flask, dilute to volume with the *Diluting Solvent*, and mix.

Procedure Separately inject equal 20-µL portions of the *Standard Preparation* and the *Sample Preparation* into the chromatograph, and record the chromatograms for a time equal to twice the retention time of Aspartame. In the chromatogram obtained from the *Sample Preparation*, the sum of the responses of all secondary peaks, other than those for 5-benzyl-3,6-dioxo-2-piperazineacetic acid and Acesulfame, is not more than the response of the Aspartame peak obtained in the chromatogram from the *Standard Preparation*.

Potassium

Standard Solutions Transfer 190.7 mg of potassium chloride, previously dried at 105° for 2 h, into a 1000-mL volumetric flask, dilute to volume with water, and mix. Transfer 100.0 mL of this solution to a second 1000-mL volumetric flask, dilute to volume with water, and mix to obtain a *Stock Solution* containing 10 µg of potassium per milliliter (equivalent to 19.07 µg of potassium chloride). Pipet 10.0-, 15.0-, and 20.0-mL aliquots of the *Stock Solution* into separate 100-mL volumetric flasks; add 2.0 mL of a 1:5 solution of sodium chloride and 1.0 mL of hydrochloric acid to each; dilute with water to volume; and mix. The *Standard Solutions* obtained contain, respectively, 1.0, 1.5, and 2.0 µg of potassium per milliliter.

Test Solution Transfer about 3.0 g of sample, accurately weighed, into a 500-mL volumetric flask, dilute to volume with water, and mix. Transfer 10 mL of this solution into a 100-mL volumetric flask and add 2.0 mL of a 1:5 sodium chloride solution and 1.0 mL of hydrochloric acid, dilute to volume with water, and mix. Filter the solution.

Procedure Concomitantly determine the absorbances of the *Standard Solutions* and the *Test Solution* at the potassium emission line of 766.5 nm, using a suitable atomic absorption spectrophotometer equipped with a potassium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbance of the *Standard Solutions* versus concentration, in micrograms per milliliter, of potassium, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, *C*, in micrograms per milliliter, of potassium in the *Test Solution*. Calculate the percent potassium in the sample taken by the formula

$$500C/W,$$

in which *W* is the quantity, in milligrams, of sample taken to prepare the *Test Solution*.

Packaging and Storage Store in well-closed containers in a cool, dry place.

DL-Aspartic Acid

DL-Aminosuccinic Acid

$$HOOCCH_2CH(NH_2)COOH$$

$C_4H_7NO_4$ Formula wt 133.10

CAS: [617-45-8]

View IR

DESCRIPTION

DL-Aspartic Acid occurs as colorless or white crystals. It is slightly soluble in water, but insoluble in alcohol and in ether. It is optically inactive and melts with decomposition at about 280°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.5% and not more than 101.5% of $C_4H_7NO_4$, calculated on the dried basis.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely.

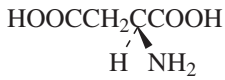
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 13.31 mg of $C_4H_7NO_4$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

L-Aspartic Acid

L-Aminosuccinic Acid



$C_4H_7NO_4$ Formula wt 133.10

CAS: [56-84-8]

View IR

DESCRIPTION

L-Aspartic Acid occurs as white crystals or as a crystalline powder. It is slightly soluble in water, but insoluble in alcohol and in ether. It melts at about 270°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_4H_7NO_4$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.25%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +24.5° and +26.0°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely.

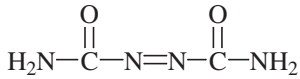
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 13.31 mg of $C_4H_7NO_4$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Azodicarbonamide

Azodicarboxylic Acid Diamide



$C_2H_4N_4O_2$ Formula wt 116.08

INS: 927a CAS: [123-77-3]

DESCRIPTION

Azodicarbonamide occurs as a yellow to orange-red, crystalline powder. It is practically insoluble in water and in most organic solvents. It is slightly soluble in dimethyl sulfoxide. It melts above 180° with decomposition.

Function Maturing agent for flour.

REQUIREMENTS

Identification A solution of 35 mg of sample in 1000 mL of water exhibits an ultraviolet absorption maximum at about 245 nm.
Assay Not less than 98.6% and not more than 100.5% of $C_2H_4N_4O_2$ after drying.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.5%.
Nitrogen Between 47.2% and 48.7%.
pH of a 2% Suspension Not less than 5.0.
Residue on Ignition Not more than 0.15%.

TESTS

Assay Transfer about 225 mg of sample, previously dried in a vacuum oven at 50° for 2 h and accurately weighed, into

a 250-mL glass-stoppered iodine flask. Add about 23 mL of dimethyl sulfoxide to the flask, washing any adhered sample down with the solvent, then stopper the flask, and place about 2 mL of the solvent in the cup or lip of the flask. Swirl occasionally, until complete solution of the sample is effected, and then loosen the stopper to drain the remainder of solvent into the flask and to rinse down any dissolved sample into the solution. Add 5.0 g of potassium iodide followed by 15 mL of water, then immediately pipet 10 mL of 0.5 *N* hydrochloric acid into the flask, and rapidly stopper. Swirl until the potassium iodide dissolves, and allow to stand for 20 to 25 min protected from light. Titrate the liberated iodine with 0.1 *N* sodium thiosulfate to the disappearance of the yellow color. Titrate with additional thiosulfate if any yellow color appears within 15 min. Perform a blank determination (see *General Provisions*) on a solution consisting of 25 mL of dimethyl sulfoxide, 5.0 g of potassium iodide, 15 mL of water, and 5 mL of 0.5 *N* hydrochloric acid, and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 5.804 mg of $C_2H_4N_4O_2$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead ion (Pb) in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in a vacuum oven at 50° for 2 h.

Nitrogen Transfer about 50 mg of sample into a 100-mL Kjeldahl flask, add 3 mL of concentrated hydriodic acid solution (57% freshly assayed), and digest the mixture with gentle heating for 1.25 h, adding sufficient water, when necessary, to maintain the original volume. Increase the heat at the end of the digestion period, and continue heating until the volume is reduced by about one-half. Cool to room temperature, add 1.5 g of potassium sulfate, 3 mL of water, and 4.5 mL of sulfuric acid, and heat until iodine fumes no longer evolve. Allow the mixture to cool, wash down the sides of the flask with water, heat until charring occurs, and again cool to room temperature. Add 40 mg of mercuric oxide to the charred material, heat until the color of the solution is pale yellow, then cool, wash down the sides of the flask with a few milliliters of water, and digest the mixture for an additional 3 h. Cool the digest, add 20 mL of ammonia-free water, 16 mL of a 50% sodium hydroxide solution, and 5 mL of a 44% sodium thiosulfate solution. Immediately connect the flask to a distillation apparatus as directed under *Nitrogen Determination*, Appendix IIIC, and distill, collecting the distillate in 10 mL of a 4% boric acid solution. Add a few drops of methyl red–methylene blue TS to the distillate, and titrate with 0.05 *N* sulfuric acid. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.05 *N* sulfuric acid is equivalent to 0.7004 mg of nitrogen.

pH of a 2% Suspension Determine as directed under *pH Determination*, Appendix IIB, using the following solution: Add 2 g of sample to 100 mL of water, and agitate the mixture with a power stirrer for 5 min.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1.5-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Balsam Peru Oil

CAS: [8007-00-9]

View IR

DESCRIPTION

Balsam Peru Oil occurs as a yellow to pale brown, slightly viscous liquid having a sweet, balsamic odor. It is obtained by extraction or distillation of Peruvian Balsam obtained from *Myroxylon pereirae* Royle Klotzsche (Fam. Leguminosae). Occasionally, crystals may occur within the liquid. It is soluble in most fixed oils, and is soluble, with turbidity, in mineral oil. It is partly soluble in propylene glycol, but it is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Between 30 and 60.
Angular Rotation Between –1° and +2°.
Ester Value Between 200 and 225.
Refractive Index Between 1.567 and 1.579 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.095 and 1.110.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed in *Ester Value* under *Esters*, Appendix VI, using about 1 g of sample, accurately weighed.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 0.5 mL of 90% alcohol and remains in solution upon dilution to 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Basil Oil, Comoros Type

Basil Oil Exotic; Basil Oil, Réunion Type

View IR

DESCRIPTION

Basil Oil, Comoros Type, occurs as a light yellow liquid with a spicy odor. It is obtained by steam distillation of the flowering tops or the entire plant of *Ocimum basilicum* L. (Fam. Lamiaceae). It may be distinguished from other types, such as basil oil, European type, by its camphoraceous odor and physicochemical constants. It is soluble in most fixed oils and, with turbidity, in mineral oil. One milliliter is soluble in 20 mL of propylene glycol with slight haziness, but it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 1.0.
Angular Rotation Between –2° and +2°.
Ester Value after Acetylation Between 25 and 45.
Refractive Index Between 1.512 and 1.520 at 20°.
Saponification Value Between 4 and 10.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.952 and 0.973.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value after Acetylation Determine as directed under *Linalool Determination*, Appendix VI, using about 2.5 g of the dry acetylated oil, accurately weighed, for the saponification. Calculate the *Ester Value after Acetylation* by the formula

$$a \times 28.05/b,$$

in which a is the volume, in milliliters, of 0.5 N alcoholic potassium hydroxide consumed in the saponification, and b is the weight of the dry acetylated oil, in grams, used in the test.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 4 mL of 80% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Basil Oil, European Type

Basil Oil, Italian Type; Sweet Basil Oil

CAS: [8015-73-4]

View IR

DESCRIPTION

Basil Oil, European Type, occurs as a pale yellow to yellow liquid with a floral-spicy odor. It is obtained by the steam distillation of the flowering tops or the entire plant of *Ocimum basilicum* L. It may be distinguished from other types, such as basil oil, Comoros type, or basil oil, Réunion type, by its more floral odor and its physicochemical constants. It is soluble in most fixed oils and, with turbidity, in mineral oil. One milliliter is soluble in 20 mL of propylene glycol with slight haziness, but it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 2.5.
Angular Rotation Between –5° and –15°.
Ester Value after Acetylation Between 140 and 180.
Refractive Index Between 1.483 and 1.493 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.900 and 0.920.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value after Acetylation Determine as directed under *Linalool Determination*, Appendix VI, using 2.5 g of the dry acetylated oil, accurately weighed, for the saponification. Calculate the *Ester Value after Acetylation* by the formula

$$a \times 28.05/b,$$

in which a is the number of milliliters of 0.5 N alcoholic potassium hydroxide consumed in the saponification, and b is the weight, in grams, of the acetylated oil used in the test.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI, using 4 mL of 80% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Bay Oil

Myrcia Oil

View IR

DESCRIPTION

Bay Oil occurs as a yellow or brown-yellow liquid with a pleasant, aromatic odor and a pungent, spicy taste. It is the volatile oil distilled from the leaves of *Pimenta acris* Kostel (Fam. Myrtaceae). It is soluble in alcohol and in glacial acetic acid. Its solutions in alcohol are acid to litmus.

Function Flavoring agent.

REQUIREMENTS

Identification
A. The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those shown in the respective spectrum in the section on *Infrared Spectra*, using the same test conditions as specified therein.
B. Shake 1 mL of sample with 20 mL of hot water, and filter. The filtrate gives not more than a slight acid reaction with litmus, and on the addition of 1 drop of ferric chloride TS yields only a transient gray-green, not a blue or purple, color.
Assay Not less than 50% and not more than 65%, by volume, of phenols.
Angular Rotation Levorotatory, but not more than –3°.
Refractive Index Between 1.507 and 1.516 at 20°.
Specific Gravity Between 0.950 and 0.990.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using in a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Beeswax, White

White Wax

INS: 901

DESCRIPTION

Beeswax, White, occurs as a yellow-white solid, somewhat translucent in thin layers, with a faint, characteristic odor, free from rancidity. It is the bleached, purified wax from the honeycomb of the bee *Apis mellifera* L. (Fam. Apidae), and it consists primarily of myricyl palmitate (myricin), cerotic acid and ester, and some high-carbon paraffins. Its specific gravity is about 0.95. Beeswax, White, is insoluble in water and sparingly soluble in cold alcohol. Boiling alcohol dissolves cerotic acid and part of the myricin. It is completely soluble in chloroform, in ether, and in fixed and volatile oils. It is partly soluble in cold carbon disulfide and is completely soluble in it at temperatures of 30° or above.

Function Surface-finishing (glazing) agent; release agent; raw material for flavoring agent.

REQUIREMENTS

Acid Value Between 17 and 24.
Carnauba Wax Passes test.
Ester Value Between 72 and 79.
Fats, Japan Wax, Rosin, and Soap Passes test.
Lead Not more than 5 mg/kg.
Melting Range Between 62° and 65°.
Saponification Cloud Test Passes test.

TESTS

Acid Value Warm about 3 g of sample, accurately weighed, in a 200-mL flask with 25 mL of absolute alcohol, previously neutralized to phenolphthalein with potassium hydroxide, until the sample is melted. Shake the mixture, add 1 mL of phenolphthalein TS, and titrate the warm solution with 0.5 *N* alcoholic potassium hydroxide to a permanent, faint pink color. Save this solution for the *Ester Value* test.
Carnauba Wax Place 100 mg of sample in a test tube, and add 20 mL of *n*-butanol. Immerse the test tube in boiling water, and shake the mixture gently until solution is complete. Transfer the test tube into a beaker of water at 60°, and allow it to cool to room temperature. A loose mass of fine, needle-like crystals separate from a clear mother liquor. Under the microscope, the crystals appear as loose needles or stellate clusters, and no amorphous masses are observed, indicating the absence of carnauba wax.
Ester Value Add 25.0 mL of 0.5 *N* alcoholic potassium hydroxide and 50 mL of alcohol to the solution resulting from the determination of *Acid Value*, heat the mixture under a reflux condenser for 4 h, and titrate the excess alkali with 0.5 *N* hydrochloric acid. Perform a residual blank titration, and calculate the *Ester Value* as the number of milligrams of

potassium hydroxide required for each gram of the sample taken for the test.

Fats, Japan Wax, Rosin, and Soap Boil 1 g of sample for 30 min with 35 mL of a 1:7 sodium hydroxide solution, maintaining the volume by the occasional addition of water, and cool the mixture. The wax separates and the liquid remains clear. Filter the cold mixture, and acidify the filtrate with hydrochloric acid. No precipitate forms.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.

Melting Range Determine as directed in *Procedure for Class II* under *Melting Range or Temperature*, Appendix IIB.

Saponification Cloud Test

Saponifying Solution Dissolve 40 g of potassium hydroxide in about 900 mL of aldehyde-free alcohol maintained at a temperature of 15° until solution is complete. Warm to room temperature, and add sufficient aldehyde-free alcohol to make 1000 mL.

Procedure Transfer 3.00 g of sample into a round-bottom, 100-mL boiling flask provided with a ground-glass joint, add 30 mL of the *Saponifying Solution*, attach a reflux condenser to the flask, and heat the mixture gently on a steam bath for 2 h. At the end of this period, remove the reflux condenser, insert a thermometer into the solution, and place the flask in an 80° water bath. Rotate the flask while both the bath and the solution cool to 65°. The solution shows no cloudiness or globule formation before this temperature is reached.

Packaging and Storage Store in well-closed containers.

Beeswax, Yellow

Yellow Wax

INS: 901 CAS: [8012-89-3]

DESCRIPTION

Beeswax, Yellow, occurs as a yellow to gray-brown solid with an agreeable, honey odor. It is the purified wax from the honeycomb of the bee *Apis mellifera* L. (Fam. Apidae) and consists primarily of myricyl palmitate (myricin), cerotic acid and ester, and some high-carbon paraffins. It is somewhat brittle when cold, and presents a dull, granular, noncrystalline fracture when broken. It becomes pliable at a temperature of about 35°. Its specific gravity is about 0.95. Beeswax, Yellow, is insoluble in water and sparingly soluble in cold alcohol. Boiling alcohol dissolves cerotic acid and part of the myricin. It is completely soluble in chloroform, in ether, and in fixed and volatile oils. It is partly soluble in cold carbon disulfide and completely soluble in it at temperatures of 30° or above.

Function Candy glaze and polish; raw material for flavoring agent.

REQUIREMENTS

Acid Value Between 18 and 24.
Carnauba Wax Passes test.
Ester Value Between 72 and 77.
Fats, Japan Wax, Rosin, and Soap Passes test.
Lead Not more than 5 mg/kg.
Melting Range Between 62° and 65°.
Saponification Cloud Test Passes test.

TESTS

Proceed as directed in the monograph for *Beeswax, White*.

Packaging and Storage Store in well-closed containers.

Bentonite

Smectite; Aluminum Silicate

INS: 558 CAS: [1302-78-9]

DESCRIPTION

Bentonite occurs commercially as powders ranging in colors and tints from off white to pale brown to gray depending on the cations present in natural deposits. It comprises natural smectite clays consisting primarily of colloidal hydrated aluminum silicates of the montmorillonite or hectorite type of minerals with varying quantities of alkalies, alkaline earths, and iron. It is insoluble in water, in alcohol, in dilute acids, and in alkalies.

Function Clarifying, filter agent.

REQUIREMENTS

Identification

A. With intense agitation, add 2 g of sample, in small portions, to 100 mL of water. Allow the mixture to stand for 12 h to ensure complete hydration. Place 2 mL of the mixture so obtained on a suitable glass slide, and allow it to air dry at room temperature to produce an oriented film. Place the slide in a vacuum desiccator over a free surface of ethylene glycol. Evacuate the desiccator, and close the stopcock so that ethylene glycol saturates the desiccator chamber. Allow the slide to stand for 12 h. Record the X-ray diffraction pattern using a copper source, and calculate the *d* values. The largest peak corresponds to a *d* value between 15.0 and 17.2 Å. Prepare a random powder specimen of sample, and determine the *d* values in the region between 1.48 and 1.54 Å. The peak is between 1.492 and 1.504 Å or between 1.510 and 1.540 Å.

B. Add 1 g of potassium nitrate and 3 g of anhydrous sodium carbonate to 0.5 g of sample contained in a metal crucible, heat until the mixture has melted, and allow it to cool. Add 20 mL of boiling water to the residue, mix, filter,

and wash the residue with 50 mL of water. Add 1 mL of hydrochloric acid and 5 mL of water to the residue, and filter. Add 1 mL of 10 *N* sodium hydroxide to the filtrate, filter, and add 3 mL of 2 *M* ammonium chloride. A gelatinous, white precipitate forms.

Arsenic Not more than 5 mg/kg.

Coarse Particles Not more than 0.5% of sample is retained on a 75-μm sieve.

Gel Formation Passes test.

Lead Not more than 0.004%.

Loss on Drying Not more than 8.0%.

Microbial Limits:

Aerobic Plate Count Not more than 1000 CFU per gram.

E. coli Negative in 25 g.

pH of a 1:50 Dispersion Between 8.5 and 10.5.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using 5.0 mL of the *Standard Arsenic Solution* and a 25-mL aliquot of the following *Sample Solution*: Transfer 8.0 g of dried sample into a 250-mL beaker containing 100 mL of 1:25 hydrochloric acid, mix, cover with a watch glass, and boil gently, stirring occasionally, for 15 min without allowing excessive foaming. Filter the hot supernatant liquid through a rapid-flow filter paper into a 200-mL volumetric flask, and wash the filter with four 25-mL portions of hot, 1:25 hydrochloric acid, collecting the washings in the volumetric flask. Cool the combined filtrates to room temperature, add 1:25 hydrochloric acid to volume, and mix.

Coarse Particles Add 100 mL of water to 20 g of sample, and mix for 15 min at not less than 5000 rpm. Transfer the mixture to a wet sieve of nominal mesh aperture (75 μm), previously dried at 100° to 105° and weighed, and wash with three 500-mL volumes of water, ensuring that any agglomerates are dispersed. Dry at 100° to 105°, and weigh. The difference in weight corresponds to the measure of coarse particles.

Gel Formation Mix 6 g of sample with 300 mg of magnesium oxide. Add the mixture, in several divided portions, to 200 mL of water contained in a blender jar with an approximately 500-mL capacity. Blend thoroughly for 5 min at high speed, transfer 100 mL of the mixture into a 100-mL graduated cylinder, and leave undisturbed for 24 h. Not more than 2 mL of supernatant liquid appears on the surface.

Lead (**Note**: The *Standard Preparation* and the *Test Preparation* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the spectrophotometer used.)

Standard Preparation On the day of use, dilute 3.0 mL of Lead Nitrate Stock Solution (see the *Flame Atomic Absorption Method* under *Lead Limit Test*, Appendix IIIB) to 100 mL with water. Each milliliter of the *Standard Preparation* contains the equivalent of 3 μg of lead.

Test Preparation Transfer 3.75 g of dried sample into a 250-mL beaker containing 100 mL of 1:25 hydrochloric acid, stir, cover with a watch glass, and boil for 15 min. Cool to room temperature, and allow the insoluble matter to settle. Decant the supernatant liquid through a rapid-flow filter paper into a 400-mL beaker. Wash the filter with four 25-mL portions of hot water, collecting the filtrate in the 400-mL beaker. Concentrate the combined extracts by gentle boiling to approximately 20 mL. If a precipitate forms, add 2 to 3 drops of nitric acid, heat to boiling, and cool to room temperature. Filter the concentrated extracts through a rapid-flow filter paper into a 50-mL volumetric flask. Transfer the remaining contents of the 400-mL beaker through the filter paper and into the flask with water. Dilute to volume with water, and mix.

Procedure Determine the absorbances of the *Test Preparation* and the *Standard Preparation* at 284 nm in a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp, deuterium arc background correction, and a single-slot burner, using an oxidizing air–acetylene flame. The absorbance of the *Test Preparation* is not greater than that of the *Standard Preparation.*

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

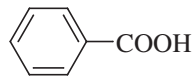
Aerobic Plate Count

E. coli

pH of a 1:50 Dispersion Disperse 4.0 g of sample in 200 mL of water, mixing vigorously to facilitate wetting, and determine as directed under *pH Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Benzoic Acid



$C_7H_6O_2$ Formula wt 122.12

INS: 210 CAS: [65-85-0]

DESCRIPTION

Benzoic Acid occurs as white crystals, scales, or needles. It begins to sublime at about 100° and is volatile with steam. One gram is soluble in 275 mL of water at 25°, in 20 mL of boiling water, in 3 mL of alcohol, in 5 mL of chloroform, and in 3 mL of ether. It is soluble in fixed and in volatile oils and is sparingly soluble in hexane.

Function Preservative; antimicrobial agent.

REQUIREMENTS

Identification Dissolve 1 g of sample in a 20:1 (v/v) mixture of water and 1 *N* sodium hydroxide, filter the solution, and

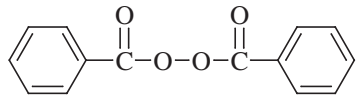
add about 1 mL of ferric chloride TS. A buff-colored precipitate forms.
Assay Not less than 99.5% and not more than 100.5% of $C_7H_6O_2$, calculated on the anhydrous basis.
Lead Not more than 2.0 mg/kg.
Readily Carbonizable Substances Passes test.
Readily Oxidizable Substances Passes test.
Residue on Ignition Not more than 0.05%.
Solidification Point Between 121° and 123°.
Water Not more than 0.7%.

TESTS

Assay Dissolve about 500 mg of sample, accurately weighed, in 25 mL of 50% alcohol previously neutralized with 0.1 *N* sodium hydroxide, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 12.21 mg of $C_7H_6O_2$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Readily Carbonizable Substances Dissolve 500 mg of sample in 5 mL of 95% sulfuric acid, and proceed as directed under *Readily Carbonizable Substances*, Appendix IIB. The resulting color is no darker than *Matching Fluid Q*.
Readily Oxidizable Substances Add 0.1 *N* potassium permanganate, dropwise, to a mixture of 100 mL of water and 1.5 mL of sulfuric acid heated to 100°, until a pink color persists for 30 s. Dissolve 1.0 g of sample in the hot solution, and titrate with 0.1 *N* potassium permanganate to a pink color that persists for 15 s. The volume of 0.1 *N* potassium permanganate consumed does not exceed 0.5 mL.
Residue on Ignition Ignite 2 g as directed in *Method I* under *Residue on Ignition (Sulfated Ash)*, Appendix IIC.
Solidification Point Determine as directed under *Solidification Point*, Appendix IIB.
Water Determine as directed in the *Karl Fischer Titrimetric Method* under *Water Determination*, Appendix IIB, using methanol in pyridine (1:2) as the solvent.

Packaging and Storage Store in well-closed containers.

Benzoyl Peroxide



$C_{14}H_{10}O_4$ Formula wt 242.23
INS: 928 CAS: [94-36-0]

DESCRIPTION

Benzoyl Peroxide occurs as a colorless, crystalline solid. It is insoluble in water, slightly soluble in alcohol, and soluble in chloroform and in ether. It melts between 103° and 106° with decomposition.

> **Caution**: Benzoyl Peroxide, especially in the dry form, is a dangerous, highly reactive oxidizing material and has been known to explode spontaneously. Observe safety precautions printed on the label of the container.

Function Bleaching agent.

REQUIREMENTS

Identification Add 50 mL of 0.5 *N* alcoholic potassium hydroxide to 500 mg of sample, heat gradually to boiling, and continue boiling for 15 min. Cool, dilute to 200 mL with water, and make the solution strongly acid with 0.5 *N* hydrochloric acid. Extract with ether, dry the extract with anhydrous sodium sulfate, and then evaporate to dryness on a steam bath. The residue of benzoic acid so obtained melts between 121° and 123° (see *Melting Range or Temperature*, Appendix IIB).
Assay Not less than 96.0% of $C_{14}H_{10}O_4$.
Lead Not more than 4 mg/kg.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 15 mL of acetone contained in a 100-mL glass-stoppered bottle, and add 3 mL of a 1:2 solution of potassium iodide. Swirl for 1 min, then immediately titrate with 0.1 *N* sodium thiosulfate (without the addition of starch TS). Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 12.11 mg of $C_{14}H_{10}O_4$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds from the residue of the following mixture, and 4 μg of lead (Pb) ion in the control: Mix 1 g of sample with 10 mL of 1 *N* sodium hydroxide, slowly evaporate to dryness on a steam bath, and cool.

Packaging and Storage Store in the original container, and observe the safety precautions printed on the label.

Bergamot Oil, Coldpressed

CAS: [8007-75-8]

FEMA: 2153

View IR

DESCRIPTION

Bergamot Oil, Coldpressed, occurs as a green to yellow-green or yellow-brown liquid with a fragrant, sweet-fruity odor. It is a volatile oil obtained by pressing, without the aid of heat, the fresh peel of the fruit of *Citrus bergamia* Risso et Poiteau (Fam. Rutaceae). It is miscible with alcohol and with glacial acetic acid. It is soluble in most fixed oils, but is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 36.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between +12° and +30°.
Refractive Index Between 1.465 and 1.468 at 20°.
Residue on Evaporation Not more than 6.0%.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.875 and 0.880.
Ultraviolet Absorbance Not less than 0.32.

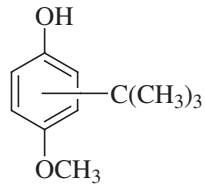
TESTS

Assay Determine as directed in *Ester Determination* under *Esters*, Appendix VI, using a 2-g sample, accurately weighed, but heat the mixture for 30 min on the steam bath. Use 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, using an Abbé or other refractometer of equal or greater accuracy.
Residue on Evaporation Determine as directed under *Residue on Evaporation*, Appendix VI, heating a sample for 5 h.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 90% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Ultraviolet Absorbance Determine as directed under *Ultraviolet Absorbance of Citrus Oils*, Appendix VI, using about 50 mg of sample, accurately weighed. The absorbance maximum occurs at 315 ± 3 nm.

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

BHA

Butylated Hydroxyanisole



$C_{11}H_{16}O_2$ Formula wt 180.25

INS: 320 CAS: [25013-16-5]

DESCRIPTION

BHA occurs as a white or slightly yellow, waxy solid. It is predominantly 3-*tert*-butyl-4-hydroxyanisole (3-BHA), with varying amounts of 2-*tert*-butyl-4-hydroxyanisole (2-BHA). It melts between 48° and 63°. It is freely soluble in alcohol and in propylene glycol, and insoluble in water.

Function Antioxidant.

REQUIREMENTS

Identification Add 2 mL of sodium borate TS and 1 mL of a 1:10,000 solution of 2,6-dichloroquinonechlorimide:absolute alcohol to 5 mL of a 1:10,000 solution of sample in 72% alcohol, and mix. A blue color appears.
Assay Not less than 98.5% of $C_{11}H_{16}O_2$.
Residue on Ignition Not more than 0.05%.

TESTS

Assay

Internal Standard Use 4-*tert*-butylphenol.

Internal Standard Solution Dissolve about 500 mg of *Internal Standard*, accurately weighed, in acetone contained in a 100-mL volumetric flask, add acetone to volume, and mix.

Standard Preparation Dissolve together accurately weighed quantities of USP Reference Standards 3-*tert*-Butyl-4-hydroxyanisole and 2-*tert*-Butyl-4-hydroxyanisole to final concentrations of 9 mg/mL and 1 mg/mL, respectively, in sufficient *Internal Standard Solution* to make 10 mL.

Assay Preparation Dissolve about 100 mg of sample, accurately weighed, in the *Internal Standard Solution* contained in a 10-mL volumetric flask, dilute to volume with the *Internal Standard Solution*, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and containing a 1.8-m × 2-mm (id) stainless-steel column, or equivalent, packed with 10% silicone GE XE-60, or equivalent. Maintain the column isothermally at a temperature between 175° and 185°, and use helium as the carrier gas at a flow rate of 30 mL/min. Chromatograph a sufficient number of injections of the *Standard Preparation*,

and record the areas as directed under *Procedure* to ensure that the relative standard deviation does not exceed 2.0% for the 3-*tert*-butyl-4-hydroxyanisole isomer and 6.0% for the 2-*tert*-butyl-4-hydroxyanisole isomer; the resolution between the isomers is not less than 1.3; and the tailing factor does not exceed 2.0.

Procedure Separately inject suitable portions (about 5 μL) of the *Standard Preparation* and the *Assay Preparation* into the gas chromatograph, and record the chromatograms. Measure the areas under the peaks for each isomer and the *Internal Standard* in each chromatogram, and calculate the quantity, (*I*), in milligrams, of each isomer in the sample taken by the equation

$$I = 10 \times C_S \times (R_U/R_S),$$

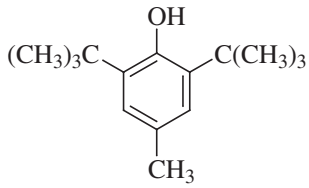
in which C_S is the concentration, in milligrams per milliliter, of the isomer in the *Standard Preparation*; R_U is the ratio of the area of the isomer to that of the *Internal Standard* in the chromatogram from the *Assay Preparation*; and R_S is the ratio of the area of the isomer to that of the *Internal Standard* in the chromatogram from the *Standard Preparation*. Calculate the weight, in milligrams, of $C_{11}H_{16}O_2$ in the sample taken by adding the quantities of the two isomers.

Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 10-g sample.

Packaging and Storage Store in well-closed containers.

BHT

Butylated Hydroxytoluene; 2,6-Di-*tert*-butyl-*p*-cresol



$C_{15}H_{24}O$ Formula wt 220.35

INS: 321 CAS: [128-37-0]

DESCRIPTION

BHT occurs as a white, crystalline solid. It is freely soluble in alcohol, and insoluble in water and in propylene glycol.

Function Antioxidant.

REQUIREMENTS

Identification Dissolve 200 mg of 3,3′-dimethoxybenzidine dihydrochloride in a mixture of 40 mL of methanol and 60 mL of 1 *N* hydrochloric acid. Add 5 mL of the resulting dianisidine solution to 10 mL of water and 2 mL of a 3:100 solution of sodium nitrite. Add this solution to 10 mL of a 1:100,000 solution of sample:methanol. An orange-red color appears within 3 min. Add 5 mL of chloroform, and shake. The chloroform layer exhibits a purple or magenta color that fades when exposed to light.

Assay Not less than 99.0% of $C_{15}H_{24}O$.

Residue on Ignition Not more than 0.002%.

TESTS

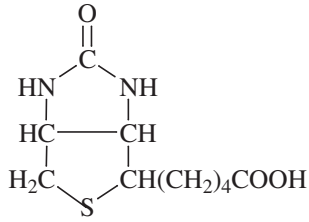
Assay The solidification point (see *Solidification Point*, Appendix IIB) of a sample is not lower than 69.2°, indicating a purity of not less than 99.0% of $C_{15}H_{24}O$.

Residue on Ignition Transfer about 50 g of sample, accurately weighed, into a tared crucible, ignite until thoroughly charred, and cool. Moisten the ash with 1 mL of sulfuric acid, and complete the ignition by heating for 15-min periods at 800° ± 25° to constant weight.

Packaging and Storage Store in well-closed containers.

Biotin

cis-Hexahydro-2-oxo-1H-thieno[3,4]imidazole-4-valeric Acid; *d*-Biotin



$C_{10}H_{16}N_2O_3S$ Formula wt 244.31

CAS: [58-85-5]

DESCRIPTION

Biotin occurs as a practically white, crystalline powder. It is stable to air and heat. One gram dissolves in about 5000 mL of water at 25° and in about 1300 mL of alcohol; it is more soluble in hot water and in dilute alkali, and it is insoluble in other common organic solvents.

Function Nutrient.

REQUIREMENTS

Identification

A. The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits relative maxima at the

same wavelengths as those of a similar preparation of USP Biotin Reference Standard.

B. A saturated solution of sample in warm water decolorizes bromine TS, added dropwise.

Assay Not less than 97.5% and not more than 100.5% of $C_{10}H_{16}N_2O_3S$.

Lead Not more than 2 mg/kg.

Melting Range Between 229° and 232°, with decomposition.

Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +89° and +93°.

TESTS

Assay Mix about 500 mg of sample, accurately weighed, with 100 mL of water, add phenolphthalein TS, and while heating and stirring continuously, slowly titrate the suspension with 0.1 *N* sodium hydroxide to a pink color. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 24.43 mg of $C_{10}H_{16}N_2O_3S$.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution in 0.1 *N* sodium hydroxide containing 500 mg of sample in each 25 mL.

Packaging and Storage Store in tight containers.

Birch Tar Oil, Rectified

View IR

DESCRIPTION

Birch Tar Oil, Rectified, occurs as a clear, dark brown liquid with a strong leather odor. It is the pyroligneous oil obtained by dry distillation of the bark and the wood of *Betula pendula* Roth and related species of *Betula* (Fam. Betulaceae) and rectified by steam distillation. It is soluble in most fixed oils, but it is insoluble in glycerin, in mineral oil, and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.886 and 0.950.

TESTS

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of absolute alcohol.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Black Pepper Oil

CAS: [8006-82-4]

View IR

DESCRIPTION

Black Pepper Oil occurs as an almost colorless to slightly green liquid with the characteristic odor of pepper and a relatively mild taste. It is the volatile oil obtained by steam distillation from the dried, unripened fruit of the plant *Piper nigrum* L. (Fam. Piperaceae). It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is sparingly soluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Angular Rotation Between –1° and –23°.

Refractive Index Between 1.479 and 1.488 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.864 and 0.884.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

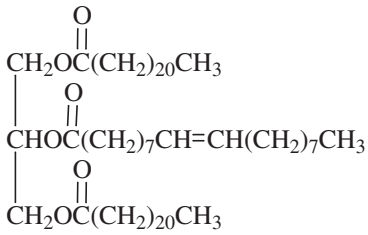
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 95% alcohol.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Bohenin

1,3-Behenic-2-oleic Glyceride



DESCRIPTION

Bohenin occurs as a white to light tan, waxy solid. It is a triglyceride containing behenic acid at the 1- and 3-positions and oleic acid at the 2-position. Behenic acid is a saturated fatty acid that occurs naturally in peanuts, most seed fats, animal milk fat, and marine oils. It is produced by the interesterification of triolein and ethyl behenate in the presence of a suitable lipase enzyme preparation. It melts at approximately 52°. It is insoluble in water; soluble in hexane, in chloroform, and in acetone; and slightly soluble in hot ethanol.

Function Tempering aid and antibloom agent in the manufacture of chocolate and chocolate coatings.

REQUIREMENTS

Identification Bohenin exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	16:0	18:0	18:1	20:0	22:0	24:0
Weight % (Range):	<1.5	<3.0	>25.0	<7.0	>58.0	<3.0

Acid Value Not more than 0.3
Diglycerides Not more than 5.0%.
Iodine Value Between 24 and 30.
Lead Not more than 0.5 mg/kg.
Peroxide Value Not more than 0.3 meq/kg
Saponification Value Between 162 and 172.
Triglycerides Not less than 95.0%.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VII.
Diglycerides and **Triglycerides**

Sample Preparation Transfer 5 g of sample, accurately weighed, into a 50-mL beaker, and warm at 80° to melt. Transfer 300 µL of melted sample into a 10-mL volumetric flask, add 9 mL of acetone, and swirl to dissolve. If crystals of Bohenin form, warm the flask to 40° in a water bath. After complete dissolution, fill the flask to 10 mL with acetone.

Standard Preparation Proceed as directed for the *Sample Preparation*, substituting a Bohenin standard (available from Laroda Fine Chemicals AB, Limhamnsgardens allé 9, 216 16 LIMHAMN Växel, Sweden) for the sample.

Procedure (See *Chromatography*, Appendix IIA.) Use a suitable high-performance liquid chromatograph equipped with differential refractometer, autosampler injection unit, mobile-phase degasser, column heating block or oven, and a computing integrator. The column is Lichrosorb RP-18 250-mm × 4.5 mm (id) (GL Science, Inc., or equivalent) and YMC-Pack ODA-A A-303 250-mm × 4.5 mm (id) (YMC Company, Ltd., or equivalent) connected in a series, or equivalent, maintained at 50°. Use 80:20 acetone:acetonitrile as the eluent, at a flow rate of 2 mL/min.

Equilibrate the column at 50° by pumping eluent at 0.9 mL/min, until a stable baseline is obtained. Using the autosampler, inject duplicate 30-µL measures of *Sample Preparation* and *Standard Preparation*.

Calculation Calculate the percent of diglycerides in the *Sample Preparation* by the following formula:

$$100(D_G/S_A)$$

in which D_G is the total sum of the peak areas at retention times between 11 and 14 min and S_A is the total sum of all peak areas.

Calculate the percent of triglycerides in the *Sample Preparation* by the following formula:

$$\%T = 100[(S_A - D_G)/S_A].$$

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Packaging and Storage Store in closed containers away from excessive heat.

Bois de Rose Oil

View IR

DESCRIPTION

Bois de Rose Oil occurs as a colorless to pale yellow liquid with a slightly camphoraceous, pleasant, floral odor. It is the volatile oil obtained by steam distillation from the chipped wood of *Aniba rosaeodora* var. *amazonica* Ducke (Fam. Lauraceae). The oils from the coastal region of Brazil and the Amazon valley tend to differ in odor and in linalool content from that produced in the Loreto province of Peru. It is soluble in most fixed oils and in propylene glycol. It is soluble in

mineral oil, occasionally with turbidity, but it is only slightly soluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 82.0% and not more than 92.0% of total alcohols, calculated as linalool ($C_{10}H_{18}O$).
Angular Rotation Between –4° and +6°.
Distillation Range Not less than 70% distills between 195° and 205°.
Refractive Index Between 1.462 and 1.470 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.868 and 0.889.

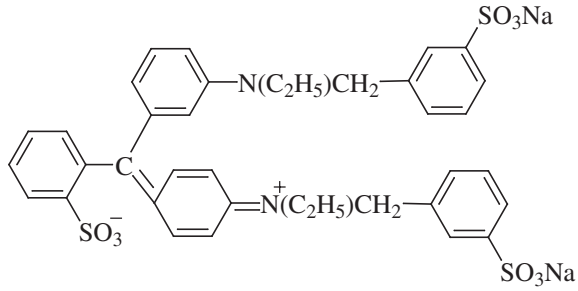
TESTS

Assay Determine as directed under *Linalool Determination*, Appendix VI, using about 1.2 g of acetylated sample, accurately weighed.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB, using 50 mL of the sample, previously dried over anhydrous sodium sulfate, and a 125-mL distillation flask.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 6 mL of 60% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Brilliant Blue[1]

Brilliant Blue FCF; CI 42090; Class: Triphenylmethane



$C_{37}H_{34}N_2O_9S_3Na_2$ Formula wt 792.86

INS: 133 CAS: [3844-45-9]

DESCRIPTION

Brilliant Blue occurs as a dark purple to bronze powder or granules. It is principally the disodium salt of ethyl[4-[*p*-[ethyl(*m*-sulfobenzyl)amino]-α-(*o*-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](*m*-sulfobenzyl) ammonium hydroxide inner salt. It dissolves in water to give a solution green-blue at neutrality, green in weak acid, and yellow in stronger acid. Addition of base to its neutral solution produces a violet color only on boiling. When dissolved in concentrated sulfuric acid, it yields a yellow solution that turns green when diluted with water. It is slightly soluble in ethanol.

Function Color.

REQUIREMENTS

Identification A freshly prepared aqueous solution containing 10 mg/L exhibits absorbance intensities (A) and wavelength maxima as follows: at pH 7, A = 1.11 at 630 nm; at pH 1, A = 0.95 at 629 nm, and A = 0.2 at 410 nm; and at pH 13, A = 1.29 at 630 nm, and A = 0.15 at 408 nm.
Assay Not less than 85.0% coloring matters.
Arsenic Not more than 3 mg/kg.
Chromium Not more than 0.0005%.
Ether Extracts (combined) Not more than 0.2%.
Lead Not more than 10 mg/kg.
Leuco Base Not more than 5.0%.

[1] To be used or sold for use to color food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Blue No. 1 can be applied only to FDA-certified batches of this color additive. Brilliant Blue is a common name given to the uncertified colorant. See the monograph entitled FD&C Blue No. 1 for directions for producing an FDA-certified batch.

Loss on Drying (Volatile Matter) at 135°, Chloride, and **Sulfate** (as sodium salts) Not more than 15.0% in combination.

Manganese Not more than 0.001%.

Subsidiary Colors Not more than 6.0%, combined, of isomeric disodium salts of ethyl[4-[*p*-[ethyl(*p*-sulfobenzyl) amino]-I-(*o*-sulfophenyl) benzylidene]-2,5-cyclohexadien-1-ylidene](*p*-sulfobenzyl) ammonium hydroxide inner salt, and ethyl[4-[*p*-[ethyl(*o*-sulfobenzyl)amino]-α-(*o*-sulfophenyl) benzylidene]-2,5-cyclohexadien-1-ylidene](*o*-sulfobenzyl) ammonium hydroxide inner salt.

Uncombined Intermediates and Products of Side Reactions

o-, m-, *and* p-*Sulfobenzaldehydes* Not more than 1.5%, combined.

N-*ethyl*,N-*(*m-*Sulfobenzyl)sulfanilic Acid* Not more than 0.3%.

Water-Insoluble Matter Not more than 0.2%.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Chloride Determine as directed in *Sodium Chloride* under *Colors*, Appendix IIIC.

Chromium Determine as directed in *Chromium* under *Colors*, Appendix IIIC.

Ether Extracts (combined) Determine as directed in *Ether Extracts* under *Colors*, Appendix IIIC.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 μg of lead (Pb) ion in the control.

Leuco Base Determine as directed in *Leuco Base* under *Colors*, Appendix IIIC, using the following *Sample Solution*: Transfer approximately 120 mg of sample, accurately weighed, into a 1-L volumetric flask, and dissolve in and dilute to volume with water.

Loss on Drying (Volatile Matter) at 135° Determine as directed in *Loss on Drying (Volatile Matter)* under *Colors*, Appendix IIIC.

Manganese A 1:20 aqueous solution gives positive tests for *Manganese*, Appendix IIIA.

Subsidiary Colors

Solvent System Use a solvent system composed of 50 mL of acetonitrile, 50 mL of isoamyl alcohol, 15 mL of 2-butanone, 5 mL of water, and 5 mL of ammonium hydroxide.

Sample Solution Transfer approximately 1 g of sample, accurately weighed, into a 100-mL volumetric flask. Fill the flask about ¾ full with water, place it in the dark for 1 h, dilute to volume with water, and mix well.

Procedure Spot 0.1 mL of the *Sample Solution* in a line across a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G, approximately 3 cm from the bottom edge. Allow the plate to dry for about 20 min in the dark, then develop with the *Solvent System* in an unlined tank equilibrated for at least 20 min before the plate is inserted. Allow the solvent front to reach within about 3 cm of the top of the plate. Dry the developed plate in the dark.

When the plate has dried, scrape off all the colored bands above the Brilliant Blue, which remains close to the origin, into a 30-mL beaker. Extract the subsidiary colors with three 6-mL portions of 95% ethanol, or until no color remains on the gel by visual inspection. Record the volume of ethanol used and the spectrum of the solution between 400 and 700 nm. Calculate the percent of subsidiary colors by the formula

$$(A \times V \times 100)/(a \times W \times b),$$

in which A is the absorbance at the wavelength maximum; V is the volume, in milliliters, of the solution; a is the absorptivity (0.126 mg/L/cm); W is the weight, in milligrams, of the sample; and b is the pathlength of the cell.

Sulfate Determine as directed in *Sodium Sulfate* under *Colors*, Appendix IIIC.

Total Color Determine the total color strength as the weight percent of the sample taken using *Methods I* and *II* in *Total Color* under *Colors*, Appendix IIIC. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 50 to 75 mg of sample, accurately weighed, into a 1-L volumetric flask, and dissolve in and dilute to volume with water. The absorptivity (a) for Brilliant Blue is 0.164 mg/L/cm at 630 nm.

Method II (Sample Preparation) Transfer approximately 0.5 g of sample, accurately weighed, into the titration flask. The stoichiometric factor (F_s) for Brilliant Blue is 2.52.

Uncombined Intermediates and Products of Side Reactions Determine as directed for *Method* I in *Uncombined Intermediates and Products of Side Reactions* under *Colors*, Appendix IIIC. Calculate the concentrations of *m*-sulfobenzaldehyde and *N*-ethyl-*N*-(3-sulfobenzyl)-sulfanilic acid using the following absorptivities:

m-Sulfobenzaldehyde, a = 0.0495 mg/L/cm at 246 nm (acid solution).

N-Ethyl-*N*-(3-sulfobenzyl)-sulfanilic acid, a = 0.078 mg/L/cm at 277 nm (alkaline solution).

Water-Insoluble Matter Determine as directed in *Water-Insoluble Matter* under *Colors*, Appendix IIIC.

Packaging and Storage Store in well-closed containers.

Brominated Vegetable Oil

DESCRIPTION

Brominated Vegetable Oil occurs as a pale yellow to dark brown, viscous, oily liquid. It is a bromine addition product of vegetable oil or oils. It is soluble in chloroform, in ether, in hexane, and in fixed oils, and is insoluble in water.

Function Flavoring agent; beverage stabilizer.

REQUIREMENTS

Identification Mix about 0.2 mL of sample with 1 g of anhydrous sodium carbonate in a suitable crucible, cover the

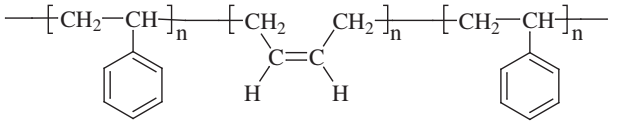
mixture with an additional 1 g of sodium carbonate, compact the mixture by gentle tapping, and heat the crucible over an open flame until the crucible turns red. Cool the crucible and its contents, dissolve the residue in 20 mL of hot water, and filter. Add 1.7 *N* nitric acid to the filtrate until effervescence ceases, then add 1 mL of silver nitrate TS. A curdy, yellow precipitate forms that is insoluble in nitric acid but soluble in an excess of stronger ammonia water.
Free Bromine Passes test.
Free Fatty Acids (as oleic acid) Not more than 2.5%.
Iodine Value Not more than 16.
Specific Gravity Within the range specified by the vendor.

TESTS

Free Bromine Dissolve 1 g of sample in 20 mL of acetone, add 1 g of sodium iodide, and allow the mixture to stand in a stoppered flask in the dark for 30 min, with occasional shaking. Add 25 mL of water and 1 mL of starch TS. No blue color appears.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using 28.2 as the equivalence factor (*e*) in the calculation for oleic acid. Titrate with the appropriate normality of sodium hydroxide solution, shaking vigorously, to the first permanent pink color of the same intensity as that of the neutralized alcohol (if the sample color interferes, titrate to a pH of 8.5, determined with a suitable instrument).
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Specific Gravity Determine by any reliable method (see *General Provisions*) at the temperature specified by the vendor.

Packaging and Storage Store in well-closed containers.

Butadiene-Styrene Rubber



DESCRIPTION

Butadiene-Styrene Rubber occurs as a synthetic liquid latex or solid rubber produced by the emulsion polymerization of butadiene and styrene, using fatty acid soaps as emulsifiers, and a suitable catalyst, molecular weight regulator (if required), and shortstop. It also occurs as a solid rubber produced by the solution copolymerization of butadiene and styrene in a hexane solution, using butyl lithium as a catalyst. Solvents and volatiles are removed by processing with hot water or by drum drying.

The latex, which has a pH between 9.5 and 11.0 and a solids content between 26% and 63%, is coagulated with or without other food-grade ingredients in a heated kettle. The coagulated mass is squeezed to drain off sera, and the coagulum is washed with hot water (with or without alkali), and it is rinsed with water until the batch is neutral. Finally, the coagulum is dried to remove residual volatiles. When butadiene-styrene rubber is purchased in the latex form, it must be washed by the preceding or an equivalent procedure. In the case of the solvent-polymerized product, solvent and volatiles are removed by processing in hot water or by drum drying.

Both of the solid forms are supplied by the manufacturer either as a slab or as a uniform, free-flowing crumb and may contain a suitable food-grade antioxidant. The crumb form, in addition, may contain a suitable food-grade partitioning agent.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

> **Note**: The following *Requirements* apply to the solid rubber as supplied by the manufacturer or to the washed and dried coagulum obtained from the latex as described above.

Identification Identify emulsion-polymerized Butadiene-Styrene Rubber latex and solid by comparing their infrared absorption spectra with the respective four typical spectra as shown in the section on *Infrared Spectra*. Prepare latex samples by first drying them at 105° for 4 h, then by dissolving them in hot toluene and evaporating on a potassium bromide plate. Prepare solid samples by dissolving them in hot toluene and evaporating on a potassium bromide plate.
Bound Styrene Between 1.0% and 50.0%.
Cadmium Not more than 1 mg/kg.
Lead Not more than 3 mg/kg.
Lithium Not more than 0.0075%.
Mercury Not more than 3 mg/kg.
Quinones Not more than 0.002%.
Residual Hexane Not more than 0.01%.
Residual Styrene Not more than 0.003%.

TESTS

Bound Styrene Determine as directed under *Bound Styrene*, Appendix IV.
Cadmium Determine as directed under *Cadmium Limit Test*, Appendix IIIB, or by the following procedure:

> **Note**: For this assay, use reagent-grade chemicals with the lowest practicable Sb, As, Bi, Cd, Cu, Pb, Hg, Ag, and Sn levels, and use only high-purity water and gases. Rinse all glass- and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse thoroughly with *High-Purity Water*.

High-Purity Water Obtain *High-Purity Water* from a mixed-bed strong-acid, strong-base ion exchange apparatus capable of producing water of more than 15-megohm resistivity.

10% Nitric Acid Solution Slowly add 100 mL of nitric acid to 500 mL of *High-Purity Water* contained in a 1000-mL volumetric flask. Dilute to volume with *High-Purity Water*.

10% Hydrochloric Acid Solution Slowly add 100 mL of hydrochloric acid to 500 mL of *High-Purity Water* contained in a 1000-mL volumetric flask. Dilute to volume with *High-Purity Water*.

Cadmium Stock Solution Use any commercially available NIST traceable 1000 ppm plasma-grade standard stock solution of cadmium.

Cadmium Calibration Standards Tare three clean, dry 4-oz polyethylene bottles (or equivalent). Add approximately 50 g of *High-Purity Water* to each. Slowly add 28 ± 1 g of concentrated nitric acid, mix thoroughly, slowly add 12 ± 1 g of concentrated hydrochloric acid, and mix thoroughly again. Using a precision micropipet, add 10, 50, and 500 µL, respectively, of *Cadmium Stock Solution* to one of each of the bottles. Dilute each solution to 100.0 ± 0.1 g with *High-Purity Water*, and mix thoroughly to obtain calibration standards with 0.1, 0.5, and 5.0 mg/kg, respectively.

Calibration Blank Solution Tare a clean, dry 4-oz polyethylene bottle (or equivalent). Add approximately 50 g of *High-Purity Water*. Slowly add 28 ± 1 g of concentrated nitric acid, mix thoroughly, slowly add 12 ± 1 g of concentrated hydrochloric acid, and mix thoroughly again. Dilute the solution to 100.0 ± 0.1 g with *High-Purity Water*, and mix thoroughly.

Note: For the solutions listed below, use a Parr Closed Digestion Vessel (catalog number 4748) with polyethylene vessel liners. Any equivalent apparatus may be used if the predigestion fortification recoveries are within the specifications noted below.

Fortification Solution Use any commercially available NIST traceable 1000 ppm plasma-grade standard stock solution of cadmium.

Sample Digestion Fortification Preparation Weigh a representative sample on a balance with 0.1-mg precision (see *Weights and Balances* under *Apparatus for Tests and Assays*, Appendix I). Transfer the sample to a digestion vessel that has been cleaned according to the manufacturer's specifications. Slowly add 5.0 mL of concentrated nitric acid to the digestion vessel, seal, and heat the vessel for 8 to 16 h at 210° ± 5°. Allow the vessel to cool to room temperature, and quantitatively transfer its contents into a clean, dry, tared 1-oz polyethylene bottle. Slowly add concentrated hydrochloric acid to achieve a final concentration of 10% (w/w), and dilute to an appropriate final mass with *High-Purity Water*.

Digestion Blank Preparation Transfer 5.0 mL of concentrated nitric acid into a digestion vessel that has been cleaned according to the manufacturer's specifications, seal, and heat the vessel for 8 to 16 h at 210° ± 5°. Allow the vessel to cool to room temperature, and quantitatively transfer its contents into a clean, dry, tared 1-oz polyethylene bottle. Slowly add concentrated hydrochloric acid to achieve a final concentration of 10% (w/w), and dilute to an appropriate final mass with *High-Purity Water*.

Digestion Fortification Preparation Prepare as directed under *Sample Preparation* (above), except immediately before heating the sample, add 25 µL of the *Fortification Solution*. Determine the recovery of the *Digestion Fortification Preparation* by analyzing the *Sample Digestion Fortification Preparation* for cadmium. Calculate the percent recovery for cadmium by subtracting the unfortified assay result from the fortified assay result and multiplying the difference, in milligrams per kilogram, by 100. The fortification level for cadmium is 1 mg/kg. Acceptable recoveries are in the 85% to 110% range.

Procedure Use an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), or equivalent instrumentation with similar capabilities. Follow the instrument manufacturer's instructions for setting instrument parameters for assay of cadmium. Select appropriate background correction points for the cadmium analyte according to the recommendations of the instrument manufacturer. Select analytical wavelengths to yield adequate sensitivity and freedom from interference.

Analyze the *Calibration Blank Solution*. Results for cadmium should indicate a concentration of less than 0.01 mg/kg. If the results are not less than 0.01 mg/kg, repeat the analysis. In the event that reanalysis is unsuccessful, take steps consistent with the manufacturer's recommendations to identify and remediate the sources of contamination or interference. Do not proceed with the analysis until the sources of contamination or interference have been identified and corrected.

Subsequently analyze all three *Cadmium Calibration Standards,* from lowest concentration to highest. Results for each of the calibration standards should indicate concentrations of 100 ± 5 mg/kg, 500 ± 25 mg/kg, and 5000 ± 250 mg/kg, respectively. If the results are not as indicated, repeat the analysis. In the event that reanalysis is unsuccessful, take steps consistent with the manufacturer's recommendations to identify and remediate the sources of contamination or interference. Do not proceed with the analysis until the sources of contamination or interference have been identified and addressed. After successful calibration of the instrument for cadmium, reanalyze the *Calibration Blank Solution* to demonstrate that there is no carryover of the cadmium.

Next, analyze the prepared samples, digestion blanks, and digestion fortification samples in groups of no more than ten. At a minimum, each group should contain a digestion blank, a prepared sample, a second replicate of the prepared sample, and that same sample prepared as a fortification sample. Analyze the *Calibration Blank Solution* followed by any of the *Cadmium Calibration Standards* between each group of ten samples.

To determine the calibration curve, aspirate the *Cadmium Calibration Standards* and the *Calibration Blank Solution*. If possible, use the calibration function incorporated in the ICP-AES instrument's soft- or firmware. If necessary, plot instrument response versus concentration of cadmium. Fit this line with a linear equation of the form $y = mx + b$, in which y is instrument response, m is the slope of the best-fit line, x is concentration, and b is the y intercept of the best-fit line. The correlation coefficient for the best-fit line should be ≥0.99. Concentrations of cadmium in the calibration blanks, calibra-

tion standards, digestion blanks, samples, and fortified samples can be directly read from the ICP-AES when using its soft- or firmware, or it can be calculated from the best-fit equation.

Lead Determine as directed under the *Cadmium Test* (above), except substitute ''lead'' for ''cadmium,'' and use the following for the *Stock Solution* and the *Standard Solutions*:

Lead Stock Solution Use any commercially available NIST traceable 1000 ppm plasma-grade standard stock solution of lead.

Lead Calibration Standards Tare three clean, dry 4-oz polyethylene bottles (or equivalent). Add approximately 50 g of *High-Purity Water* to each. Slowly add 28 ± 1 g of concentrated nitric acid, mix thoroughly, slowly add 12 ± 1 g of concentrated hydrochloric acid, and mix thoroughly again. Using a precision micropipet, add 10, 50, and 500 µL, respectively, of *Lead Stock Solution* to one of each of the bottles. Dilute each solution to 100.0 ± 0.1 g with *High-Purity Water*, and mix thoroughly to obtain calibration standards with 0.1, 0.5, and 5.0 mg/kg, respectively.

Lithium

Atomic Absorption Spectrophotometer Use a suitable instrument, equipped with a lithium hollow-cathode lamp, capable of measuring the radiation absorbed by lithium in the 670-nm spectral band.

Standard Solution Transfer 399.3 mg of reagent-grade lithium carbonate to a 1000-mL volumetric flask, dissolve in a minimal amount of 1:1 hydrochloric acid:water, dilute to volume with water, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute to volume with water, and mix. Finally, transfer 10.0 mL of this solution to a second 100-mL volumetric flask, add 1.0 mL of hydrochloric acid, dilute to volume with water, and mix. This solution contains 75 µg of lithium per 100 mL.

Sample Solution Accurately weigh 1 g of a solid-rubber sample, wrap it tightly in ashless filter paper, and place it in a tared platinum crucible. Heat in an oven at 100° for 15 min, and then transfer to a muffle furnace programmed to reach 500° within 1 to 3 h after introduction of the sample. Remove the crucible from the furnace 15 to 20 min after 500° has been reached, and cool in a desiccator. Quantitatively transfer the contents of the crucible to a 100-mL volumetric flask, using 1 mL of hydrochloric acid and water, dilute to volume with water, and mix.

Procedure Following the manufacturer's instructions for operating the atomic absorption spectrophotometer, aspirate a suitable portion of the *Standard Solution* through the flame. In a similar manner, aspirate a suitable portion of the *Sample Solution*. Any absorbance produced by the *Sample Solution* should not exceed that produced by the *Standard Solution*.

Mercury Determine as directed under the *Cadmium Test* (above), except substitute ''mercury'' for ''cadmium,'' and use the following for the *Stock Solution* and the *Standard Solutions*:

Mercury Stock Solution Use any commercially available NIST traceable 1000 ppm plasma-grade standard stock solution of mercury.

Mercury Calibration Standards Tare three clean, dry 4-oz polyethylene bottles (or equivalent). Add approximately 50 g of *High-Purity Water* to each. Slowly add 28 ± 1 g of concentrated nitric acid, mix thoroughly, slowly add 12 ± 1 g of concentrated hydrochloric acid, and mix thoroughly again. Using a precision micropipet, add 10, 50, and 500 µL, respectively, of *Mercury Stock Solution* to one of each of the bottles. Dilute each solution to 100.0 ± 0.1 g with *High-Purity Water*, and mix thoroughly to obtain calibration standards with 0.1, 0.5, and 5.0 mg/kg, respectively.

Quinones Determine as directed under *Quinones*, Appendix IV.

Residual Hexane (**Note**: The isooctane, 2,2,4-trimethylpentane, used in this test should be of chromatographic-grade quality.)

Internal Standard Stock Solution Transfer 150 mg of *n*-nonane, accurately weighed, to a 50-mL volumetric flask, dilute to volume with isooctane, and mix.

Dilute Internal Standard Solution Pipet 10.0 mL of *Internal Standard Stock Solution* into a 100-mL volumetric flask, dilute to volume with isooctane, and mix. Pipet 5.0 mL of this solution into a 250-mL volumetric flask, dilute to volume with isooctane, and mix. Each milliliter of the final solution contains 6 µg of *n*-nonane.

Hexane Standard Solution Transfer 150 mg of *n*-hexane, accurately weighed, to a 50-mL volumetric flask, dilute to volume with isooctane, and mix. Pipet 1.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with isooctane, and mix. Finally, pipet 10.0 mL of this solution and 10.0 mL of *Internal Standard Stock Solution* into a 50-mL volumetric flask, dilute to volume with isooctane, and mix.

Sample Preparation Accurately weigh 1.5 g of a solid-rubber sample, transfer it into a 4-oz bottle, and pipet 25.0 mL of the *Dilute Internal Standard Solution* into the bottle. Stopper the bottle, and shake mechanically overnight to dissolve the rubber. Add 50 mL of methanol to precipitate the polymer, and shake vigorously for 15 min. Allow the mixture to settle, and decant the liquid phase into a 250-mL separator. Wash the polymer with 25 mL of methanol, and add the wash to the separator. Add 50 to 75 mL of cold water to the separator, and shake vigorously for 1 min, venting periodically to release any pressure. Allow the phases to separate, drain off the bottom (aqueous) phase, and rewash the isooctane phase with a second 50-mL portion of cold water. Shake again, allow to separate, and drain off the bottom layer. Transfer 10 mL of the isooctane phase to a 20-mL vial for the analysis.

Procedure Use a gas chromatograph equipped with a flame-ionization detector and a 3-m × 3-mm stainless steel column, or equivalent, packed with 60- to 80-mesh Chromosorb P containing 15% didecyl phthalate and capable of separating hexane, isooctane, and *n*-nonane, or equivalent. Maintain the column isothermally at 120°. Set the injection port temperature to 240° and the detector to 250°. Use helium as the carrier gas, flowing at a rate of 30 mL/min. Use a digital integrator or computer for data acquisition, although any mode (other than triangulation and planimetry) that gives accurate and reliable measurement of the peak areas is satisfactory.

Obtain chromatograms of duplicate 5-µL portions of the *Hexane Standard Solution*, and measure the areas under the hexane and *n*-nonane peaks. In a similar manner, obtain chromatograms of duplicate 5-µL portions of the *Sample Preparation*, and measure the areas under the hexane and *n*-nonane peaks. The peak area ratio of hexane to *n*-nonane (i.e., sum of hexane areas divided by sum of *n*-nonane areas) produced by the *Sample Preparation* does not exceed that produced by the *Hexane Standard Solution*.

Residual Styrene Determine as directed under *Residual Styrene*, Appendix IV.

Packaging and Storage Store in well-closed containers.

Butane

n-Butane

$CH_3CH_2CH_2CH_3$

C_4H_{10} Formula wt 58.12

CAS: [106-97-8]

DESCRIPTION

Butane occurs as a colorless, flammable gas. One volume of water dissolves 0.15 volume; 1 volume of alcohol dissolves 18 volumes; 1 volume of ether dissolves 25 or 30 volumes, respectively, at 17° and 770 mm Hg. Its boiling temperature is –0.5°.

Function Propellant; aerating agent.

REQUIREMENTS

Caution: Butane is highly flammable and explosive. Observe precautions and perform sampling and analytical operations in a well-ventilated fume hood.

Identification

A. The infrared absorption spectrum of sample exhibits maxima, among others, at about the following wavelengths, in µm: 3.4 (vs), 6.8 (s), 7.2 (m), and 10.4 (m).

B. The vapor pressure of a test sample, obtained as directed in the *Sampling Procedure* (below) and determined at 21° by means of a suitable pressure gauge, is between 205 and 235 kPa absolute (30 and 34 psia, respectively).

Assay Not less than 97.0% of C_4H_{10}.

Acidity of Residue Passes test.

High-Boiling Residue Not more than 5 mg/kg.

Sulfur Compounds Passes test.

Water Not more than 10 mg/kg.

TESTS

Sampling Procedure Use a stainless steel sampling cylinder equipped with a stainless steel valve and having a capacity of not less than 200 mL and a pressure rating of 240 psi or more. Dry the cylinder with the valve open at 110° for 2 h, and evacuate the hot cylinder to less than 1 mm of mercury. Close the valve, and cool and weigh the cylinder. Tightly connect one end of a charging line to the sample container, and loosely connect the other end to the sampling cylinder. Carefully open the sample container, and allow the sample to flush out the charging line through the loose connection. Avoid excessive flushing that causes moisture to freeze in the charging line and connections. Tighten the fitting on the sampling cylinder, and open its valve, allowing the sample to flow into the evacuated cylinder. Continue sampling until the desired amount of sample is obtained, then close the sample container valve, and finally, close the sampling cylinder valve.

Caution: Do not overload the sampling cylinder.

Weigh the charged sampling cylinder again, and calculate the sample weight.

Assay

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a thermal-conductivity detector and containing a 6-m × 3-mm aluminum column, or equivalent, packed with 10 weight percent tetraethylene glycol dimethyl ether liquid phase on a support of crushed firebrick (GasChrom R, or equivalent), which has been calcined or burned with a clay binder above 900° and silanized, or equivalent. Use helium as the carrier gas at a flow rate of 50 mL/min, and maintain the temperature of the column at 33°.

System Suitability The peak responses obtained for the sample in the chromatograms from duplicate determinations agree within 1%.

Procedure Connect one sample cylinder to the chromatograph through a suitable sampling valve and a flow control valve downstream from the sampling valve. Flush the liquid sample through the sampling valve, taking care to avoid trapping gas or air in the valve. Inject a suitable volume, typically 2 µL, of sample into the chromatograph, and record the chromatogram.

Calculation Calculate the purity of the sample using the following formula:

$$(100 \times B)/(x + y + z \ \ldots)$$

in which B is the sample response and x, y, z . . . represent the sum of all the responses in the chromatogram.

Acidity of Residue Add 10 mL of water to the residue obtained in *High-Boiling Residue* (below), mix by swirling for about 30 s, add 2 drops of methyl orange TS, insert the stopper in the tube, and shake vigorously. No pink or red color appears in the aqueous layer.

High-Boiling Residue Prepare a cooling coil from copper tubing [about 6.1 m × 6 mm (od)] to fit into a suitable vacuum-jacketed flask. Immerse the cooling coil in a mixture of dry ice and acetone in a vacuum-jacketed flask, and connect one end of the tubing to a sample cylinder (see *Sampling Procedure*, above). Carefully open the sample cylinder valve, flush the cooling coil with about 50 mL of the liquified sample, and discard this portion of liquid. Continue delivering liquid

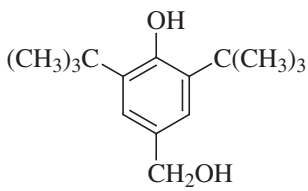
from the cooling coil, and collect it in a previously chilled 1000-mL sedimentation cone until the cone is filled to the 1000-mL mark (approximately 600 g). Allow the liquid to evaporate, using a warm water bath maintained at about 40° to reduce evaporating time. When all of the liquid has evaporated, rinse the sedimentation cone with two 50-mL portions of pentane, and combine the rinsings in a tared 150-mL evaporating dish. Transfer 100 mL of the pentane solvent to a second, tared 150-mL evaporating dish, place both evaporating dishes on a water bath, evaporate to dryness, and heat the dishes in an oven at 100° for 60 min. Cool the dishes in a desiccator, and weigh. Repeat the heating for 15-min periods until successive weighings are within 0.1 mg. The weight of the residue obtained from the sample is the difference between the weights of the residues in the two evaporating dishes. Calculate the milligrams per kilogram of high-boiling residue based on a sample weight of 600 g.

Sulfur Compounds Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose. The gas is free from the characteristic odor of sulfur compounds.

Water Determine as directed under *Water Determination*, Appendix IIB, but use the following modifications: (a) Provide the closed-system titrating vessel with an opening and pass through it a coarse-porosity gas dispersion tube connected to a sampling cylinder. (b) Dilute the reagent with anhydrous methanol to give a water equivalence factor of between 0.2 and 1.0 mg/mL; age this diluted solution for not less than 16 h before standardization. (c) Obtain a 100-g sample as directed in the *Sampling Procedure* (above), and introduce the sample into the titration vessel through the gas dispersion tube at a rate of about 100 mL of gas per minute; if necessary, heat the sampling cylinder gently to maintain this flow rate.

Packaging and Storage Store in tight cylinders protected from heat.

Butylated Hydroxymethylphenol



$C_{15}H_{24}O_2$ Formula wt 236.35

DESCRIPTION

Butylated Hydroxymethylphenol occurs as a nearly white, crystalline solid. It is freely soluble in alcohol, and insoluble in water and in propylene glycol.

Function Antioxidant.

REQUIREMENTS

Identification Butylated Hydroxymethylphenol may be identified by its solidification point, as determined in the *Assay*.

Assay Not less than 98.0% of $C_{15}H_{24}O_2$.

TESTS

Assay Determine as directed under *Solidification Point*, Appendix IIIB. The sample's solidification point is not lower than 140°, indicating a purity of not less than 98.0%, by weight, of $C_{15}H_{24}O_2$.

Packaging and Storage Store in well-closed containers.

1,3-Butylene Glycol

Butane-1,3-diol

$$CH_2OHCH_2CHOHCH_3$$

$C_4H_{10}O_2$ Formula wt 90.12

CAS: [107-88-0]

DESCRIPTION

1,3-Butylene Glycol occurs as a clear, colorless, hygroscopic, viscous liquid. It is miscible with water, with acetone, and with ether in all proportions, but is immiscible with fixed oils. It dissolves most essential oils and synthetic flavoring substances.

Function Solvent for flavoring agents.

REQUIREMENTS

Assay Not less than 99.0% of $C_4H_{10}O_2$.
Distillation Range Between 200° and 215°.
Lead Not more than 2 mg/kg.
Specific Gravity Between 1.004 and 1.006 at 20°.

TESTS

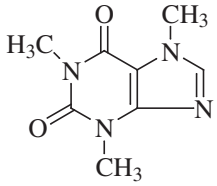
Assay Prepare an acetylating reagent, within one week of use, by mixing 3.4 mL of water and 130 mL of acetic anhydride with 1000 mL of anhydrous pyridine. For the *Assay*, pipet 20 mL of this reagent into a 250-mL iodine flask, and add about 1 g of sample, accurately weighed. Attach a dry reflux condenser to the flask, and reflux for 1 h. Allow the flask to cool to room temperature, then rinse the condenser with 50 mL of chilled (10°) carbon dioxide-free water, allowing the water to drain into the flask. Stopper the flask, cool to below 20°, add

phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide, swirling the contents of the flask continuously during the titration. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.5 *N* sodium hydroxide is equivalent to 2.253 mg of $C_4H_{10}O_2$.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in well-closed containers.

Caffeine

1,3,7-Trimethylxanthine



$C_8H_{10}N_4O_2$ Formula wt, anhydrous 194.19
$C_8H_{10}N_4O_2 \cdot H_2O$ Formula wt, monohydrate 212.21

CAS: anhydrous [58-08-2]

View IR

DESCRIPTION

Caffeine occurs as a white powder or as white, glistening needles, usually matted together. It may be compacted or compressed into free-flowing granules or pellets. It is odorless and has a bitter taste. Caffeine is anhydrous or contains one molecule of water of hydration. Its solutions are neutral to litmus. The hydrate is efflorescent in air, and 1 g is soluble in about 50 mL of water, in 75 mL of alcohol, in about 6 mL of chloroform, and in 600 mL of ether.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or hydrous.
Identification

A. Dissolve about 5 mg of sample in 1 mL of hydrochloric acid contained in a porcelain dish, add 50 mg of potassium chlorate, and evaporate on a steam bath to dryness. Invert the dish over a vessel containing a few drops of 6 *N* ammonium hydroxide. The residue acquires a purple color, which disappears on the addition of a solution of a fixed alkali.

B. The infrared absorption spectrum of a mineral oil dispersion of the sample, previously dried at 80° for 4 h, exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Caffeine Reference Standard.
Assay Not less than 98.5% and not more than 101.0% of $C_8H_{10}N_4O_2$, calculated on the anhydrous basis.
Lead Not more than 1 mg/kg.
Melting Range *Anhydrous*: Between 235° and 237.5°.
Other Alkaloids Passes test.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.1%.
Water *Anhydrous*: Not more than 0.5%; *Hydrous*: Not more than 8.5%.

TESTS

Assay Dissolve about 170 mg of finely powdered sample, accurately weighed, in 5 mL of glacial acetic acid with warming. Cool, add 10 mL of acetic anhydride and 20 mL of toluene, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically. Each milliliter of 0.1 *N* perchloric acid is equivalent to 19.42 mg of $C_8H_{10}N_4O_2$.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Lead Determine as directed for *Method I* in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 3-g sample.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB, using a sample previously dried at 80° for 4 h.
Other Alkaloids Add a few drops of mercuric–potassium iodide TS to 5 mL of a 1:50 aqueous solution. No precipitate forms.
Readily Carbonizable Substances Dissolve 500 mg of sample in 5 mL of 95% sulfuric acid. The resulting color is no darker than that of *Matching Fluid D* under *Readily Carbonizable Substances*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store hydrous caffeine in tight containers and anhydrous caffeine in well-closed containers.

Calcium Acetate

$Ca(C_2H_3O_2)_2$ Formula wt 158.17
INS: 263 CAS: [62-54-4]

DESCRIPTION

Calcium Acetate occurs as a fine, white, bulky powder. It is freely soluble in water and slightly soluble in alcohol.

Function Buffer; stabilizer; firming agent.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Calcium* and for *Acetate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of $Ca(C_2H_3O_2)_2$, calculated on the anhydrous basis.
Chloride Not more than 0.05%.
Fluoride Not more than 0.005%.
Lead Not more than 2 mg/kg.
Sulfate Not more than 0.1%.
Water Not more than 7.0%.

TESTS

Assay Dissolve about 300 mg of sample, accurately weighed, in 150 mL of water containing 2 mL of 2.7 *N* hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 7.909 mg of $Ca(C_2H_3O_2)_2$.
Chloride Determine as directed in *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 40-mg sample does not exceed that produced by a control containing 20 μg of chloride (Cl) ion.
Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB, except in the *Procedure*, use 10 mL of 1 *N* hydrochloric acid to dissolve the sample.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Sulfate Determine as directed in *Sulfate Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 200-mg sample does not exceed that produced by a control containing 200 μg of sulfate (SO_4) ion.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Calcium Acid Pyrophosphate

$CaH_2P_2O_7$ Formula wt 216.04

CAS: [35405-51-7]

DESCRIPTION

Calcium Acid Pyrophosphate occurs as a fine, white, acidic powder. It is insoluble in water, but it is soluble in dilute hydrochloric and nitric acids.

Function Leavening agent; nutrient.

REQUIREMENTS

Identification

A. Dissolve about 100 mg of sample by warming it with a mixture of 5 mL of 2.7 *N* hydrochloric acid and 5 mL of water; add dropwise, while shaking, 2.5 mL of 6 *N* ammonium hydroxide; and then add 5 mL of ammonium oxalate TS. A white precipitate forms.

B. Dissolve 100 mg of sample in 100 mL of 1.7 *N* nitric acid. Add 0.5 mL of this solution to 30 mL of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and then add 0.5 mL of the solution to 30 mL of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 95.0% and not more than 100.5% of $CaH_2P_2O_7$.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Lead Not more than 2 mg/kg.
Loss on Ignition Not more than 10.0%.

TESTS

Assay Dissolve about 300 mg of sample, accurately weighed, in 10 mL of 2.7 *N* hydrochloric acid. Add about 120 mL of water and a few drops of methyl orange TS, and boil for 30 min. Keep the volume and pH of the solution constant during the boiling period by adding hydrochloric acid or water if necessary. Add 2 drops of methyl red TS and 30 mL of ammonium oxalate TS, then add, dropwise, with constant stirring, a mixture of equal volumes of 6 *N* ammonium hydroxide and water until the pink color of the indicator just disappears. Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through a sintered-glass filter crucible using gentle suction. Wash the precipitate in the beaker with about 30 mL of cold (below 20°) wash solution, prepared by diluting 10 mL of ammonium oxalate TS to 1000 mL with water. Allow the precipitate to settle, and pour the supernatant liquid through the filter. Repeat this washing by decantation three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter with two 10-mL portions of cold (below 20°) water. Place the sintered-glass filter crucible in the beaker, and add 10 mL of water and 50 mL of cold, 1:6 sulfuric acid. Add 35 mL of 0.1 *N* potassium permanganate from a buret, and stir until the color disappears. Heat to about 70°, and complete the titration with 0.1 *N* potassium permanganate. Each milliliter of 0.1 *N* potassium permanganate is equivalent to 5.40 mg of $CaH_2P_2O_7$.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 5 mL of 2.7 *N* hydrochloric acid.
Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB, using 1.0 g of sample, accurately weighed.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Ignition Transfer about 1 g of sample, accurately weighed, into a suitable tared crucible, ignite at 800° ± 25° for 30 min, cool in a desiccator, and weigh.

Packaging and Storage Store in well-closed containers.

Calcium Alginate

Algin

$[(C_6H_7O_6)_2Ca]_n$ Formula wt, calculated 195.16
Formula wt, actual (avg.) 219.00

INS: 404 CAS: [9005-35-0]

DESCRIPTION

Calcium Alginate occurs as a white to yellow, fibrous or granular powder. It is the calcium salt of alginic acid (see the monograph for *Alginic Acid*). It is insoluble in water, but it is soluble in alkaline solutions or in solutions of substances that combine with the calcium. It is insoluble in organic solvents.

Function Stabilizer; thickener; emulsifier.

REQUIREMENTS

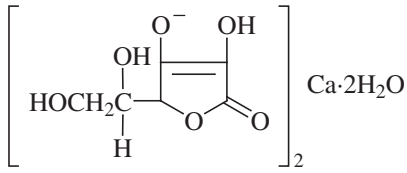
Identification Place about 5 mg of sample into a test tube, add 5 mL of water, 1 mL of a freshly prepared 1:100 solution of naphtholresorcinol:ethanol, and 5 mL of hydrochloric acid. Heat the mixture to boiling, boil gently for about 3 min, and then cool to about 15°. Transfer the contents of the test tube into a 30-mL separator with the aid of 5 mL of water, and extract with 15 mL of isopropyl ether. Perform a blank determination (see *General Provisions*), and make any necessary correction. The isopropyl ether extract from the sample exhibits a deeper purple hue than that from the blank.
Assay A sample yields not less than 18% and not more than 21% of carbon dioxide (CO_2), corresponding to between 89.6% and 104.5% of Calcium Alginate (equiv wt 219.00), calculated on the dried basis.
Arsenic Not more than 3 mg/kg.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 15.0%.

TESTS

Assay Determine as directed under *Alginates Assay*, Appendix IIIC. Each milliliter of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 27.38 mg of Calcium Alginate (equiv wt 219.00).
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Packaging and Storage Store in well-closed containers.

Calcium Ascorbate



$C_{12}H_{14}CaO_{12}\cdot 2H_2O$ Formula wt 426.34

INS: 302 CAS: [5743-27-1]

DESCRIPTION

Calcium Ascorbate occurs as a white to slightly yellow, crystalline powder. It is soluble in water, slightly soluble in alcohol, and insoluble in ether. The pH of a 1:10 aqueous solution is between 6.8 and 7.4.

Function Antioxidant.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Calcium*, Appendix IIIA, and it decolorizes dichlorophenol–indophenol TS.
Assay Not less than 98.0% and not more than 100.5% of $C_{12}H_{14}CaO_{12}\cdot 2H_2O$.
Lead Not more than 2 mg/kg.
Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +95° and +97°.
Oxalate Passes test.

TESTS

Assay Dissolve about 300 mg of sample, accurately weighed, in 50 mL of water in a 250-mL Erlenmeyer flask, and immediately titrate with 0.1 *N* iodine to a pale yellow color that persists for at least 30 s. Each milliliter of 0.1 *N* iodine is equivalent to 10.66 mg of $C_{12}H_{14}CaO_{12}\cdot 2H_2O$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 1 g of sample in each 20 mL.

Oxalate Add 2 drops of glacial acetic acid and 5 mL of a 1:10 calcium acetate solution to a solution of 1 g of sample in 10 mL of water. The solution remains clear after standing for 5 min.

Packaging and Storage Store in tight containers, preferably in a cool, dry place.

Calcium Bromate

$Ca(BrO_3)_2 \cdot H_2O$ Formula wt 313.90

INS: 924b CAS: [10102-75-7]

DESCRIPTION

Calcium Bromate occurs as a white, crystalline powder. It is very soluble in water.

Function Maturing agent; oxidizing agent.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution in 2.7 *N* hydrochloric acid imparts a transient yellow-red color to a nonluminous flame.

B. Add sulfurous acid dropwise to a 1:20 aqueous solution. A yellow color develops that disappears upon the addition of an excess of sulfurous acid.

Assay Not less than 99.8% and not more than 100.5% of $Ca(BrO_3)_2 \cdot H_2O$.

Lead Not more than 4 mg/kg.

TESTS

Assay Dissolve about 900 mg of sample, accurately weighed, in 50 mL of water in a 250-mL glass-stoppered Erlenmeyer flask. Add 3 g of potassium iodide, followed by 3 mL of hydrochloric acid. Allow the mixture to stand for 5 min, add 100 mL of cold water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 26.16 mg of $Ca(BrO_3)_2 \cdot H_2O$.

Lead

Sample Solution Dissolve 2 g of sample in 10 mL of water, add 10 mL of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 5 mL of hydrochloric acid, again evaporate to dryness, and then dissolve the residue in 40 mL of water.

Procedure Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 20-mL portion of the *Sample Solution*, and 4 μg of lead (Pb) ion in the control.

Packaging and Storage Store in well-closed containers.

Calcium Carbonate

$CaCO_3$ Formula wt 100.09

INS: 170(i) CAS: [471-34-1]

DESCRIPTION

Calcium Carbonate occurs as a fine, white or colorless, microcrystalline powder. It is stable in air, and it is practically insoluble in water and in alcohol. The presence of any ammonium salt or carbon dioxide increases its solubility in water, but the presence of any alkali hydroxide reduces the solubility.

Function pH control agent; nutrient; dough conditioner; firming agent; yeast nutrient.

REQUIREMENTS

Identification A sample dissolves, with effervescence, in 1 *N* acetic acid, in 2.7 *N* hydrochloric acid, and in 1.7 *N* nitric acid, and the resulting solutions, after boiling, give positive tests for *Calcium*, Appendix IIIA.

Assay Not less than 98.0% and not more than 100.5% of $CaCO_3$ after drying.

Acid-Insoluble Substances Not more than 0.2%.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Lead Not more than 3 mg/kg.

Loss on Drying Not more than 2%.

Magnesium and Alkali Salts Not more than 1%.

TESTS

Assay Transfer about 200 mg of sample, previously dried at 200° for 4 h and accurately weighed, into a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 mL of 2 *N* hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the sample. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute the contents to about 100 mL with water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 5.004 mg of $CaCO_3$.

Acid-Insoluble Substances Suspend 5 g of sample in 25 mL of water, agitate while cautiously adding 25 mL of 1:2 hydrochloric acid, and add water to make a volume of about 200 mL. Heat the solution to boiling, cover, digest on a steam bath for 1 h, cool, and filter. Wash the precipitate with water until the last washing shows no chloride with silver nitrate TS, and then ignite it. The weight of the residue does not exceed 10 mg.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of 2.7 *N* hydrochloric acid.

Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB.

Lead

Sample Solution Cautiously dissolve 5 g of sample in 25 mL of 1:2 hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in about 15 mL of water, and dilute to 25 mL (1 mL = 200 mg).

Procedure Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 20-mL portion of the *Sample Solution*, and 12 μg of lead (Pb) ion in the control.

Alternatively, determine as directed in the *APDC Extraction Method* under *Lead Limit Test*.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 200° for 4 h.

Magnesium and Alkali Salts Mix 1 g of sample with 40 mL of water, carefully add 5 mL of hydrochloric acid, mix, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add 6 *N* ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-mL cylinder, dilute to 100 mL with water, and let it stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper, and place 50 mL of the clear filtrate in a platinum dish. Add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 5 mg.

Packaging and Storage Store in well-closed containers.

Calcium Chloride

$CaCl_2$ Formula wt, anhydrous 110.98

$CaCl_2 \cdot 2H_2O$ Formula wt, dihydrate 147.01

CAS: anhydrous [10043-52-4]

INS: 509 CAS: dihydrate [10035-04-8]

DESCRIPTION

Calcium Chloride occurs as white, hard fragments, granules, or powder. It is anhydrous or contains two molecules of water of hydration. It is deliquescent. It is soluble in water and slightly soluble in alcohol. The pH of a 1:20 aqueous solution is between 4.5 and 11.0.

Function Firming agent.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or the dihydrate.

Identification A 1:10 aqueous solution gives positive tests for *Calcium* and for *Chloride*, Appendix IIIA.

Assay *Anhydrous*: Not less than 93.0% and not more than 100.5% of $CaCl_2$; *Dihydrate*: Not less than 99.0% and not more than 107.0% of $CaCl_2 \cdot 2H_2O$.

Acid-Insoluble Matter *Anhydrous*: Not more than 0.02%; no particles per kilogram of sample greater than 2 mm in any dimension.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.004%.

Lead Not more than 5 mg/kg.

Magnesium and Alkali Salts *Anhydrous*: Not more than 5.0%; *Dihydrate*: Not more than 4.0%.

TESTS

Assay Transfer about 1.5 g of sample, accurately weighed, into a 250-mL volumetric flask, dissolve it in a mixture of 100 mL of water and 5 mL of 2.7 *N* hydrochloric acid, dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a suitable container, and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 5.55 mg of $CaCl_2$ or 7.35 mg of $CaCl_2 \cdot 2H_2O$.

Acid-Insoluble Matter *Anhydrous*: Place a 32-mm (od) lintine disk filter[1] in a suitable filter assembly comprising a 2.5-L screw-cap bottle cut in half horizontally and fitted with a rubber washer with a 35-mm od and a 25-mm id, followed by the lintine disk, a 20-mesh stainless steel screen with a 35-mm od, and a bottle cap with a 25-mm hole in the top. With the filter at the bottom, wash the assembly with 100 mL of 1:300 acetic acid, followed by 100 mL of water. Remove the disk from the assembly, place it on a watch glass, and dry the combination at 105° for 2 h.

Dissolve 1 kg of sample in 3 L of water containing 10 mL of glacial acetic acid. Allow the solution to cool, and filter it through the lintine disk. Rinse the walls of the filter assembly so that all insoluble matter is transferred to the disk, and wash with 100 mL of water. Place the disk on the same watch glass mentioned above, and dry at 105° for 2 h, being careful at all times not to lose any particles that may be on the disk. The difference in the two weights is the weight of the acid-insoluble matter.

Place the disk under a low-power magnifier (4× to 10× magnification). Using a millimeter rule, measure the largest dimension of each particle (or as many as may be necessary) on the disk. No particles greater than 2 mm in any dimension are present.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of water.

Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB.

[1]Available from Filter Fabrics, Inc., 814 E. Jefferson, Goshen, IN 46526; 219-533-3114.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 20 mL of water, and 5 μg of lead (Pb) ion in the control.
Magnesium and Alkali Salts Dissolve 1.0 g of sample in about 50 mL of water, add 500 mg of ammonium chloride, mix, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add 6 *N* ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-mL cylinder, dilute with water to 100 mL, let it stand for 4 h or overnight, then decant the clear, supernatant liquid through a dry filter paper. Add 0.5 mL of sulfuric acid to 50 mL of the clear filtrate in a platinum dish, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 25 mg for the anhydrous or 20 mg for the dihydrate.

Packaging and Storage Store in tight containers.

Calcium Chloride Solution

DESCRIPTION

Calcium Chloride Solution occurs as a clear to slightly turbid, colorless or slightly colored liquid at room temperature. It is nominally available in a concentration range of about 35% to 45% of $CaCl_2$.

Function Sequestrant; firming agent.

REQUIREMENTS

Identification When diluted to a concentration of about 1:10 ($CaCl_2$ basis), a sample gives positive tests for *Calcium* and for *Chloride*, Appendix IIIA.
Assay Not less than 90.0% and not more than 110.0%, by weight, of the labeled amount of calcium chloride, expressed as $CaCl_2$.
Alkalinity [as $Ca(OH)_2$] Not more than 0.3%.
Fluoride Not more than 0.004%, calculated on the amount of $CaCl_2$ as determined in the *Assay*.
Lead Not more than 4 mg/kg, calculated on the amount of $CaCl_2$ as determined in the *Assay*.
Magnesium and Alkali Salts Not more than 5.0%, calculated on the amount of $CaCl_2$ as determined in the *Assay*.

TESTS

Assay Transfer an accurately weighed amount of *Sample Solution*, equivalent to about 1 g of $CaCl_2$, into a 250-mL volumetric flask, add 5 mL of 2.7 *N* hydrochloric acid and 100 mL of water to dissolve, dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a suitable container, and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 5.55 mg of $CaCl_2$.
Alkalinity [as $Ca(OH)_2$] Dilute an accurately weighed amount of *Sample Solution*, equivalent to about 5 g of $CaCl_2$, to 50 mL with water, add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid. Each milliliter of 0.1 *N* hydrochloric acid is equivalent to 3.71 mg of $Ca(OH)_2$.
Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB, using as the sample an accurately weighed amount of *Sample Solution* equivalent to 1 g of $CaCl_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using as the sample an accurately weighed amount of *Sample Solution* equivalent to 1 g of $CaCl_2$ diluted to 10 mL with water, and 4 μg of lead (Pb) ion in the control.
Magnesium and Alkali Salts Using an accurately weighed amount of *Sample Solution*, equivalent to 1.0 g of $CaCl_2$, dilute to 50 mL with water, add 500 mg of ammonium chloride, mix, and boil for about 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add 6 *N* ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture into a 100-mL cylinder, dilute to 100 mL with water, let it stand for 4 h or overnight, and then decant the clear, supernatant liquid through a dry filter paper. Add 0.5 mL of sulfuric acid to 50 mL of the clear filtrate in a platinum dish, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts are completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 25 mg.

Packaging and Storage Store in tight containers.

Calcium Citrate

Tricalcium Citrate

$$\left[CH_2(COO)C(OH)(COO)CH_2COO \right]_2 Ca_3$$

$Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$ Formula wt 570.50

INS: 333 CAS: [5785-44-4]

DESCRIPTION

Calcium Citrate occurs as a fine, white powder. It is very slightly soluble in water, but it is insoluble in alcohol.

Function Sequestrant; buffer; firming agent.

REQUIREMENTS

Identification

A. Dissolve 500 mg of sample in 10 mL of water and 2.5 mL of 1.7 *N* nitric acid, add 1 mL of mercuric sulfate TS, heat to boiling, and then add potassium permanganate TS. A white precipitate forms.

B. Completely ignite 500 mg of sample at as low a temperature as possible, cool, and dissolve the residue in a mixture of 10 mL of water and 1 mL of glacial acetic acid. Filter, and add 10 mL of ammonium oxalate TS to the filtrate. A voluminous, white precipitate forms that is soluble in hydrochloric acid.

Assay Not less than 97.5% and not more than 100.5% of $Ca_3(C_6H_5O_7)_2$ after drying.

Fluoride Not more than 0.003%.

Lead Not more than 2 mg/kg.

Loss on Drying Between 10.0% and 14.0%.

TESTS

Assay Dissolve about 350 mg of sample, previously dried at 150° for 4 h and accurately weighed, in a mixture of 10 mL of water and 2 mL of 2.7 *N* hydrochloric acid, and dilute to about 100 mL with water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 8.300 mg of $Ca_3(C_6H_5O_7)_2$.

Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB, using a 1-g sample, accurately weighed. Use 1.0, 5.0, and 10.0 mL of the *Sodium Fluoride Solution* (equivalent to 5.0, 25.0, and 50.0 mg/kg of fluoride, respectively) to prepare the *Calibration Curve*, and use 10 mL of water and only 10 mL of 1 *N* hydrochloric acid to dissolve the sample as directed under *Procedure*.

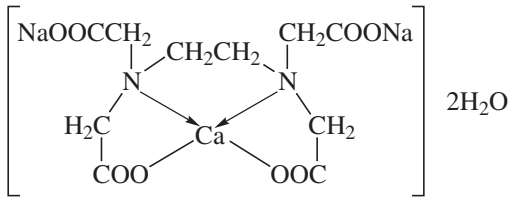
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample for 4 h at 150°.

Packaging and Storage Store in well-closed containers.

Calcium Disodium EDTA

Calcium Disodium Ethylenediaminetetraacetate; Calcium Disodium (Ethylenedinitrilo)tetraacetate; Calcium Disodium Edetate



$C_{10}H_{12}CaN_2Na_2O_8 \cdot 2H_2O$ Formula wt 410.30

INS: 385 CAS: [23411-34-9]

DESCRIPTION

Calcium Disodium EDTA occurs as white, crystalline granules or as a white to off white powder. It is slightly hygroscopic and is stable in air. It is freely soluble in water.

Function Preservative; sequestrant.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution responds to the oxalate test for *Calcium* and to the flame test for *Sodium*, Appendix IIIA.

B. The infrared absorption spectrum of a mineral oil dispersion of sample exhibits maxima only at the same wavelengths as those of a similar preparation of USP Edetate Calcium Disodium Reference Standard.

C. Add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS to 5 mL of water contained in a test tube. Add about 50 mg of sample to the deep red solution so obtained, and mix. The deep red color disappears.

Assay Not less than 97.0% and not more than 102.0% of $C_{10}H_{12}CaN_2Na_2O_8$, calculated on the anhydrous basis.

Lead Not more than 4 mg/kg.

Magnesium-Chelating Substances Passes test.

Nitrilotriacetic Acid Not more than 0.1%.

pH of a 1:100 Solution Between 6.5 and 7.5.

Water Not more than 13.0%.

TESTS

Assay Transfer about 1.2 g of sample, accurately weighed, into a 250-mL beaker, and dissolve in 75 mL of water. Add 25 mL of 1 *N* acetic acid and 1.0 mL of diphenylcarbazone TS, and titrate slowly with 0.1 *M* mercuric nitrate to the first appearance of a purple color. Each milliliter of 0.1 *M* mercuric nitrate is equivalent to 37.43 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

Lead Determine as directed for the *Dithizone Method* under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, but use 70%

perchloric acid instead of 30% hydrogen peroxide to decompose the sample.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

The resulting solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 4 μg of lead (Pb) ion in the control.

Magnesium-Chelating Substances Transfer 1 g of sample, accurately weighed, into a small beaker, and dissolve it in 5 mL of water. Add 5 mL of a buffer solution prepared by dissolving 67.5 g of ammonium chloride in 200 mL of water, adding 570 mL of ammonium hydroxide, and diluting with water to 1000 mL. Then add 5 drops of eriochrome black TS to the buffered solution, and titrate with 0.1 *M* magnesium acetate to the appearance of a deep wine red color. Not more than 2.0 mL is required.

Nitrilotriacetic Acid

Mobile Phase Add 10 mL of a 1:4 solution of tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 *M* phosphoric acid to a pH of 7.5 ± 0.1. Transfer the solution into a 1000-mL volumetric flask, add 90 mL of methanol, dilute to volume with water, mix, filter through a membrane filter (0.5-μm or finer porosity), and de-gas.

Cupric Nitrate Solution Prepare an aqueous solution containing about 10 mg of cupric nitrate per milliliter.

Stock Standard Solution Transfer about 100 mg of nitrilotriacetic acid, accurately weighed, into a 10-mL volumetric flask, add 0.5 mL of ammonium hydroxide, and mix. Dilute to volume with water, and mix.

Standard Preparation Transfer 1.0 g of sample into a 100-mL volumetric flask, add 100 μL of *Stock Standard Solution*, dilute to volume with *Cupric Nitrate Solution*, and mix. Sonicate, if necessary, to achieve complete solution.

Test Preparation Transfer 1.0 g of sample into a 100-mL volumetric flask, dilute to volume with *Cupric Nitrate Solution*, and mix. Sonicate, if necessary, to achieve complete solution.

Chromatographic System (See *Chromatography*, Appendix IIIA.) Set up the system with reference to *High-Performance Liquid Chromatography*. The chromatograph has a 254-nm detector and a 15-cm × 4.6-mm column that contains 5- to 10-mm porous microparticles of silica bonded to octylsilane (Zorbax 8, or equivalent). Set the flow rate to about 2 mL/min. Chromatograph three replicate injections of the *Standard Preparation*, and record the peak responses as directed under *Procedure*. The relative standard deviation is not more than 2.0%, and the resolution factor between nitrilotriacetic acid and Calcium Disodium EDTA is not less than 4.0.

Procedure Separately inject equal volumes (about 50 μL) of the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention times are about 3.5 min for nitrilotriacetic acid and 9 min for Calcium Disodium EDTA. The response of the nitrilotriacetic acid peak of the *Test Preparation* does not exceed the difference between the nitrilotriacetic acid peak responses obtained from the *Standard Preparation* and the *Test Preparation*.

pH of a 1:100 Solution Determine as directed under *pH Determination*, Appendix IIB.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Calcium Gluconate

$$\left[CH_2OH(CHOH)_4COO\right]_2Ca$$

$C_{12}H_{22}CaO_{14}$ Formula wt, anhydrous 430.38
$C_{12}H_{22}CaO_{14}\cdot H_2O$ Formula wt, monohydrate 448.39
CAS: anhydrous [299-28-5]

INS: 578

DESCRIPTION

Calcium Gluconate occurs as white, crystalline granules or powder. It is anhydrous or contains one molecule of water of hydration. It is stable in air. One gram dissolves slowly in about 30 mL of water at 25° and in about 5 mL of boiling water. It is insoluble in alcohol and in many other organic solvents. Its solutions are neutral to litmus.

Function Firming agent; stabilizer; texturizer.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or the monohydrate.

Identification

A. A 1:50 aqueous solution gives positive tests for *Calcium*, Appendix IIIA.

B. Dissolve a quantity of sample in water, heating in a water bath at 60° if necessary, to obtain a *Test Solution* containing 10 mg/mL. Similarly, prepare a *Standard Solution* of USP Potassium Gluconate Reference Standard in water, diluting to 10 mg/mL. To prepare a *Spray Reagent*, dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 *N* sulfuric acid to volume, and mix. Apply separate 5-μL portions of the *Test Solution* and the *Standard Solution* on a suitable thin-layer chromatographic plate (see *Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent system consisting of a 50:30:10:10 mixture of alcohol, water, ammonium hydroxide, and ethyl acetate until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray Reagent*. After spraying, heat the plate at 110° for about 10 min. The principal spot obtained

from the *Test Solution* corresponds in color, size, and R_f value to that obtained from the *Standard Solution*.
Assay *Anhydrous*: Not less than 98.0% and not more than 102.0% of $C_{12}H_{22}CaO_{14}$, calculated on the dried basis; *Monohydrate*: Not less than 98.0% and not more than 102.0% of $C_{12}H_{22}CaO_{14}\cdot H_2O$, calculated on the as-is basis.
Lead Not more than 2 mg/kg.
Loss on Drying *Anhydrous*: Not more than 3.0%; *Monohydrate*: Not more than 2.0%.
Sucrose and Reducing Sugars Not more than 1.0%.

TESTS

Assay Dissolve about 800 mg of sample, accurately weighed, in 100 mL of water containing 2 mL of 2.7 *N* hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 21.52 mg of $C_{12}H_{22}CaO_{14}$ or 22.42 mg of $C_{12}H_{22}CaO_{14}\cdot H_2O$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 16 h.
Sucrose and Reducing Sugars Transfer 1.0 g of sample into a 250-mL conical flask, and add 20 mL of hot water to dissolve the sample. Cool, add 25 mL of alkaline cupric citrate TS, cover the flask, and boil gently for 5 min, accurately timed. Cool rapidly to room temperature, add 25 mL of 0.6 *N* acetic acid, 10.0 mL of 0.1 *N* iodine, and 10 mL of 2.7 *N* hydrochloric acid. Immediately titrate with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate consumed is equivalent to 2.7 mg of reducing substances (as dextrose).

Packaging and Storage Store in well-closed containers.

Calcium Glycerophosphate

$C_3H_7CaO_6P$ Formula wt 210.14

INS: 383 CAS: [27214-00-2]

DESCRIPTION

Calcium Glycerophosphate occurs as a fine, white powder. It is somewhat hygroscopic. One gram dissolves in about 50 mL of water at 25°. It is more soluble in water at a lower temperature, and citric acid increases its solubility in water. It is insoluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification A saturated sample solution gives positive tests for *Calcium*, Appendix IIIA.
Assay Not less than 98.0% and not more than 100.5% of $C_3H_7CaO_6P$, after drying.
Alkalinity Passes test.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 12.0%.

TESTS

Assay Accurately weigh about 2 g of sample, previously dried at 150° for 4 h, and dissolve it in 100 mL of water and 5 mL of 2.7 *N* hydrochloric acid. Transfer the solution into a 250-mL volumetric flask, dilute to volume with water, and mix well. Pipet 50.0 mL of this solution into a suitable container, and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 10.51 mg of $C_3H_7CaO_6P$.
Alkalinity A solution of 1 g of sample in 60 mL of water requires not more than 1.5 mL of 0.1 *N* sulfuric acid for neutralization, using 3 drops of phenolphthalein TS as indicator.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying the sample at 150° for 4 h.

Packaging and Storage Store in tight containers.

Calcium Hydroxide

Slaked Lime

$Ca(OH)_2$ Formula wt 74.10

INS: 526 CAS: [1305-62-0]

DESCRIPTION

Calcium Hydroxide occurs as a white powder. One gram dissolves in 630 mL of water at 25°, and in 1300 mL of

boiling water. It is soluble in glycerin and in a saturated solution of sucrose but insoluble in alcohol.

Function Buffer; neutralizing agent; firming agent.

REQUIREMENTS

Identification

A. Mix a sample with from 3 to 4 times its weight of water. The sample forms a smooth magma. The clear, supernatant liquid from the magma is alkaline to litmus.

B. Mix 1 g of sample with 20 mL of water, and add sufficient glacial acetic acid to effect solution. The resulting solution gives positive tests for *Calcium*, Appendix IIIA.

Assay Not less than 95.0% and not more than 100.5% of $Ca(OH)_2$.

Acid-Insoluble Substances Not more than 0.5%.

Arsenic Not more than 3 mg/kg.

Carbonate Passes test.

Fluoride Not more than 0.005%.

Lead Not more than 2 mg/kg.

Magnesium and Alkali Salts Not more than 4.8%.

TESTS

Assay Transfer about 1.5 g of sample, accurately weighed, into a beaker, and gradually add 30 mL of 2.7 *N* hydrochloric acid. When solution is complete, transfer it into a 500-mL volumetric flask, rinse the beaker thoroughly, add the rinsings to the flask, dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a suitable container, and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 3.705 mg of $Ca(OH)_2$.

Acid-Insoluble Substances Dissolve 2 g of sample in 30 mL of 1:3 hydrochloric acid, and heat to boiling. Filter the mixture through a suitable tared, porous-bottom porcelain crucible, wash the residue with hot water until the last washing is free from chloride, ignite at 800° ± 25° for 45 min, cool, and weigh.

> **Note**: Avoid exposing the crucible to sudden temperature changes.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 15 mL of 2.7 *N* hydrochloric acid.

Carbonate Mix 2 g of sample with 50 mL of water, and add an excess of 2.7 *N* hydrochloric acid. The solution produces no more than a slight effervescence.

Fluoride Determine as directed under the *Fluoride Limit Test*, Appendix IIIB, using 1.0 g of sample, accurately weighed.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 15 mL of 2.7 *N* hydrochloric acid, and 5 µg of lead (Pb) ion in the control.

Magnesium and Alkali Salts Dissolve 500 mg of sample in a mixture of 30 mL of water and 10 mL of 2.7 *N* hydrochloric acid, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS; then add 6 *N* ammonium hydroxide, dropwise, until the mixture is just alkaline; and cool. Transfer the mixture into a 100-mL cylinder, dilute to 100 mL with water, let it stand for 4 h or overnight, then decant the clear, supernatant liquid through a dry filter paper. Add 0.5 mL of sulfuric acid to 50 mL of the clear filtrate contained in a tared platinum dish, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue at 800° ± 25° to constant weight.

Packaging and Storage Store in tight containers.

Calcium Iodate

$Ca(IO_3)_2 \cdot H_2O$ Formula wt 407.90

INS: 916 CAS: [7789-80-2]

DESCRIPTION

Calcium Iodate occurs as a white powder. It is slightly soluble in water, and insoluble in alcohol.

Function Maturing agent; dough conditioner.

REQUIREMENTS

Identification Add 1 drop of starch TS and a few drops of 20% hypophosphorous acid to 5 mL of a saturated solution of sample. A transient blue color appears.

Assay Not less than 99.0% and not more than 101.0% of $Ca(IO_3)_2 \cdot H_2O$.

Lead Not more than 4 mg/kg.

TESTS

Assay Dissolve about 600 mg of sample, accurately weighed, in 10 mL of 70% perchloric acid and 10 mL of water, heating gently if necessary, and dilute with water to 250.0 mL.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Transfer 50.0 mL of this solution to a 250-mL glass-stoppered Erlenmeyer flask, add 1 mL of 70% perchloric acid and 5 g of potassium iodide, stopper the flask, and swirl briefly. Let the solution stand for 5 min, then titrate with 0.1 *N* sodium thiosulfate, adding starch TS just before the endpoint is

reached. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 3.398 mg of $Ca(IO_3)_2 \cdot H_2O$.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Packaging and Storage Store in well-closed containers.

Calcium Lactate

2-Hydroxypropanoic Acid Calcium Salt

$$\left[CH_3CH(OH)COO\right]_2Ca \cdot xH_2O$$

$C_6H_{10}CaO_6 \cdot xH_2O$ Formula wt, anhydrous 218.22

INS: 327 CAS: [814-80-2]

DESCRIPTION

Calcium Lactate occurs as a white to cream-colored, crystalline powder or granules. It contains up to five molecules of water of crystallization. The pentahydrate is somewhat efflorescent and at 120° becomes anhydrous. It is soluble in water and practically insoluble in alcohol.

Function Buffer; dough conditioner; yeast nutrient.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Calcium* and for *Lactate*, Appendix IIIA.

Assay Not less than 98.0% and not more than 101.0% of $C_6H_{10}CaO_6$, calculated on the dried basis.

Acidity Passes test (about 0.45%, as lactic acid).

Fluoride Not more than 0.0015%.

Lead Not more than 2 mg/kg.

Loss on Drying *Pentahydrate*: Between 22.0% and 27.0%; *Trihydrate*: Between 15.0% and 20.0%; *Monohydrate*: Between 5.0% and 8.0%; *Dried Form*: Not more than 3.0%.

Magnesium and Alkali Salts Not more than 1%.

TESTS

Assay Dissolve an accurately weighed amount of sample, equivalent to about 350 mg of $C_6H_{10}CaO_6$, in 150 mL of water containing 2 mL of 2.7 *N* hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration with the disodium EDTA to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 10.91 mg of $C_6H_{10}CaO_6$.

Acidity Dissolve 1 g of sample in 20 mL of water, add 3 drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Not more than 0.5 mL of titrant is required.

Fluoride Determine as directed in *Method I* (3.3-g sample) or *Method III* (1.0-g sample) under *Fluoride Limit Test*, Appendix IIIB.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 3-g sample.

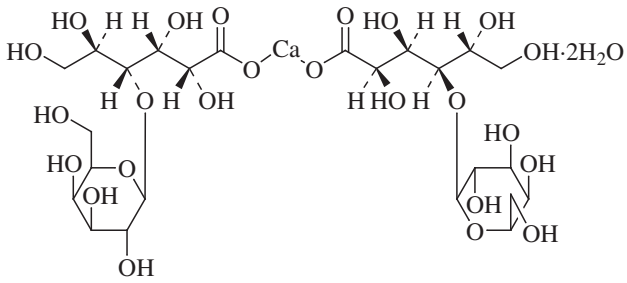
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying 1.5 g of sample at 120° for 4 h.

Magnesium and Alkali Salts Mix 1 g of sample with 40 mL of water, carefully add 1 mL of hydrochloric acid, boil for 1 min, and rapidly add 40 mL of oxalic acid TS. Immediately add 2 drops of methyl red TS, then add 6 *N* ammonium hydroxide, dropwise from a buret, until the mixture is just alkaline, and cool to room temperature. Transfer the mixture into a 100-mL graduate, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper, transfer 50 mL of the clear filtrate to a tared platinum dish, and add 0.5 mL of sulfuric acid. Evaporate to a small volume on a steam bath, then carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of the ammonium salts. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 5 mg.

Packaging and Storage Store in tight containers.

Calcium Lactobionate

Calcium 4-(β,D-Galactosido)-D-gluconate



$C_{24}H_{42}CaO_{24}$ Formula wt, anhydrous 754.66

INS: 399 CAS: [5001-51-4]

DESCRIPTION

Calcium Lactobionate occurs as a white to cream-colored, free-flowing powder. It readily forms double salts, such as the chloride, bromide, and gluconate. It is anhydrous when obtained by spray-drying, or the dihydrate when obtained by crystallization. It is freely soluble in water, but insoluble in

alcohol and in ether. It decomposes at about 120°. The pH of a 1:10 aqueous solution is between 6.5 and 7.5.

Function Firming agent in dry pudding mixes; nutrient.

REQUIREMENTS

Labeling Indicate whether the product has been obtained through spray-drying or from crystallization.

Identification

A. The infrared absorption spectrum of a potassium bromide dispersion of the sample, previously dried at 105° for 8 h, exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Calcium Lactobionate Reference Standard.

B. A sample gives positive tests for *Calcium*, Appendix IIIA.

Calcium Content Not less than 5.05% and not more than 5.55% of calcium (Ca), calculated on the dried basis.

Halides Not more than 0.04%.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 8.0%.

Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +23° and +25°.

Reducing Substances Not more than 1.0%.

Sulfate Not more than 0.7%.

TESTS

Calcium Content Dissolve about 1.5 g of sample, accurately weighed, in 100 mL of water containing 2 mL of 2.7 *N* hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of calcium (Ca).

Halides Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. A 1.2-g sample shows no more turbidity than 0.7 mL of 0.020 *N* hydrochloric acid.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 3-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 8 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 500 mg of sample, calculated on the anhydrous basis, in each 10 mL.

Reducing Substances Transfer 1.0 g of sample into a 250-mL conical flask, dissolve it in 20 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil the contents gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 *N* acetic acid, 10.0 mL of 0.1 *N* iodine, and 10 mL of 3 *N* hydrochloric acid, and titrate with 0.1 *N* sodium thiosulfate, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination (see *General Provisions*), make any necessary correction, and note the difference in volumes of 0.1 *N* sodium thiosulfate required. Each milliliter of the difference in volume of 0.1 *N* sodium thiosulfate consumed is equivalent to 2.7 mg of reducing substances (as dextrose).

Sulfate Transfer about 25 g of sample, accurately weighed, into a 600-mL beaker, dissolve it in 200 mL of water, adjust the solution to a pH between 4.5 and 6.5 with 2.7 *N* hydrochloric acid, and filter if necessary. Heat the filtrate or clear solution to just below the boiling point, then while stirring vigorously, add 10 mL of barium chloride TS, boil gently for 5 min, and allow the solution to stand for at least 2 h, or, preferably, overnight. Collect the precipitate of barium sulfate on a suitable, tared crucible, wash until free from chloride, dry, and ignite at 600° to constant weight. The weight of barium sulfate so obtained, multiplied by 0.412, represents the weight of sulfate (SO_4) in the sample taken.

Packaging and Storage Store in well-closed containers.

Calcium Lignosulfonate

CAS: [8061-52-7]

DESCRIPTION

Calcium Lignosulfonate occurs as a brown, amorphous polymer. It is obtained from the spent sulfite and sulfate pulping liquor of wood or from the sulfate (kraft) pulping process. It may contain up to 30% reducing sugars. It is soluble in water, but not in any of the common organic solvents. The pH of a 1:100 aqueous solution is between approximately 3 and 11.

Function Binder; dispersant.

REQUIREMENTS

Identification

A. A 0.15-g/L aqueous solution gives positive tests for *Calcium*, Appendix IIIA.

B. Dissolve 100 mg of sample in 50 mL of water. Add 1 mL each of 10% acetic acid and 10% sodium nitrite solutions to this solution. Mix the solution by swirling, and allow it to stand for 15 min at room temperature. A brown color appears.

C. The ultraviolet absorption spectrum of a 0.1-g/L aqueous solution at pH 5 exhibits a peak between 275 and 280 nm.

Assay Not less than 5.0% sulfonate sulfur.

Calcium Not more than 7.0%.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 10.0%.

Reducing Sugars Not more than 30.0%.

Residue on Ignition Not more than 20.0%.

Viscosity of a 50% Solution Not more than 3000 centipoises.

TESTS

Assay (as Sulfonate Sulfur) Dissolve 1.0 g of sample, accurately weighed, in 400 mL of water contained in a beaker. Direct a gentle stream of nitrogen gas over the liquid's surface. Add 10 mL of nitric acid, and swirl the solution thoroughly until the reaction subsides. Add 10 mL of 70% perchloric acid, and swirl thoroughly again.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Place the uncovered beaker on a hot plate, and heat the contents vigorously until the center of the bottom of the beaker becomes clear. Remove the beaker, and cool to room temperature. Add 5 mL of hydrochloric acid, and heat again until white fumes evolve. After cooling, dilute the solution to approximately 100 mL with water, adjust to pH 6 ± 0.2 with 10% sodium hydroxide, and heat the solution to boiling. Add 15 mL of 10% barium chloride solution, and leave the solution overnight in a fresh beaker in a steam bath at 90° to 95°. Filter through ashless filter paper (Whatman No. 42, or equivalent), and wash the precipitate with 200 mL of warm water. Transfer the paper and precipitate to a tared crucible. Heat the crucible slowly on a Bunsen burner to expel moisture. Place the crucible and contents in a muffle furnace at 850° for 1 h. Let the crucible cool in a desiccator, and then weigh the residue to the nearest 0.0001 g. Calculate the percent of sulfonate sulfur by the formula

$$(R/S) \times 13.7,$$

in which R is the weight, in grams, of the residue; S is the weight, in grams, of the sample taken; and 13.7 is the conversion factor.

Calcium

Strontium Chloride Solution While stirring, add 164.7 g of 60% perchloric acid to 500 mL of water contained in a 1-L beaker.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Then, while stirring, add 15.2 g of strontium chloride hexahydrate, stirring until solution is complete. Transfer the solution into a 1-L volumetric flask, and dilute to volume at room temperature with water. Mix the contents by inverting the stoppered flask several times.

Standard Solution Dilute a certified Calcium Standard Solution (NIST, or equivalent), quantitatively and stepwise, with water to obtain a *Standard Solution* containing 0.7 mg of calcium per milliliter of water. Store the *Standard Solution* in polyethylene bottles because of its instability in glass.

Sample Solution Dilute 1 g of sample, previously dried and accurately weighed, to 10.0 mL with water. If the initial *Sample Solution* is not particle free, filter through a 0.45-µm disposable Millipore filter, discarding the first few milliliters of filtrate. Pipet 5 mL of *Strontium Chloride Solution* into a 50-mL volumetric flask, and add 5.0 mL of the filtrate. Dilute to volume with water, and mix well.

Procedure Using a suitably calibrated atomic absorption spectrophotometer and following the manufacturer's instructions for optimum operation of the spectrophotometer, determine the absorbance of the *Standard Solution* and the *Sample Solution* at 422.7 nm. The absorbance of the *Sample Solution* is not greater than that of the *Standard Solution*.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying* Appendix IIC, drying a sample at 105° for 24 h.

Reducing Sugars (**Note**: The *Copper Reagent Solution* used in this test must be prepared several days in advance of use.)

Lead Subacetate Solution Dissolve 80 g of lead subacetate in 220 mL of water. Stir overnight, and filter through Whatman No. 42 filter paper, or equivalent. Dilute the supernatant solution to a specific gravity of 1.254 with freshly boiled water.

Copper Reagent Solution Dissolve 28 g of anhydrous dibasic sodium phosphate and 40 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) in 700 mL of water. Add 100 mL of 1 *N* sodium hydroxide and 8 g of copper sulfate pentahydrate, followed by 180 g of anhydrous sodium sulfate. Add 0.7134 g of potassium iodate, and dilute to 1 L. Allow to stand for several days, then filter the clear top part of the solution through a medium-porosity sintered-glass funnel.

Dextrose Standard Solution Dissolve 140 mg of dried dextrose, accurately weighed, in 500 mL of water.

Dibasic Sodium Phosphate Solution Dissolve 19 g of sodium phosphate, dibasic, heptahydrate, in 100 mL of water.

Procedure Dissolve 1 g of sample, accurately weighed, in 150 mL of water, and adjust the pH to between 6.9 and 7.2 with sodium hydroxide solution or acetic acid. Add *Lead Subacetate Solution* in increments until no further precipitation is observed. Bring the volume to 250.0 mL with water, and mix well. Centrifuge the mixture, pipet 10 mL of the supernatant into a 50-mL volumetric flask, and dilute to about 35 mL with water. Add 2 mL or more of *Dibasic Sodium Phosphate Solution* until no further precipitation forms. Dilute to 50 mL with water, and mix. Centrifuge at 2100 × gravity for 10 min. Pipet 5 mL of the supernatant solution into a test tube containing exactly 5 mL of *Copper Reagent Solution*, and mix. Loosely plug the tube, and place it in a boiling water bath for 40 min ± 10 s. At the end of the heating period, cool the tube immediately in cold water. Add 2 mL of 2.5% potassium iodide solution and 1.5 mL of 2 *N* sulfuric acid. Mix well, and titrate with 0.005 *N* sodium thiosulfate, using starch as the indicator, and note the volume of 0.005 *N* sodium thiosulfate consumed as V_S. Run a corresponding blank titration, V_R, using 5 mL of water and 5 mL of *Copper Reagent Solution*.

Repeat the entire procedure with the dextrose standard (5 mL of *Dextrose Standard Solution* and 5 mL of *Copper Reagent Solution*), noting the volume of 0.005 *N* sodium thiosulfate consumed as V_D. Run a corresponding blank titration, V_B, using 5 mL of water and 5 mL of *Copper Reagent Solution*.

Calculate the percent of reducing sugars by the formula

$$35 \times (V_B - V_S)/(V_B - V_D),$$

in which $V_B - V_S$ is the number of milliliters of 0.005 *N* sodium thiosulfate consumed by the 5-mL aliquot of sample, and $V_B - V_D$ is the number of milliliters of 0.005 *N* sodium thiosulfate consumed by 5 mL of *Dextrose Standard Solution*.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Viscosity of a 50% Solution Dissolve 200 g of sample, calculated on the dried basis and accurately weighed, in 200 mL of water contained in a 500-mL beaker. Equilibrate the solution at 25°, and measure its viscosity with a Brookfield viscometer A (model LVG, or equivalent), using a number 2 spindle at 20 rpm.

Packaging and Storage Store in well-closed containers.

Calcium Oxide

Lime

CaO Formula wt 56.08

INS: 529 CAS: [1305-78-8]

DESCRIPTION

Calcium Oxide occurs as hard, white or gray-white masses or granules, or as a white to gray-white powder. One gram dissolves in about 840 mL of water at 25° and in about 1740 mL of boiling water. It is soluble in glycerin but insoluble in alcohol.

Function pH control agent; nutrient; dough conditioner; yeast food.

REQUIREMENTS

Identification Slake 1 g of sample with 20 mL of water, and add glacial acetic acid until the sample is dissolved. The resulting solution gives positive tests for *Calcium*, Appendix IIIA.

Assay Not less than 95.0% and not more than 100.5% of CaO after ignition.

Acid-Insoluble Substances Not more than 1%.

Alkalies or Magnesium Not more than 3.6%.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.015%.

Lead Not more than 2 mg/kg.

Loss on Ignition Not more than 10.0%.

TESTS

Assay Ignite about 1 g of sample to constant weight, and dissolve the ignited sample, accurately weighed, in 20 mL of 2.7 *N* hydrochloric acid. Cool the solution, dilute to 500.0 mL with water, and mix. Pipet 50.0 mL of this solution into a suitable container, and add 50 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 2.804 mg of CaO.

Acid-Insoluble Substances Slake 5 g of sample, then mix it with 100 mL of water and sufficient hydrochloric acid, added dropwise, to effect solution. Boil the solution, cool, add hydrochloric acid, if necessary, to make the solution distinctly acid, and filter through a tared glass filter crucible. Wash the residue with water until free of chlorides, dry at 105° for 1 h, cool, and weigh.

Alkalies or Magnesium Dissolve 500 mg of sample in 30 mL of water and 15 mL of 2.7 *N* hydrochloric acid. Heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously. Add 2 drops of methyl red TS, and neutralize the solution with 6 *N* ammonium hydroxide to precipitate the calcium completely. Heat the mixture on a steam bath for 1 h, cool, dilute to 100 mL with water, mix well, and filter. Add 0.5 mL of sulfuric acid to 50 mL of the filtrate, then evaporate to dryness, and ignite to constant weight in a tared platinum crucible at 800° ± 25°.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 15 mL of 2.7 *N* hydrochloric acid.

Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB, using a 1.0-g sample, accurately weighed.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 15 mL of 2.7 *N* hydrochloric acid, and 5 μg of lead (Pb) ion in the control.

Loss on Ignition Ignite 1 g of sample to constant weight in a tared platinum crucible at 1100° ± 50°.

Packaging and Storage Store in tight containers.

Calcium Pantothenate

D-Calcium Pantothenate; Dextro Calcium Pantothenate

$$\left[HOCH_2C(CH_3)_2CH(OH)CONH(CH_2)_2COO\right]_2Ca$$

$C_{18}H_{32}CaN_2O_{10}$ Formula wt 476.54

CAS: [137-08-6]

DESCRIPTION

Calcium Pantothenate occurs as a slightly hygroscopic, white powder. It is the calcium salt of the dextrorotatory isomer of pantothenic acid. It is stable in air. One gram dissolves in about 3 mL of water. It is soluble in glycerin, but is practically insoluble in alcohol, in chloroform, and in ether.

Function Nutrient.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Calcium*, Appendix IIIA.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample, previously dried at 105° for 3 h, exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Calcium Pantothenate Reference Standard.

C. Boil 50 mg of sample in 5 mL of 1 *N* sodium hydroxide for 1 min, cool, and add 5 mL of 1 *N* hydrochloric acid and 2 drops of ferric chloride TS. A strong yellow color appears.

Assay Not less than 97.0% and not more than 103.0% of Dextrorotatory Calcium Pantothenate ($C_{18}H_{32}CaN_2O_{10}$) after drying.

Alkalinity Passes test.

Alkaloids Passes test.

Calcium Content Not less than 8.2% and not more than 8.6% of calcium (Ca) after drying.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 5.0%.

Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +25.0° and +27.5° after drying.

TESTS

Assay (Use low-actinic glassware throughout this procedure.)

Mobile Phase Transfer 2.0 mL of phosphoric acid into a 2-L volumetric flask, and dilute to volume with water. Filter the solution through a 0.45-μm pore-size disk.

Internal Standard Preparation Transfer about 80 mg of *p*-hydroxybenzoic acid, accurately weighed, into a 1000-mL volumetric flask, dissolve in 5 mL of alcohol, dilute to volume with *Mobile Phase*, and mix.

Standard Preparation Transfer about 15 mg of USP Calcium Pantothenate Reference Standard, previously dried at 105° for 3 h and accurately weighed, into a 25-mL volumetric flask. Dilute to volume with *Internal Standard Preparation*, and mix.

Sample Preparation Proceed as directed for the *Standard Preparation*, using an accurately weighed amount of sample equivalent to about 15 mg of Calcium Pantothenate that has been previously dried at 105° for 3 h.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with an ultraviolet detector that measures at 210 nm. Under typical conditions, the instrument contains a 15-cm × 3.9-mm (id) column packed with octadecylsilanized silica (10-μm μBondapak C 18, or equivalent.) The flow rate is about 1.5 mL/min.

System Suitability Three replicate injections of the *Standard Preparation* show a relative standard deviation of not more than 2.0%.

Procedure Separately inject equal volumes (about 10 μL) of the *Standard Preparation* and the *Sample Preparation* into the chromatograph, record the chromatograms, and measure the peak responses obtained for the *Sample Preparation* and the *Standard Preparation*. The relative retention times are 0.5 for Calcium Pantothenate and 1.0 for *p*-hydroxybenzoic acid. Calculate the quantity, in milligrams, of $C_{18}H_{32}CaN_2O_{10}$ in the portion of sample taken by the formula

$$25C(R_U/R_S),$$

in which C is the concentration, in milligrams per milliliter, of USP Calcium Pantothenate Reference Standard in the *Standard Preparation*, and R_U and R_S are the ratios of the peak responses obtained for Calcium Pantothenate and *p*-hydroxybenzoic acid from the *Sample Preparation* and the *Standard Preparation*, respectively.

Alkalinity Dissolve 1 g of sample in 15 mL of recently boiled and cooled water in a small flask. As soon as solution is complete, add 1.0 mL of 0.1 *N* hydrochloric acid, then add 0.05 mL of phenolphthalein TS, and mix. No pink color appears within 5 s.

Alkaloids Dissolve 200 mg of sample in 5 mL of water, and add 1 mL of 2.7 *N* hydrochloric acid and 2 drops of mercuric–potassium iodide TS. No turbidity develops within 1 min.

Calcium Content Dissolve about 950 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 100 mL of water containing 2 mL of 2.7 *N* hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of calcium (Ca).

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 500 mg of sample, previously dried at 105° for 3 h.

Packaging and Storage Store in tight containers.

Calcium Pantothenate, Calcium Chloride Double Salt

Calcium Chloride Double Salt of DL- or D-Calcium Pantothenate

$C_{18}H_{32}CaN_2O_{10}\cdot CaCl_2$ Formula wt 587.52

CAS: [6363-38-8]

DESCRIPTION

Calcium Pantothenate, Calcium Chloride Double Salt occurs as a white, free-flowing, fine powder. It is a chemical complex

composed of approximately equimolecular quantities of dextrorotatory (D) or racemic (DL) calcium pantothenate and calcium chloride. It is freely soluble in water, but insoluble in alcohol. Its solutions in water are alkaline to litmus.

Function Nutrient.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Calcium*, Appendix IIIA.

B. Dissolve 50 mg of sample in 5 mL of 1 *N* sodium hydroxide, and filter. Add 1 drop of cupric sulfate TS to the filtrate. A deep blue color appears.

C. Stir 1.0 g of dried sample with 15 mL of dimethylformamide for 5 min. Centrifuge the mixture, transfer 2.0 mL of the clear supernatant liquid to a weighing dish, evaporate it under vacuum on a steam bath, and dry the residue in an oven at 105° for 1 h. The weight, in grams, of the residue, composed of uncombined calcium pantothenate and calcium chloride, multiplied by 750 equals the percentage of uncomplexed material in the sample. It does not exceed 10.0% of the weight of the sample.

Assay Not less than 45.0% and not more than 55.0% of Calcium Pantothenate ($C_{18}H_{32}CaN_2O_{10}$) after drying.

Arsenic Not more than 3 mg/kg.

Calcium Content Between 12.4% and 13.6% of calcium (Ca) after drying.

Chloride Content (as Cl) Between 10.5% and 12.1% of chloride after drying.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 5.0%.

TESTS

Assay Determine as directed in the monograph for *Calcium Pantothenate*.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 25 mL of water.

Calcium Content Determine as directed in the monograph for *Calcium Pantothenate*.

Chloride Content (as Cl) Transfer about 1 g of sample, previously dried in vacuum for 1 h and accurately weighed, into a 250-mL beaker, and add sufficient water to make 100 mL. Equip a pH meter with glass and silver electrodes, and set it on the ''+ millivolt'' scale. Insert the electrodes and a motor-driven, glass stirring rod into the sample beaker. Add 1 to 2 drops of methyl orange TS. Stir, and add, dropwise, 10% nitric acid until a pink color appears, then add 10 mL in excess. Titrate the solution with 0.1 *N* silver nitrate to a reading of +1.0 millivolt on the pH meter. Each milliliter of 0.1 *N* silver nitrate is equivalent to 3.545 mg of chloride.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in vacuum at 100° for 1 h.

Packaging and Storage Store in tight containers.

Calcium Pantothenate, Racemic

$C_{18}H_{32}CaN_2O_{10}$ Formula wt 476.54

CAS: [6381-63-1]

DESCRIPTION

Calcium Pantothenate, Racemic, occurs as a white, slightly hygroscopic powder. It is a mixture of the calcium salts of the dextrorotatory (D) and levorotatory (DL) isomers of pantothenic acid. It is optically inactive. It is stable in air and freely soluble in water. It is soluble in glycerin, and is practically insoluble in alcohol, in chloroform, and in ether. Its solutions are neutral or alkaline to litmus.

> **Note**: The physiological activity of Racemic Calcium Pantothenate is approximately one-half that of the dextrorotatory isomer.

Function Nutrient.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Calcium*, Appendix IIIA.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample, previously dried at 105° for 3 h, exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Calcium Pantothenate Reference Standard.

C. Boil 50 mg of sample in 5 mL of 1 *N* sodium hydroxide for 1 min, cool, and add 5 mL of 1 *N* hydrochloric acid and 2 drops of ferric chloride TS. A strong yellow color appears.

Assay Not less than 97.0% and not more than 103.0% of Calcium Pantothenate ($C_{18}H_{32}CaN_2O_{10}$) after drying.

Alkalinity Passes test.

Alkaloids Passes test.

Calcium Content Not less than 8.2% and not more than 8.6% of calcium (Ca) after drying.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 5.0%.

Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between –0.05° and +0.05° after drying.

TESTS

Assay Determine as directed under *Assay* in the monograph for *Calcium Pantothenate*.

Alkalinity Dissolve 1 g of sample in 15 mL of recently boiled and cooled water in a small flask. As soon as solution is complete, add 1.6 mL of 0.1 *N* hydrochloric acid, then add 0.05 mL of phenolphthalein TS, and mix. No pink color appears within 5 s.

Alkaloids Dissolve 200 mg of sample in 5 mL of water, and add 1 mL of 2.7 *N* hydrochloric acid and 2 drops of mercuric–potassium iodide TS. No turbidity develops within 1 min.

Calcium Content Dissolve about 950 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 100 mL of water containing 2 mL of 2.7 *N* hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of calcium (Ca).
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 500 mg of sample, previously dried at 105° for 3 h, in each 10-mL portion.

Packaging and Storage Store in tight containers.

Calcium Peroxide

CaO_2 Formula wt 72.08

INS: 930 CAS: [1305-79-9]

DESCRIPTION

Calcium Peroxide occurs as a white or yellow powder or granular material. It decomposes in moist air, is practically insoluble in water, and dissolves in acids, forming hydrogen peroxide. A 1:100 aqueous slurry has a pH of about 12.

Function Dough conditioner; oxidizing agent.

REQUIREMENTS

Identification Cautiously dissolve 250 mg of sample in 5 mL of glacial acetic acid, and add a few drops of a saturated solution of potassium iodide. Iodine is liberated. Add 20 mL of water and sufficient sodium thiosulfate TS to remove the iodine color. The resulting solution gives positive tests for *Calcium*, Appendix IIIA.
Assay Not less than 60.0% of CaO_2.
Fluoride Not more than 0.005%.
Lead Not more than 4 mg/kg.

TESTS

Assay Transfer about 1 g of sample, accurately weighed, into an Erlenmeyer flask, add 30 mL of water and 30 mL of 85% phosphoric acid:water (1:1 v/v), and titrate immediately with 0.5 *N* potassium permanganate to the first faint pink color that persists for 1 min. Each milliliter of 0.5 *N* potassium permanganate is equivalent to 18.02 mg of CaO_2.

Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB, using 1.0 g of sample, accurately weighed.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 10 mL of the following solution and 4 µg of lead (Pb) ion in the control: Transfer 4.0 g of sample, accurately weighed, into a 250-mL beaker, cautiously add 50 mL of nitric acid, and evaporate just to dryness on a steam bath. Add 20 mL of nitric acid, repeat the evaporation, cool, and dissolve the residue in sufficient water containing 4 drops of nitric acid to make 40.0 mL.

Packaging and Storage Store in tight containers, and avoid contact with readily oxidizable materials. Observe the safety precautions printed on the label of the original container.

Calcium Phosphate, Dibasic

Dicalcium Phosphate

$CaHPO_4$ Formula wt, anhydrous 136.06
$CaHPO_4 \cdot 2H_2O$ Formula wt, dihydrate 172.09

INS: 341(ii) CAS: anhydrous [7757-93-9]
CAS: dihydrate [7789-77-7]

DESCRIPTION

Calcium Phosphate, Dibasic, occurs as a white powder. It is anhydrous or contains two molecules of water of hydration. It is stable in air. It is insoluble in alcohol, is practically insoluble in water, but is readily soluble in dilute hydrochloric and nitric acids.

Function Leavening agent; dough conditioner; nutrient; yeast food.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or the dihydrate.
Identification

A. Dissolve about 100 mg of sample by warming it with a mixture of 5 mL of 2.7 *N* hydrochloric acid and 5 mL of water. Add 2.5 mL of 6 *N* ammonium hydroxide, dropwise, with shaking, and then add 5 mL of ammonium oxalate TS. A white precipitate forms.

B. Add 10 mL of ammonium molybdate TS to 10 mL of a warm 1:100 aqueous solution in a slight excess of nitric acid. A yellow precipitate of ammonium phosphomolybdate forms.

Assay *Anhydrous or Dihydrate*: Not less than 97.0% and not more than 105.0%.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Lead Not more than 2 mg/kg.
Loss on Ignition *Anhydrous*: Between 7.0% and 8.5%; *Dihydrate*: Between 24.5% and 26.5%.

TESTS

Assay Dissolve, with the aid of gentle heat if necessary, about 250 mg of sample, accurately weighed, in a mixture of 5 mL of hydrochloric acid and 3 mL of water contained in a 250-mL beaker equipped with a magnetic stirrer, and cautiously add 125 mL of water. With constant stirring, add, in the order named, 0.5 mL of triethanolamine, 300 mg of hydroxy naphthol blue indicator, and from a 50-mL buret, about 23 mL of 0.05 *M* disodium ethylenediaminetetraacetate. Add a 45:100 sodium hydroxide solution until the initial red color changes to clear blue, continue to add it dropwise until the color changes to violet, then add an additional 0.5 mL. The pH is between 12.3 and 12.5. Continue the titration, dropwise, with the 0.05 *M* disodium ethylenediaminetetraacetate to the appearance of a clear blue endpoint that persists for not less than 60 s. Each milliliter of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 6.803 mg of $CaHPO_4$ or to 8.604 mg of $CaHPO_4 \cdot 2H_2O$.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 5 mL of 2.7 *N* hydrochloric acid.

Fluoride (**Note**: Prepare and store all solutions in plastic containers.)

Buffer Solution Dissolve 73.5 g of sodium citrate in water to make 250 mL of solution.

Standard Solution Dissolve an accurately weighed quantity of USP Sodium Fluoride RS quantitatively in water to obtain a solution containing 1.1052 mg/mL. Transfer 20.0 mL of the resulting solution into a 100-mL volumetric flask containing 50 mL of *Buffer Solution*, dilute to volume with water, and mix. Each milliliter of this solution contains 100 μg of fluoride ion.

Sample Solution Transfer 2.0 g of sample to a beaker containing a plastic-coated stirring bar, add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer Solution* and sufficient water to make 100 mL.

Electrode System Use a fluoride-specific, ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV.

Standard Response Line Transfer 50.0 mL of *Buffer Solution* and 2.0 mL of hydrochloric acid into a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in millivolts. Continue stirring, and at 5-min intervals, add 100 μL, 100 μL, 300 μL, and 500 μL of *Standard Solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 μg/mL) versus potential, in millivolts.

Procedure Rinse and dry the electrodes, insert them into the *Sample Solution*, stir for 5 min, and read the potential, in millivolts. From the measured potential and the *Standard Response Line*, determine the concentration, *C*, in micrograms per milliliter, of fluoride ion in the *Sample Solution*. Calculate the percentage of fluoride in the sample taken by the formula

$$C \times 0.005.$$

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Ignition Ignite about 3 g of sample, accurately weighed, preferably in a muffle furnace, at 800° to 825° to constant weight.

Packaging and Storage Store in tightly closed containers.

Calcium Phosphate, Monobasic

Monocalcium Phosphate; Calcium Biphosphate; Acid Calcium Phosphate

$Ca(H_2PO_4)_2$ Formula wt, anhydrous 234.05
$Ca(H_2PO_4)_2 \cdot H_2O$ Formula wt, monohydrate 252.07

CAS: anhydrous [7758-23-8]
INS: 341(i) CAS: monohydrate [10031-30-8]

DESCRIPTION

Calcium Phosphate, Monobasic, occurs as white crystals or granules or as a granular powder. It is anhydrous or contains one molecule of water of hydration, but because of its deliquescent nature, more than the calculated amount of water may be present. It is sparingly soluble in water and is insoluble in alcohol.

Function Buffer; dough conditioner; firming agent; leavening agent; nutrient; yeast food; sequestrant.

REQUIREMENTS

Labeling Indicate the state of hydration.

Identification

A. Dissolve 100 mg of sample by warming it in a mixture of 2 mL of 2.7 *N* hydrochloric acid and 8 mL of water. Add 5 mL of ammonium oxalate TS. A white precipitate forms.

B. Add ammonium molybdate TS to a warm solution of sample in a slight excess of nitric acid. A yellow precipitate of ammonium phosphomolybdate forms.

Assay *Anhydrous*: Not less than 16.8% and not more than 18.3% of Ca; *Monohydrate*: Not less than 15.9% and not more than 17.7% of Ca.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Lead Not more than 2 mg/kg.

Loss on Drying *Monohydrate*: Not more than 1%.

Loss on Ignition *Anhydrous*: Between 14.0% and 15.5%.

TESTS

Assay Accurately weigh a portion of sample equivalent to about 475 mg of Calcium Phosphate, Monobasic, Anhydrous $[Ca(H_2PO_4)_2]$, dissolve it in 10 mL of 2.7 *N* hydrochloric acid, add a few drops of methyl orange TS, and boil for 5 min, keeping the volume and pH of the solution constant during the boiling period by adding hydrochloric acid or water

as necessary. Add 2 drops of methyl red TS and 30 mL of ammonium oxalate TS, then while constantly stirring, add, dropwise, a mixture of equal volumes of 6 *N* ammonium hydroxide and water until the pink color of the indicator just disappears. Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through a sintered-glass crucible, using gentle suction. Wash the precipitate in the beaker with about 30 mL of cold (below 20°) wash solution, prepared by diluting 10 mL of ammonium oxalate TS to 1000 mL with water. Allow the precipitate to settle, and pour the supernatant liquid through the filter. Repeat this washing by decantation three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter with two 10-mL portions of cold (below 20°) water. Place the sintered-glass crucible in the beaker, and add 100 mL of water and 50 mL of cold 1:6 sulfuric acid. Add 35 mL of 0.1 *N* potassium permanganate from a buret, and stir until the color disappears. Heat to about 70°, and complete the titration with 0.1 *N* potassium permanganate. Each milliliter of 0.1 *N* potassium permanganate is equivalent to 2.004 mg of Ca.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 5 mL of 2.7 *N* hydrochloric acid.

Fluoride *Anhydrous*: Determine as directed in *Method II* under the *Fluoride Limit Test*, Appendix IIIB. *Monohydrate*: Proceed as directed under *Fluoride* in the monograph for *Calcium Phosphate, Dibasic*.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying *Monohydrate*: Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 60° for 3 h.

Loss on Ignition *Anhydrous*: Ignite, preferably in a muffle furnace, about 3 g of sample, accurately weighed, at 800° for 30 min.

Packaging and Storage Store in well-closed containers.

Calcium Phosphate, Tribasic

Tricalcium Phosphate; Precipitated Calcium Phosphate; Calcium Hydroxyapatite

$Ca_3(PO_4)_2$ Formula wt 310.18
$Ca_5OH(PO_4)_3$ Formula wt 502.31
$Ca_{10}(OH)_2(PO_4)_6$ Formula wt 1004.61

INS: 341(iii) CAS: [7758-87-4]
CAS: [1306-06-5]
CAS: [62974-97-4]

DESCRIPTION

Calcium Phosphate, Tribasic, occurs as a white powder that is stable in air. It consists of a variable mixture of calcium phosphates. It is insoluble in alcohol and almost insoluble in water, but it dissolves readily in dilute hydrochloric and nitric acids.

Function Anticaking agent; buffer; nutrient; clouding agent.

REQUIREMENTS

Identification

A. Add ammonium molybdate TS to a warm solution of sample in a slight excess of nitric acid. A yellow precipitate forms.

B. Dissolve about 100 mg of sample by warming it with 5 mL of 2.7 *N* hydrochloric acid and 5 mL of water; while shaking, add 1 mL of 6 *N* ammonium hydroxide, dropwise; and then add 5 mL of ammonium oxalate TS. A white precipitate forms.

Assay Not less than 34.0% and not more than 40.0% of calcium (Ca).

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.0075%.

Lead Not more than 2 mg/kg.

Loss on Ignition Not more than 10.0%.

TESTS

Assay Determine as directed in the monograph for *Calcium Phosphate, Dibasic*, using a 150-mg sample, accurately weighed. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of Ca.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 5 mL of 2.7 *N* hydrochloric acid.

Fluoride (**Note**: Prepare and store all solutions in plastic containers.)

Buffer Solution, *Standard Solution*, and *Electrode System* Prepare as directed under *Fluoride* in the monograph for *Calcium Phosphate, Dibasic*.

Standard Response Line Prepare as directed under *Fluoride* in the monograph for *Calcium Phosphate, Dibasic*, except use 3.0 mL of hydrochloric acid instead of 2.0 mL.

Procedure Determine as directed under *Fluoride* in the monograph for *Calcium Phosphate, Dibasic*, except use 3.0 mL of hydrochloric acid instead of 2.0 mL.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Ignition Ignite, preferably in a muffle furnace, about 3 g of sample, accurately weighed, at 800° to 825° to constant weight.

Packaging and Storage Store in well-closed containers.

Calcium Propionate

$(CH_3CH_2COO)_2Ca$

$C_6H_{10}CaO_4$ Formula wt 186.22

INS: 282 CAS: [4075-81-4]

DESCRIPTION

Calcium Propionate occurs as white crystals or as a crystalline solid. The pH of a 1:10 aqueous solution is between 7.5 and 10.5. One gram dissolves in about 3 mL of water.

Function Preservative; mold inhibitor.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Calcium*, Appendix IIIA.

B. Upon ignition at a relatively low temperature, a sample yields an alkaline residue that effervesces with acids.

Assay Not less than 98.0% and not more than 100.5% of $C_6H_{10}CaO_4$, calculated on the anhydrous basis.

Fluoride Not more than 0.003%.

Insoluble Substances Not more than 0.2%.

Lead Not more than 2 mg/kg.

Magnesium (as MgO) Passes test (about 0.4%).

Water Not more than 5.0%.

TESTS

Assay Dissolve about 400 mg of sample, accurately weighed, in 100 mL of water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 9.311 mg of $C_6H_{10}CaO_4$.

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, Appendix IIIB, using a 1.0-g sample.

Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, filter through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° to constant weight.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Magnesium (as MgO) Place 400.0 mg of sample, 5 mL of 2.7 *N* hydrochloric acid, and about 10 mL of water in a small beaker, and dissolve the sample by heating on a hot plate. Evaporate the solution to a volume of about 2 mL, and cool. Transfer the residual liquid into a 100-mL volumetric flask, dilute to volume with water, and mix. Dilute 7.5 mL of this solution to 20 mL with water, add 2 mL of 1 *N* sodium hydroxide and 0.05 mL of a 1:1000 solution of Titan yellow (Clayton yellow), mix, allow to stand for 10 min, and shake. Any color does not exceed that produced by 1.0 mL of *Magnesium Standard Solution* (see *Solutions and Indicators)* in the same volume as that of a control containing 2.5 mL of the sample solution (corresponding to 10 mg of sample) and the quantities of the reagents used in the test.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tightly closed containers.

Calcium Pyrophosphate

$Ca_2P_2O_7$ Formula wt 254.10

INS: 450(vi) CAS: [7790-76-3]

DESCRIPTION

Calcium Pyrophosphate occurs as a fine, white powder. It is insoluble in water, but is soluble in dilute hydrochloric and nitric acids.

Function Buffer; neutralizing agent; nutrient.

REQUIREMENTS

Identification

A. Dissolve about 100 mg of sample by warming it with a mixture of 5 mL of 2.7 *N* hydrochloric acid and 5 mL of water; while shaking, add 2.5 mL of 6 *N* ammonium hydroxide, dropwise, and then add 5 mL of ammonium oxalate TS. A white precipitate forms.

B. Dissolve 100 mg of sample in 100 mL of 1.7 *N* nitric acid. Add 0.5 mL of this solution to 30 mL of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and then add 0.5 mL of the solution to 30 mL of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 96.0% of $Ca_2P_2O_7$.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Lead Not more than 2 mg/kg.

Loss on Ignition Not more than 1%.

TESTS

Assay Dissolve about 300 mg of sample, accurately weighed, in 10 mL of 2.7 *N* hydrochloric acid, add about 120 mL of water and a few drops of methyl orange TS, and boil for 30 min, keeping the volume and pH of the solution constant during the boiling period by adding hydrochloric acid or water, if necessary. Add 2 drops of methyl red TS and 30 mL of ammonium oxalate TS, then, while constantly stirring, add, dropwise, a mixture of equal volumes of 6 *N* ammonium hydroxide and water until the pink color of the indicator just

disappears. Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through a sintered-glass crucible, using gentle suction. Wash the precipitate in the beaker with about 30 mL of cold (below 20°) wash solution, prepared by diluting 10 mL of ammonium oxalate TS to 1000 mL. Allow the precipitate to settle, and pour the supernatant liquid through the filter. Repeat this washing by decantation three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter with two 10-mL portions of cold (below 20°) water. Place the sintered-glass crucible in the beaker, and add 100 mL of water and 50 mL of cold 1:6 sulfuric acid. Add 35 mL of 0.1 *N* potassium permanganate from a buret, and stir until the color disappears. Heat to about 70°, and complete the titration with 0.1 *N* potassium permanganate. Each milliliter of 0.1 *N* potassium permanganate is equivalent to 6.35 mg of $Ca_2P_2O_7$.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 5 mL of 2.7 *N* hydrochloric acid.

Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB, using 1.0 g of sample, accurately weighed.

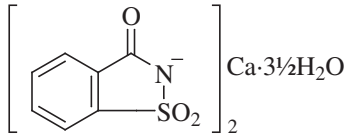
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Ignition Ignite about 1 g of sample, accurately weighed, preferably in a muffle furnace at 800° to 825° for 30 min.

Packaging and Storage Store in well-closed containers.

Calcium Saccharin

1,2-Benzisothiazolin-3-one 1,1-Dioxide Calcium Salt



$C_{14}H_8CaN_2O_6S_2 \cdot 3\frac{1}{2}H_2O$ Formula wt 467.48

INS: 954 CAS: anhydrous [6485-34-3]

DESCRIPTION

Calcium Saccharin occurs as white crystals or as a white, crystalline powder. One gram is soluble in 1.5 mL of water.

Function Nonnutritive sweetener.

REQUIREMENTS

Identification

A. Dissolve about 100 mg of sample in 5 mL of a 1:20 solution of sodium hydroxide, evaporate to dryness, and gently fuse the residue over a small flame until ammonia no longer evolves. After the residue has cooled, dissolve it in 20 mL of water, neutralize the solution with 2.7 *N* hydrochloric acid, and filter. Add 1 drop of ferric chloride TS to the filtrate. A violet color appears.

B. Mix 20 mg of sample with 40 mg of resorcinol, cautiously add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 mL of water and an excess of 1 *N* sodium hydroxide. A fluorescent green liquid results.

C. A 1:10 aqueous solution gives positive tests for *Calcium*, Appendix IIIA.

D. Add 1 mL of hydrochloric acid to 10 mL of a 1:10 aqueous solution. A crystalline precipitate of saccharin forms. Wash the precipitate well with cold water, and dry at 105° for 2 h. The saccharin thus obtained melts between 226° and 230° (see *Melting Range or Temperature*, Appendix IIB).

Assay Not less than 98.0% and not more than 101.0% of $C_{14}H_8CaN_2O_6S_2$, calculated on the anhydrous basis.

Benzoate and Salicylate Passes test.

Lead Not more than 2 mg/kg.

Readily Carbonizable Substances Passes test.

Selenium Not more than 0.003%.

Toluenesulfonamides Not more than 0.0025%.

Water Not more than 15.0%.

TESTS

Assay With the aid of 10 mL of water, quantitatively transfer about 500 mg of sample, accurately weighed, into a separator. Add 2 mL of 2.7 *N* hydrochloric acid, and extract the precipitated saccharin, first with 30 mL, then with five 20-mL portions, of a solvent comprising 9:1 (v/v) chloroform:alcohol. Filter each extract through a small filter paper moistened with the solvent mixture, and evaporate the combined filtrates to dryness on a steam bath with the aid of a current of air. Dissolve the residue in 75 mL of hot water, cool, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 20.22 mg of $C_{14}H_8CaN_2O_6S_2$.

Benzoate and Salicylate Add 3 drops of ferric chloride TS to 10 mL of a 1:20 aqueous solution previously acidified with 5 drops of glacial acetic acid. No precipitate or violet color appears.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Readily Carbonizable Substances Determine as directed under *Readily Carbonizable Substances*, Appendix II, using 200 mg of sample dissolved in 5 mL of 95% sulfuric acid and kept at 48° to 50° for 10 min. The color is no darker than that of *Matching Fluid A*.

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Toluenesulfonamides

Methylene Chloride Use a suitable grade (such as that obtainable from Burdick & Jackson Laboratories, Inc.), equiv-

alent to the product obtained by distillation in an all-glass apparatus.

Internal Standard Stock Solution Transfer 100.0 mg of 95% *n*-tricosane (obtainable from Chemical Samples Co.) into a 10-mL volumetric flask, dissolve in and dilute to volume with *n*-heptane, and mix.

Stock Standard Preparation Transfer 20.0 mg each of reagent-grade *o*-toluenesulfonamide and *p*-toluenesulfonamide into a 10-mL volumetric flask, dissolve in and dilute to volume with methylene chloride, and mix.

Diluted Standard Preparations Pipet 0.1, 0.25, 1.0, 2.5, and 5.0 mL, respectively, of the *Stock Standard Preparation* into five 10-mL volumetric flasks. Pipet 0.25 mL of the *Internal Standard Stock Solution* into each flask, dilute each to volume with methylene chloride, and mix. These solutions contain, respectively, 20, 50, 200, 500, and 1000 μg/mL of each toluenesulfonamide, plus 250 μg of *n*-tricosane.

Test Preparation (See *Chromatography*, Appendix IIA.) Dissolve 2.00 g of sample in 8.0 mL of 5% sodium bicarbonate solution, and mix the solution thoroughly with 10.0 g of chromatographic siliceous earth (Celite 545, Johns-Manville, or equivalent). Transfer the mixture into a 250- × 25-mm chromatographic tube, or equivalent, having a fritted-glass disk and a Teflon stopcock at the bottom and a reservoir at the top. Pack the contents of the tube by tapping the column on a padded surface, and then by tamping firmly from the top. Place 100 mL of methylene chloride in the reservoir, and adjust the stopcock so that 50 mL of eluate is collected in 20 to 30 min. Add 25 μL of *Internal Standard Stock Solution* to the eluate, mix, and then concentrate the solution to a volume of 1.0 mL in a suitable concentrator tube fitted with a modified Snyder column, using a Kontes tube heater maintained at 90°.

Procedure Inject 2.5 μL of the *Test Preparation* into a suitable gas chromatograph equipped with a flame-ionization detector and a 3-m × 2-mm (id) glass column, or equivalent, packed with 3% phenyl methyl silicone (OV-17, Applied Science Laboratories, Inc., or equivalent) on 100- to 120-mesh, silanized, calcined, diatomaceous silica (Gas-Chrom Q, Applied Science, or equivalent).

> **Caution**: The glass column should extend into the injector for on-column injection and into the detector base to avoid contact with metal.

Maintain the column at 180°. Set the injection port temperature to 225° and the detector to 250°. Use helium as the carrier gas with a flow rate of 30 mL/min. Set the instrument attenuation so that 2.5 μL of the *Diluted Standard Preparation* containing 200 μg/mL of each toluenesulfonamide gives a response of 40% to 80% of full-scale deflection. Record the chromatogram, note the peaks for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and the *n*-tricosane internal standard, and calculate the areas for each peak by suitable means. The retention times for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane are about 5, 6, and 15 min, respectively.

In a similar manner, obtain the chromatograms for 2.5-μL portions of each of the five *Diluted Standard Preparations*, and for each solution, determine the areas of the *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane peaks. From the values thus obtained, prepare standard curves by plotting the concentration of each toluenesulfonamide, in micrograms per milliliter, versus the ratio of the respective toluenesulfonamide peak area to that of *n*-tricosane. From the standard curve, determine the concentration, in micrograms per milliliter, of each toluenesulfonamide in the *Test Preparation*. Divide each value by 2 to convert the result to milligrams per kilogram of the toluenesulfonamide in the 2-g sample taken for analysis.

> **Note**: If the toluenesulfonamide content of the sample is greater than about 500 mg/kg, the impurity may crystallize out of the methylene chloride concentrate (see *Test Preparation*). Although this level of impurity exceeds that permitted by the specification, the analysis may be completed by diluting the concentrate with methylene chloride containing 250 μg of *n*-tricosane per milliliter, and by applying appropriate dilution factors in the calculation. Care must be taken to redissolve completely any crystalline toluenesulfonamide to give a homogeneous solution.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Calcium Silicate

INS: 552 CAS: [1344-95-2]

DESCRIPTION

Calcium Silicate occurs as a white to off white, free-flowing powder that remains so after absorbing relatively large amounts of water or other liquids. It is a hydrous or anhydrous silicate with varying proportions of CaO and SiO_2. It is insoluble in water, but it forms a gel with mineral acids. The pH of a 1:20 aqueous slurry is between 8.4 and 12.5.

Function Anticaking agent; filter aid.

REQUIREMENTS

Identification

A. Mix about 500 mg of sample with 10 mL of 2.7 *N* hydrochloric acid, filter, and neutralize the filtrate to litmus paper with 6 *N* ammonium hydroxide. The neutralized filtrate gives positive tests for *Calcium*, Appendix IIIA.

B. Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with a sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a weblike structure.

Fluoride Not more than 10 mg/kg.

Lead Not more than 5 mg/kg.

The following additional *Requirements* should conform to the representations of the vendor: *Calcium Oxide*, *Loss on Drying*, *Loss on Ignition*, and *Silicon Dioxide*.

TESTS

Assay for Silicon Dioxide Transfer about 400 mg of sample, accurately weighed, into a beaker, add 5 mL of water and 10 mL of perchloric acid, and heat until dense, white fumes of perchloric acid evolve.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Cover the beaker with a watch glass, and continue to heat for 15 min longer. Allow to cool, add 30 mL of water, filter, and wash the precipitate with 200 mL of hot water. Retain the combined filtrate and washings for use in the *Assay for Calcium Oxide* (below). Transfer the filter paper and its contents into a platinum crucible, heat slowly to dryness, and then heat sufficiently to char the filter paper. After cooling, add a few drops of sulfuric acid, and then ignite at about 1300° to constant weight. Moisten the residue with 5 drops of sulfuric acid, add 15 mL of hydrofluoric acid, heat cautiously on a hot plate until all of the acid is driven off, and ignite to constant weight at a temperature not lower than 1000°.

> **Caution**: Handle hydrofluoric acid in an appropriate fume hood.

Cool in a desiccator, and weigh. The loss in weight is equivalent to the amount of SiO_2 in the sample taken.

Assay for Calcium Oxide Using 1 *N* sodium hydroxide, neutralize to litmus the combined filtrate and washings retained in the *Assay for Silicon Dioxide* (above), and add, while stirring, about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret. Add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 2.804 mg of CaO.

Fluoride

0.2 N *EDTA/0.2* N *TRIS Solution* Transfer 18.6 g of disodium ethylenediaminetetraacetate (EDTA) and 6.05 g of tris (hydroxymethyl)aminomethane (TRIS), both accurately weighed, into a 250-mL beaker. Add 200 mL of hot, deionized water, and stir until dissolved. Adjust the pH to 7.5 to 7.6 by adding 5 *N* sodium hydroxide. Cool the solution, and adjust the pH to 8.0 with 5 *N* sodium hydroxide. Transfer the solution into a 250-mL volumetric flask, and dilute to volume with deionized water. Mix well, and store in a plastic container.

Fluoride Stock Solution (1000 mg/kg F) Dissolve 2.210 g of sodium fluoride, accurately weighed, in 50 mL of deionized water. Transfer the solution into a 1-L volumetric flask, and dilute to volume. Store this solution and all fluoride solutions in plastic containers.

100 mg/kg Fluoride Solution Pipet 10 mL of *Fluoride Stock Solution* into a 100-mL volumetric flask, and dilute to volume with deionized water.

On the day of use prepare the following:

10 mg/kg Fluoride Solution Pipet 10 mL of *100 mg/kg Fluoride Solution* into a 100-mL volumetric flask, and dilute to volume with deionized water.

1 mg/kg Fluoride Solution Pipet 1 mL of *100 mg/kg Fluoride Solution* into a 100-mL volumetric flask, and dilute to volume with deionized water.

Sample Solution Transfer 5 g of sample, accurately weighed, into a 150-mL Teflon beaker. Add 40 mL of deionized water and 20 mL of 1 *N* hydrochloric acid. Heat to near boiling for 1 min while stirring continuously. Cool in an ice bath, transfer into a 100-mL volumetric flask, and dilute to volume with deionized water. The sample does not dissolve completely.

Calibration Curve Pipet 20 mL of *10 mg/kg Fluoride Solution* and 20 mL of *1 mg/kg Fluoride Solution* into separate 100-mL plastic beakers. Add 10 mL of *0.2* N *EDTA/0.2* N *TRIS Solution* to each beaker. Measure the potential, in millivolts, of each solution with a suitable fluoride-selective, ion-indicating electrode and a calomel reference electrode connected to a pH meter capable of measuring potentials with a reproducibility of ± 0.2 mV (Orion model 96-09 combination fluoride electrode, or equivalent). Generate a standard curve by plotting the logarithms of the fluoride ion concentrations, in milligrams per kilogram, of the *Fluoride Solutions* versus the potential, in millivolts, or calibrate an Orion Expandable Ion Analyzer EA-940 (or an equivalent instrument) for a direct concentration reading.

Procedure Pipet a 20-mL aliquot of *Sample Solution* into a 100-mL plastic beaker, add 10 mL of *0.2* N *EDTA/0.2* N *TRIS Solution*, and measure the solution potential as described under *Calibration Curve* (above). From the measured potential of the *Sample Solution*, calculate the concentration, in milligrams per kilogram, of fluoride ion from the *Calibration Curve*.

Lead

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade Lead Nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL with water, and mix. Each milliliter of this solution contains 100 µg of lead (Pb) ion. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute stepwise and quantitatively an accurately measured volume of *Lead Nitrate Stock Solution* with water to obtain the *Standard Lead Solution*, which contains 0.25 µg/mL of lead (Pb) ion.

Sample Solution Transfer 5.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 4, or equivalent, filter paper into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter paper into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Procedure Set a suitable atomic absorption spectrophotometer to a wavelength of 217 nm. Adjust the instrument to zero absorbance against water. Read the absorbance of the *Standard Lead Solution*.

Aspirate the *Sample Solution* into the spectrophotometer, and measure the absorbance in the same manner. The absorbance obtained from the *Sample Solution* is not greater than that obtained from the *Standard Lead Solution*.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Loss on Ignition Transfer about 1 g of sample, previously dried at 105° for 2 h and accurately weighed, into a suitable tared crucible, and ignite at 900° to constant weight.

Packaging and Storage Store in well-closed containers.

Calcium Sorbate

2,4-Hexadienoic Acid, Calcium Salt

$$\left[CH_3CH{=}CHCH{=}CHCOO\right]_2Ca$$

$C_{12}H_{14}CaO_4$ Formula wt 262.32

INS: 203 CAS: [7492-55-9]

DESCRIPTION

Calcium Sorbate occurs as a fine, white crystalline powder. It decomposes at about 400°. It is sparingly soluble in water and practically insoluble in organic solvents, in fats, and in oils.

Function Antimicrobial agent; preservative.

REQUIREMENTS

Identification

A. Ignite 1 g of sample at 800°. Cool, and slake with 10 mL of water. Add glacial acetic acid until the sample is dissolved, and filter if necessary. The resulting solution gives positive tests for *Calcium*, Appendix IIIA.

B. Place 200 mg of sample in 5 mL of methanol. Add 0.1 mL of 1 *N* sodium hydroxide, and dissolve in 95 mL of water. After the addition of a few drops of bromine TS, the color disappears.

Assay Not less than 98.0% and not more than 101.0% of $C_{12}H_{14}CaO_4$, calculated on the dried basis.

Acidity (as sorbic acid) Passes test (approximately 1%).

Alkalinity [as $Ca(OH)_2$] Passes test (approximately 0.5%).

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 1.0%.

TESTS

Assay Dissolve about 150 mg of sample, accurately weighed, in 50 mL of glacial acetic acid in a 250-mL glass-stoppered Erlenmeyer flask, warming if necessary to effect solution. Cool to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to a blue-green endpoint that persists for at least 30 s.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Two milliliters of 0.1 *N* perchloric acid is equivalent to 26.23 mg of $C_{12}H_{14}CaO_4$.

Acidity or Alkalinity Add some drops of methanol, 30 mL of water, and several drops of phenolphthalein TS to 1 g of sample. If the mixture is colorless, titrate with 0.1 *N* sodium hydroxide to a pink color that persists for 15 s. Not more than 1.0 mL is required. If the mixture is pink, titrate with 0.1 *N* hydrochloric acid. Not more than 1.35 mL is required to discharge the pink color.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.

Packaging and Storage Store in tight containers.

Calcium Stearate

CAS: [1592-23-0]

DESCRIPTION

Calcium Stearate occurs as a fine, white to yellow-white, bulky powder. It is a compound of calcium with a mixture of solid organic acids obtained from edible sources and consists chiefly of variable proportions of calcium stearate and calcium palmitate. It is unctuous and free from grittiness. It is insoluble in water, in alcohol, and in ether.

Function Anticaking agent; binder; emulsifier.

REQUIREMENTS

Identification

A. Heat 1 g of sample with a mixture of 25 mL of water and 5 mL of hydrochloric acid. Fatty acids are liberated, floating as an oily layer on the surface of the liquid. The water layer gives positive tests for *Calcium*, Appendix IIIA.

B. Mix 25 g of sample with 200 mL of hot water, then add 60 mL of 2 *N* sulfuric acid, and heat the mixture, while stirring frequently, until the fatty acids separate cleanly as a transparent layer. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm them on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the

water layer, then melt the acids, filter into a dry beaker, and dry at 105° for 20 min. The solidification point of the fatty acids so obtained is not below 54° (see *Solidification Point*, Appendix IIB).
Assay Not less than 9.0% and not more than 10.5% of CaO, calculated on the dried basis.
Free Fatty Acids (as stearic acid) Not more than 3.0%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 4.0%.

TESTS

Assay Boil about 1.2 g of sample, accurately weighed, with 50 mL of 0.1 *N* hydrochloric acid for 10 min, or until the fatty acid layer is clear, adding water if necessary to maintain the original volume. Cool, filter, and wash the filter and flask thoroughly with water until the last washing is not acid to litmus. Neutralize the filtrate to litmus with 1 *N* sodium hydroxide. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 2.804 mg of CaO.
Free Fatty Acids (as stearic acid) Transfer 2 g of sample, accurately weighed, into a dry, 125-mL Erlenmeyer flask containing 50 mL of acetone, fit an air-cooled reflux condenser onto the neck of the flask, boil the mixture on a steam bath for 10 min, and cool. Filter through two layers of Whatman No. 42, or equivalent, filter paper, and wash the flask, residue, and filter with 50 mL of acetone. Add phenolphthalein TS and 5 mL of water to the filtrate, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*) using 100 mL of acetone and 5 mL of water, and make any necessary correction. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 28.45 mg of stearic acid ($C_{18}H_{36}O_2$).
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° to constant weight, using 2-h increments of heating.

Packaging and Storage Store in well-closed containers.

Calcium Stearoyl Lactylate

Calcium Stearoyl-2-Lactylate; Calcium Stearoyl Lactate

INS: 482(i) CAS: [5793-94-2]

DESCRIPTION

Calcium Stearoyl Lactylate occurs as a cream-colored powder. It is a mixture of calcium salts of stearoyl lactic acid, with minor proportions of other salts of related acids. It is slightly soluble in hot water.

Function Dough conditioner; stabilizer; whipping agent.

REQUIREMENTS

Identification

A. Heat 1 g of sample with a mixture of 25 mL of water and 5 mL of hydrochloric acid. Fatty acids are liberated, floating as an oily layer on the surface of the liquid. The water layer gives positive tests for *Calcium*, Appendix IIIA.

B. Mix 25 g of sample with 200 mL of hot water, then add 60 mL of 2 *N* sulfuric acid, and heat the mixture, while stirring frequently, until the fatty acids separate cleanly as a transparent layer. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm them on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, then melt the acids, filter into a dry beaker, and dry at 105° for 20 min. The solidification point of the fatty acids so obtained is not below 54° (see *Solidification Point*, Appendix IIB).
Acid Value Between 50 and 86.
Calcium Content Between 4.2% and 5.2%.
Ester Value Between 125 and 164.
Lead Not more than 2 mg/kg.
Total Lactic Acid Between 32.0% and 38.0%.

TESTS

Acid Value Transfer about 1 g of sample, accurately weighed, to a 125-mL volumetric flask, add 25 mL of alcohol, previously neutralized in phenolphthalein TS, and heat on a hot plate until the sample is dissolved. Cool, add 5 drops of phenolphthalein TS, and titrate rapidly with 0.1 *N* sodium hydroxide to the appearance of the first pink color that persists for at least 30 s. Calculate the acid value by the formula

$$56.1V \times N/W,$$

in which V is the volume, in milliliters, N is the normality of the sodium hydroxide solution, and W is the weight, in grams, of the sample taken. Retain the neutralized solution for the determination of *Ester Value*.
Calcium Content

Stock Lanthanum Solution Transfer 5.86 g of lanthanum oxide (La_2O_3) into a 100-mL volumetric flask, wet with a few milliliters of water, slowly add 25 mL of hydrochloric acid, and swirl until the lanthanum oxide is completely dissolved. Dilute to volume with water, and mix.

Stock Calcium Solution Transfer 124.8 mg of calcium carbonate ($CaCO_3$) previously dried at 200° for 4 h, into a 100-mL volumetric flask, carefully dissolve in 2 mL of 2.7 *N* hydrochloric acid, dilute to volume with water, and mix. This 500-mg/kg calcium solution is commercially available.

Standard Preparations Transfer 10.0 mL of the *Stock Lanthanum Solution* into each of three 50-mL volumetric flasks. Using a microliter syringe, transfer 0.20 mL of the *Stock Calcium Solution* into the first flask, 0.40 mL into the

second flask, and 0.50 mL into the third flask. Dilute each flask to volume with water, and mix. The flasks contain 2.0, 4.0, and 5.0 μg of calcium per milliliter, respectively. Prepare these solutions fresh daily.

Sample Preparation Transfer about 250 mg of sample, accurately weighed, into a 30-mL beaker. While heating, dissolve the sample in 10 mL of alcohol, and quantitatively transfer the solution into a 25-mL volumetric flask. Wash the beaker with two 5-mL portions of alcohol, adding the washings to the flask, dilute to volume with alcohol, and mix. Transfer 5.0 mL of the *Stock Lanthanum Solution* to a second 25-mL volumetric flask. Using a microliter syringe, transfer 0.25 mL of the alcoholic solution of the sample to the second flask, dilute to volume with water, and mix.

Procedure Concomitantly determine the absorbance of each *Standard Preparation* and of the *Sample Preparation* at 422.7 nm, with a suitable atomic absorption spectrophotometer, following the operating parameters as recommended by the manufacturer of the instrument. Plot the absorbance of the *Standard Preparations* versus concentration of calcium, in micrograms per milliliter, and from the curve so obtained determine the concentration, *C*, in micrograms per milliliter, of calcium in the *Sample Preparation*. Calculate the quantity, in milligrams, of calcium in the sample taken by the formula

$$2.5C.$$

Ester Value Prepare an alcoholic potassium hydroxide solution by dissolving 11.2 g of potassium hydroxide in 250 mL of alcohol and diluting with 25 mL of water. Add 10.0 mL of this solution to the neutralized solution retained in the test for *Acid Value* (above). Add 5 drops of phenolphthalein TS, connect a suitable condenser, and reflux for 2 h. Cool, add 5 additional drops of phenolphthalein TS, and titrate the excess alkali with 0.1 *N* sulfuric acid. Perform a blank determination (see *General Provisions*) using 10.0 mL of the alcoholic potassium hydroxide solution, and make any necessary correction. Calculate the ester value by the formula

$$56.1(B - S) \times (N/W),$$

in which $B - S$ represents the difference between the volumes of 0.1 *N* sulfuric acid required for the blank and the sample, respectively, *N* is the normality of the sulfuric acid, and *W* is the weight, in grams, of the sample taken.

Lead Determine as directed in the *Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Total Lactic Acid

Standard Solution Dissolve 1.067 g of lithium lactate ($LiC_3H_5O_3$) in sufficient water to make 1000.0 mL.

Standard Curve Transfer 10.0 mL of the *Standard Solution* into a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer 1.0, 2.0, 4.0, 6.0, and 8.0 mL of the diluted *Standard Solution* into separate 100-mL volumetric flasks, dilute each flask to volume with water, and mix. These standards represent 1, 2, 4, 6, and 8 μg of lactic acid per milliliter, respectively. Transfer 1.0 mL of each solution into separate test tubes, and continue as directed in the *Procedure*, beginning with ''Add 1 drop of cupric sulfate TS. . . .'' After color development and reading the absorbance values, construct a *Standard Curve* by plotting absorbance versus micrograms of lactic acid.

Test Preparation Transfer about 200 mg of sample, accurately weighed, into a 125-mL Erlenmeyer flask, add 10 mL of 0.5 *N* alcoholic potassium hydroxide and 10 mL of water, attach an air condenser, and reflux gently for 45 min. Wash the sides of the flask and the condenser with about 40 mL of water, and heat on a steam bath until no odor of alcohol remains. Add 6 mL of 1:2 sulfuric acid, heat until the fatty acids are melted, then cool to about 60°, and add 25 mL of petroleum ether. Swirl the mixture gently, and transfer quantitatively to a separator. Collect the water layer in a 100-mL volumetric flask, and wash the petroleum ether layer with two 20-mL portions of water, adding the washings to the volumetric flask. Dilute to volume with water, and mix. Transfer 1.0 mL of this solution into a second 100-mL volumetric flask, dilute to volume with water, and mix.

Procedure Transfer 1.0 mL of the *Test Preparation* into a test tube, and transfer 1.0 mL of water to a second test tube to serve as the blank. Treat each tube as follows: Add 1 drop of cupric sulfate TS, swirl gently, and then rapidly add 9.0 mL of sulfuric acid from a buret. Loosely stopper the tube, and heat in a water bath at 90° for exactly 5 min. Cool immediately to below 20° in an ice bath for 5 min, add 3 drops of *p*-phenylphenol TS, shake immediately, and heat in a water bath at 30° for 30 min, shaking the tube twice during this time to disperse the reagent. Heat the tube in a water bath at 90° for exactly 90 s, and then cool immediately to room temperature in an ice water bath. Determine the absorbance of the solution in a 1-cm cell, at 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Obtain the weight, in micrograms, of lactic acid in the portion of the *Test Preparation* taken for the *Procedure* by means of the *Standard Curve*.

Packaging and Storage Store in tight containers in a cool, dry place.

Calcium Sulfate

$CaSO_4$ Formula wt, anhydrous 136.14
$CaSO_4 \cdot 2H_2O$ Formula wt, dihydrate 172.18

INS: 516 CAS: anhydrous [7778-18-9]
CAS: dihydrate [10101-41-4]

DESCRIPTION

Calcium Sulfate occurs as a fine, white to slightly yellow-white powder. It is anhydrous or contains two molecules of water of hydration.

Function Nutrient; yeast food; dough conditioner; firming agent; sequestrant.

REQUIREMENTS

Identification Dissolve about 200 mg of sample by warming it with a mixture of 4 mL of 2.7 *N* hydrochloric acid and 16 mL of water. A white precipitate forms when 5 mL of ammonium oxalate TS is added to 10 mL of the solution. Upon the addition of barium chloride TS to the remaining 10 mL, a white precipitate forms that is insoluble in hydrochloric and nitric acids.
Assay Not less than 98.0% of $CaSO_4$, calculated on the dried basis.
Fluoride Not more than 0.003%.
Lead Not more than 2 mg/kg.
Loss on Drying *Anhydrous*: Not more than 1.5%; *Dihydrate*: Between 19.0% and 23.0%.
Selenium Not more than 0.003%.

TESTS

Assay Dissolve 250 mg of sample, accurately weighed, in 100 mL of water and 4 mL of 2.7 *N* hydrochloric acid, boil to effect solution, and cool. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 25 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 6.807 mg of $CaSO_4$.
Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB, using 1.67 g of sample, accurately weighed.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample to constant weight at 250°.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Packaging and Storage Store in well-closed containers.

Cananga Oil

CAS: [68606-83-7]

View IR

DESCRIPTION

Cananga Oil occurs as a light to deep yellow liquid with a harsh, floral odor suggestive of ylang ylang. It is the oil obtained by distillation from the flowers of the tree *Cananga odorata* Hook f. et Thoms (Fam. Anonaceae). It is soluble in most fixed oils and in mineral oil, but it is practically insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between –15° and –30°.
Refractive Index Between 1.495 and 1.505 at 20°.
Saponification Value Between 10 and 40.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.904 and 0.920.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed under *Saponification Value*, Appendix VI, using about 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 0.5 mL of 95% alcohol, usually becoming cloudy on further dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Candelilla Wax

INS: 902 CAS: [8006-44-8]

View IR

DESCRIPTION

Candelilla Wax occurs as a hard, yellow-brown, opaque to translucent wax. It is a purified wax obtained from the leaves of the candelilla plant, *Euphorbia antisyphilitica* (Fam. Euphorbiaceae). Its specific gravity is about 0.983. It is soluble in chloroform and in toluene, but insoluble in water.

Function Masticatory substance in chewing gum base; surface-finishing agent.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Between 12 and 22.
Lead Not more than 3 mg/kg.
Melting Range Between 68.5° and 72.5°.
Saponification Value Between 43 and 65.

TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV, using 10 μg of lead (Pb) ion in the control.
Melting Range Determine as directed in *Procedure for Class II* under *Melting Range or Temperature*, Appendix IIB.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Packaging and Storage Store in well-closed containers.

Canola Oil

Low Erucic Acid Rapeseed Oil; LEAR Oil

CAS: [120962-03-0]

DESCRIPTION

Canola Oil occurs as a light yellow oil. It is typically obtained by a combination of mechanical expression followed by *n*-hexane extraction, from the seed of the plant *Brassica juncea*, *Brassica napus*, or *Brassica rapa* (Fam. Cruciferae). The plant varieties are those producing oil-bearing seeds with a low erucic acid ($C_{22:1}$) content. It is a mixture of triglycerides composed of both saturated and unsaturated fatty acids. It is refined, bleached, and deodorized to substantially remove free fatty acids; phospholipids; color; odor and flavor components; and miscellaneous, other non-oil materials. It can be hydrogenated to reduce the level of unsaturated fatty acids for functional purposes in foods. It is a liquid at 0° and above.

Function Cooking or salad oil; component of margarine or shortening; coating agent; texturizer.

REQUIREMENTS

Labeling Hydrogenated Canola Oil less than fully hydrogenated must be labeled as Partially Hydrogenated Canola Oil.
Identification Unhydrogenated Canola Oil exhibits the following composition profile of fatty acids, determined as directed under *Fatty Acid Composition*, Appendix VII.

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<0.2	<6.0	<1.0	<2.5	>50	<40.0
Fatty Acid:	18:3	20:0	20:1	22:0	22:1	24:0	24:1
Weight % (Range):	<14	<1.0	<2.0	<0.5	<2.0	<0.2	<0.2

Acid Value Not more than 6.
Cold Test Passes test.
Color (AOCS-Wesson) Not more than 1.5 red/15 yellow.
Erucic Acid Not more than 2.0%.
Free Fatty Acids (as oleic acid) Not more than 0.05%.
Iodine Value Between 110 and 126.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 14.0%.
Peroxide Value Not more than 10 meq/kg.
Refractive Index Between 1.465 and 1.467 at 40°.
Saponifiable Value Between 178 and 193.
Stability Not less than 7 h.
Sulfur Not more than 10 mg/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
Cold Test Determine as directed under *Cold Test*, Appendix VII.
Color (AOCS Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII, using a 133.4-mm cell.
Erucic Acid Determine as part of *Fatty Acid Composition*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Linolenic Acid Determine as directed under *Fatty Acid Composition*, Appendix VII.
Peroxide Value Accurately weigh about 10 g of sample, add 30 mL of a 3:2 mixture of glacial acetic acid:chloroform, and mix. Add 1 mL of a saturated solution of potassium iodide, and mix for 1 min. Add 100 mL of water, begin titrating with 0.05 *N* sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value, as milliequivalents of peroxide per kilogram of sample, by the formula

$$S \times N \times 1000/W,$$

in which *S* is the net volume, in milliliters, of sodium thiosulfate solution required for the sample; *N* is the exact normality of the sodium thiosulfate solution; and *W* is the weight, in grams, of the sample taken.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponifiable Value Determine as directed under *Saponification Value*, Appendix VII.
Stability Determine as directed under *Stability*, Appendix VII.
Sulfur Organosulfur compounds present in the sample react with Raney nickel to produce nickel sulfides. Nickel sulfides

are treated with a strong acid to produce hydrogen sulfide, which is trapped and titrated with mercuric acetate using a dithizone indicator.

Caution: This test requires the use of the following hazardous substances: mercuric acetate, spongy nickel, and dibenzyl disulfide. Conduct the test in a fume hood.

Apparatus Fit a 125-mL, round-bottom boiling flask with a cylindrical filling funnel (20 mL with open top), an ST PTFE metering valve stopcock, and a gas inlet tube (see the figure for Raney Nickel Reduction Apparatus in Appendix IIIC under *Sulfur* (*by Oxidative Microcoulometry*). Fit a water-jacketed distillation column with hooks on top of the boiling flask. Fit a piece of glass tubing with ground ST inner joints with hooks to the distillation column, and connect the distillation column and a gas dispersion tube with ST outer joints with hooks.

Dibenzyl Disulfide Solution Accurately weigh 0.75 g of dibenzyl disulfide, and place in a 250-mL volumetric flask. Dilute to volume with methyl isobutyl ketone, and mix.

Sulfur Standard Accurately weigh five 250.0-g samples of food-grade peanut oil. Transfer 0.0, 1.0, 2.0, 3.0, and 4.0 mL of the *Dibenzyl Disulfide Solution* into the peanut oil samples; the samples contain 0, 3, 6, 9, and 12 mg/kg of sulfur, respectively.

Raney Nickel Preparation (**Caution**: Raney nickel is pyrophoric when dry.) Raney nickel is produced by reacting nickel–aluminum alloy with sodium hydroxide. Each Raney nickel pellet is prepared individually, and each is enough catalyst for one determination. To produce one Raney nickel pellet, accurately weigh 1 g of nickel–aluminum alloy powder (50% Ni, 50% Al), place it in a 50-mL centrifuge tube, and chill it in an ice bath. Slowly add 5 mL of water to the tube, and let it stand for 10 min. Then, slowly add 10 mL of 2.5 *N* sodium hydroxide, and allow the mixture to react for 30 min. Cap the tube, and place it in a 50° water bath for 2 h. Centrifuge the mixture at 1000 rpm for 10 min, and discard the supernatant liquid. Wash the pellet twice with 15 mL of water and twice with 15 mL of isopropanol, centrifuging between each wash. Store the catalyst under isopropanol for no longer than 2 weeks.

Note: Properly dispose of unused *Raney Nickel Preparation* by transferring it to a 250-mL Erlenmeyer flask, and placing it in a fume hood. Add 20 mL of 60% (w/v) hydrochloric acid, and allow complete digestion of the catalyst.

Caution: Hydrogen gas evolves during the digestion process.

Dithizone Indicator Solution Dissolve 10 mg of dithizone (diphenylthiocarbazone) with acetone in a 10-mL volumetric flask, and fill to volume.

Mercuric Acetate Titrant (**Caution**: Mercuric acetate is a strong irritant when ingested or inhaled or upon dermal exposure.) Transfer 3.82 g of mercuric acetate into a 1000-mL volumetric flask containing 950 mL of water. Add 12.2 mL of glacial acetic acid, dilute to volume with water, and mix. Transfer 10.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix. The titrant solution contains 0.0012 *M* mercuric acetate.

Titration Reagent Blank Add 50.0 mL of 1 *N* sodium hydroxide and 50.0 mL of acetone to a 250-mL beaker, and mix. Add 0.5 mL of the *Dithizone Indicator Solution*, and titrate with *Mercuric Acetate Titrant* until the color changes from bright amber to red. Record the volume of titrant used.

Procedure Place 15 to 20 g of sample, accurately weighed, on the bottom of the boiling flask. Discard the isopropanol from the *Raney Nickel Preparation*, add 10 mL of 95% isopropanol, mix, and add the mixture to the sample. Attach the water condenser and the nitrogen line to the boiling flask, and adjust the gas flow to 4 psi through the sample. Place a heating mantle under the flask. Immerse the bubbler in a 250-mL beaker containing 50.0 mL of 1 *N* sodium hydroxide, and stir slowly. Boil the sample for 90 min. Add 50 mL of acetone and 0.5 mL of *Dithizone Indicator Solution* to the 250-mL beaker. Add 20 mL of 60% hydrochloric acid into the filling funnel fitted onto the boiling flask, and adjust the nitrogen flow to 2 to 3 psi. Position the stir bar directly under the bubbler for maximum dispersion of the hydrogen sulfide bubbles. Slowly add the solution of 60% hydrochloric acid to the boiling flask. Begin the titration with *Mercuric Acetate Titrant* until the bright amber color changes to red. Add enough hydrochloric acid to turn the solution in the boiling flask green, and then let it boil for 15 min. Continue the titration throughout the boiling stage, making sure to rinse the inside of the bubbler with the solution in the beaker by turning off the nitrogen flow until the solution rises to the top of the vertical tube (the solution usually returns to amber during the first rinse). Rinse the tube a second time. Continue the titration, and record the volume of titrant used to the nearest 0.01 mL.

Calculation The concentration of sulfur in the sample, in milligrams per kilogram, is calculated by the following formula:

$$(V_S - V_B) \times K/W,$$

in which V_S is the volume, in milliliters, of titrant to the endpoint for the sample; V_B is the volume, in milliliters, of titrant to the endpoint for the blank (usually about 0.10 mL); K is a constant determined from the calibration of the *Sulfur Standard* (expressed as micrograms of sulfur per milliliter of titrant); and W is the weight, in grams, of the sample taken.

The *Sulfur Standards* are analyzed, in duplicate, to determine the constant, K, and are calculated by the following formula:

$$K = W \times C/(V_S - V_B),$$

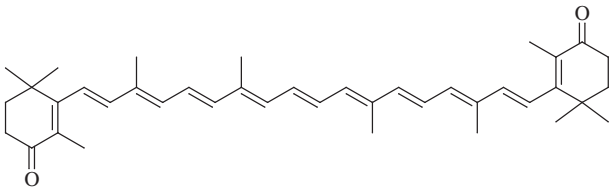
in which W is the weight, in grams, of the *Sulfur Standard*; C is the concentration, in milligrams per kilogram, of the *Sulfur Standard*; V_S is the volume, in milliliters, of titrant for the *Sulfur Standard*; and V_B is the volume, in milliliters, of titrant for the *Titration Reagent Blank*.

Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of a 1:1 chloroform:methanol mixture to dissolve the sample.

Packaging and Storage Store in tightly closed containers, ensuring no contact with metals, filled to the top or flushed with nitrogen gas.

Canthaxanthin

4,4'-Diketo-β-carotene; Cantha; β-Carotene-4,4′-dione



$C_{40}H_{52}O_2$ Formula wt 564.85

INS: 161g CAS: [514-78-3]

DESCRIPTION

Canthaxanthin occurs as a dark, crystalline powder. It is soluble in chloroform, very slightly soluble in acetone, but insoluble in water. It melts at about 207° to 212° with decomposition.

Function Color.

REQUIREMENTS

Identification The absorption spectrum of *Sample Solution B*, prepared as directed in the *Assay* (below), exhibits a maximum near 470 nm.
Assay Not less than 96.0% and not more than 101.0% of $C_{40}H_{52}O_2$.
Arsenic Not more than 3 mg/kg.
Lead Not more than 10 mg/kg.
Mercury Not more than 1 mg/kg.
Residue on Ignition Not more than 0.2%.

TESTS

Assay (**Note**: Carry out all work in low-actinic glassware and in subdued light.)

Sample Solution A Transfer about 50 mg of sample, accurately weighed, into a 100-mL volumetric flask, dissolve in 10 mL of acid-free chloroform, immediately dilute to volume with cyclohexane, and mix. Pipet 5 mL of this solution into a second 100-mL volumetric flask, dilute to volume with cyclohexane, and mix.

Sample Solution B Pipet 5 mL of *Sample Solution A* into a 50-mL volumetric flask, dilute to volume with cyclohexane, and mix.

Procedure Using a suitable spectrophotometer, determine the absorbance of *Sample Solution B* in a 1-cm cell at the wavelength of maximum absorption at about 470 nm using cyclohexane as the blank. Calculate the quantity, in milligrams, of $C_{40}H_{52}O_2$ in the sample taken by the formula

$$20{,}000A/220,$$

in which A is the absorbance of the solution and 220 is the absorptivity of pure Canthaxanthin.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB.
Residue on Ignition Ignite 1 g of sample as directed under *Residue on Ignition*, Appendix IIC, using a silica crucible and moistening the residue with 2 mL of nitric acid and 1 mL of sulfuric acid.

Packaging and Storage Store in tight, light-resistant containers under inert gas.

Caramel

Caramel Color

INS: 150 CAS: [8028-89-5]

DESCRIPTION

Caramel usually occurs as a dark brown to black liquid or solid. It is a complex mixture of compounds, some of which are in the form of colloidal aggregates. Caramel is manufactured by heating carbohydrates, either alone or in the presence of food-grade acids, alkalies, and/or salts. Caramel is produced from commercially available, food-grade nutritive sweeteners consisting of fructose, dextrose (glucose), invert sugar, sucrose, malt syrup, molasses, and/or starch hydrolysates and fractions thereof. The acids that may be used are food-grade sulfuric, sulfurous, phosphoric, acetic, and citric acids; the alkalies are ammonium, sodium, potassium, and calcium hydroxides; and the salts are ammonium, sodium, and potassium carbonate, bicarbonate, phosphate (including mono- and dibasic), sulfate, and bisulfite. Food-grade antifoaming agents such as polyglycerol esters of fatty acids may be used as processing aids during its manufacture. Caramel is soluble in water.

Four distinct classes of Caramel can be distinguished by the reactants used in their manufacture and by specific identification tests:

Class I (Plain Caramel, Caustic Caramel) Prepared by heating carbohydrates with or without acids or alkalis; no ammonium or sulfite compounds are used.

Class II (Caustic Sulfite Caramel) Prepared by heating carbohydrates with or without acids or alkalis in the presence of sulfite compounds; no ammonium compounds are used.

Class III (Ammonia Caramel) Prepared by heating carbohydrates with or without acids or alkalis in the presence of ammonium compounds; no sulfite compounds are used.

Class IV (Sulfite Ammonia Caramel) Prepared by heating carbohydrates with or without acids or alkalis in the presence of both sulfite and ammonium compounds.

All of these Caramels shall meet the criteria established for Caramel in this monograph.

Function Color.

REQUIREMENTS

Ammoniacal Nitrogen[1] Not more than 0.6%, calculated on an equivalent color basis.
Arsenic[2] Not more than 1 mg/kg.
Color Intensity[3] Between 0.01 and 0.6 absorbance units (a.u.).
Lead[2] Not more than 2 mg/kg.
Mercury[2] Not more than 0.1 mg/kg.
4-Methylimidazole[1] Not more than 0.025%, calculated on an equivalent color basis.
Sulfur Dioxide[1] Not more than 0.2%, calculated on an equivalent color basis.
Total Nitrogen[1] Not more than 3.3%, calculated on an equivalent color basis.
Total Sulfur[1] Not more than 3.5%, calculated on an equivalent color basis.

TESTS

Ammoniacal Nitrogen Transfer 25.0 mL of 0.1 *N* sulfuric acid into a 500-mL receiving flask, and connect it to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser so that the condenser delivery tube is immersed beneath the surface of the acid solution in the receiving flask. Transfer about 2 g of sample, accurately weighed, into an 800-mL long-neck Kjeldahl digestion flask, and add 2 g of carbonate-free magnesium oxide, 200 mL of water, and several boiling chips to the flask. Swirl the digestion flask to mix the contents, and quickly connect it to the distillation apparatus. Heat the digestion flask to boiling, and collect about 100 mL of distillate in the receiving flask. Wash the tip of the delivery tube with a few milliliters of water, collecting the washings in the receiving flask; add 4 or 5 drops of methyl red TS; titrate with 0.1 *N* sodium hydroxide; and record the volume, in milliliters, as *S*. Conduct a blank determination (see *General Provisions*), and record the milliliters of 0.1 *N* sodium hydroxide required as *B*. Calculate the percent ammoniacal nitrogen (equivalent color basis) by the formula

$$[(B - S) \times 0.0014 \times 100/W] \times 0.1/A_{610},$$

in which 0.0014 is the milliequivalent weight of nitrogen for 0.1 *N* sodium hydroxide**;** *W* is the weight, in grams, of the sample taken; 0.1 is the basis of color equivalency; and A_{610} is the color intensity as-is.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 1.0 mL of the *Standard Arsenic Solution* (1 g As) in the control.

Color Intensity (**Note**: Because *Color Intensity* is expressed on a solids basis, determine *Total Solids* first.)

Total Solids

Liquid Samples Use fine quartz sand that passes a No. 40, but not a No. 60, sieve and that has been prepared by digestion with hydrochloric acid, washed acid free, dried, and ignited. Mix 30.0 g of the prepared sand, accurately weighed, with 1.5 to 2.0 g of sample, accurately weighed, and dry to constant weight at 60° under reduced pressure (50 mm Hg). Record the final weight of the sand plus the sample solids. Calculate the percent total solids as follows:

$$[(W_F - W_S)/W_C] \times 100,$$

in which W_F is the final weight, in grams, of the sand plus the sample solids; W_S is the weight, in grams, of the prepared sand taken; and W_C is the weight, in grams, of the sample taken.

Solid Samples, Powdered or Granular Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 60° under reduced pressure (50 mm Hg) to constant weight. Calculate the percent total solids by the formula

$$[(W_D - W_B)/(W_S - W_B)] \times 100,$$

in which W_D is the weight, in grams, of the bottle and sample after drying; W_B is the weight, in grams, of the empty bottle; and W_S is the weight, in grams, of the bottle and sample before drying.

Color Intensity

Color Intensity Transfer 100 mg of sample into a 100-mL volumetric flask, dilute to volume with water, and mix. Centrifuge if the solution is cloudy. Determine the absorbance (A_{610}) of the clear solution in a 1-cm cell at 610 nm with a suitable spectrophotometer previously standardized using water as the reference. Calculate the color intensity by the formula

$$(A_{610} \times 100)/S,$$

in which *S* is the percent total solids.

Lead (**Note**: For this test, use reagent-grade chemicals with as low a lead content as is practicable as well as high-purity water and gases. Before use in this analysis, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse them thoroughly with

[1]These tests are calculated on an equivalent color basis that permits the values to be expressed in terms of a Caramel having a color intensity standardized to 0.1 a.u.

[2]These tests are calculated on an as-is basis.

[3]*Color Intensity* is defined as the absorbance of a 0.1% (w/v) solution of Caramel in water measured in a 1-cm cell at 610 nm and is expressed on a *Total Solids* basis.

high-purity water, preferably obtained from a mixed-bed, strong-acid, strong-base ion-exchange cartridge capable of producing water with an electrical resistivity of 12 to 15 megohms.)

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL with water, and mix. Prepare and store this solution in glass containers that are free from lead salts. Each milliliter of this solution contains 100 μg of lead (Pb) ion.

Standard Lead Solution On the day of use, transfer 50.0 mL of *Lead Nitrate Stock Solution* into a 500-mL volumetric flask containing 50 mL of water, add 5 mL of nitric acid, dilute to volume with water, and mix. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 μg of lead (Pb) ion.

Standard Solutions Prepare a series of lead standard solutions serially diluted from the *Standard Lead Solution*. Pipet 2, 5, 10, and 20 mL, respectively, of *Standard Lead Solution* into separate 100-mL volumetric flasks, add 1 mL of nitric acid, dilute to volume, and mix. The *Standard Solutions* contain, respectively, 0.20, 0.50, 1.00 and 2.00 μg of lead per milliliter.

Sample Solution Transfer about 25 g of sample, accurately weighed, into an ashing vessel. [Suitable ashing vessels have approximately a 100-mL capacity and are flat-bottom platinum crucibles or dishes, Vycor or quartz tall-form beakers, or Vycor evaporating dishes (Corning Glass Works No. 13180, or equivalent). Discard Vycor vessels when the inner surfaces become etched.] Dry the sample overnight at 120° in a forced-draft oven. (The sample must be absolutely dry to prevent flowing or spattering in the furnace.) Place the sample in a furnace equipped with a pyrometer to control the temperature over a range of 260° to 600°, with a variation of less than 10°, and set it at 250°. Slowly, in 50° increments, raise the temperature to 350°, and hold at this temperature until smoking ceases. Increase the temperature to 500° in approximately 75° increments (the sample must not ignite). Ash for 16 h or overnight at 500°. Remove the sample from the furnace, and allow it to cool. The ash should be white and essentially carbon free. If the ash still contains excess carbon particles (i.e., the ash is gray rather than white), proceed as follows: Wet with a minimal amount of water followed by the dropwise addition of 0.5 to 3 mL of nitric acid. Dry on a hot plate. Transfer the ash to a furnace set at 250°, slowly increase the temperature to 500°, and continue heating for 1 to 2 h. Repeat the nitric acid treatment and ashing, if necessary, to obtain a carbon-free residue.

> **Note**: Local overheating or deflagration may result if the sample still contains much intermingled carbon and especially if much potassium is present in the ash.

Dissolve the residue in 5 mL of 1 *N* nitric acid, warming on a steam bath or hot plate for 2 to 3 min to aid solution. Filter, if necessary, and decant through S&S 589 Black Ribbon paper, or equivalent, into a 50-mL volumetric flask. Repeat with two 5-mL portions of 1 *N* nitric acid, filter, and add the washings to the original filtrate. Dilute to volume with 1 *N* nitric acid, and mix to prepare the *Sample Solution*.

Similarly, prepare duplicate reagent blanks for each *Standard Solution* and *Sample Solution*, including any additional water and nitric acid if used for sample ashing.

> **Note**: Do not ash nitric acid in a furnace because the lead contaminant will be lost.

Evaporate nitric acid to dryness in an ashing vessel on a steam bath or hot plate, and then proceed as above, beginning at ''Dissolve the residue in 5 mL of 1 *N* nitric acid, warming on a steam bath. . . .''

> **Note**: Complete the sample preparation and the analysis on the same day.

Aqueous Butyl Acetate Use spectral-grade butyl acetate, and saturate it with water.

APDC Solution Transfer 2.00 g of APDC (ammonium 1-pyrrolidinedithiocarbamate) (Aldrich Chemical, or equivalent) into a 100-mL volumetric flask, dilute to volume with water, and mix. Remove insoluble free acid and other impurities normally present by two to three extractions with 10-mL portions of *Aqueous Butyl Acetate.*

Lead-Free Citric Acid Solution Dissolve 10 g of citric acid in 30 mL of water. While stirring, slowly add ammonium hydroxide until the pH is between 8.0 and 8.5, using short-range pH paper as an external indicator. Transfer the solution to a separatory funnel, and extract with 10-mL portions of *Dithizone Extraction Solution* (under *Lead Limit Test*, Appendix IIIB), until the dithizone solution retains its green color or remains unchanged. Drain the final dithizone layer, plus about 1 mL of the aqueous layer, into a beaker, and while stirring, slowly add 1:1 nitric acid until the pH is between 3.5 and 4, again using short-range pH paper as an external indicator. Transfer this solution into a 100-mL volumetric flask (through a filter, if necessary), dilute to volume with water, and mix thoroughly.

Pipet 20 mL each of the *Standard Solutions*, the *Sample Solution*, and the appropriate reagent blanks into separate 60-mL separatory funnels. Treat each solution as follows: Add 4 mL of *Citric Acid Solution* and 2 to 3 drops of bromocresol green TS. The solution should be yellow. Adjust the pH to about 5.4, using ammonium hydroxide initially and then ammonium hydroxide diluted with 4 volumes of water in the vicinity of the color change (the first permanent appearance of light blue). Add 4 mL of *APDC Solution*, stopper, and shake for 30 to 60 s. Pipet 5.0 mL of *Aqueous Butyl Acetate*, stopper the separatory funnel, and shake vigorously for 30 to 60 s. Let stand until the layers separate clearly, and drain and discard the lower aqueous phase. If an emulsion forms or the solvent layer is cloudy, drain the solvent layer into a 15-mL centrifuge tube, cover with aluminum foil or Parafilm (or equivalent), and centrifuge for about 1 min at 2000 rpm.

Procedure Use an atomic absorption spectrophotometer equipped with a 4-in., single-slot burner head. Set the instrument to previously determined optimum conditions for organic solvent aspiration (3 to 5 mL/min) and at a wavelength of 283.3 nm. Use an air–acetylene flame adjusted for maximum lead absorption with a fuel-lean flame. Aspirate the blanks, the *Standard Solutions*, and the *Sample Solution*, flushing with water and then with *Aqueous Butyl Acetate* between

measurements. Record the absorbance of the *Standard Solutions* and the *Sample Solution* (containing butyl acetate), and correct for the blanks. Prepare the *Standard Curve* by plotting the absorbance of each *Standard Solution* against its concentration, in 1 g of lead per milliliter. (This concentration, in butyl acetate, is four times that in the aqueous standard.) From the *Standard Curve*, determine the concentration, *C*, in 1 g/mL, of the *Sample Solution*. Calculate the quantity, in milligrams per kilogram, of lead in the sample by the formula

$$12.5 \times C/W,$$

in which *W* is the weight, in grams, of the sample taken.

Mercury

Standard Preparation Prepare as directed under *Mercury Limit Test*, Appendix IIIB, using 1.0 mL of the stock solution, equivalent to 1 μg of mercury, instead of the 2.0 mL specified therein.

Sample Preparation Transfer 5 g of sample into a 250-mL Erlenmeyer flask, and continue as directed in the second full paragraph for *Sample Solution* under *Arsenic Limit Test*, Appendix IIIB, beginning with ''add 5 mL of sulfuric acid and a few glass beads. . . .'' After the sample has been digested and the solution diluted to 35 mL, as directed therein, add 1 mL of a 1:25 solution of potassium permanganate, and mix.

Procedure Continue as directed in *Procedure* under *Mercury Limit Test*, Appendix IIIB. Any absorbance produced by the *Sample Preparation* is not more than half that produced by the *Standard Preparation*, indicating not more than 0.1 mg of mercury per kilogram of sample.

4-Methylimidazole (4-MeI)

4-Methylimidazole Stock Solution Purify reagent-grade 4-methylimidazole (Aldrich, or equivalent) by redistillation (b.p. 92° to 93°, 0.05 mm Hg), and then prepare a stock solution by transferring 50 mg of the distillate, accurately weighed, into a 50-mL volumetric flask and diluting to volume with tetrahydrofuran (acetone is also an acceptable solvent). Mix thoroughly, and store in a refrigerator.

Standard Solutions Pipet 1.0-, 1.5-, 2.0-, 2.5-, 3.0-, 3.5-, 4.0-, and 5.0-mL portions of the *4-Methylimidazole Stock Solution* into separate 10-mL volumetric flasks, dilute each to volume with the same solvent used to prepare the stock solution, and mix. The standards thus prepared represent 4-methylimidazole concentrations (w/v) of 100, 150, 200, 250, 300, 350, 400, and 500 mg/L, respectively. Store the *Standard Solutions* in a refrigerator, and use within 1 month.

Sample Preparation Place a plug of fine glass wool in the base of a 300- × 22-mm (id) chromatographic tube having a Teflon stopcock. The column bed, approximately 150 mm tall, should be of uniform consistency, yet open enough to allow elution to occur readily. Transfer 10.0 g of sample, accurately weighed, into a 250-mL polypropylene beaker, and mix thoroughly with 5.0 g of 3 *N* sodium hydroxide. The pH of the mixture should exceed 12. Add 20.0 g of chromatographic siliceous earth (Johns-Manville Celite 545, or equivalent) to the beaker, and thoroughly mix with a wide-blade, stainless steel spatula until a homogeneous, semidry mixture is obtained. Homogeneity is obtained when the color is uniform and no dark clumps are seen. Quantitatively transfer the mixture into the column. Place a plug of glass wool on top of the column, and then allow the column to fall a short distance vertically to help settle the contents.

Rinse the sample beaker with methylene chloride, and pour the contents into the column with the stopcock open. Allow the methylene chloride to pass down the column until it reaches the stopcock. Close the stopcock and allow the methylene chloride to remain in contact with the column bed for 5 min. Open the stopcock, and pass methylene chloride through the column at a rate of 5 mL/min. Collect 200 mL of eluate in a 300-mL round-bottom flask. While maintaining the flask at a temperature of 35° in a water bath, remove the bulk of the solvent from the eluate by rotary vacuum evaporation (350 to 390 mm Hg). Reduce the volume to about 1 mL. During the concentration step, watch the flask carefully to ensure that no loss of sample occurs by bumping. Use a disposable Pasteur pipet to quantitatively transfer the extract residue to a 5-mL volumetric flask, rinsing the flask several times with small (approximately 0.7 mL) portions of the same solvent used to prepare the original solutions (tetrahydrofuran or acetone) until the dilution mark is reached. Mix thoroughly.

Procedure (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph equipped with a hydrogen flame-ionization detector, and a silanized 1-m × 4-mm (id) glass column, or equivalent, packed with 90- to 100-mesh Anakrom ABS, or equivalent, containing 7.5% Carbowax 20M and 2% potassium hydroxide, or equivalent. Maintain the column at 190° (isothermal). Set the injection point temperature to 200° and the detector temperature to 250°. Use nitrogen as the carrier gas, with a flow rate of 50 mL/min.

Use an autosampler to inject 5.0 μL of each *Standard Solution*, and determine their concentrations.

> **Note**: If using manual injections, avoid fractionation in the syringe needle, and ensure that 5.0 μL is injected by using the solvent-flush technique with the solvent used to prepare the *Standard Solutions*.

For each *Standard Solution* chromatogram, obtain the corrected peak area. If not using an integrator, calculate the corrected peak area by multiplying the peak height, in millimeters, by the peak width at one-half height, in millimeters, by the proper attenuation and range factors, depending on the particular apparatus and operating parameters used. Plot each corrected peak area thus obtained versus its respective concentration of 4-methylimidazole to obtain the standard curve. In the same manner, chromatograph a 5.0-μL portion of the *Sample Preparation*, calculate the peak area corresponding to any 4-methylimidazole contained in the sample, and by reference to the standard curve, obtain the content of the 4-methylimidazole in the sample.

Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X, using 0.5 g of sample, accurately weighed.

Total Nitrogen Determine as directed in *Method II* under *Nitrogen Determination*, Appendix IIIC.

Total Sulfur Place 1 to 3 g of magnesium oxide or an equivalent quantity of magnesium nitrate, hexahydrate (6.4 to 19.2 g); 1 g of powdered sucrose; and 50 mL of nitric acid into the largest casserole available that fits in an electric muffle furnace. Transfer about 5 g of sample, accurately weighed,

into the casserole when the expected amount of sulfur is 2.5% or less, or 1 g of sample when the expected amount is greater than 2.5%. Place the same quantities of reagents in another casserole for the blank. Evaporate on a steam bath to the consistency of paste. Place the casserole in a cold electric muffle furnace, gradually heat to 525°, and hold at that temperature until all nitrogen dioxide fumes are driven off. Cool the casserole, add 100 mL of water to dissolve the sample, and neutralize to pH 7 with hydrochloric acid, using short-range pH indicator paper as an external indicator. Add an additional 2 mL of hydrochloric acid, filter the solution into a suitable beaker, heat to boiling, and while stirring, slowly add 20 mL of barium chloride TS to the hot solution. Boil the contents of the beaker for 5 min, and allow to stand overnight. Filter the contents of the beaker through a tight, ashless filter paper, and quantitatively transfer the precipitate to the paper. Thoroughly wash the paper and the precipitate with hot water, and then transfer the paper to a tared crucible previously ignited for 1 h at 800° in a muffle furnace. Dry the paper in the crucible for 1 h at 105°, and then carefully char it, with free access to air, at low heat over a burner. Gradually increase the heat to burn away the paper, and ignite the crucible and contents for 1 h at 800°. Cool and weigh, and calculate the percent sulfur by the formula

$$[(W_S - W_B)/S] \times 13.74 \times 0.1/A_{610},$$

in which W_S is the weight, in grams, of the ignited residue of barium sulfate from the sample determination; W_B is the weight, in grams, of the ignited residue from the blank determination; and S is the weight, in grams, of the sample taken.

Packaging and Storage Store in well-closed containers and avoid exposure to excessive heating and, for solid products, excessive humidity.

ADDITIONAL INFORMATION

Identification of Classes The four classes of Caramel may be distinguished from each other by the following methods:

Class I Not more than 50% of the color is bound by DEAE (diethylaminoethyl) cellulose, and not more than 50% of the color is bound by phosphoryl cellulose.

Class II More than 50% of the color is bound by DEAE cellulose, and it exhibits an absorbance ratio (at equal concentrations) of more than 50.

Class III Not more than 50% of the color is bound by DEAE cellulose, and more than 50% of the color is bound by phosphoryl cellulose.

Class IV More than 50% of the color is bound by DEAE cellulose, and it exhibits an absorbance ratio (at equal concentrations) of not more than 50.

Identification Tests for Classes

Absorbance Ratio (**Note**: For the purposes of this test, *Absorbance Ratio* is defined as the absorbance of Caramel, at equal concentrations, at 280 nm divided by the absorbance at 560 nm.) Transfer 100 mg of sample into a 100-mL volumetric flask with the aid of water, dilute to volume, mix, and centrifuge if the solution is cloudy. Pipet 5.0 mL of the clear solution into a 100-mL volumetric flask, dilute to volume with water, and mix. Using a spectrophotometer equipped with a monochromator to provide a band width of 2 nm or less and of such quality that the stray-light characteristic is 0.5% or less, standardized with water as a reference, determine the absorbance of the 0.1% solution in a 1-cm cell at 560 nm and that of the 1:20 diluted solution at 280 nm. Calculate the absorbance ratio of the sample by the formula

$$(A_{C1} \times 20)/A_{C2},$$

in which A_{C1} is the sample absorbance at 280 nm, 20 is the dilution factor, and A_{C2} is the sample absorbance at 560 nm.

Color Bound by DEAE Cellulose (**Note**: For the purposes of this monograph, *Color Bound by DEAE Cellulose* is defined as the percent decrease in absorbance of a Caramel solution at 560 nm after treatment with DEAE cellulose.)

Special Reagent Use DEAE cellulose of 1.0 meq/g capacity. DEAE cellulose of higher or lower capacities may be used in proportionately higher or lower quantities.

Procedure Prepare a *Sample Solution* of approximately 0.5 absorbance unit at 560 nm by transferring an appropriate amount of sample into a 100-mL volumetric flask with the aid of 0.025 *N* hydrochloric acid. Dilute to volume with 0.025 *N* hydrochloric acid, and centrifuge or filter if the solution is cloudy. Add 140 mg of *Special Reagent* to a 20-mL aliquot of the *Sample Solution*, mix thoroughly for several minutes, centrifuge or filter, and collect the clear supernatant liquid. Determine the absorbance of the *Sample Solution* and of the supernatant liquid in a 1-cm cell at 560 nm, with a suitable spectrophotometer previously standardized using 0.025 *N* hydrochloric acid as a reference. Calculate the percent of color bound by DEAE cellulose by the formula

$$100[(X_1 - X_2)/X_1],$$

in which X_1 is the absorbance of the *Sample Solution* at 560 nm, and X_2 is the absorbance of the supernatant liquid at 560 nm.

Color Bound by Phosphoryl Cellulose (**Note**: For the purposes of this monograph, *Color Bound by Phosphoryl Cellulose* is defined as the percent decrease in absorbance of a Caramel solution at 560 nm after treatment with phosphoryl cellulose.)

Special Reagent Use phosphoryl cellulose (cellulose phosphate) of 1.2 meq/g capacity. Phosphoryl cellulose of higher or lower capacities may be used in proportionately higher or lower quantities.

Procedure Transfer 200 to 300 mg of sample into a 100-mL volumetric flask, dilute to volume with 0.025 *N* hydrochloric acid, and centrifuge or filter if the solution is cloudy. Add 1.42 g of *Special Reagent* to a 40-mL aliquot of the *Sample Solution*, mix thoroughly for several minutes, centrifuge or filter, and collect the clear supernatant liquid. Determine the absorbance of the *Sample Solution* and of the supernatant liquid in a 1-cm cell at 560 nm with a suitable spectrophotometer previously standardized using 0.025 *N* hydrochloric acid as a reference. Calculate the percent color bound by phosphoryl cellulose by the formula

$$100[(X_1 - X_2)/X_1],$$

in which X_1 is the absorbance of the *Sample Solution* at 560 nm, and X_2 is the absorbance of the supernatant liquid at 560 nm.

2-Acetyl-4-(5)-tetrahydroxybutylimidazole (THI) [Class III (Ammonia Caramel) is the only class of Caramel color found to contain THI.] Because some countries have a THI limit of 25 ppm on an equivalent color basis, the following method for determining THI is provided.

2,4-Dinitrophenylhydrazine Hydrochloride (DNPH) Add 5 g of reagent-grade 2,4-dinitrophenylhydrazine to 10 mL of hydrochloric acid contained in a 100-mL Erlenmeyer flask, and gently shake the latter until the free base (red) is converted to the hydrochloride (yellow). Add 100 mL of ethanol, and heat the mixture on a steam bath until all of the solids have dissolved. Cool to room temperature, and after the solution has crystallized, filter off the hydrochloride. Wash the hydrochloride with ether, dry at room temperature, and store in a desiccator. Upon storage, the hydrochloride slowly converts to the free base. The latter can be removed by washing with purified (peroxide-free) dimethoxyethane.

DNPH Hydrochloride Reagent Prepare the reagent by mixing 0.5 g of *DNPH Hydrochloride* with 15 mL of 5% methanol in dimethoxyethane for 30 min. Store in a refrigerator at 4°. When properly prepared and stored, this reagent is stable for at least 3 months.

THI–DNPH Standard Add 0.5 g of *DNPH Hydrochloride* to 1 mL of hydrochloric acid, followed by 10 mL of ethyl alcohol, and heat on a steam bath until solution. Add 100 mg of THI to the hot solution—crystallization begins in a few minutes. Filter off the THI–DNPH when the suspension reaches room temperature. Obtain the *THI–DNPH Standard* by recrystallizing the THI–DNPH with a hydrochloric acid–ethyl alcohol mixture (1 drop of hydrochloric acid per 5 mL of ethyl alcohol). The yield is 70% to 80% based on the THI used. When stored in the refrigerator, the *THI–DNPH Standard* is stable for at least 1 year.

Stock THI–DNPH Solution Dissolve about 10 mg of *THI–DNPH Standard*, accurately weighed, in a 100-mL volumetric flask, and dilute to volume with absolute carbonyl-free methanol. Dilute a portion of this solution tenfold with methanol. The THI concentration, in milligrams per liter, of the *Stock THI–DNPH Solution* is 0.47 times that of the THI–DNPH. When stored in the refrigerator, the *Stock THI–DNPH Solution* is stable for at least 20 weeks.

Cation-Exchange Resin (Strong) Dowex 50 AG × 8, proton form, 100- to 200-mesh.

Cation-Exchange Resin (Weak) Amberlite CG AG 50 I, proton form, 100- to 200-mesh. (Sediment two or three times before use.)

Carbonyl-Free Methanol Add 5 g of Girard's Reagent P (Aldrich, or equivalent) and 0.2 mL of hydrochloric acid to 500 mL of methanol, and reflux for 2 h. Distill the refluxed methanol through a short Vigreux column, and store in tightly closed bottles.

Purified Dimethoxyethane Use distillation to purify dimethoxyethane from 2,4-dinitrophenylhydrazine in the presence of acid, and redistill it from sodium hydroxide. Immediately before use, pass it through a column of neutral alumina to remove peroxides.

Combination Columns Connect a dropping funnel and two small columns, one fitted above the other, with 14.5-mm standard ground-glass joints. Use a 100-mL dropping funnel, equipped with a Teflon stopcock, as the solvent reservoir. Fill the upper column [150 × 12.5 mm (id), filling height max 9 cm, bed height 50 to 60 mm; or 200 × 10 mm (id), filling height max 14 cm, bed height 80 to 90 mm], equipped with a 1-mm (id) capillary outlet, with *Cation-Exchange Resin (Weak)*. Fill the lower column [175 mm × 10 mm (id), bed height 60 mm], equipped with a 1-mm (id) capillary outlet and a Teflon stopcock, with *Cation-Exchange Resin (Strong)*.

Sample Preparation Dissolve 200 to 250 mg of sample, accurately weighed, in 3 mL of water. Quantitatively transfer the solution to the upper part of the combination column. Elute with water until a total of about 100 mL of water has passed through the column. Disconnect the upper column, and elute the lower column with 0.5 *N* hydrochloric acid. Discard the first 10.0 mL of eluate, and subsequently collect a volume of 35 mL. Concentrate the solution to dryness at 40° (15 mm Hg), then dissolve the syrup residue in 250 µL of carbonyl-free methanol, and add 250 µL of *DNPH Hydrochloride Reagent*. Transfer the reaction mixture (sample) to a septum-capped vial, and store for 5 h at room temperature.

Procedure Prepare a series of *THI–DNPH Standard Solutions* serially diluted from the *Stock THI–DNPH Solution*. Pipet 1, 2, and 5 mL, respectively, of the *Stock THI–DNPH Solution*, into separate 10-mL volumetric flasks, and dilute to volume with absolute, carbonyl-free methanol. Prepare a standard curve by injecting 5 µL of the *Stock THI–DNPH Solution*, and the serially diluted *THI–DNPH Standard Solutions* into a 250-mm × 4-mm (id), 10-1m LiChrosorb RP-8 HPLC column (Alltech Associates, Inc., or equivalent) fitted with an ultraviolet detector set at 385 nm. The mobile phase is 50:50 (v/v) methanol:0.1 *M* phosphoric acid. Inject 5 µL of sample into the column. Adjustments in the mobile phase composition may be needed as column characteristics vary among manufacturers. At a mobile phase flow rate of 2 mL/min and column dimensions of 250 × 4.6 mm, elute THI–DNPH at about 6.3 ± 0.1 min. Measure the peak areas. Calculate the amount of THI in the sample from the standard curve. (For THI limits greater than 25 mg/kg, prepare a series of *Standard THI–DNPH Solutions* in a range encompassing the expected THI concentration in the sample.)

Caraway Oil

CAS: [8000-42-8]

View IR

DESCRIPTION

Caraway Oil occurs as a colorless to pale yellow liquid with the characteristic odor and taste of caraway. It is a volatile

oil distilled from the dried, ripe fruit of *Carum carvi* L. (Fam. Umbelliferae).

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 50.0%, by volume, of ketones as carvone.
Angular Rotation Between +70° and +80°.
Refractive Index Between 1.484 and 1.488 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.900 and 0.910.

TESTS

Assay Determine as directed in the *Neutral Sulfite Method* under *Aldehydes and Ketones,* Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 8 mL of 80% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Carbon, Activated

DESCRIPTION

Carbon, Activated, occurs as a black substance, varying in particle size from coarse granules to a fine powder. It is a solid, porous, carbonaceous material prepared by carbonizing and activating organic substances. The raw materials, which include sawdust, peat, lignite, coal, cellulose residues, coconut shells, and petroleum coke, may be carbonized and activated at a high temperature with or without the addition of inorganic salts in a stream of activating gases such as steam or carbon dioxide. Alternatively, carbonaceous matter may be treated with a chemical activating agent such as phosphoric acid or zinc chloride, and the mixture carbonized at an elevated temperature, followed by removal of the chemical activating agent by water washing. Activated Carbon is insoluble in water and in organic solvents.

Function Decolorizing agent; taste- and odor-removing agent; purification agent in food processing.

REQUIREMENTS

Identification

A. Place about 3 g of powdered sample in a glass-stoppered Erlenmeyer flask containing 10 mL of dilute hydrochloric acid (5%), boil for 30 s, and cool to room temperature. Add 100 mL of iodine TS, stopper, and shake vigorously for 30 s. Filter through Whatman No. 2 filter paper, or equivalent, discarding the first portion of filtrate. Compare 50 mL of the subsequent filtrate with a reference solution prepared by diluting 10 mL of iodine TS to 50 mL with water, but not treated with carbon. The color of the carbon-treated iodine solution is no darker than that of the reference solution, indicating the adsorptivity of the sample.

B. Ignite a portion of the sample in air. Carbon monoxide and carbon dioxide are produced, and an ash remains.

Cyanogen Compounds Passes test.
Higher Aromatic Hydrocarbons Passes test.
Iodine Number Not less than 400.
Lead Not more than 10 mg/kg.
Water Extractables Not more than 4.0%.
The following additional *Requirements* should conform to the representations of the vendor: *Loss on Drying* and *Residue on Ignition*.

TESTS

Cyanogen Compounds Mix 5 g of sample with 50 mL of water and 2 g of tartaric acid, and distill the mixture, collecting 25 mL of distillate below the surface of a mixture of 2 mL of 1 *N* sodium hydroxide and 10 mL of water contained in a small flask placed in an ice bath. Dilute the distillate to 50 mL with water, and mix. Add 12 drops of ferrous sulfate TS to 25 mL of the diluted distillate, heat almost to boiling, cool, and add 1 mL of hydrochloric acid. No blue color is produced.
Higher Aromatic Hydrocarbons Extract 1 g of sample with 12 mL of cyclohexane in a continuous-extraction apparatus for 2 h. Place the extract in a Nessler tube and a solution of 100 μg of quinine sulfate in 1000 mL of 0.1 *N* sulfuric acid in a matching Nessler tube. The extract shows no more color or fluorescence than does the solution when observed under ultraviolet light.
Iodine Number[1]

Hydrochloric Acid Solution (5% by weight) Add 70 mL of concentrated hydrochloric acid to 550 mL of water, and mix well.

Potassium Iodate Solution (0.1000 *N)* Dry 4 or more grams of primary standard-grade potassium iodate (KIO_3) at 110° ± 5° for 2 h, and cool to room temperature in a desiccator.

[1]Portions of this test are adapted from ''ASTM D 4607-94(1999)—Standard Test Method for Determination of Iodine Number of Activated Carbon.'' The original ASTM method is available in its entirety from ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428; phone: 610-832-9585; fax: 610-832-9555; email: service@astm.org; website: <www.astm.org>.

Dissolve 3.5667 ± 0.1 mg of the dry potassium iodate in about 100 mL of water. Quantitatively transfer to a 1-L volumetric flask, dilute to volume with water, and mix thoroughly. Store in a glass-stoppered bottle.

Starch Solution Mix 1.0 ± 0.5 g of starch with 5 to 10 mL of cold water to make a paste. Continue to stir while adding an additional 25 ± 5 mL of water to the starch paste. Pour the mixture, while stirring, into 1 L of boiling water, and boil for 4 to 5 min. Make this solution fresh daily.

Sodium Thiosulfate Solution (0.100 *N*) Dissolve 24.820 g of sodium thiosulfate in approximately 75 ± 25 mL of freshly boiled water, and add 0.10 ± 0.01 g of sodium carbonate. Quantitatively transfer the mixture to a 1-L volumetric flask, and dilute to volume with water. Allow the solution to stand for a minimum of 4 days before standardizing. Store the solution in an amber bottle.

To standardize the solution, perform the following in triplicate: Pipet 25.0 mL of *0.1000* N *Potassium Iodate Solution* into a wide-mouthed Erlenmeyer flask. Add 2.00 ± 0.01 g of potassium iodide, and shake the flask to dissolve the potassium iodide crystals. Pipet 5.0 mL of concentrated hydrochloric acid into the flask, and titrate the free iodine with *Sodium Thiosulfate Solution* to a light yellow color. Add a few drops of *Starch Solution*, and continue the titration until 1 drop produces a colorless solution. Determine the *Sodium Thiosulfate Solution* normality using the following formula:

$$(P \times R)/S,$$

in which P is the volume, in milliliters, of *0.1000* N *Potassium Iodate Solution*; R is the normality of the *0.1000* N *Potassium Iodate Solution*; and S is the volume, in milliliters, of *Sodium Thiosulfate Solution*. Average the three normality results; repeat the test if the range of values exceeds 0.003 *N*.

Standard Iodine Solution (0.100 ± 0.001 *N*) Transfer 12.700 g of iodine and 19.100 g of potassium iodide (KI), accurately weighed, into a beaker, and mix. Add 2 to 5 mL of water, and stir well. While stirring, continue to add small increments, approximately 5 mL each, of water until the total volume is 50 to 60 mL. Allow the solution to stand a minimum of 4 h to ensure crystal dissolution, stirring occasionally. Quantitatively transfer the solution to a 1-L volumetric flask, and dilute to volume with water. The iodide-to-iodine weight ratio must be 1.5:1. Store the solution in an amber bottle.

Note: Standardize this solution just before use.

To standardize this solution, perform the following in triplicate. Pipet 25.0 mL into a 250-mL wide-mouthed Erlenmeyer flask. Titrate with the standardized *Sodium Thiosulfate Solution* until a light yellow color develops. Add a few drops of *Starch Solution*, and continue the titration until 1 drop produces a colorless solution. Determine the *Iodine Solution* normality using the following formula:

$$(S \times N_1)/I,$$

in which S is the volume, in milliliters, of the standardized *Sodium Thiosulfate Solution*; N_1 is the normality of the standardized *Sodium Thiosulfate Solution*; and I is the volume, in milliliters, of *Iodine Solution*. Average the three normality results; repeat the test if the range of values exceeds 0.003 *N*. The standardized *Iodine Solution* concentration must be 0.100 ± 0.001 *N*. If it is not, repeat all of the steps starting from, ''Transfer 12.700 g of iodine. . . .''

Procedure This procedure applies to both powdered and granular sample. A representative sample of powdered sample may need additional grinding until 60 wt % (or more) passes through a 325-mesh screen and 95 wt % (or more) passes through a 100-mesh screen. Grind a representative sample sufficiently to pass through the screens as described above. Dry the ground sample, and cool to room temperature in a desiccator.

Three dosages of sample must be estimated to determine the iodine number. Weigh the three dosages (M) of dry carbon to the nearest milligram. Transfer each to one of three clean, dry 250-mL Erlenmeyer flasks equipped with ground glass stoppers.

Pipet 10.0 mL of *Hydrochloric Acid Solution* into each flask, stopper each flask, and swirl gently until the carbon is completely wetted. Loosen the stoppers to vent the flasks, place on a hot plate in a fume hood, and bring the contents to a boil. Allow to boil gently for 30 ± 2 s to remove any sulfur (which may interfere with the test results). Remove the flasks from the hot plate and cool to room temperature.

Standardize and then pipet 100.0 mL of *Iodine Solution* into each flask.

Note: Stagger the addition of standardized *Iodine Solution* to the three flasks so that no delays are encountered in handling.

Immediately stopper the flasks, and shake the contents vigorously for 30 ± 1 s. Quickly filter each mixture by gravity through one sheet of folded filter paper (Whatman No. 2V, or equivalent) into one of three beakers.

Note: Prepare the filtration equipment in advance to avoid delays in filtering the samples.

For each filtrate, use the first 20 to 30 mL to rinse a pipet, and discard the rinse portions. Use clean beakers to collect the remaining filtrates. Mix each filtrate by swirling the beaker, and pipet 50.0 mL of each filtrate into one of three clean 250-mL Erlenmeyer flasks. Titrate each filtrate with standardized *Sodium Thiosulfate Solution* until a pale yellow color develops. Add 2 mL of *Starch Solution*, and continue the titration with standardized *Sodium Thiosulfate Solution* until 1 drop produces a colorless solution. Record the volume (S) of standardized *Sodium Thiosulfate Solution* used.

Calculation The capacity of a carbon for any adsorbate depends on the concentration of the adsorbate. The concentrations of the standard iodine solution and filtrate must be known to determine an appropriate carbon weight to produce final concentrations agreeing with the definition of iodine number. The amount of sample to be used in the determination is governed by the activity of the sample. If filtrate normalities (C) are not within the range of 0.008 *N* to 0.040 *N*, repeat the procedure using different sample weights.

Once filtrate normalities are set within the specified range, perform the following calculations for each carbon dosage:

$$A = (N_2)(12693.0),$$

in which N_2 is the normality of the standardized *Iodine Solution*.

$$B = (N_1)(126.93),$$

in which N_1 is the normality of the standardized *Sodium Thiosulfate Solution*.

Calculate the dilution factor (DF) using the equation

$$DF = (I + H)/F,$$

in which I is the volume, in milliliters, of *Iodine Solution* used in the standardization procedure; H is the volume, in milliliters, of *Hydrochloric Acid Solution* used; and F is the volume, in milliliters, of filtrate used.

Calculate the weight, in milligrams, of iodine adsorbed per gram of sample (X/M) by the equation

$$X/M = [A - (DF)(B)(S)]/M,$$

in which S is the volume, in milliliters, of standardized *Sodium Thiosulfate Solution* used, and M is the weight, in grams, of the sample.

Calculate the normality of the residual filtrate (C) as follows:

$$C = (N_1 \times S)/F.$$

Using logarithmic paper, plot X/M (as the ordinate) versus C (as the abscissa) for each of the three carbon dosages. Calculate the least squares fit for the three points, and plot. The iodine number is the X/M value at a residual iodine concentration (C) of 0.02 N. The regression coefficient for the least squares fit should be greater than 0.995.

Carbon dosages may be estimated initially by using three values of C (usually 0.01, 0.02, and 0.03) as follows:

$$M = [A - (DF)(C)(126.93)(50)]/E,$$

in which M is the weight, in grams, of the carbon dosage and E is the nominal iodine number of the sample. If new carbon dosages have been determined, repeat the *Procedure* and *Calculations*.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, testing a 20-mL portion of the filtrate obtained in the test for *Water Extractables* (below) and using 10 µg of lead ion (Pb) in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 120° for 4 h.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 500-g sample.

Water Extractables Transfer 5.00 g of sample into a 250-mL flask provided with a reflux condenser and a Bunsen valve. Add 100 mL of water and several glass beads, and reflux for 1 h. Cool slightly, and filter through Whatman No. 2, or equivalent, filter paper, discarding the first 10 mL of filtrate. Cool the subsequent filtrate to room temperature, and pipet 25.0 mL into a tared crystallization dish.

> **Note**: Retain the remainder of the filtrate for the *Lead* test.

Evaporate the filtrate in the dish to incipient dryness on a hot plate, never allowing the solution to boil. Dry for 1 h at 100° in a vacuum oven, cool, and weigh.

Packaging and Storage Store in well-closed containers.

Carbon Dioxide

CO_2 Formula wt 44.01

INS: 290 CAS: [124-38-9]

DESCRIPTION

Carbon Dioxide occurs as a colorless gas. One liter of Carbon Dioxide weighs about 1.98 g at 0° and a pressure of 760 mm Hg. Under a pressure of about 59 atmospheres, it may be condensed to a liquid, a portion of which forms a white solid (''dry ice'') upon rapid vaporization. Solid Carbon Dioxide evaporates without melting upon exposure to air. One volume of the gas dissolves in about 1 volume of water, forming a solution that is acid to litmus.

Function Propellant and aerating agent; carbonating agent; direct-contact freezing agent.

REQUIREMENTS

Identification

A. Pass 100 ± 5 mL of sample, released from the vapor phase of the contents of the container, through a carbon dioxide detector tube (see *Detector Tubes* under *Solutions and Indicators*) at the rate specified for the tube. The indicator change extends throughout the entire indicating range of the tube.

B. The sample, when passed through barium hydroxide TS, forms a precipitate that dissolves with effervescence in acetic acid.

Assay Not less than 99.5% of CO_2, by volume.

Carbonyl Sulfide Not more than 0.5 ppm, by volume.

Hydrogen Sulfide Not more than 0.5 ppm, by volume.

Nitric Oxide (NO) and Nitrogen Dioxide (NO_2) Not more than 5 ppm total, by volume.

Nonvolatile Hydrocarbons Not more than 10 mg/kg.

Sulfur Dioxide Not more than 5 ppm, by volume.

Volatile Hydrocarbons (as methane) Not more than 0.005%, by volume.

Water Passes test.

TESTS

> **Note**: The following *Tests* are designed to reflect the quality of Carbon Dioxide in both its vapor and its liquid phases, which are present in previously unopened cylinders. Reduce the container pressure by means of a regulator.

Withdraw the samples for the *Tests* with the least possible release of gas consistent with proper purging of the sampling apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination of or changes to the samples. Perform *Tests* in the sequence in which they are listed.

The various detector tubes called for in the respective *Tests* are listed under *Detector Tubes* in *Solutions and Indicators*.

Assay (**Note**: Sampling for this *Assay* may be done from the vapor phase for convenience, but this results in more residual volume. If the specification of 0.5 mL is exceeded from the vapor phase, a liquid sample may be taken.) Assemble a 100-mL gas buret provided with a leveling bulb and a two-way stopcock to a gas absorption pipet of suitable capacity by connecting the pipet to one of the buret outlets. Fill the buret with slightly acidified water (turned pink with methyl orange), and fill the pipet with potassium hydroxide solution (1:2). By manipulating the leveling bulb and leveling water, draw the potassium hydroxide solution to fill the pipet and capillary connection up to the stopcock, and then fill the buret with the leveling water, and draw it through the other stopcock opening in such a manner that all gas bubbles are eliminated from the system. Draw into the buret 100.0 mL of sample taken from the liquid phase as directed below in the test for *Nitric Oxide and Nitrogen Dioxide*. By raising the leveling bottle, force the measured sample into the pipet. The absorption may be facilitated by rocking the pipet or by flowing the sample between pipet and buret. Draw any residual gas into the buret, and measure its volume. Not more than 0.5 mL of gas remains.

Carbonyl Sulfide

Standard Preparation Obtain a standard gas mixture of 50 ppm carbonyl sulfide in helium from a specialty gas supplier.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a Sievers 350 (or equivalent)[1] Chemiluminescence Detector (SCD) and a 30-m × 0.53-mm id, 5-mm DB-5 capillary column (J&W Scientific Company, or equivalent). Set the carrier gas, helium, at a head-pressure of 5 psig. Set the injection port at 100°, and the split ratio at 1:1. Set the column temperature at 30°. The retention time for carbonyl sulfide is approximately 3 min. Operate the SCD with 190 mL/min of hydrogen and 396 mL/min of air. Optimize the gas flows and probe position of the SCD for maximum sensitivity.

Procedure Inject, in triplicate, 5.00 mL of the *Standard Preparation* into the gas chromatograph, record the chromatograms, and average the peak area responses. The relative standard deviation does not exceed 5.0%.

Similarly, inject, in triplicate, 5.00 mL of sample, average the peak area responses, and calculate the ppm v/v in the sample by the equation

$$\text{ppm} = S(A_U/A_S),$$

in which S is the calculated ppm of carbonyl sulfide in the *Standard Preparation* (approximately 0.5 ppm), A_U is the average of the sample peak area responses, and A_S is the average area of the *Standard Preparation* area responses.

Hydrogen Sulfide Pass 50 mL of sample released from the vapor phase through a hydrogen sulfide detector tube (Dräger #672804, 0.5 to 15 ppm, or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 0.5 ppm, for the volume of carbon dioxide specified in this test.

Nitric Oxide (NO) and Nitrogen Dioxide (NO_2) Position the sample container so that when its valve is opened, the liquid phase can be sampled (generally this requires that the cylinder be inverted). Attach a section of tubing long enough to act as a vaporizer for the small quantity of liquid to be sampled. Connect one end of a nitric oxide–nitrogen dioxide detector tube (see *Detector Tubes*) to the tubing and the other end to a gas flow meter. Pass 500 mL of the liquid sample through the tube at a suitable rate. No frost should reach the tube inlet from the expanding sample. The indicator change corresponds to not more than 5 ppm.

Nonvolatile Hydrocarbons Pass a sample of liquid Carbon Dioxide from a storage container or sample cylinder through a commercial carbon dioxide snow horn directly into an open, clean container. Collect the resulting Carbon Dioxide snow in this container. Weigh 500 g of this sample and transfer it into a clean beaker. Allow the Carbon Dioxide solid to sublime completely, with a watch-glass placed over the beaker to prevent ambient contamination. Wash the beaker with a residue-free solvent, and transfer the solvent from the beaker to a clean, tared watch-glass or petri dish with two additional rinses of the beaker with the solvent. Allow the solvent to evaporate, using heating to 104°, until the watch-glass or petri dish is at a constant weight. Determine the weight of the residue by difference. The weight of the residue does not exceed 5 mg (10 mg/kg).

Sulfur Dioxide Pass 1050 ± 50 mL of sample, taken from the liquid phase as described in the test for *Nitric Oxide and Nitrogen Dioxide*, through a sulfur dioxide detector tube (see *Detector Tubes*) at the rate specified for the tube. The indicator change corresponds to not more than 5 ppm.

Volatile Hydrocarbons

Standard Preparation Obtain a standard gas mixture of 50 ppm methane in helium from a specialty gas supplier.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame ionization detector and a 1.8-m × 3-mm (od) metal column, or equivalent, packed with 80- to 100-mesh HayeSep Q (or equivalent). Use helium as the carrier gas at a flow rate of 30 mL/min. Maintain both the injector temperature and the detector temperatures at 230°. Program the column temperature according to the following steps: Hold it at 70° for 1 min, then increase to 200° at a rate of 20°/min, and then hold at 200° for 10 min. The parameters for the detector are sensitivity range: 10^{-12} A/mV; attenuation: 32. The concentration of volatile hydrocarbons is reported in methane equivalents. The various gas chromatographic responses, excluding the Carbon Dioxide response, are summed to yield the total

[1] Any sulfur-selective detector may be used; e.g., electrolytic conductivity, flame photometric, or sulfur chemiluminescence. The detector must be capable of detecting less than 0.1 ppm v/v of carbonyl sulfide with a signal-to-noise ratio of 10:1.

volatile hydrocarbon concentration. The composition of hydrocarbons present will vary from sample to sample. Typical retention times are methane: 0.4 min; carbon dioxide: 0.8 min; hexane: 14.4 min.

Procedure Inject in triplicate 1.00 mL of the *Standard Preparation* into the gas chromatograph, and average the peak area responses. The relative standard deviation should not exceed 5.0%. Similarly, inject in triplicate 1.00 mL of sample, sum the average peak areas of the individual peaks, *except for the Carbon Dioxide peaks*, and calculate the ppm v/v in the sample by the equation

$$\text{ppm} = S(A_U/A_S),$$

in which S is the calculated ppm of methane in the *Standard Preparation* (approximately 50 ppm), A_U is the sum of the averages of the individual peak area responses in the sample, and A_S is the average of the *Standard Preparation* area responses.

Water Pass 24,000 mL of the gas sample through a suitable water-absorption tube (see *Detector Tubes*), not less than 100 mm long, which previously has been flushed with about 500 mL of sample and weighed. Regulate the flow so that about 60 min will be required for passage of the gas. The gain in weight of the absorption tube does not exceed 1.0 mg.

Packaging and Storage Store in metal cylinders.

Cardamom Oil

CAS: [8000-66-6]

View IR

DESCRIPTION

Cardamom Oil occurs as a colorless or very pale yellow liquid with the aromatic, penetrating, and somewhat camphoraceous odor of cardamom and a pungent, strongly aromatic taste. It is the volatile oil distilled from the seed of *Elettaria cardamomum* (L.) Maton (Fam. Zingiberaceae). It is affected by light. It is miscible with alcohol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between +22° and +44°.
Refractive Index Between 1.462 and 1.466 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.917 and 0.947.

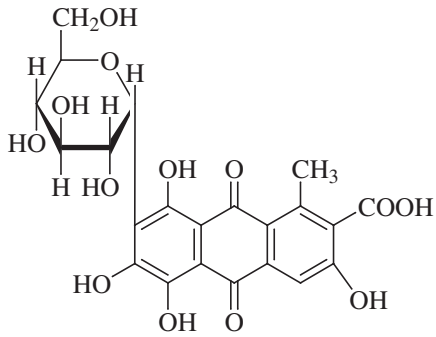
TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index* Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 5 mL of 70% alcohol. The solution may be clear or hazy.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Carmine

Carminic Acid



$C_{22}H_{20}O_{13}$ Formula wt 492.39

INS: 120 CAS: [1390-65-4]

DESCRIPTION

Carmine occurs as bright red, friable pieces or as a dark red powder. It is the aluminum or the calcium–aluminum lake, on an aluminum hydroxide substrate, of the coloring principles obtained by an aqueous extraction of cochineal. Cochineal consists of the dried female insects *Dactylopius coccus costa* (*Coccus cacti* L.), enclosing young larvae; the coloring principles thus derived consist mainly of carminic acid ($C_{22}H_{20}O_{13}$). It is soluble in alkali solutions, slightly soluble in hot water, and practically insoluble in cold water and in dilute acids.

Before use in food, Carmine must be pasteurized or otherwise treated to destroy all viable *Salmonella* microorganisms. According to the pertinent U.S. color additive regulation (**21** *CFR* 73.100), ‘‘. . . pasteurization or such other treatment is deemed to permit the adding of safe and suitable substances (other than chemical preservatives) that are essential to the method of pasteurization or other treatment used.’’

Carminic acid crystallizes from water as bright red crystals that darken at 130° and decompose at 250°; it is freely soluble

in water, in alcohol, in ether, in concentrated sulfuric acid, and in solutions of alkali hydroxides; it is insoluble in petroleum ether and in chloroform. Its aqueous solutions at pH 4.8 are red-orange to yellow, and at 6.2 are dark red to violet.

Note: The specifications and tests in this monograph refer to Carmine without any added substances for pasteurization or any other such treatment.

Function Color.

REQUIREMENTS

Identification Mix 333 mg of sample with 44 mL of water, 0.15 mL of a 1:10 sodium hydroxide solution, and 0.2 mL of ammonium hydroxide, warm to dissolve, and dilute to volume with water in a 500-mL volumetric flask. Pipet 10.0 mL of this solution into a 250-mL volumetric flask, dilute to volume with water, and mix. The resulting solution exhibits absorption maxima at 520 nm and 550 nm when determined in a 1-cm cell with a suitable spectrophotometer against a water blank, and the absorbance at 520 nm is not less than 0.30.
Assay Not less than 50.0% of carminic acid ($C_{22}H_{20}O_{13}$), calculated on the dried basis.
Arsenic Not more than 1 mg/kg.
Ash Not more than 12.0%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 20.0%.
Microbial Limits:
Salmonella Negative in 25 g.
Protein Not more than 25%.

TESTS

Assay Dissolve about 0.100 g of sample (~52% carminic acid content), accurately weighed, in 30 mL of 2 *N* hydrochloric acid, and heat to a boil for 30 s. After cooling, dilute to a volume of 1 L.

Note: If a black or brown precipitate forms, filter the solution.

With a suitable spectrophotometer, determine the absorbance of this solution in a 1-cm cell at the wavelength of maximum absorbance (about 494 nm), using a 1:3 aqueous dilution of 2 *N* hydrochloric acid as the blank. To obtain accurate results, the absorbance must be in the range of 0.650 to 0.750. Adjust the starting weight as necessary to achieve this absorbance. Calculate the percent carminic acid in the sample taken by the formula

$$100A/13.9W,$$

in which A is the absorbance of the sample solution and W is the weight, in grams, of the sample taken.
Arsenic Transfer 3.0 g of sample into a 500-mL Kjeldahl flask equipped with a steam trap, add 5 g of ferrous sulfate and 75 mL of hydrochloric acid, and mix. Connect the flask with the steam trap and with a condenser, the delivery tube of which consists of a large-sized straight adapter and extends to slightly above the bottom of a 500-mL Erlenmeyer flask containing 100 mL of water. Begin heating the Kjeldahl flask, and collect about 40 mL of distillate in the Erlenmeyer flask. Pour the distillate mixture into a 600-mL beaker, add 20 mL of bromine water, and heat on a hot plate until the volume is reduced to about 2 mL. Transfer the residual liquid into a 125-mL arsine generator flask (see Appendix IIIB, Fig. 11) with the aid of 35 mL of water, and continue as directed in the *Procedure* under *Arsenic Limit Test*, Appendix IIIB, beginning with ''Add 20 mL of 1:5 sulfuric acid. . . .''
Ash Transfer about 1 g of sample into a tared, previously ignited and cooled porcelain crucible, and ignite red-hot with a Meker burner to constant weight.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 1-g sample at 135° for 3 h.
Microbial Limits (**Note:** The current method for the following test may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):
Salmonella
Protein Determine the nitrogen content (N) of the sample as directed in *Method II* under *Nitrogen Determination*, Appendix IIIC. Calculate the protein content, in percent, by the formula

$$6.25\ N/W)100,$$

in which 6.25 is the conversion factor from nitrogen to protein; N is the weight, in milligrams, of nitrogen; and W is the weight, in milligrams, of sample taken.

Packaging and Storage Store in well-closed containers in a cool, dry place.

Carnauba Wax

INS: 903 CAS: [8015-86-9]

DESCRIPTION

Carnauba Wax occurs as a hard, brittle substance with a resinous fracture and a color ranging from light brown to pale yellow. It is a purified wax obtained from the leaf buds and leaves of the Brazilian wax palm *Copernicia cereferia* (Arruda) Mart. [synonym *C. prunifera* (Muell.)]. Its specific gravity is about 0.997. It is partially soluble in boiling alcohol, is soluble in chloroform and in ether, but is insoluble in water.

Function Anticaking agent; surface-finishing (glazing) agent; release agent; carrier for flavors.

REQUIREMENTS

Acid Value Between 2 and 7.
Ester Value Between 71 and 88.

Lead Not more than 5 mg/kg.
Melting Range Between 80° and 86°.
Residue on Ignition Not more than 0.25%.
Saponification Value Between 78 and 95.
Unsaponifiable Matter Between 50.0% and 55.0%.

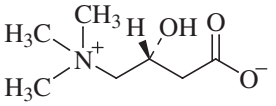
TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Ester Value Subtract the *Acid Value* from the *Saponification Value* (below) to obtain the *Ester Value*.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Melting Range Determine as directed in *Procedure for Class II* under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Heat a 2-g sample in a tared, open, porcelain or platinum dish over an open flame. It volatilizes without emitting an acrid odor. Ignite as directed under *Residue on Ignition*, Appendix IIC.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 5 g of sample, accurately weighed.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.

Packaging and Storage Store in well-closed containers.

L-Carnitine

4-Amino-3-hydroxybutyric Acid Trimethylbetaine; Levocarnitine; 4-Trimethylamino-3-hydroxybutyrate; (*R*)-3-Carboxy-2-hydroxy-*N*,*N*,*N*-trimethyl-1-propanaminium Hydroxide, Inner Salt



$C_7H_{15}NO_3$ Formula wt 161.20

CAS: [541-15-1]

DESCRIPTION

L-Carnitine occurs as white crystals or as a white, crystalline, hygroscopic powder. It is freely soluble in water, in alcohol, in alkaline solutions, and in dilute mineral acids. It is practically insoluble in acetone and in ethyl acetate. It decomposes without melting at about 185° to 195°.

Function Nutrient.

REQUIREMENTS

Identification

A. Dissolve about 1 g of sample in 10 mL of water and 10 mL of 1 *N* hydrochloric acid, and add 5 mL of sodium tetraphenylborate TS. A white precipitate forms.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample, previously dried in vacuum at 60° for 5 h, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Levocarnitine Reference Standard.

Assay Not less than 97.0% and not more than 103.0% of $C_7H_{15}NO_3$, calculated on the anhydrous basis.
Chloride Not more than 0.4%.
Lead Not more than 1 mg/kg.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between –29.0° and –32.0°, calculated on the anhydrous basis.
pH Between 5.5 and 9.5, in a 1:20 aqueous solution.
Potassium Not more than 0.2%.
Residue on Ignition Not more than 0.5%.
Sodium Not more than 0.1%.
Water Not more than 4.0%.

TESTS

Assay Dissolve about 1.0 g of sample, accurately weighed, in water contained in a 250-mL flask.

> **Note**: Avoid atmospheric moisture uptake during weighing.

Titrate with 1.0 *N* hydrochloric acid to a potentiometric endpoint. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 1.0 *N* hydrochloric acid is equivalent to 161.2 mg of $C_7H_{15}NO_3$.

Chloride

Test Solution Dissolve about 100 mg of sample, accurately weighed, in 30 to 40 mL of water, and mix. Add 10% nitric acid dropwise until the solution is neutral to litmus. Add an additional 1 mL of 10% nitric acid, and dilute with water to a total volume of 50 mL.

Chloride Reference Solution Transfer by micropipet 0.56 mL of 0.02 *N* hydrochloric acid solution to 30 to 40 mL of water in a 50-mL flask, add 1 mL of 10% nitric acid, and dilute with water to a volume of 50 mL.

Procedure Add 1 mL of 0.1 *N* silver nitrate to both the *Test Solution* and the *Chloride Reference Solution*. Mix, allow to stand for 5 min protected from direct sunlight, and compare the two solutions. The turbidity of the *Test Solution* is not greater than that of the *Chloride Reference Solution*.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of a previously dried sample in 100 mL of water.
pH Determine as directed under *pH Determination*, Appendix IIB.

Potassium (**Note**: The *Standard Solution* and the *Test Solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the spectrophotometer.)

Standard Solution Transfer 5.959 g of potassium chloride, previously dried at 105° for 2 h and accurately weighed, into a 250-mL volumetric flask, dilute to volume with water, and mix. This solution contains 12.5 mg of potassium per milliliter. Quantitatively dilute an accurately measured volume of this solution with water, stepwise if necessary, to obtain a solution containing 31.25 μg of potassium per milliliter.

Test Solutions Transfer 62.5 mg of sample into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix to obtain a stock solution. Place 0, 2.0, and 4.0 mL of the *Standard Solution* into three separate 25-mL volumetric flasks. Add 20.0 mL of the stock solution to each flask, dilute to volume with water, and mix. These solutions contain 0 (*Test Solution A*), 2.5 (*Test Solution B*), and 5.0 (*Test Solution C*) μg/mL of potassium.

Procedure Concomitantly determine the absorbance values of the *Test Solutions* at the potassium emission line at 766.7 nm with a suitable atomic absorption spectrophotometer equipped with an air–acetylene flame, using water as the blank. Plot the absorbance values of the *Test Solutions* versus their contents of potassium, in micrograms per milliliter; draw the straight line best fitting the three points; and extrapolate the line until it intersects with the concentration axis. From the intercept, determine the amount, in micrograms, of potassium in each milliliter of *Test Solution A*. Calculate the percent potassium in the portion of sample taken by multiplying the concentration, in micrograms per milliliter, of potassium found in *Test Solution A* by 0.2.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Sodium (**Note**: The *Standard Solution* and the *Test Solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the spectrophotometer.)

Standard Solution Transfer 6.355 g of sodium chloride, previously dried at 105° for 2 h and accurately weighed, into a 250-mL volumetric flask, dilute to volume with water, and mix. This solution contains 10.0 mg of sodium per milliliter. Quantitatively dilute an accurately measured volume of this solution with water, stepwise if necessary, to obtain a solution containing 250 μg of sodium per milliliter.

Test Solutions Transfer 4.0 g of sample into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix to obtain a stock solution. Add 0, 2.0, and 4.0 mL of the *Standard Solution* to three separate 25-mL volumetric flasks. Add 20.0 mL of the stock solution to each flask, dilute to volume with water, and mix. These solutions contain 0 (*Test Solution A*), 20.0 (*Test Solution B*), and 40.0 (*Test Solution C*) μg/mL of sodium.

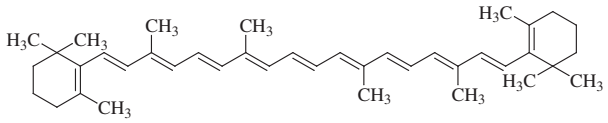
Procedure Concomitantly determine the absorbance values of the *Test Solutions* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer equipped with an air–acetylene flame, using water as the blank. Plot the absorbance values of the *Test Solutions* versus their contents of sodium, in micrograms per milliliter; draw the straight line best fitting the three points; and extrapolate the line until it intersects with the concentration axis. From the intercept, determine the amount, in micrograms, of sodium in each milliliter of *Test Solution A*. Calculate the percent sodium in the portion of sample taken by multiplying the concentration, in micrograms per milliliter, of sodium found in *Test Solution A* by 0.003125.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

β-Carotene

Carotene



$C_{40}H_{56}$ Formula wt 536.88

INS: 160a(ii) CAS: [7235-40-7]

DESCRIPTION

Beta-Carotene occurs as red crystals or as crystalline powder. It is insoluble in water and in acids and alkalies, but is soluble in carbon disulfide and in chloroform. It is sparingly soluble in ether, in solvent hexane, and in vegetable oils, and is practically insoluble in methanol and in ethanol. It melts between 176° and 182°, with decomposition.

Function Nutrient; color.

REQUIREMENTS

Identification

A. Determine the absorbance of *Sample Solution B* (prepared for the *Assay*) at 455 nm and at 483 nm. The ratio A_{455}/A_{483} is between 1.14 and 1.18.

B. Determine the absorbance of *Sample Solution B* at 455 nm and that of *Sample Solution A* at 340 nm. The ratio A_{455}/A_{340} is not lower than 1.5.

Assay Not less than 96.0% and not more than 101.0% of $C_{40}H_{56}$, calculated on the dried basis.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 0.2%.

Residue on Ignition Not more than 0.2%.

TESTS

Assay (**Note**: Carry out all work in low-actinic glassware and in subdued light.)

Sample Solution A Transfer about 50 mg of sample, accurately weighed, into a 100-mL volumetric flask, dissolve it in 10 mL of acid-free chloroform, immediately dilute to volume with cyclohexane, and mix. Pipet 5 mL of this solution into a second 100-mL volumetric flask, dilute to volume with cyclohexane, and mix.

Sample Solution B Pipet 5 mL of *Sample Solution A* into a 50-mL volumetric flask, dilute to volume with cyclohexane, and mix.

Procedure Determine the absorbance of *Sample Solution B* in a 1-cm cell at the wavelength of maximum absorption at about 455 nm, with a suitable atomic absorption spectrophotometer, using cyclohexane as the blank. Calculate the quantity, in milligrams, of $C_{40}H_{56}$ in the sample taken by the formula

$$20{,}000A/250,$$

in which A is the absorbance of the solution, and 250 is the absorptivity of pure β-Carotene.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in a vacuum over phosphorus pentoxide at 40° for 4 h.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in a cool place in tight, light-resistant containers under inert gas.

Carrot Seed Oil

CAS: [8015-88-1]

FEMA: 2244

View IR

DESCRIPTION

Carrot Seed Oil occurs as a light yellow to amber liquid having a pleasant, aromatic odor. It is the volatile oil obtained by steam distillation from the crushed seeds of *Daucus carota* L. (Fam. Umbelliferae). It is soluble in most fixed oils, and is soluble, with opalescence, in mineral oil. It is practically insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 5.0.
Angular Rotation Between –4° and –30°.
Refractive Index Between 1.483 and 1.493 at 20°.
Saponification Value Between 9 and 58.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.900 and 0.943.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed under *Saponification Value*, Appendix VI, using about 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 0.5 mL of 90% alcohol. The solution may become opalescent upon further dilution up to 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from glass or aluminum or that are lined with tin.

Cascarilla Oil

Sweetwood Bark Oil

CAS: [8007-06-5]

View IR

DESCRIPTION

Cascarilla Oil occurs as a light yellow to brown amber liquid with a pleasant, spicy odor. It is the volatile oil obtained by steam distillation of the dried bark of *Croton cascarilla* Benn. and of *Croton eluteria* Benn. (Fam. Euphorbiaceae). It is soluble in most fixed oils and in mineral oil, but it is practically insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those shown in the respective spectrum in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Between 3 and 10.
Angular Rotation Between –1° and +8°.
Ester Value after Acetylation Between 62 and 88.

Refractive Index Between 1.488 and 1.494 at 20°.
Saponification Value Between 8 and 20.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.892 and 0.914.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value after Acetylation Determine as directed under *Total Alcohols*, Appendix VI, using about 2 g of the dried, acetylated sample oil, accurately weighed. Calculate the *Ester Value after Acetylation* by the formula

$$A \times 28.05/B,$$

in which A is the number of milliliters of 0.5 N alcoholic potassium hydroxide consumed in the saponification, and B is the weight, in grams, of the acetylated sample oil.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed under *Saponification Value*, Appendix VI, using 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 0.5 mL of 90% alcohol and remains in solution on dilution to 10 mL.
Specific Gravity Determine by any reliable method (see *General Requirements*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Casein and Caseinate Salts

CAS: [9000-71-9]

DESCRIPTION

Casein occurs as an off white to cream colored, granular or fine powder. It is derived from the coagulum formed by treating skim milk with a food-grade acid (*Acid Casein*), enzyme (*Rennet Casein*), or other food-grade precipitating agent. After the precipitation, Casein is separated from the soluble milk fraction, washed, and dried. Chemically, Casein is a mixture of at least 20 electrophoretically distinct phosphoproteins. The main fractions—designated α-casein, β-casein, and κ-casein—are known to be mixtures, rather than single proteins. Casein contains all the amino acids known to be essential for human nutrition. It is insoluble in water and alcohol, but it can be dissolved by aqueous alkalies to form Caseinate Salts.

Caseinate Salts occur as white to cream colored granules or powders. They are soluble or dispersible in water. They are prepared by treatment of Casein with food-grade alkalies, neutralizing agents, enzymes, buffers, or sequestrants. Common counter-ions are NH_4^+, Ca^{++}, Mg^{++}, K^+, and Na^+.

Function Binder; extender; clarifying agent; emulsifier; stabilizer.

REQUIREMENTS

Assay *Acid Casein*: Not less than 90.0% protein; *Rennet Casein*: Not less than 86.0% protein; *Caseinate Salts*: Not less than 84.0% protein, calculated on the dried basis.
Fat Not more than 2.25%.
Free Acid (Casein only) Passes test.
Lactose Not more than 2.0%.
Lead Not more than 1 mg/kg.
Loss on Drying Not more than 12.0%.

TESTS

Assay Determine as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the percent protein (P) by the equation

$$P = N \times 6.38,$$

in which N is the percent nitrogen.
Fat Transfer 1 g of sample, accurately weighed, into a fat-extraction flask, add 10 mL of water, and shake until homogeneous (warm if necessary). Add approximately 1 mL of ammonium hydroxide, and heat in a water bath for 15 min at 60° to 70°, shaking occasionally. Add 10 mL of alcohol, and mix well. Add 25 mL of peroxide-free ether, stopper, and shake vigorously for 1 min; allow to cool, if necessary; add 25 mL of petroleum ether; and repeat the vigorous shaking. Allow the layers to separate and clarify, or centrifuge to expedite the process. Decant the organic layer into a suitable flask or dish, and repeat the extraction twice with 15 mL each of ether and petroleum ether for each extraction. Evaporate the combined ether extractions on a steam bath, and dry the residue to a constant weight at 102°, or 70° to 75° at less than 50 mm Hg. Calculate the percent fat (F) by the equation

$$F = (R \times 100)/S,$$

in which R is the weight, in grams, of the residue, and S is the weight, in grams, of the sample.
Free Acid Transfer 10 g of finely ground sample, accurately weighed, into a 500-mL conical flask. Add 200 mL of freshly boiled water maintained at 60°, swirl, and stopper. Place the flask in an 80° water bath for 30 min, shaking at 10-min intervals. Cool to room temperature, and filter. Transfer a 100.0-mL portion of the clear filtrate, accurately weighed, into a 250-mL conical flask, add 0.5 mL of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide to a pink endpoint that persists for 30 s. Not more than 2.7 mL of 0.1 N sodium hydroxide is consumed.

Lactose

Phenol Reagent Heat a mixture of 8 g of phenol and 2 g of water until the crystals dissolve.

Lactose Solution Transfer approximately 2 g of lactose monohydrate, accurately weighed, into a 100-mL volumetric flask; dissolve in and dilute to volume with water.

Sample Solution Transfer approximately 1 g of sample, accurately weighed, into a 150-mL beaker. If the sample is *Acid Casein*, add 0.10 g of sodium hydrogen carbonate. If the sample is *Rennet Casein*, add 0.10 g of sodium tripolyphosphate. Add 25 mL of water, and dissolve the sample by gently swirling while warming to 60° to 70° on a hot plate. Cool the solution to ambient temperature, and add 15 mL of water, 8 mL of 0.1 *N* hydrochloric acid, and 1 mL of a 10% solution of acetic acid. Mix well by swirling, and after 5 min, add 1 mL of 1 *M* sodium acetate, and mix well.

After the precipitate has settled, filter and discard the first 5 mL of filtrate. Pipet 2 mL of the remaining filtrate into a test tube, add 0.2 mL of *Phenol Reagent*, and mix well. Add 5 mL of sulfuric acid by using an automatic dispenser or by other means that permit mixing within 1 to 2 s. Ensure that the solution has been thoroughly mixed, and allow it to stand for 15 min, then cool to 20° in a water bath for 5 min.

Standard Solutions Transfer 10 mL of *Lactose Solution* into a 100-mL volumetric flask, dissolve in and dilute to volume with water (*Diluted Lactose Solution*). Transfer, respectively, 1, 2, 3, and 4 mL of *Diluted Lactose Solution* into four 100-mL volumetric flasks, and dilute to volume with water. These dilutions (*Standard Dilutions of Lactose*) contain 20, 40, 60, and 80 μg of lactose per milliliter of solution, respectively. Into each of five test tubes add, in sequence, 2 mL of water and, respectively, 3 mL of each of the *Standard Dilutions of Lactose*. Add *Phenol Reagent* and sulfuric acid to each test tube as described under *Sample Solution*.

Apparatus Use a suitable spectrophotometer capable of operating in the visible range.

Calibration Determine the absorbance of each *Standard Solution* in a 1-cm pathlength cell at 490 nm against the water blank. Calculate the slope of the curve obtained by plotting absorbance versus micrograms per milliliter of lactose. The slope of the curve is the absorptivity (a) of the lactose–reagent product.

Procedure Determine the absorbance of the *Sample Solution* at 490 nm against a blank prepared using identical reagents.

Calculation Calculate the percent lactose (L) in the sample by the equation

$$L = (A \times 0.00475)/(a \times m),$$

in which A is the absorbance of the *Sample Solution* at 490 nm; the numerical factor accounts for dilution and conversion to percent from micrograms per milliliter; a is the absorptivity calculated under *Calibration*; and m is the weight, in grams, of the sample taken.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 102° for 3 h.

Packaging and Storage Store in well-closed containers.

Cassia Oil

Cinnamon Oil

FEMA: 2258 CAS: [8007-80-5]

View IR

DESCRIPTION

Cassia Oil occurs as a yellow or brown liquid having the characteristic odor and taste of cassia cinnamon. It is the volatile oil obtained by steam distillation from the leaves and twigs of *Cinnamomum cassia* Blume (Fam. Lauraceae), rectified by distillation, and consisting mainly of cinnamic aldehyde. Upon aging or exposure to air it darkens and thickens. It is soluble in glacial acetic acid and in alcohol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 80.0%, by volume, of total aldehydes.
Angular Rotation Between –1° and +1°.
Chlorinated Compounds Passes test.
Refractive Index Between 1.602 and 1.614 at 20°.
Rosin or Rosin Oils Passes test.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.045 and 1.063.

TESTS

Assay Determine as directed in *Neutral Sulfite Method* under *Aldehydes and Ketones*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Chlorinated Compounds Determine as directed under *Chlorinated Compounds*, Appendix VI.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Rosin or Rosin Oils Shake a 2-mL sample in a test tube with 5 to 10 mL of solvent hexane, allow the liquids to separate, decant the hexane layer, which is just slightly colored, into another test tube, and shake it with an equal volume of 1:1000 cupric acetate solution. The mixture does not turn green.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight, light-resistant containers. Avoid exposure to excessive heat.

Castor Oil

Ricinus Oil

INS: 1503 CAS: [8001-79-4]

DESCRIPTION

Castor Oil occurs as a pale yellow or almost colorless, transparent, viscous liquid. It is the fixed oil obtained from the seed of *Ricinus communis* L. (Fam. Euphorbiaceae) and consists mainly of the triglyceride of ricinoleic acid. It is soluble in alcohol, and is miscible with absolute alcohol, with glacial acetic acid, with chloroform, and with ether.

Function Antisticking agent; release agent; component of protective coatings.

REQUIREMENTS

Identification

A. A sample is only partly soluble in solvent hexane (distinction from *most other fixed oils*), but it yields a clear liquid with an equal volume of alcohol (*foreign fixed oils*).

B. Castor Oil exhibits the following composition profile of fatty acids determine as directed under *Fatty Acid Composition*, Appendix VII.

Fatty Acid:	16:0	18:0	18:1	18:3	18:0 di-OH
Weight % (Range):	0.9–1.6	1.0–1.8	3.7–6.7	0.2–0.6	0.4–1.3
Fatty Acid:	18:1-OH	20:0			
Weight % (Range):	83.6–89.0	0.2–0.5			

Free Fatty Acids Passes test.
Hydroxyl Value Between 160 and 168.
Iodine Value Between 83 and 88.
Lead Not more than 0.1 mg/kg.
Saponification Value Between 176 and 185.
Specific Gravity Between 0.952 and 0.966.

TESTS

Free Fatty Acids Dissolve about 10 g of sample, accurately weighed, in 50 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 *N* sodium hydroxide) contained in a flask. Add 1 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains pink after shaking for 30 s. Not more than 7 mL of 0.1 *N* sodium hydroxide is required for a 10.0-g sample.
Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII, using about 300 mg of sample, accurately weighed.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 3 g of sample, accurately weighed.
Specific Gravity Determine as directed under *Specific Gravity*, Appendix VII.

Packaging and Storage Store in tight containers, and avoid exposure to excessive heat.

Cedar Leaf Oil

Thuja Oil; White Cedar Leaf Oil

FEMA: 2267 CAS: [8007-20-3]

View IR

DESCRIPTION

Cedar Leaf Oil occurs as a colorless to yellow liquid having a strong camphoraceous and sage odor. It is the volatile oil obtained by steam distillation from the fresh leaves and branch ends of the eastern arborvitae, *Thuja occidentalis* L. (Fam. Cupressaceae). It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 60.0% of ketones, calculated as thujone ($C_{10}H_{16}O$).
Angular Rotation Between –7° and –14°.
Refractive Index Between 1.456 and 1.460 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.906 and 0.916.

TESTS

Assay Accurately weigh about 1 g of sample, and determine as directed in the *Hydroxylamine Method* under *Aldehydes and Ketones*, Appendix VI, using 76.10 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 70% alcohol, occasionally becoming cloudy on dilution to 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from glass or that are lined with tin.

Celery Seed Oil

View IR

DESCRIPTION

Celery Seed Oil occurs as a yellow to green-brown liquid with a pleasant, aromatic odor. It is the volatile oil obtained by steam distillation of the fruit or seed of *Apium graveolens* L. It is soluble in most fixed oils with the formation of a flocculent precipitate, and in mineral oil with turbidity. It is partly soluble in propylene glycol, but it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 4.5.
Angular Rotation Between +48° and +78°.
Refractive Index Between 1.480 and 1.490 at 20°.
Saponification Value Between 25 and 65.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.870 and 0.910.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed under *Saponification Value*, Appendix VI, using 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 8 mL of 90% alcohol, usually with turbidity.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Cellulose Gel

Cellulose, Microcrystalline

INS: 460 CAS: [9004-34-6]

DESCRIPTION

Cellulose Gel occurs as a fine, white or almost white powder. It is purified, partially depolymerized cellulose prepared by treating *alpha*-cellulose, obtained as a pulp from fibrous plant material, with mineral acids. It consists of free-flowing, nonfibrous particles that may be compressed into self-binding tablets that disintegrate rapidly in water. It is insoluble in water, in dilute acids, in dilute sodium hydroxide solutions, and in most organic solvents.

Function Anticaking agent; binding agent; dispersing agent.

REQUIREMENTS

Identification

A. Sift 20 g of sample for 5 min on an air-jet sieve equipped with a screen having 38-μm openings. If more than 5% is retained on the screen, mix 30 g of sample with 270 mL of water; otherwise, mix 45 g of sample with 255 mL of water. Mix for 5 min in a single-speed, high-speed (equal to or greater than 18,000 rpm) power blender (use a Waring Blender, Model 700G, or equivalent) that has a clover-shaped jar design. The jar and blades meet the following specifications: The jar has a 7.0-cm id at the bottom and a 9.2-cm id at the top and an overall height of 21.9 cm, and the four blades are arranged so that two of the blades are pointed up and two are pointed down. Transfer 100 mL of the dispersion into a 100-mL graduated cylinder, and allow it to stand for 3 h. A white, opaque, bubble-free dispersion that does not form a supernatant liquid at the surface is obtained.

B. Add a few drops of iodine TS to 20 mL of the dispersion obtained in *Identification Test A*, and mix. No purple to blue or blue color appears.

Assay Not less than 97.0% and not more than 102.0% of carbohydrate, calculated as cellulose on the dried basis.

Lead Not more than 2 mg/kg.
Loss on Drying Not more than 7.0%.
pH Between 5.0 and 7.5.
Residue on Ignition Not more than 0.05%.
Water-Soluble Substances Not more than 0.24%.

TESTS

Assay With the aid of about 25 mL of water, transfer about 125 mg of sample, accurately weighed, into a 300-mL Erlenmeyer flask. Add 50.0 mL of 0.5 *N* potassium dichromate, mix, then carefully add 100 mL of sulfuric acid, and heat to boiling. Remove the mixture from the heat, allow it to stand at room temperature for 15 min, cool it in a water bath, and transfer it into a 250-mL volumetric flask. Dilute almost to volume with water, cool to 25°, then dilute to volume with water, and mix. Titrate a 50.0-mL aliquot with 0.1 *N* ferrous ammonium sulfate, using 2 or 3 drops of orthophenanthroline TS as the indicator, and record the volume required, in milliliters, as *S*. Perform a blank determination (see *General Provisions*), and record the volume of 0.1 *N* ferrous ammonium sulfate required, in milliliters, as *B*. Calculate the percent cellulose in the sample by the formula

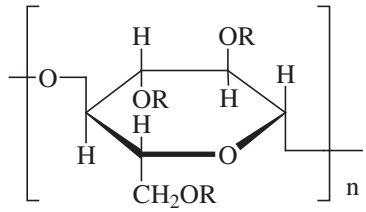
$$(B - S) \times 338/W,$$

in which *W* is the weight, in milligrams, of sample taken, corrected for *Loss on Drying* (below).
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample to constant weight at 105°.
pH Determine as directed under *pH Determination*, Appendix IIB, using the supernatant liquid from the following procedure: Shake about 5 g of sample with 40 mL of water for 20 min, and centrifuge.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.
Water-Soluble Substances Shake 5 g of sample with 80 mL of water for 10 min. Filter the mixture through Whatman No. 42, or equivalent, filter paper into a tared beaker, evaporate the filtrate to dryness on a steam bath, dry at 105° for 1 h, cool, and weigh.

Packaging and Storage Store in well-closed containers.

Cellulose Gum

Sodium Carboxymethylcellulose; CMC; Modified Cellulose



in which R = H or CH_2COONa

INS: 466 CAS: [9004-32-4]

DESCRIPTION

Cellulose Gum occurs as a white to cream colored powder or as granules. The powder is hygroscopic. It readily disperses in water to form colloidal solutions. It is insoluble in most solvents. A 1:100 aqueous suspension has a pH between 6.5 and 8.5.

Function Thickener; stabilizer.

REQUIREMENTS

Identification

A. While stirring to produce a uniform dispersion, add about 1 g of powdered sample to 50 mL of warm water. Continue stirring until a colloidal solution is produced, and then cool to room temperature. Save part of this solution for *Identification Test B*. Add 10 mL of cupric sulfate TS to about 10 mL of the solution. A fluffy, blue-white precipitate forms.

B. The solution from *Identification Test A* gives positive tests for *Sodium*, Appendix IIIA.

Assay Not less than 99.5% and not more than 100.5% of Cellulose Gum, calculated on the dried basis.
Degree of Substitution Not less than 0.2 and not more than 1.50 carboxymethyl groups (—CH_2COOH) per anhydroglucose unit after drying.
Lead Not more than 3 mg/kg.
Loss on Drying Not more than 10.0%.
Sodium Not more than 12.4% after drying.
Viscosity of a 2%, Weight in Weight, Solution Not less than 25 centipoises.

TESTS

Assay Calculate the percent Cellulose Gum by subtracting from 100 the percents of *Sodium Chloride* and *Sodium Glycolate* determined as follows:

Sodium Chloride Transfer about 5 g of sample, accurately weighed, into a 250-mL beaker, add 50 mL of water and 5 mL of 30% hydrogen peroxide, and heat on a steam bath for 20 min, stirring occasionally to ensure complete dissolution.

Cool, add 100 mL of water and 10 mL of nitric acid, and titrate with 0.05 *N* silver nitrate to a potentiometric endpoint, using a silver/calomel (AgCl) electrode set, and stirring constantly. Calculate the percent sodium chloride in the sample by the formula

$$(584.4 \times V \times N)/(100 - b)W,$$

in which 584.4 is an equivalence factor for sodium chloride; *V* and *N* represent the volume, in milliliters, and the normality, respectively, of the silver nitrate; *b* is the percent *Loss on Drying*, determined separately (below); and *W* is the weight, in grams, of the sample.

Sodium Glycolate Sample Solution Transfer about 500 mg of sample, accurately weighed, into a 100-mL beaker, moisten thoroughly with 5 mL of glacial acetic acid, followed by 5 mL of water, and stir with a glass rod until solution is complete (usually about 15 min). While stirring, slowly add 50 mL of acetone, then add 1 g of sodium chloride, and stir for several minutes to ensure complete precipitation of the Cellulose Gum. Filter through a soft, open-textured paper, previously wetted with a small amount of acetone, and collect the filtrate in a 100-mL volumetric flask. Use an additional 30 mL of acetone to facilitate transfer of the solids and to wash the filter cake, then dilute to volume with acetone, and mix.

Standard Solution Prepare a series of *Standard Solutions* as follows: Transfer 100 mg of glycolic acid, previously dried in a desiccator at room temperature overnight and accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Use this solution within 30 days. Transfer 1.0, 2.0, 3.0, and 4.0 mL, respectively, of this solution into separate 100-mL volumetric flasks, add sufficient water to each flask to make 5 mL, then add 5 mL of glacial acetic acid, and dilute to volume with acetone.

Procedure Transfer 2.0 mL of the *Sample Solution* and 2.0 mL of each *Standard Solution* into separate 25-mL volumetric flasks, and prepare a blank flask with 2.0 mL of a solution containing 5% each of glacial acetic acid and water in acetone. Place the uncovered flasks in a boiling water bath for exactly 20 min to remove the acetone, remove from the bath, and cool. Add to each flask 5.0 mL of 2,7-dihydroxynaphthalene TS, mix thoroughly, add an additional 15 mL, and again mix thoroughly. Cover the mouth of each flask with a small piece of aluminum foil. Place the flasks upright in a boiling water bath for 20 min, then remove from the bath, cool, dilute to volume with sulfuric acid, and mix.

Using a suitable spectrophotometer, determine the absorbance of each solution at 540 nm against the blank, and prepare a standard curve using the absorbance obtained from each of the *Standard Solutions*.

Calculation From the standard curve and the absorbance of the *Sample Solution*, determine the weight (*w*), in milligrams, of glycolic acid in the sample, and calculate the percent sodium glycolate in the sample by the formula

$$(12.9 \times w)/(100 - b)W,$$

in which 12.9 is a factor converting glycolic acid to sodium glycolate; *b* is the percent *Loss on Drying*, determined separately; and *W* is the weight, in grams, of the sample.

Degree of Substitution Transfer about 200 mg of sample, previously dried at 105° to constant weight, and accurately weighed, into a 250-mL, glass-stoppered Erlenmeyer flask. Add 75 mL of glacial acetic acid, connect the flask with a water-cooled condenser, and reflux gently on a hot plate for 2 h. Cool, transfer the solution to a 250-mL beaker with the aid of 50 mL of glacial acetic acid, and titrate with 0.1 *N* perchloric acid in dioxane while stirring with a magnetic stirrer.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Determine the endpoint potentiometrically with a pH meter equipped with a standard glass electrode and a calomel electrode modified as follows: Discard the aqueous potassium chloride solution contained in the electrode, rinse and fill with the supernatant liquid obtained by shaking thoroughly 2 g each of potassium chloride and silver chloride (or silver oxide) with 100 mL of methanol, then add a few crystals of potassium chloride and silver chloride (or silver oxide) to the electrode.

Record the milliliters of 0.1 *N* perchloric acid versus mV (0- to 700-mV range), and continue the titration to a few milliliters beyond the endpoint. Plot the titration curve, and read the volume (*A*), in milliliters, of 0.1 *N* perchloric acid at the inflection point.

Calculate the degree of substitution by the formula

$$(16.2\ A/G)/[1.000 - (8.0\ A/G)],$$

in which 16.2 is one-tenth of the molecular weight of one anhydroglucose unit; *A* is the volume, in milliliters, of 0.1 *N* perchloric acid required; *G* is the weight, in milligrams, of the sample taken; and 8.0 is one-tenth of the molecular weight of one sodium carboxymethyl group.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, using 2 g of sample and 6 μg of lead (Pb) ion in the control.

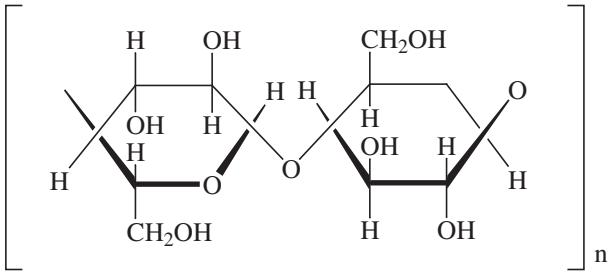
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample to constant weight at 105°.

Sodium From the weight of the sample and the number of milliliters of 0.1 *N* perchloric acid consumed in the determination of *Degree of Substitution* (above), calculate the percent sodium. Each milliliter of 0.1 *N* perchloric acid is equivalent to 2.299 mg of sodium (Na).

Viscosity of a 2%, Weight in Weight, Solution Determine as directed under *Viscosity of Cellulose Gum*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Cellulose, Powdered



INS: 460(ii) CAS: [9004-34-6]

DESCRIPTION

Cellulose, Powdered, occurs as a white substance and consists of fibrous particles that may be compressed into self-binding tablets that disintegrate rapidly in water. It exists in various grades, exhibiting degrees of fineness ranging from a dense, free-flowing powder to a coarse, fluffy, nonflowing material. It is purified, mechanically disintegrated cellulose prepared by processing bleached cellulose obtained as a pulp from such fibrous materials as wood or cotton. It is insoluble in water, in dilute acids, and in nearly all organic solvents. It is slightly soluble in 1 *N* sodium hydroxide.

Function Anticaking agent; binding agent; bulking agent; dispersing agent; filter aid; texturizing agent; thickening agent.

REQUIREMENTS

Identification

A. Mix approximately 30 g of sample with 270 mL of water in a high-speed (approximately 12,000 rpm) power blender for 5 min. The mixture will be either a free-flowing suspension or a heavy, lumpy suspension that flows poorly (if at all), settles only slightly, and contains many trapped air bubbles. The mixture is not slimy. If a free-flowing suspension is obtained, transfer 100 mL of it into a 100-mL graduated cylinder, and allow it to settle for 1 h. The solids settle in the cylinder and a supernatant liquid appears above the layer of sample. (Save the mixture for *Identification Tests D* and *E*.)

B. Boil 10 g of sample with 90 mL of water for 5 min, filter while hot through ashless, fine quantitative paper (S & S 589 Blue Ribbon, or equivalent), and add 2 drops of iodine TS to the filtrate. The color does not change from yellow-red.

C. Add 2 to 5 mg of sample to 20 mL of a 0.1% solution of anthrone in 75% sulfuric acid, and heat on a steam bath. The solution turns blue-green within 5 min.

D. Place a few drops of the stirred mixture from *Identification Test A* on a microscope slide, and insert a coverglass. Observe at 100 magnifications with a microscope. Fibers and fiber fragments are visible, regardless of the degree of fineness of the sample.

E. Dilute 10 mL of the stirred mixture from *Identification Test A* to 1000 mL with water, and filter 125 mL of the dilution through a Büchner funnel using Whatman No. 4 filter paper, or equivalent. Rinse the pad with 25 mL of acetone, and dry (paper included) at 105°. Transfer the powder to a tared weighing bottle, weigh, then transfer to a 50-mL Erlenmeyer flask, and seal with a rubber stopper. Record the weight, in milligrams, of the sample as *w*. Prepare 0.167 *M* and 1.0 *M* solutions of cupriethylenediamine (CED), determining the volumes of each as follows: 0.12 × *w* equals the milliliters of 0.167 *M* CED to use, and 0.08 × *w* equals the milliliters of 1.0 *M* CED to use. Add a few 3-mm glass beads and the calculated volume of 0.167 *M* CED, blow nitrogen over the surface of the solution, and shake for 2 min. Add the calculated volume of 1.0 *M* CED, again introduce the nitrogen, and shake vigorously for at least 3 min. A dark blue solution, clear under microscopic examination, appears.

Assay Not less than 97.0% and not more than 102.0% of carbohydrate, calculated as Cellulose.

Ash (Total) Not more than 0.3%.

Chloride Not more than 0.05%.

Lead Not more than 3 mg/kg.

Loss on Drying Not more than 7.0%.

pH Between 5.0 and 7.5.

Sulfur (Total) Not more than 0.01%.

Water-Soluble Substances Not more than 1.5%.

TESTS

Assay With the aid of about 25 mL of water, transfer about 125 mg of sample, accurately weighed, into a 300-mL Erlenmeyer flask. Add 50.0 mL of 0.5 *N* potassium dichromate, mix, then carefully add 100 mL of sulfuric acid, and heat to boiling. Remove the flask from the heat, allow the solution to stand at room temperature for 15 min, cool it in a water bath, and transfer the solution to a 250-mL volumetric flask. Dilute with water almost to volume, cool to 25°, dilute to volume with water, and mix. Titrate a 50-mL aliquot with 0.1 *N* ferrous ammonium sulfate, using 2 or 3 drops of orthophenanthroline TS. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the normality, *N*, of the ferrous ammonium sulfate solution by the formula

$$(0.1 \times 50)/B,$$

in which *B* is the volume, in milliliters, of ferrous ammonium sulfate solution required in the blank titration. Calculate the percent Cellulose in the sample by the formula

$$6.75(B - S) \times N/2W,$$

in which *S* is the volume, in milliliters, of ferrous ammonium sulfate solution used in the sample titration, and *W* is the weight, in grams, of the sample taken, corrected for moisture content (see *Loss on Drying*, below).

Ash (Total) Heat 3 g of sample at 550° ± 50° until completely charred, then ignite at 800° ± 25° until free from carbon, cool in a desiccator, and weigh.

Chloride Transfer about 5 g of sample, accurately weighed, into a 500-mL conical flask, add 250 mL of water, and reflux the mixture for 1 h. Filter through Whatman No. 4 filter paper, or equivalent, and reflux the sample with 200 mL of water

for 30 min. Filter as before, and combine the filtrates and hot water rinses. Add 1 mL of nitric acid, heat to boiling, and slowly add 5 mL of a 5% solution of silver nitrate. After the precipitate has coagulated, cool, and filter through a sintered-glass filtering funnel. Wash with a 1:100 nitric acid solution until free from silver nitrate, rinse with water, dry at 130°, and weigh. Perform a blank determination (see *General Provisions*) to obtain the corrected weight of the sample precipitate, each milligram of which is equivalent to 0.247 mg of chloride.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 3-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample to constant weight at 105°.

pH Determine as directed under *pH Determination*, Appendix IIB, using the supernatant liquid from the following preparation: Mix 10 g of sample with 90 mL of water, allow to stand with occasional stirring for 1 h.

Sulfur (Total) Transfer about 5 g of sample, previously dried at 105° to constant weight and accurately weighed, into a 300-mL conical flask, and add 50 mL of 2:3 perchloric acid:nitric acid (v/v).

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Heat on a hot plate under a hood, and boil until all organic matter has been destroyed and copious fumes of perchloric acid evolve. If the organic matter chars and cannot be destroyed quickly by further heating for a short time, add 10 to 20 mL of the acid mixture, and continue the treatment until a clear, syrupy residue is obtained.

> **Note**: All of the nitric acid must be driven from the flask, because it will otherwise form a double salt with the barium sulfate formed later.

Allow the mixture to cool for a few min, then add 200 mL of hot water, and heat again to boiling. (If the mixture is cloudy, filter, and rinse the filter with a small amount of hot water before boiling.) As soon as the mixture is boiling gently, carefully run in 20 mL of barium chloride TS, boil for a few minutes longer, and allow to stand for at least 12 h on a steam bath. Filter any barium sulfate onto an ashless filter paper, and rinse with five portions of boiling water to remove traces of perchloric acid. Place the paper in a tared platinum dish, dry in an oven at 105°, and ignite at 800° ± 25° for 1 h. Perform a blank determination (see *General Provisions*) to obtain the corrected weight of the sample precipitate, each milligram of which is equivalent to 0.137 mg of sulfur.

Water-Soluble Substances Mix 6 g of sample with 90 mL of recently boiled and cooled water, and allow to stand with occasional stirring for 10 min. Filter through Whatman No. 2 filter paper, or equivalent, discard the first 10 mL of filtrate, and pass the filtrate through the same filter a second time, if necessary, to obtain a clear filtrate. Evaporate a 15-mL portion of the filtrate to dryness in a tared evaporating dish on a steam bath, dry at 105° for 1 h, cool in a desiccator, and weigh.

Packaging and Storage Store in well-closed containers.

Chamomile Oil, English Type

CAS: [8015-92-7]

View IR

DESCRIPTION

Chamomile Oil, English Type, occurs as a light blue or light green-blue liquid with a strong, aromatic odor, characteristic of the flowers. The color may change with age to green-yellow or yellow-brown. It is the oil obtained by steam distillation of the dried flowers of the so-called English or Roman Chamomile, *Anthemis nobilis* L. (Fam. Asteraceae). It is soluble in most fixed oils, and it is almost completely soluble in mineral oil. It is soluble, with slight haziness, in propylene glycol, but it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Acid Value Not more than 15.0.

Ester Value Between 250 and 310.

Refractive Index Between 1.440 and 1.450 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.892 and 0.910.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.

Ester Value Determine as directed under *Ester Value*, Appendix VI, using about 1 g of sample, accurately weighed.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 80% alcohol, sometimes with a slight precipitate.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Chamomile Oil, German Type

Chamomile Oil, Hungarian Type

View IR

DESCRIPTION

Chamomile Oil, German Type, occurs as a deep blue or blue-green liquid with a strong, characteristic odor and a bitter, aromatic taste. When the oil is exposed to light or air, the blue color changes to green and finally to brown. It is the oil obtained by steam distillation of the flowers and stalks of *Matricaria chamomilla* L. (Fam. Asteraceae). Upon cooling, the oil may become viscous. It is soluble in most fixed oils and in propylene glycol. It is insoluble in glycerin and in mineral oil.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Between 5 and 50.
Ester Value Not more than 40.
Ester Value after Acetylation Between 65 and 155.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.910 and 0.950.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Ester Value Determine as directed under *Ester Value*, Appendix VI, using about 5 g of sample, accurately weighed.
Ester Value after Acetylation Determine as directed under *Ester Value*, Appendix VI, except acetylate a 10-mL sample as directed under *Total Alcohols*, Appendix VI, and use about 1.5 g of the dried, acetylated oil, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. The oil does not usually dissolve clearly in 95% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Chlorine

Cl_2 Formula wt 70.91

INS: 925 CAS: [7782-50-5]

DESCRIPTION

Chlorine occurs as a green-yellow gas, normally packaged as a liquid under pressure in containers approved by the U.S. Department of Transportation. At 60 °F, it has a vapor pressure of 70.91 psig. Its vapor density is about 2.5 times that of air. About 0.8 lb (0.362 kg) is soluble in 100 lb (45.4 kg) of water at 60 °F under atmospheric pressure.

> **Caution**: Chlorine gas is a respiratory irritant. Large amounts cause coughing, labored breathing, and irritation of the eyes. In extreme cases, the difficulty in breathing may cause death due to suffocation. Liquid Chlorine causes skin and eye burns on contact. (Safety precautions to be observed in handling the material are specified in the *Chlorine Manual*, available from The Chlorine Institute, Inc., Suite 506, 2001 L Street, N.W., Washington, D.C. 20036, <www.chl2.com>.)

Function Antimicrobial agent; bleaching agent; oxidizing agent.

REQUIREMENTS

Identification Cautiously pass a few milliliters of sample gas through 10 mL of 1 *N* sodium hydroxide that has previously been chilled in an ice bath. The resulting solution gives positive tests for *Chloride*, Appendix IIIA, and it darkens starch iodide paper.
Assay Not less than 99.5%, by volume.
Lead Not more than 10 mg/kg.
Mercury Not more than 1 mg/kg.
Moisture Not more than 0.015%, by weight.
Residue Not more than 0.015%, by weight, of nonvolatile matter.

TESTS

Assay Determine by ASTM Method E 412-93, ''Assay of Liquid Chlorine (Zinc Amalgam Method).''

> **Sample Solution for the Determination of Lead and Mercury** Dissolve the residue obtained under *Residue* (below) in 2.5 mL of freshly prepared aqua regia, and dilute with water to a volume, in milliliters, equivalent to the weight, in grams, of the initial sample. One milliliter of the final dilution is equivalent to 1 g of sample.

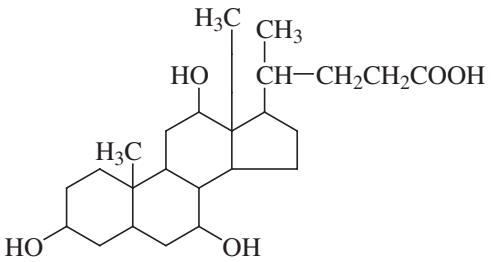
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 1.0-mL portion of the *Sample Solution* mixed with 5 mL of water and 11 mL of 2.7 *N* hydrochloric acid, and 10 μg of lead (Pb) ion in the control.

Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB, testing the *Sample Preparation* prepared as follows: Transfer 2.0 mL of the *Sample Solution* into a 50-mL beaker, add 10 mL of water, 1 mL of 1:5 sulfuric acid, and 1 mL of a 1:25 solution of potassium permanganate, cover the beaker with a watch glass, boil for a few seconds, and cool.
Moisture and Residue Determine by ASTM Method E 410-92, ''Moisture and Residue in Liquid Chlorine.''

Packaging and Storage Store in suitable pressure containers, observing applicable U.S. Department of Transportation regulations pertaining to shipping containers.

Cholic Acid

Cholalic Acid; 3,7,12-Trihydroxycholanic Acid



$C_{24}H_{40}O_5$ Formula wt 408.58

INS: 1000 CAS: [81-25-4]

DESCRIPTION

Cholic Acid occurs as colorless plates or as a white, crystalline powder. One gram dissolves in about 30 mL of alcohol or acetone and in about 7 mL of glacial acetic acid. It is very slightly soluble in water.

Function Emulsifier.

REQUIREMENTS

Identification Add 1 mL of a 1:100 furfural solution to 1 mL of a 1:5000 solution in 50% acetic acid. Cool in an ice bath for 5 min, add 15 mL of 1:2 sulfuric acid, mix, and warm in a water bath at 70° for 10 min. Immediately cool in an ice bath, and stir for 2 min. A blue color appears.
Assay Not less than 98.0% of $C_{24}H_{40}O_5$, calculated on the dried basis.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 0.5%.
Melting Range Between 197° and 202°.
Optical (Specific) Rotation $[\alpha]_D^{25°}$: Not less than +37°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 400 mg of sample, accurately weighed, into a 250-mL Erlenmeyer flask, add 20 mL of water and 40 mL of alcohol, cover with a watch glass, heat gently on a steam bath until dissolved, and cool. Add 5 drops of phenolphthalein TS, and using a 10-mL microburet, titrate with 0.1 *N* sodium hydroxide to the first pink color that persists for 15 s. Perform a blank determination (see *General Provisions*) and make any necessary correction. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 40.86 mg of $C_{24}H_{40}O_5$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 140° under a vacuum of not more than 5 mm Hg for 4 h.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution of sample in alcohol containing 200 mg of sample in each 10 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in tight containers.

Choline Bitartrate

(2-Hydroxyethyl)trimethylammonium-L-(+)-tartrate Salt

$$[HOCH_2CH_2N^+(CH_3)_3]C_4H_5O_6^-$$

$C_9H_{19}NO_7$ Formula wt 253.25

INS: 1001(v) CAS: [87-67-2]

DESCRIPTION

Choline Bitartrate occurs as a white, hygroscopic, crystalline powder. It is freely soluble in water, slightly soluble in alcohol, and insoluble in ether and in chloroform.

Function Nutrient.

REQUIREMENTS

Identification

A. Dissolve 500 mg of sample in 2 mL of water, add 3 mL of 1 *N* sodium hydroxide, and heat to boiling. The odor of trimethylamine is detectable.

B. Dissolve 500 mg of sample in 2 mL of iodine TS. A red-brown precipitate forms immediately. Add 5 mL of 1 *N* sodium hydroxide. The precipitate dissolves, and the solution

becomes clear yellow. Heat the solution. A pale yellow precipitate forms.

C. Add 1 mL of a 1:100 aqueous solution and 2 mL of a 1:50 solution of potassium ferrocyanide to 2 mL of cobaltous chloride TS. An emerald green color develops immediately.

Assay Not less than 98.0% of $C_9H_{19}NO_7$, calculated on the anhydrous basis.

1,4-Dioxane Passes test.

Lead Not more than 2 mg/kg.

Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Between 17.5° and 18.5°.

Residue on Ignition Not more than 0.1%.

Water Not more than 0.5%.

TESTS

Assay Transfer about 500 mg of sample, accurately weighed, into a 250-mL Erlenmeyer flask, add 50 mL of glacial acetic acid, and warm on a steam bath until solution is complete. Cool, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to a green endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 25.36 mg of $C_9H_{19}NO_7$.

1,4-Dioxane Determine as directed under *1,4-Dioxane*, Appendix IIIB.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 400 mg of sample per milliliter of water.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water Determine by drying a sample in a vacuum desiccator over phosphorus pentoxide for 4 h, or as directed under *Water Determination*, Appendix IIB, using a 2-g sample dissolved in 50 mL of methanol.

Packaging and Storage Store in tight containers.

Choline Chloride

(2-Hydroxyethyl)trimethylammonium Chloride

$$\left[HOCH_2CH_2N^+(CH_3)_3\right]Cl^-$$

$C_5H_{14}ClNO$ Formula wt 139.65

INS: 1001(iii) CAS: [67-48-1]

DESCRIPTION

Choline Chloride occurs as colorless or white crystals or as a crystalline powder. It is hygroscopic, and is very soluble in water and in alcohol.

Function Nutrient.

REQUIREMENTS

Identification

A. A sample responds to *Identification Tests A*, *B*, and *C* in the monograph for *Choline Bitartrate*.

B. A 1:20 aqueous solution gives positive tests for *Chloride*, Appendix IIIA.

Assay Not less than 98.0% and not more than 100.5% of $C_5H_{14}ClNO$, calculated on the anhydrous basis.

1,4-Dioxane Passes test.

Lead Not more than 2 mg/kg.

Residue on Ignition Not more than 0.05%.

Water Not more than 0.5%.

TESTS

Assay Transfer about 300 mg of sample, accurately weighed, into a 250-mL Erlenmeyer flask, add 50 mL of glacial acetic acid, and warm on a steam bath until solution is complete. Cool, add 10 mL of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to a green endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 13.96 mg of $C_5H_{14}ClNO$.

1,4-Dioxane Determine as directed under *1,4-Dioxane Limit Test*, Appendix IIIB.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 4-g sample.

Water Determine by drying a sample in a vacuum desiccator over phosphorus pentoxide for 4 h, or determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Cinnamon Bark Oil, Ceylon Type

CAS: [8015-91-6]

FEMA: 2290

View IR

DESCRIPTION

Cinnamon Bark Oil, Ceylon Type, occurs as a yellow liquid with an odor of cinnamon and a spicy burning taste. It is the volatile oil obtained by steam distillation from the dried inner bark of the clipped cinnamon shrub *Cinnamomum zeylanicum* Nees (Fam. Lauraceae). It is soluble in most fixed oils and in propylene glycol. It is insoluble in glycerin and in mineral oil.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 55.0% and not more than 78.0% of aldehydes, calculated as cinnamic aldehyde (C_9H_8O).
Angular Rotation Between –2° and 0°.
Refractive Index Between 1.573 and 1.591 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.010 and 1.030.

TESTS

Assay Determine as directed under *Aldehydes*, Appendix VI, using about 2.5 g of sample, accurately weighed, and using 66.10 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from glass or aluminum or that are lined with tin.

Cinnamon Leaf Oil

CAS: [8015-91-6]

FEMA: 2292

View IR

DESCRIPTION

Cinnamon Leaf Oil occurs as a light to dark brown liquid with a spicy cinnamon–clove odor and taste. It is the volatile oil obtained by steam distillation from the leaves and twigs of the true cinnamon shrub *Cinnamomum zeylanicum* Nees (Fam. Lauraceae). The commercial oils, according to the geographical origin, are designated as either Cinnamon Leaf Oil, Ceylon, or Cinnamon Leaf Oil, Seychelles, and the two types differ in physical and chemical properties. Cinnamon Leaf Oil is soluble in most fixed oils and in propylene glycol. It is soluble, with cloudiness, in mineral oil, but is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

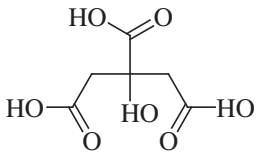
Labeling Indicate whether it is the Ceylon or Seychelles type.
Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay *Ceylon Type*: Not less than 80.0% and not more than 88.0%, by volume, of phenols; *Seychelles Type*: Not less than 87.0% and not more than 96.0%, by volume, of phenols.
Angular Rotation *Ceylon Type*: Between –2° and +1°; *Seychelles Type*: Between –2° and 0°.
Refractive Index *Ceylon Type*: Between 1.529 and 1.537; *Seychelles Type*: Between 1.533 and 1.540 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity *Ceylon Type*: Between 1.030 and 1.050; *Seychelles Type*: Between 1.040 and 1.060.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI, using a measure of filtered sample prepared as follows: Shake a suitable quantity of the oil with about 2% of powdered tartaric acid, and filter.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. *Ceylon Type*: One milliliter of sample dissolves in 1.5 mL of 70% alcohol. *Seychelles Type*: One milliliter of sample dissolves in 1 mL of 70% alcohol. The solutions may cloud upon further dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from glass or aluminum or that are lined with tin.

Citric Acid



$C_6H_8O_7$ Formula wt, anhydrous 192.13
$C_6H_8O_7 \cdot H_2O$ Formula wt, monohydrate 210.14

INS: 330 CAS: anhydrous [77-92-9]
CAS: monohydrate [5949-29-1]

DESCRIPTION

Citric Acid occurs as colorless, translucent crystals or as a white, granular to fine, crystalline powder. It is anhydrous or contains one molecule of water of hydration. The hydrous form is efflorescent in dry air. It is odorless and has a strongly acid taste. One gram is soluble in about 0.5 mL of water, in about 2 mL of alcohol, and in about 30 mL of ether.

Function Sequestrant; dispersing agent; acidifier; flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or hydrous.
Identification A 1:10 aqueous solution gives positive tests for *Citrate*, Appendix IIIA.
Assay Not less than 99.5% and not more than 100.5% of $C_6H_8O_7$, calculated on the anhydrous basis.
Lead Not more than 0.5 mg/kg.
Oxalate Passes test.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.05%.
Tridodecylamine (for solvent-extracted Citric Acid only) Not more than 0.1 mg/kg.
Water *Anhydrous*: Not more than 0.5%; *Monohydrate*: Not more than 8.8%.

TESTS

Assay Dissolve about 3 g of sample, accurately weighed, in 40 mL of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sodium hydroxide is equivalent to 64.04 mg of $C_6H_8O_7$.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Oxalate Neutralize 10 mL of a 1:10 aqueous solution with 6 *N* ammonium hydroxide, add 5 drops of 2.7 *N* hydrochloric acid, cool, and add 2 mL of calcium chloride TS. No turbidity forms.
Readily Carbonizable Substances Transfer 1.00 ± 0.01 g of finely powdered sample into a 150-mm × 18-mm (od) tube previously rinsed with 10 mL of 98% sulfuric acid at 90° or used exclusively for this test. Add 10 ± 0.1 mL of 98% sulfuric acid, carefully agitate the tube until solution is complete, and immerse the tube in a water bath at 90° ± 1° for 1 h. Occasionally remove the tube from the water bath, and carefully agitate it to ensure that the sample is dissolved and gaseous decomposition products are allowed to escape to the atmosphere. Cool the tube to ambient temperature, carefully shake it to ensure that all gases are removed, and using an adequate spectrophotometer, measure the absorbance and transmission of the solution at 470 nm in a 1-cm cell. The absorbance does not exceed 0.52, and the transmission is equal to or exceeds 30%.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 4-g sample.
Tridodecylamine (for solvent-extracted Citric Acid only)

Buffered Indicator Solution Prepare a mixture consisting of 700 mL of 0.1 *M* citric acid (anhydrous, reagent grade), 200 mL of 0.2 *M* disodium phosphate, and 50 mL each of 0.2% bromophenol blue and of 0.2% bromocresol green in spectrograde methanol.

No-Indicator Buffer Solution Prepare a mixture consisting of 700 mL of 0.1 *M* citric acid (anhydrous, reagent grade), 200 mL of 0.2 *M* disodium phosphate, and 100 mL of spectrograde methanol.

Amine Stock Solution Transfer between 40 and 45 mg of tridodecyl(trilauryl)amine, accurately weighed, into a 500-mL volumetric flask, dilute to volume with isopropyl alcohol, and mix. Discard after 3 weeks.

Standard Amine Solution Using a graduated 5-mL pipet, transfer an amount of *Amine Stock Solution* equivalent to 400 μg of tridodecylamine into a 100-mL volumetric flask, dilute to volume with isopropyl alcohol, and mix. Prepare this solution fresh on the day of use.

Sample Solution Either dissolve 160 g of anhydrous sample in 320 mL of water, or dissolve 174 g of monohydrate sample in 306 mL of water.

Procedure Dissolve 160 g of anhydrous, reagent-grade citric acid in 320 mL of water, and divide the solution equally between two 250-mL separators, S_1 and S_2. Add 5 mL of *No-Indicator Buffer Solution* to S_1. Add 2.0 mL of *Standard Amine Solution* and 5 mL of *Buffered Indicator Solution* to S_2. Divide the *Sample Solution* equally between two additional 250-mL separators, S_3 and S_4. Add 5 mL of *No-Indicator Buffer Solution* to S_3, and 5 mL of *Buffered Indicator Solution* to S_4.

Add 20 mL of a spectrograde chloroform:*n*-heptane (1:1 v/v) to each of the four separators, shake for 15 min on a mechanical shaker, and allow the phases to separate for 45 min. Drain all except the last few drops of the lower (aqueous) phases, and discard. Add 25 mL of 0.05 *N* sulfuric acid to the organic phases in each separator, hand-shake for 30 s, and allow the phases to separate for 30 min. Drain all except

the last few drops of the lower (organic) phases through dry Whatman No. 40, or equivalent, paper, and collect the aqueous filtrates in separate, small, glass-stoppered containers.

Using a suitable spectrophotometer standardized before analysis, determine the absorbance of each solution against chloroform:heptane (1:1 v/v) in a 5-cm cell at 400 nm. The net absorbance of the sample ($S_4 - S_3$) is not greater than that of the standard ($S_2 - S_1$).

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Clary Oil

Clary Sage Oil

CAS: [8016-63-5]

View IR

DESCRIPTION

Clary Oil occurs as a pale yellow to yellow liquid with a herbaceous odor and a winy bouquet. It is the oil obtained by steam distillation from the flowering tops and leaves of the clary sage plant, *Salvia sclarea* L. (Fam. Labiatae). It is soluble in most fixed oils, and in mineral oil up to 3 volumes, but it becomes opalescent on further dilution. It is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 48.0% and not more than 75.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Acid Value Not more than 2.5.
Angular Rotation Between –6° and –20°.
Refractive Index Between 1.458 and 1.473 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.886 and 0.929.

TESTS

Assay Determine as directed under *Ester Determination*, Appendix VI, using about 2 g of sample, accurately weighed, and using 98.15 as the equivalence factor (*e*) in the calculation.
Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 90% alcohol, becoming opalescent on further dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Clove Leaf Oil

CAS: [8015-97-2]

View IR

DESCRIPTION

Clove Leaf Oil occurs as a pale yellow liquid with a sharp, spicy, peppery odor and taste. It is the volatile oil obtained by steam distillation of the leaves of *Eugenia caryophyllata* Thunberg (*Eugenia aromatica* L. Baill.) (Fam. Myrtaceae). It is soluble in propylene glycol and in most fixed oils with slight opalescence, and it is relatively insoluble in glycerin and in mineral oil.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 84.0% and not more than 88.0%, by volume, of phenols.
Angular Rotation Between –2° and 0°.
Refractive Index Between 1.531 and 1.535 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.036 and 1.046.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI, using a measure of filtered sample prepared as follows: Shake a suitable quantity of sample with 2% powdered tartaric acid for about 2 min, and filter. Modify the test by heating the flask in a boiling water bath for 10 min after shaking the sample with 1 *N* potassium hydroxide. Remove from the boiling water bath, cool, and proceed as directed.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol. A slight opalescence may occur when additional solvent is added.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Clove Oil

Clove Bud Oil

CAS: [8000-34-8]

View IR

DESCRIPTION

Clove Oil occurs as a colorless or pale yellow liquid with a sharp, spicy odor and taste. It is the volatile oil obtained by steam distillation from the dried flowerbuds of *Eugenia caryophyllata* Thunberg (*Eugenia aromatica* L. Baill.) (Fam. Myrtaceae). It darkens and thickens upon aging or exposure to air.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 85.0%, by volume, of phenols.
Angular Rotation Between –1.5° and 0°.
Phenols Passes test.
Refractive Index Between 1.527 and 1.535 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.038 and 1.060.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Phenols Shake 1 mL of sample with 20 mL of hot water. The water shows no more than a scarcely perceptible acid reaction with blue litmus paper. Cool the mixture, pass the water layer through a wetted filter, and treat the clear filtrate with 1 drop of ferric chloride TS. The mixture has only a transient gray-green color, but not a blue or violet color.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in aluminum or tin- or epoxy-phenolic-lined, tight, light-resistant containers, and avoid exposure to excessive heat.

Clove Stem Oil

CAS: [8015-98-3]

View IR

DESCRIPTION

Clove Stem Oil occurs as a yellow to light brown liquid with a sharp, spicy odor and taste. It is the volatile oil obtained by steam distillation from the dried stems of the buds of *Eugenia caryophyllata* Thunberg (*Eugenia aromatica* L. Baill.) (Fam. Myrtaceae). It is soluble in fixed oils and in propylene glycol, but it is relatively insoluble in glycerin and in mineral oil.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 89.0% and not more than 95.0%, by volume, of phenols.
Angular Rotation Between –1.5° and 0°.
Refractive Index Between 1.534 and 1.538 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.048 and 1.056.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI, using a measure of filtered sample prepared as follows: Shake a suitable quantity of the sample oil with about 2% powdered tartaric acid for about 2 min, and filter. Modify the test by heating the flask in a boiling water bath for 10 min after shaking the sample oil with 1 *N* potassium hydroxide. Remove the flask from the boiling water bath, cool, and proceed as directed.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mL tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Cocoa Butter Substitute

DESCRIPTION

Cocoa Butter Substitute occurs as a white, waxy solid that is predominantly a mixture of triglycerides derived primarily from palm, safflower, sunflower, or coconut oils. The resulting products may be used directly or with cocoa butter in all proportions for the preparation of coatings. In contrast to many edible oils and hard butters, Cocoa Butter Substitute has an abrupt melting range, changing from a rather firm, plastic solid below 32° to a liquid at about 33.8° to 35.5°.

Function Coating agent; texturizer.

REQUIREMENTS

Identification Cocoa Butter Substitute exhibits the following typical composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	≤12	12:0	14:0	16:0	16:1
Weight % (Range):	0.0	0.0	0.0	21–24	0.0
Fatty Acid:	18:0	18:1	18:2	≥20	
Weight % (Range):	40–44	31–35	0.5–1.5	0.3–0.7	

Color (AOCS-Wesson) Not more than 2.5 red.
Free Fatty Acids (as oleic acid) Not more than 1.0%.
Hexane Not more than 5 mg/kg.
Iodine Value Between 30 and 33.
Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.
Residual Catalyst (as F) Not more than 0.5 mg/kg.
Total Glycerides Not less than 98.0% of total.
  Monoglycerides Not more than 1.0%.
  Diglycerides Not more than 7.0%.
  Triglycerides Not less than 90.0%.
Unsaponifiable Matter Not more than 1.0%.
Water Not more than 0.1%.

TESTS

Color (AOCS-Wesson) Determine as directed under *Color* (AOCS-Wesson), Appendix VII.

Free Fatty Acids (as oleic acid) Using the diglyceride fraction under *Total Glycerides* (below), determine as directed under *Free Fatty Acids*, Appendix VII, except add 2 mL of phenolphthalein TS, and titrate with the appropriate normality of sodium hydroxide. Use the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Hexane

Standard Preparation Using a micropipet, transfer and dissolve 34 µL of hexane in 45 g of cold-pressed cottonseed oil that has not been extracted with hexane. As directed under *Procedure* (below), analyze aliquots of 0.1, 0.25, 0.5, and 5.0 mg; the aliquots correspond to 2, 5, 10, and 100 mg/kg, respectively, of residual hexane in a 25-mg sample.

Assay Preparation Pack the lower half of 8.5-cm × 9.5-mm (od) borosilicate glass tubing (inlet liner) with glass wool that has been heated at 200° for 16 h to expel volatiles. Transfer 25 mg of sample, accurately weighed, into the glass tubing, and cover it with a small plug of treated glass wool.

Standard Curve Chromatograph aliquots of each *Standard Preparation* as directed under *Procedure*. Measure the peak areas for each *Standard Preparation*. Plot a standard curve using the concentration, in milligrams per kilogram, of each *Standard Preparation* versus its corresponding peak area, and draw the best straight line. To ensure that the relative standard deviation does not exceed 2.0%, chromatograph a sufficient number of replicates of each *Standard Preparation*, and record the areas as directed under *Procedure* (below).

Procedure (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph that is equipped with independent dual flame-ionization detectors and a 0.6-m × 6.35-mm (od) stainless-steel U-tube, or equivalent, packed with Porapak P, or equivalent. Maintain the inlet temperature at 110° and the detectors at 200°. Hold the column oven initially at 70° for 2 min followed by a linear temperature gradient at 5°/min to 180° and a final hold at 180° for 10 min or until the column is clean. Use helium as the carrier gas at a flow rate of 60 mL/min, hydrogen as the fuel gas at a flow rate of 52 mL/min for each flame, and air as the scavenger gas for both flames at a flow rate of 500 mL/min. Insert the *Assay Preparation* into the inlet liner of the gas chromatograph, immediately sealing the base of the inlet and the lower lip of the glass tubing with a silicone O-ring (Applied Science Laboratories, Inc., or equivalent) previously heated at 200° for 2 h to remove volatile impurities. Immediately close the inlet liner with the septum and septum liner. Allow the carrier gas to flow through the *Assay Preparation*, chromatograph, and record the chromatograms. Using the peak area of hexane eluting from the *Assay Preparation* at the same time as the *Standard Preparation*, read directly from the *Standard Curve* the concentration, *C*, of hexane, in milligrams per kilogram, of the *Assay Preparation*. Calculate the quantity of hexane, in milligrams per kilogram, in the sample taken by the formula

$$25C/W,$$

in which *W* is the weight, in milligrams, of the sample introduced into the gas chromatograph.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Peroxide Value Determine as directed in *Method II* under *Peroxide Value*, Appendix VII.
Residual Catalyst (as F) Determine as directed in *Method I* under *Fluoride Limit Test*, Appendix IIIB, beginning with ''. . . and 30 mL of water in a 125-mL distillation flask having a side arm and trap,'' using the ashed residue of the following as the sample: Transfer 30 g of sample, accurately weighed, into a 250-mL distillation flask having a side arm and a trap. Connect the flask with a condenser, and fit it with a thermometer and a capillary tube. Both of these should reach nearly to the bottom of the flask so that they extend into the liquid during the distillation. Add 0.2 g of silver sulfate, three boiling beads, and 25 mL of 1:1 sulfuric acid:water to the flask. Connect a dropping funnel or a steam generator to the capillary tube. Distill until the temperature reaches 135°. Then, through the capillary, add water from the funnel or introduce steam, as necessary, to maintain the temperature as close as possible to 135° until 250 mL of distillate has been collected in a beaker. Cool the distillate. Add 3 mL of 30% hydrogen peroxide to remove any sulfites, let it stand for 5 min, and evaporate the distillate in a dish containing 15 mL of saturated calcium hydroxide suspension. Ash the residue at 600° for 4 h.

The total volume of sodium fluoride TS required for the solutions from both *Distillate A* and *Distillate B* should not exceed 0.75 mL.
Total Glycerides Determine as directed under *Total Monoglycerides*, Appendix VII, except save all three elution fractions to determine the percentages of *Monoglycerides*, *Diglycerides*, and *Triglycerides*.

Note: Use toluene instead of benzene.

The diglyceride fraction also contains free fatty acids, the percentage of which is determined under *Free Fatty Acids*. Calculate the percentage of *Total Glycerides* (*G*), which is the sum of the percentages of *Monoglycerides*, *Diglycerides*, and *Triglycerides*, by the following formulas:

$$M = W_M 100/W_U,$$
$$D = (W_D 100/W_U) - F,$$
$$T = W_T 100/W_U,$$
$$G = M + D + T,$$

in which M is the percentage of monoglycerides; W_M is the weight, in grams, of monoglycerides; W_U is the weight, in grams, of the sample taken; D is the percentage of diglycerides; W_D is the weight, in grams, of diglycerides; F is the percentage of free fatty acids; T is the percentage of triglycerides; and W_T is the weight, in grams, of triglycerides.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of a 1:1 solution of chloroform:methanol to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Coconut Oil (Unhydrogenated)

CAS: [8001-31-8]

DESCRIPTION

Coconut Oil (Unhydrogenated) occurs as a viscous, white to light yellow-tan liquid. It is obtained from the kernel of the fruit of the coconut palm *Cocos nucifera* (Fam. Palmae). The crude oil obtained by mechanically pressing dried coconut meat (copra) is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and other non-oil materials. Compared with many natural fats, Coconut Oil (Unhydrogenated) has an abrupt melting range, changing from a rather firm, plastic solid at about 21° or below to a liquid at about 27°.

Function Coating agent; emulsifying agent; texturizer.

REQUIREMENTS

Identification Coconut Oil (Unhydrogenated) exhibits the following typical composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	6:0	8:0	10:0	12:0	14:0	16:0	16:1
Weight % (Range):	0–0.8	5–9	4–8	44–52	15–21	8–11	0–1
Fatty Acid:	18:0	18:1	18:2	20:0			
Weight % (Range):	1–4	5–8	0–2.5	0–0.4			

Arsenic Not more than 0.5 mg/kg.
Color (AOCS-Wesson) Not more than 20 yellow/2.0 red.
Free Fatty Acids *Oleic Acid*: Not more than 0.1%; *Lauric Acid*: Not more than 0.07%.
Iodine Value Between 6 and 11.
Lead Not more than 0.1 mg/kg.
Melting Range Between 23.5° and 27°.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared using 2 g of sample, accurately weighed. The absorbance caused by any red color from the solution of the sample does not exceed that produced by 1.0 mL of *Standard Arsenic Solution* (1 µg As) when treated in the same manner and under the same conditions as the sample.
Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factors (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Free fatty acids as lauric acid, $e = 20.0$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Melting Range Determine as directed under *Melting Range*, Appendix VII.
Peroxide Value Determine as directed in *Method II* under *Peroxide Value*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Cognac Oil, Green

Wine Yeast Oil

CAS: [8016-21-5]

View IR

DESCRIPTION

Cognac Oil, Green, occurs as a green to blue-green liquid with the characteristic aroma of cognac. It is the volatile oil obtained by steam distillation from wine lees. It is soluble in most fixed oils and in mineral oil. It is very slightly soluble in propylene glycol, and it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Between 32 and 70.
Angular Rotation Between –1° and +2°.
Ester Value Between 200 and 245.
Refractive Index Between 1.427 and 1.430 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.864 and 0.870.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed in *Ester Value* under *Esters*, Appendix VI, using about 1 g of sample, accurately weighed.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 80% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Copaiba Oil

CAS: [8013-97-6]

View IR

DESCRIPTION

Copaiba Oil occurs as a colorless to slightly yellow liquid with the characteristic odor of copaiba balsam and an aromatic, slightly bitter and pungent taste. It is the volatile oil obtained by steam distillation of copaiba balsam, an exudate from the trunk of various South American species of *Copaifera* L. (Fam. Leguminosae). It is soluble in alcohol, in most fixed oils, and in mineral oil. It is insoluble in glycerin and practically insoluble in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between –7° and –33°.
Gurjun Oil Passes test.
Refractive Index Between 1.493 and 1.500 at 20°.
Specific Gravity Between 0.880 and 0.907.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Gurjun Oil Add 5 or 6 drops of sample to 10 mL of glacial acetic acid containing 5 drops of nitric acid. No purple color appears within 2 min, indicating the absence of gurjun oil.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Copper Gluconate

$$\left[CH_2OH(CHOH)_4COO\right]_2Cu$$

$C_{12}H_{22}CuO_{14}$ Formula wt 453.84

CAS: [527-09-3]

DESCRIPTION

Copper Gluconate occurs as a fine, light blue powder. It is very soluble in water, and is very slightly soluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Copper*, Appendix IIIA.

B. Dissolve a quantity of sample in water, heating in a water bath at 60° if necessary, to obtain a *Test Solution* containing 10 mg/mL. Similarly, prepare a *Standard Solution* of USP Potassium Gluconate Reference Standard in water, diluting to 10 mg/mL. Apply separate 5-µL portions of the *Test Solution* and the *Standard Solution* on a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent system consisting of a mixture of alcohol, water, ammonium hydroxide, and ethyl acetate (50:30:10:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry it at 110° for 20 min. Allow it to cool, and spray it with a spray reagent prepared as follows: Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 *N* sulfuric acid to volume, and mix. After spraying, heat the plate at 110° for about 10 min. The principal spot obtained from the *Test Solution* corresponds in color, size, and R_f value to that obtained from the *Standard Solution*.

Assay Not less than 98.0% and not more than 102.0% of $C_{12}H_{22}CuO_{14}$.

Lead Not more than 5 mg/kg.

Reducing Substances Not more than 1.0%.

TESTS

Assay Dissolve about 1.5 g of sample, accurately weighed, in 100 mL of water in a 250-mL Erlenmeyer flask, add 2 mL of glacial acetic acid and 5 g of potassium iodide, mix well, and titrate with 0.1 *N* sodium thiosulfate to a light yellow color. Add 2 g of ammonium thiocyanate, mix, then add 3 mL of starch TS and continue titrating to a milk-white endpoint. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 45.38 mg of $C_{12}H_{22}CuO_{14}$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 1-g sample in 25 mL of water, and 5 µg of lead (Pb) ion in the control.

Reducing Substances Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, dissolve it in 10 mL of water, add 25 mL of alkaline cupric citrate TS, and cover the flask with a small beaker. Boil gently for exactly 5 min, and cool rapidly to room temperature. Add 25 mL of a 1:10 solution of acetic acid, 10.0 mL of 0.1 *N* iodine, 10 mL of 2.7 *N* hydrochloric acid, and 3 mL of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in milligrams, of reducing substances (as D-glucose) by the formula

$$27(V_1N_1 - V_2N_2),$$

in which 27 is an empirically determined equivalence factor for D-glucose; V_1 and N_1 are the volume and normality, respectively, of the iodine solution; and V_2 and N_2 are the volume and normality, respectively, of the sodium thiosulfate solution.

Packaging and Storage Store in well-closed containers.

Copper Sulfate

Cupric Sulfate

$CuSO_4$ Formula wt, anhydrous 159.6

$CuSO_4 \cdot 5H_2O$ Formula wt, pentahydrate 249.68

INS: 519

CAS: anydrous [7758-98-7]

CAS: pentahydrate [7758-99-8]

DESCRIPTION

Copper Sulfate occurs as blue crystals, crystalline granules, or powder. It effloresces slowly in dry air and is freely soluble in water, soluble in glycerin, and slightly soluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification A 1:20 solution gives positive tests for *Copper* and for *Sulfate*, Appendix IIIA.

Assay Not less than 98.0% and not more than 102.0% of $CuSO_4 \cdot 5H_2O$.

Iron Not more than 0.01%.

Lead Not more than 4 mg/kg.

Substances Not Precipitated by Hydrogen Sulfide Not more than 0.3%.

TESTS

Assay Dissolve about 1 g of sample, accurately weighed, in 50 mL of water, add 4 mL of glacial acetic acid and 3 g

of potassium iodide, mix well, and titrate with 0.1 *N* sodium thiosulfate to a light yellow color. Add 2 g of ammonium thiocyanate, mix, and then add 3 mL of starch TS, and continue titrating to a milky white endpoint. Perform a blank titration (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate used is equivalent to 24.97 mg of $CuSO_4 \cdot 5H_2O$.

Iron Add 2 mL of hydrochloric acid and 0.1 mL of nitric acid to the residue from *Substances Not Precipitated by Hydrogen Sulfide* (below), cover with a watch glass, and digest on a steam bath for 20 min. Remove the watch glass, and evaporate to dryness. Dissolve the residue in 1 mL of hydrochloric acid, and dilute to 60 mL with water. Dilute 5 mL of this solution to 40 mL with water, add 2 mL of hydrochloric acid, and dilute to 50 mL with water. Add 40 mg of ammonium peroxydisulfate crystals and 10 mL of ammonium thiocyanate TS, and mix thoroughly. Any red color produced within 1 h shall not exceed that produced by 0.033 mg of iron in an equal volume of solution containing the reagents used in the test.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Substances Not Precipitated by Hydrogen Sulfide Dissolve 5 g of sample in 200 mL of 1:100 sulfuric acid, heat to 70°, and pass hydrogen sulfide through the solution until the copper is completely precipitated. Dilute to 250 mL, mix thoroughly, allow the precipitate to settle, and filter. Evaporate 200 mL of the filtrate to dryness in a tared dish, ignite at 800° ± 25° for 15 min, cool, and weigh.

Packaging and Storage Store in tight containers.

Coriander Oil

CAS: [8008-52-4]

View IR

DESCRIPTION

Coriander Oil occurs as a colorless or pale yellow liquid with the characteristic odor and taste of coriander. It is the volatile oil obtained by steam distillation from the dried ripe fruit of *Coriandrum sativum* L. (Fam. Umbelliferae).

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Angular Rotation Between +8° and +15°.

Refractive Index Between 1.462 and 1.472 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.863 and 0.875.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 70% alcohol.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight containers protected from light. Avoid exposure to excessive heat.

Corn Oil (Unhydrogenated)

CAS: [8001-30-7]

DESCRIPTION

Corn Oil (Unhydrogenated) occurs as an amber-colored oil. It is obtained from the corn plant *Zea mays* (Fam. Gramineae), usually by solvent extraction of the corn germ. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and other non-oil materials. It is a liquid at 21° to 27°, but traces of wax, unless they are removed by winterization, may cause the oil to cloud when cooled to low temperature. It is free from visible foreign material (other than wax) at 21° to 27°.

Function Coating agent; emulsifying agent; texturizer.

REQUIREMENTS

Identification Corn Oil exhibits the following typical composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<1.0	8.0–19	<0.5	0.5–4.0	19–50	38–65
Fatty Acid:	18:3	20:0	20:1	22:0	22:1	24:0	
Weight % (Range):	<2.0	<1.0	<0.5	<0.3	<0.1	<0.4	

Arsenic Not more than 0.5 mg/kg.

Color (AOCS-Wesson) Not more than 5.0 red.

Free Fatty Acids (as oleic acid) Not more than 0.1%.

Iodine Value Between 120 and 130.

Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 2.0%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 4 g of sample, accurately weighed. The absorbance caused by any red color from the solution of the sample does not exceed that produced by 2.0 mL of *Standard Arsenic Solution* (2 µg As) when treated in the same manner and under the same conditions as the sample.
Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Linolenic Acid Determine as directed under *Fatty Acid Composition*, Appendix VII.
Peroxide Value Determine as directed in *Method II* under *Peroxide Value*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Costus Root Oil

CAS: [8023-88-9]

View IR

DESCRIPTION

Costus Root Oil occurs as a light yellow to brown, viscous liquid with a peculiar, persistent odor reminiscent of violet, orris, and vetivert. It is the volatile oil obtained by steam distillation from the dried, triturated roots of the herbaceous perennial plant *Saussurea lappa* Clarke (Fam. Compositae) or by a solvent extraction procedure followed by vacuum distillation of the resinoid extract. It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 42.
Angular Rotation Between +10° and +36°.
Ester Value Between 90 and 150.
Refractive Index Between 1.512 and 1.523 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.995 and 1.039.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed under *Ester Value*, Appendix VI, using about 1 g of sample, accurately weighed.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 0.5 mL of 90% alcohol, but the solution becomes cloudy upon further dilution, and paraffin crystals may occasionally separate.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Cottonseed Oil (Unhydrogenated)

CAS: [8001-29-4]

DESCRIPTION

Cottonseed Oil (Unhydrogenated) occurs as a dark red-brown oil. It is obtained from the seed of the cotton plant *Gossypium hirsutum* (American) or *Gossypium barbadense* (Egyptian) by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is liquid at 21° to 27°, clouds at 21°, and partially solidifies at storage temperatures

below 10° to 16°. It is free from visible foreign material at 23° to 27°.

Function Cooking or salad oil; component of margarine or shortening; tenderizer; carrier; stabilizer; thickener; coating agent; texturizer.

REQUIREMENTS

Identification Cottonseed Oil (Unhydrogenated) exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<14:0	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	0.5–2.0	17–29	<1.5	1.0–4.0	13–44	40–63
Fatty Acid:	18:3	20:0	20:1	22:0	22:1	24:0	
Weight % (Range):	0.1–2.1	<0.5	<0.5	<0.5	<0.5	<0.5	

Color (AOCS-Wesson) Not more than 70 yellow/4.5 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 99 and 119.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 2.1%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

$$\text{Free fatty acids as oleic acid, } e = 28.2.$$

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Linolenic Acid Determine as directed under *Fatty Acid Composition*, Appendix VII.
Peroxide Value Accurately weigh about 10 g of sample, add 30 mL of a 3:2 mixture of glacial acetic acid:chloroform, and mix. Add 1 mL of a saturated solution of potassium iodide, and mix for 1 min. Add 100 mL of water, begin titrating immediately with 0.05 *N* sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value, as milliequivalents of peroxide per kilogram of sample, by the formula

$$S \times N \times 1000/W,$$

in which *S* is the net volume, in milliliters, of sodium thiosulfate solution required for the sample; *N* is the exact normality of the sodium thiosulfate solution; and *W* is the weight, in grams, of sample taken.

Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Cubeb Oil

CAS: [8007-87-2]

View IR

DESCRIPTION

Cubeb Oil occurs as a colorless or light green to blue-green liquid with a spicy odor and a slightly acrid taste. It is the volatile oil obtained by steam distillation from the mature, unripe, sun-dried fruit of the perennial vine *Piper cubeba* L. (Fam. Piperaceae). It is soluble in most fixed oils and in mineral oil, but it is insoluble in glycerin and propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 2.0.
Angular Rotation Between –12° and –43°.
Refractive Index Between 1.492 and 1.502 at 20°.
Saponification Value Not more than 8.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.898 and 0.928.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed under *Saponification Value*, Appendix VI, using about 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 10 mL of 90% alcohol.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Cumin Oil

CAS: [8014-13-9]

View IR

DESCRIPTION

Cumin Oil occurs as a light yellow to brown liquid with a strong and somewhat disagreeable odor. It is the volatile oil obtained by steam distillation from the plant *Cuminum cyminum* L. (Fam. Umbelliferae). It is relatively soluble in most fixed oils and in mineral oil. It is very soluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 45.0% and not more than 54.0% of aldehydes, calculated as cuminaldehyde ($C_{10}H_{12}O$).
Angular Rotation Between +3° and +8°.
Refractive Index Between 1.500 and 1.506 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.905 and 0.925.

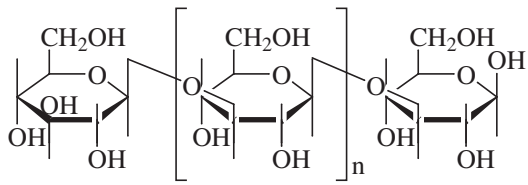
TESTS

Assay Determine as directed under *Aldehydes*, Appendix VI, using about 1 g of sample, accurately weighed, and 74.10 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand for 30 min at room temperature before titrating.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 8 mL of 80% alcohol. The solution may become hazy on the addition of more alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Curdlan

Beta-1,3-glucan



$(C_6H_{10}O_5)_n$

CAS: [54724-00-4]

DESCRIPTION

Curdlan occurs as a white to nearly white powder. It is a high-molecular-weight polymer of glucose (β-1,3-glucan) produced by pure-culture fermentation of a carbohydrate by a nonpathogenic and nontoxigenic strain of *Agrobacterium biobar* 1 (formerly *Alcaligenes faecalis* var. *myxogenes*) or *Agrobacterium radiobacter*. Curdlan consists of β-(1,3)-linked glucose residues and has the unusual property of forming an elastic gel when its aqueous suspension is heated to a temperature above 54°. It is insoluble in water, but is soluble in alkaline solutions.

Function Firming agent; gelling agent; stabilizer; thickener.

REQUIREMENTS

Identification

A. Add 5 mL of sulfuric acid TS to 10 mL of a 2% aqueous suspension of sample, heat in a boiling water bath for 30 min, and cool. Neutralize the mixture with barium carbonate, and centrifuge it at 900 g for 10 min. Add 1 mL of the supernatant to 5 mL of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide forms.

B. Heat a 2% aqueous suspension of sample in a boiling water bath for 10 min, and cool. A firm gel forms.

C. Suspend 0.2 g of sample in 5 mL of water, add 1 mL of 3 *N* sodium hydroxide, and shake. The sample dissolves.
Assay Not less than 80% (calculated as anhydrous glucose).
Gel Strength (2% aqueous suspension) Not less than 600 g/cm^2.
Lead Not more than 0.5 mg/kg.
Loss on Drying Not more than 10%.
Microbial Limits
Aerobic Plate Count Not more than 1000 CFU per gram.
E. coli Negative in 1 g.

Nitrogen Not more than 0.3%.
pH (1% aqueous suspension) Between 6.0 and 7.5.
Residue on Ignition Not more than 6%.

TESTS

Assay

Sample Solution Transfer about 100 mg of sample, accurately weighed, into a 100-mL volumetric flask, and dissolve in about 90 mL of 0.1 *N* sodium hydroxide. Add 0.1 *N* sodium hydroxide to volume, and mix well. Transfer 5 mL of the solution into a 100-mL volumetric flask, add water to volume, and mix well. Add 1 mL of a 5:100 solution of reagent-grade phenol and 5 mL of sulfuric acid TS to 1 mL of the solution, shake vigorously, and cool in ice-cold water. Prepare a blank and a *Reference Standard Solution* in the same manner, using 0.1 mL of water and 100 mg of reagent-grade glucose, respectively.

Procedure Determine the absorbance of the *Sample Solution* and the *Reference Standard Solution* in 1-cm cells at 490 nm with a suitable spectrophotometer, using the blank solution to zero it.

Calculation Calculate the percent Curdlan in the sample taken using the following equation:

$$\text{Curdlan } (\%) = (A/A_R) \times (0.9 \times W_R/W) \times 100,$$

in which A is the absorbance of the *Sample Solution*; A_R is the absorbance of the *Reference Standard Solution*; 0.9 is the molecular weight of anhydrous glucose divided by the molecular weight of glucose; W_R is the weight, in milligrams, of the reagent-grade glucose used to make the *Reference Standard Solution*; and W is the weight, in milligrams, of the sample.

Gel Strength (2% aqueous suspension)

Procedure Place 200 mg of sample into the tube of a Potter-Elvehjem homogenizer, add 10 mL of water, and homogenize at about 1500 g for 5 min. Transfer the suspension into a 16-mm × 150-mm test tube, de-aerate in vacuum for 3 min, and heat in a boiling water bath for 10 min to form a gel. Cool in running water, let it stand for 30 min, and remove the gel from the test tube. Accurately cut the gel at distances of 20 mm and 30 mm from the bottom to obtain a section 10 mm long. Determine the gel strength using a Rheo Meter Model CR-200D (Sun Scientific Co., Ltd., Japan; Load cell: 1000 g; set to a measurement mode 4) or an equivalent instrument capable of uniaxial compression and having a load cell sensitivity of 500 to 1000 g. Use a cylindrical stainless steel plunger with a 0.5-cm diameter. Lower the plunger into the gel at 250 mm/min. The resulting force-time curve is recorded and used for gel strength calculation.

Calculation Calculate gel strength by the following equation:

$$\text{Gel strength (g force/cm}^2) = f/0.196 \text{ cm}^2,$$

in which f is the force on the force-time curve that shows a sharp yielding downward trend associated with rupture of the gel, and 0.196 is the area, in centimeters squared, of the plunger.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in a vacuum for 5 h at 60°.
Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):
Aerobic Plate Count
E. coli
Nitrogen Determine as directed in *Method II* under *Nitrogen Determination*, Appendix IIIC, using a 1-g sample.
pH (1% aqueous suspension) Determine as directed under *pH Determination*, Appendix IIB, using a 1% aqueous suspension.
Residue on Ignition Determine as directed for *Method I* under *Residue on Ignition*, Appendix IIC, using a 1-g sample.

Packaging and Storage Store in airtight containers.

beta-Cyclodextrin

β-Cyclodextrin; BCD

$(C_6H_{10}O_5)_7$ Formula wt 1135.0

INS: 459 CAS: [7585-39-9]

DESCRIPTION

Beta-Cyclodextrin occurs as a white, fine, crystalline solid, frequently a fine, crystalline powder. It is a nonreducing cyclic compound consisting of seven alpha-(1,4) linked D-glucopyranosyl units. It is slightly soluble in water.

Function Encapsulating agent; stabilizer.

REQUIREMENTS

Identification

A. The infrared absorption spectrum of a potassium bromide dispersion of sample exhibits relative maxima at the same wavelengths as those of a similar preparation of USP beta-Cyclodextrin Reference Standard.

B. The retention time of the major peak in the chromatogram of *Assay Preparation* corresponds to that in the chromatogram of *Standard Preparation*, obtained as directed in the *Assay* (below).

Assay Not less than 98.0% and not more than 101.0% of $(C_6H_{10}O_5)_7$ as beta-Cyclodextrin, calculated on the anhydrous basis.
Lead Not more than 1 mg/kg.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +160° and +164°, calculated on the anhydrous basis.

Reducing Sugars (dextrose equivalent) Not more than 1.0%, calculated on the anhydrous basis.
Residue on Ignition Not more than 0.1%.
Toluene Not more than 1 mg/kg.
Trichloroethylene Not more than 1 mg/kg.
Water Not more than 14.0%.

TESTS

Assay

Mobile Phase Prepare a filtered and degassed 65:35 mixture of acetonitrile:water.

Internal Standard Solution Transfer 2.0 g of glycerol to a 100-mL volumetric flask. Dilute to volume with water, and mix. Filter through a 0.45-μm membrane filter. Use fresh or store in a freezer, thaw in hot water, and mix.

Standard Preparation Transfer 100 mg of USP beta-Cyclodextrin Reference Standard, accurately weighed, into a 10-mL volumetric flask. Dilute to volume with water, and mix. Use fresh or store in a freezer, thaw in hot water, and mix. Mix 1.0 mL of this solution with 1.0 mL of *Internal Standard Solution*.

System Suitability Preparation Transfer 50 mg each of USP alpha-Cyclodextrin Reference Standard and USP beta-Cyclodextrin Reference Standard, accurately weighed, into a 10-mL volumetric flask. Dilute to volume, and mix. Filter through a 0.45-μm membrane filter.

Assay Preparation Transfer 1 g of beta-Cyclodextrin, accurately weighed, into a 100-mL volumetric flask, dilute to volume with water, and mix. Filter this solution through a 0.45-μm membrane filter. Mix 1.0 mL of the filtered solution with 1.0 mL of *Internal Standard Solution*.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a liquid chromatograph equipped with a refractive index detector that can be maintained at a constant temperature of 25°, a 25-cm × 4.6-mm (id) column packed with 10-μm porous silica gel bonded with aminopropylsilane (Alltech 35643, or equivalent), and a guard column that contains the same packing. Maintain the column at a constant temperature of 25° ± 2°, and the flow rate at about 2.0 mL/min. Inject 20 μL of *System Suitability Preparation* into the chromatograph, and record the peak responses as directed under *Procedure*. The relative standard deviation for replicate injections is not more than 2.0%, and the alpha-Cyclodextrin and beta-Cyclodextrin peaks exhibit baseline separation, the relative retention times being about 0.8 and 1.0, respectively.

Procedure Separately inject about 20 μL of *Assay Preparation* and *Standard Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in milligrams, of $(C_6H_{10}O_5)_7$ in the portion of beta-Cyclodextrin taken by the formula

$$100 \times C \times (R_U/R_S),$$

in which C is the concentration, in milligrams per milliliter, of anhydrous beta-Cyclodextrin in the *Standard Preparation*, as determined from the concentration of USP beta-Cyclodextrin Reference Standard corrected for moisture content by a titrimetric water determination, and R_U and R_S are the peak response ratios of the beta-Cyclodextrin peak to the internal standard peak obtained from the *Assay Preparation* and the *Standard Preparation*, respectively.

Lead Determine as directed in *Method II* for the *Flame Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 1 g of cyclodextrin dissolved in 100 mL of water.

Reducing Sugars Determine as directed under *Reducing Sugars Assay*, Appendix X, using 60 to 120 mg of sample, accurately weighed.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, using a 1- to 2-g sample.

Toluene

Toluene Stock Solution Transfer 100 mg, accurately weighed, or 115.3 μL, accurately measured, of toluene into a 100-mL volumetric flask, and dilute to volume with methanol. This solution contains 1000 μg of toluene in each milliliter.

Toluene Standard Solutions Prepare five working standard solutions with concentrations of 10, 50, 100, 250, and 500 μg/mL by quantitatively diluting *Toluene Stock Solution* with methanol.

α,α,α-Trifluorotoluene Stock Solution Transfer 80 mg, accurately weighed, or 67.3 μL, accurately measured, of α,α,α-trifluorotoluene into a 100-mL volumetric flask, and dilute to volume with methanol. The resulting solution contains 800 μg of α,α,α-trifluorotoluene in each milliliter.

α,α,α-Trifluorotoluene Standard Solutions Prepare three working standard solutions with concentrations of 80, 400, and 800 μg/mL by quantitatively diluting *α,α,α-Trifluorotoluene Stock Solution* with methanol. These are the surrogate standards.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph connected to a purge and trap apparatus (see below). The gas chromatograph is equipped with a photoionization detector and a 30-m × 0.53-mm (id) fused silica open tubular column, or equivalent, with a 1.5-μm crossbonded 5% diphenyl, 95% dimethyl polysiloxane (Restek RTX-5, or equivalent) stationary phase. Use ultra-high-purity helium as the carrier gas at a flow rate of 20 mL/min. Program the column temperature according to the following steps: Hold it at 40° for 2 min, then increase it to 180° at a rate of 20°/min, and hold it at 180° for 2 min.

Purge and Trap Apparatus The purging apparatus uses disposable 15- × 150-mm test tubes. Use ultra-high-purity helium to purge the sample for 15 min at a flow rate of 60 mL/min. Maintain at 100° all lines that the sample vapor passes through in the purge module.

The trap consists of a 30.5-cm × 2.7-mm (id) stainless steel tube, with a packing of a porous polymer based on 2,6-diphenyl-*p*-phenylene oxide (Tenax, or equivalent). The length of the packing in the tube is 24 cm. The entire void volume of the trap is at the vented end of the trap column. Maintain the trap at 100°. Recondition the trap for a subsequent run by baking it for 5 min at 190°.

Procedure

Calibration Place an empty purge tube into the purging apparatus. Fill a 5-mL syringe with 0.5 *N* sodium hydroxide, and inject into this solution an accurately measured 5-µL aliquot of 10 µg/mL *Toluene Standard Solution*. Introduce this solution into the purge tube. Start the instrument for the run, automated if desired, by purging for 15 min, then heating the trap at 180° for 3 min. Repeat this sequence for each of the *Toluene Standard Solutions* and the α,α,α-*Trifluorotoluene Standard Solutions*.

Plot standard curves of the standard concentration (C_S), in micrograms per milliliter, versus detector response (r_S) for the *Toluene Standard Solutions* and α,α,α-*Trifluorotoluene Standard Solutions*.

Determination To prepare the *Sample Preparation*, transfer 500 mg of sample, accurately weighed, and about 0.1 g of salicylic acid into a purge tube, and attach the purge tube to the purging apparatus. Add 5 µL of 400-µg/mL α,α,α-*Trifluorotoluene Standard Solution* to a 5-mL syringe filled with 0.5 *N* sodium hydroxide. Add the contents of the syringe to the sample in the purge apparatus, and start the run as described under *Calibration*. The procedure is valid only when the detector response of the surrogate standard in the *Sample Preparation* is within ± 15% of the value from the standard curve for α,α,α-trifluorotoluene. Calculate the concentration, in micrograms per gram (numerically equivalent to milligrams per kilogram), of toluene in the sample taken by the formula

$$5C_S/W_S,$$

in which C_S is the concentration, in micrograms per milliliter, of toluene from the toluene standard curve based on the detector response for toluene obtained from the *Sample Preparation*, and W_S is the weight, in grams, of sample taken for the assay.

Trichloroethylene

Purge and Trap Apparatus The apparatus[1] comprises three sections: the sample purge, the trap, and the desorber. The sample purge accepts 5-mL samples with a water column not less than 3 cm deep, and the gaseous headspace between the water column and the trap has a total volume of not more than 15 mL. The purge gas is passed through the water column as finely divided bubbles with a diameter of less than 3 mm at the origin and is introduced not more than 5 mm from the base of the water column.

Use a trap not shorter than or narrower than 25 cm × 2.67 mm (id). Pack the trap to contain the indicated minimum lengths of adsorbents in the following order, beginning at the trap inlet: 7.7 cm of 2,6-diphenylene oxide polymer (TENAX GC, or equivalent), 7.7 cm of silica gel, and 7.7 cm of coconut charcoal.

The desorber is capable of rapidly heating the trap to 250°, which is the maximum temperature to be used.

Condition the assembled trap before use at 225° overnight with an inert gas at a flow rate of not less than 20 mL/min. Before daily use, condition the trap for 15 min at 225°.

Standard Solution Transfer 50 mg of reagent-grade trichloroethylene, accurately weighed, to a 50-mL volumetric flask. Dilute with methanol to volume, and mix.

Diluted Standard Solution Accurately transfer 0.5, 1.0, 2.0, 3.0, and 5.0 mL of the *Standard Solution* into five 50-mL volumetric flasks, and dilute to volume with water. These *Diluted Standard Solutions* correspond to trichloroethylene concentrations of 10.2, 20.4, 40.8, 61.2, and 102 ng/µL.

Chromatographic System (See *Chromatography*, Appendix IIA.) Connect the purge and trap apparatus to the gas chromatograph with a flame-ionization detector. Use a gas chromatograph equipped with a 30-m × 0.32-mm (id) capillary column coated with a 1-µm film thickness of dimethylpolysiloxane oil (such as DB-1, OV-1, or equivalent). Hold the column temperature initially at 40° for 3 min, then program it to rise to 220° at 4°/min. Set the detector temperature to 280°. Use helium as the carrier gas, and nitrogen as the purge gas, at a flow rate of 40 mL/min.

Procedure Introduce exactly 20 µL of each *Diluted Standard Solution* on the inner wall of the sample purge. Desorb according to equipment instructions, and record the peak areas. Prepare a calibration graph by plotting the peak area responses versus the weight of trichloroethylene introduced into the purge.

Introduce about 250 mg of sample (*W*) accurately weighed, on the fritted sparger of the sample purge. Purge and desorb according to equipment instructions. Record the peak area of trichloroethylene, and read the corresponding weight (*X*) of trichloroethylene from the calibration curve. Calculate the amount of trichloroethylene by the formula

$$\text{trichloroethylene (mg/kg)} = X\text{ (ng)}/W\text{ (mg)}.$$

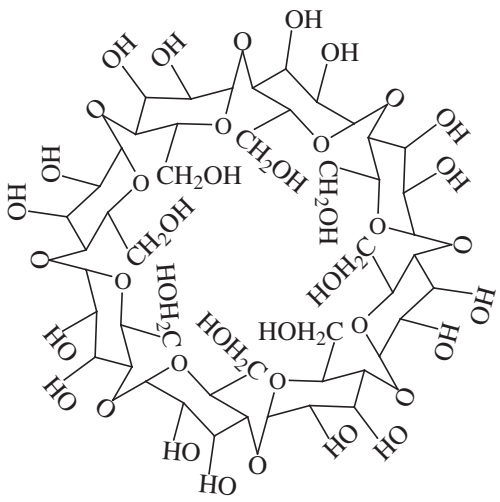
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers in a dry place.

[1]The apparatus used is based on that described in the U.S. Environmental Protection Agency Test Method for Purgeable Halocarbons—Method 601.

gamma-Cyclodextrin

γ-Cyclodextrin; gamma-CD; Cyclooctaamylose; Cyclomaltooctaose



$(C_6H_{10}O_5)_8$ Formula wt 1297.14

CAS: [17465-86-0]

View IR

DESCRIPTION

Gamma-Cyclodextrin occurs as a white or almost white crystalline solid. It is a nonreducing cyclic saccharide consisting of eight α-1,4-linked D-glucopyranosyl units manufactured by the action of cyclomaltodextrin glucanotransferase on hydrolyzed starch followed by purification of the gamma-Cyclodextrin. It is freely soluble in water and is very slightly soluble in ethanol.

Function Stabilizer; emulsifier; carrier.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.0% as $(C_6H_{10}O_5)_8$, calculated on the anhydrous basis.

Iodine Reaction Passes test.

Lead Not more than 1 mg/kg.

Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +174° and +180° in a 1% solution.

Reducing Sugars (as glucose) Not more than 0.5%.

Residue on Ignition Not more than 0.1%.

Volatile Organic Compounds Not more than 20 mg/kg.

Water Not more than 11.0%.

TESTS

Assay

Sample Solution Transfer 1.0 g of sample, accurately weighed, into a 100-mL flask, dilute to volume with water, and mix.

Chromatographic System Determine as directed under *Chromatography*, Appendix IIA, but use a liquid chromatograph equipped with a differential refractometer detector and a 30-cm × 7.8-mm (id) column packed with 25-μm diameter beads of silver bonded to sulfonated divinyl benzene–styrene copolymer (Aminex HPX-42A, Bio-Rad Laboratories, or equivalent). Maintain the column at a constant temperature of 65° ± 10°, and the flow rate at 0.3 to 1.0 mL/min. Use deionized water as the mobile phase.

Procedure Inject about 20 μL of the *Sample Solution* into the chromatograph, record the chromatogram, and measure the responses for all peaks.

Calculation Calculate the content of gamma-Cyclodextrin in the sample by the peak area percentage method using the following equation:

$$A = (B/C) \times 100,$$

in which A is the percentage of gamma-Cyclodextrin in the sample, B is the peak area of gamma-Cyclodextrin in the chromatogram, and C is the sum of the areas of all peaks recorded in the chromatogram.

Iodine Reaction Place 0.2 g of sample in a test tube, and add 2 mL of a 0.1 *N* iodine solution. Heat the mixture in a water bath, and allow to cool at room temperature. A clear, brown solution forms.

Lead Reflux about 5 g of sample, accurately weighed, with 30 mL of nitric acid for 1 h. Remove the reflux condenser, and attach a condenser to the flask. Continue to heat, and collect the distilled nitric acid. Allow the residue to cool, add 20 mL of water, and allow it to cool again. Add 2 mL of orthophosphoric acid, dilute to 100 mL with water, and determine the lead content of the solution as directed for *Method I* in *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB.

Reducing Sugars (as glucose) Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, dissolve in 10 mL of water, add 25 mL of alkaline cupric citrate TS, and cover the flask with a small beaker. Boil gently for exactly 5 min, and cool rapidly to room temperature. Add 25 mL of 10% acetic acid solution, 10.0 mL of 0.1 *N* iodine, 10 mL of dilute hydrochloric acid TS, and 3 mL of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the content of reducing substances (as D-glucose) (R) by the equation

$$\%R = [(V_1N_1 - V_2N_2) \times 2.7]/W,$$

in which V_1 and N_1 are the volume, in milliliters, and the normality, respectively, of the iodine solution; V_2 and N_2 are the volume, in milliliters, and the normality, respectively, of the sodium thiosulfate solution; 2.7 is an empirically determined equivalence factor for D-glucose; and W is the weight, in grams, of the sample.

Residue on Ignition Determine as directed in *Method I (for Solids)* under *Residue on Ignition (Sulfated Ash)*, Appendix IIC.

Volatile Organic Compounds Dissolve 50 g of sample in about 700 mL of water in a 1-L round-bottom flask, and add a magnetic stirrer. Attach the flask to the lower part of a Bleidner

apparatus (see Figure 1), and connect a 100-mL round-bottom flask containing about 70 mL of hexane and a few boiling stones to the other side of the apparatus. Fill the Bleidner apparatus with equal amounts of water and hexane, and place a reflux condenser on the top. Heat both flasks with heating mantels to boiling. Using the magnetic stirrer, stir the contents of the 1-L flask well. Keep the content of the two flasks boiling for 8 h. After cooling, remove the 100-mL flask, transfer the contents to a 100-mL volumetric flask, and fill that flask to volume with hexane.

Analyze the hexane solution as directed under *Gas Chromatography*, Appendix IIA, with the following conditions: Use a gas chromatograph equipped with a flame-ionization detector and a 30-m × 0.32-mm (id) column with a stationary phase consisting of 0.25-μm, cross-bonded, 95% dimethyl 5% diphenyl polysiloxane (JBW Scientific DB-5.625, or equivalent). Set the injector to 280°, and hold the temperature at 70° for 4 min, and then increase it to 250° at intervals of 10° per min. Use nitrogen gas as a carrier with a flow rate of 70 mL per minute. The detection is FID at 280°.

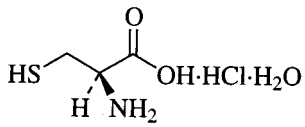
Calculate the area(s) under the peak for each volatile organic compound, and convert it to milligrams per kilograms of gamma-Cyclodextrin using the response factor for 8-cyclohexadecen-1-one. The response factor is determined from a calibration curve using 8-cyclohexadecen-1-one concentrations of 0.1 to 6 mg/100 mL of hexane.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers in a dry place.

L-Cysteine Monohydrochloride

L-2-Amino-3-mercaptopropanoic Acid Monohydrochloride



$C_3H_7NO_2S·HCl$ Formula wt, anhydrous 157.62
$C_3H_7NO_2S·HCl·H_2O$ Formula wt, monohydrate 175.63
CAS: anhydrous [52-89-1]
INS: 920 CAS: monohydrate [7048-04-6]

View IR

DESCRIPTION

L-Cysteine Monohydrochloride occurs as a white, crystalline powder. It is freely soluble in water and in alcohol. The anhydrous form melts with decomposition at about 175°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits maxima only at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same conditions as specified therein.

Assay Not less than 98.0% and not more than 101.5% of $C_3H_7NO_2S·HCl$, calculated on the dried basis.

Lead Not more than 5 mg/kg.

Loss on Drying Not less than 8.0% and not more than 12.0%.

Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +5.0° and +8.0°; or $[\alpha]_D^{25°}$: Between +4.9° and +7.9°, calculated on the dried basis.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 300 mg of sample, previously dried as directed under *Loss on Drying* (below) and accurately weighed, into a 250-mL glass-stoppered flask. Add 20 mL of water, 4 g of potassium iodide, 5 mL of 2.7 *N* hydrochloric acid, and 25.0 mL of 0.1 *N* iodine. Stopper the flask, allow the mixture to stand for 30 min in a dark place, and titrate the excess iodine with 0.1 *N* sodium thiosulfate. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* iodine is equivalent to 15.76 mg of $C_3H_7NO_2S·HCl$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at room temperature for 24 h in a vacuum desiccator using a suitable desiccant and maintaining a pressure of not more than 5 mm Hg.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 8 g of undried sample in sufficient 1 *N* hydrochloric acid to make 100 mL.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

L-Cystine

3,3'-Dithiobis(2-aminopropanoic acid)

$HOOCCH(NH_2)CH_2SSCH_2CH(NH_2)COOH$

$C_6H_{12}N_2O_4S_2$ Formula wt 240.30
INS: 921 CAS: [56-89-3]

View IR

DESCRIPTION

L-Cystine occurs as colorless to white crystals. It is soluble in diluted mineral acids and in alkaline solutions. It is very slightly soluble in water and in alcohol.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_{12}N_2O_4S_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.2%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between −215° and −225°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Determine as directed under *Nitrogen Determination*, Appendix IIIC, using a 200-mg sample. Percent L-Cystine equals percent $N \times 8.58$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 2 g of a previously dried sample in sufficient 1 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed containers.

Dammar Gum

Dammar Resin; Damar Gum; Damar Resin; Dammar

CAS: [9000-16-2]

DESCRIPTION

Crude Dammar Gum occurs as irregular, white to yellow to brown tears, fragments, or powder, sometimes admixed with fragments of bark. Refined grades are white to yellow and are free of fragments of ligneous matter. Dammar Gum is the dried exudate from trees of the *Agathis*, *Hopea*, or *Shorea* genera. It consists of a complex mixture of acidic and neutral terpenoid compounds together with polysaccharide material. It is insoluble in water and in ethanol and is soluble in toluene and in limonene. A chloroform solution of Dammar Gum is dextrorotatory.

Function Stabilizer; glazing agent.

REQUIREMENTS

Identification Prepare a 10% solution of sample in chloroform, and spot 20 µL of it on a thin-layer plate coated with a 0.2-mm layer of silica (Merck F254, or equivalent) in a previously equilibrated chamber. Elute with a 30:25 mixture of diethyl ether:heptane. In a suitable fume hood, spray the plate with sulfuric acid, and dry it at 180° for 3 min. Two dark spots are observed at R_f with values of 0.8 and 0.7, and with the ratio of the faster-moving spot to the second spot being about 1.1.
Acid Number Between 20 and 40.
Ash (Total) Not more than 0.5%.
Iodine Value Between 10 and 40.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 6.0%.
Melting Range Between 90° and 95°.
Softening Point Between 86° and 90°.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX, but modified as follows: Add 30 mL of toluene and 30 mL of neutral ethanol to an accurately weighed 5-g sample. Titrate with 0.5 *N* alcoholic potassium hydroxide, using phenolphthalein TS as the indicator.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 18 h.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Softening Point Determine as directed for the *Ring-and-Ball Method* under *Softening Point*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Decanoic Acid

Capric Acid

$$CH_3(CH_2)_8COOH$$

$C_{10}H_{20}O_2$ Formula wt 172.27

CAS: [334-48-5]

FEMA: 2364

View IR

DESCRIPTION

Decanoic Acid occurs as white crystals having a sour, fatty, rancid odor. It is soluble in most organic solvents and practically insoluble in water.

Function Component in the manufacture of other food-grade additives; defoaming agent; flavoring agent.

REQUIREMENTS

Acid Value Between 320 and 329.
Iodine Value Not more than 0.6.
Residue on Ignition Not more than 0.1%.
Titer (Solidification Point) Between 27° and 32°.
Unsaponifiable Matter Not more than 0.2%.
Water Not more than 0.2%.

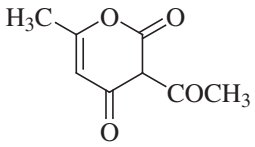
TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 10-g sample.
Titer (Solidification Point) Determine as directed under *Solidification Point*, Appendix IIB.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Dehydroacetic Acid

3-Acetyl-6-methyl-1,2-pyran-2,4(3H)-dione;
Methylacetopyronone



$C_8H_8O_4$ Formula wt 168.15

CAS: [520-45-6]

DESCRIPTION

Dehydroacetic Acid occurs as a white or nearly white, crystalline powder. It is soluble in aqueous solutions of fixed alkalies, and is very slightly soluble in water. One gram of sample dissolves in about 35 mL of alcohol and in 5 mL of acetone.

Function Antimicrobial agent; preservative.

REQUIREMENTS

Identification The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Dehydroacetic Acid Reference Standard.
Assay Not less than 98.0% and not more than 100.5% of $C_8H_8O_4$, calculated on the dried basis.
Lead No more than 0.5 mg/kg.
Loss on Drying Not more than 1%.
Melting Range Between 109° and 111°.
Residue on Ignition Not more than 0.1%.

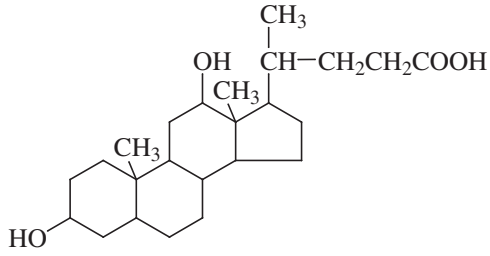
TESTS

Assay Transfer about 500 mg of sample, accurately weighed, into a 250-mL Erlenmeyer flask, dissolve it in 75 mL of neutral alcohol, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for at least 30 s. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 16.82 mg of $C_8H_8O_4$.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 80° for 4 h.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed containers.

Desoxycholic Acid

Deoxycholic Acid; 13α,12α-Dihydroxycholanic Acid



$C_{24}H_{40}O_4$ Formula wt 392.58

CAS: [83-44-3]

DESCRIPTION

Desoxycholic Acid occurs as a white, crystalline powder. It is practically insoluble in water, slightly soluble in chloroform

and in ether, soluble in acetone and in solutions of alkali hydroxides and carbonates, and freely soluble in alcohol.

Function Emulsifier.

REQUIREMENTS

Identification Add 2 drops of benzaldehyde and 3 drops of 75% sulfuric acid to about 10 mg of sample, heat at 50° for 5 min, and then add 10 mL of glacial acetic acid. A green color appears. (Cholic acid produces a brown color.)
Assay Not less than 98.0% and not more than 102.0% of $C_{24}H_{40}O_4$, calculated on the dried basis.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 1%.
Melting Range Between 172° and 175°.
Residue on Ignition Not more than 0.2%.

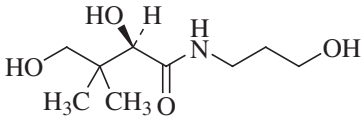
TESTS

Assay Transfer about 500 mg of sample, accurately weighed, into a 250-mL Erlenmeyer flask, and add 20 mL of water and 40 mL of alcohol. Cover the flask with a watch glass, heat the mixture gently on a steam bath until the sample is dissolved, and allow the mixture to cool to room temperature. Add a few drops of phenolphthalein TS to the solution, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for 15 s. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 39.26 mg of $C_{24}H_{40}O_4$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 140° under a vacuum of not more than 5 mm Hg for 4 h.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight containers.

Dexpanthenol

D(+)-Pantothenyl Alcohol; Panthenol



$C_9H_{19}NO_4$ Formula wt 205.25

CAS: [81-13-0]

DESCRIPTION

Dexpanthenol occurs as a clear, viscous, somewhat hygroscopic liquid. It is the dextrorotatory isomer of the alcohol analogue of pantothenic acid. Some crystallization may occur on standing. It is freely soluble in water, in alcohol, in methanol, and in propylene glycol. It is soluble in chloroform and in ether, and is slightly soluble in glycerin. Its solutions are alkaline to litmus.

Function Nutrient.

REQUIREMENTS

Identification

A. Add 5 mL of 1 *N* sodium hydroxide and 1 drop of cupric sulfate TS to 1 mL of a 10% aqueous solution, and shake vigorously. A deep-blue color develops.

B. Add 1 mL of 1 *N* hydrochloric acid to 1 mL of a 1% aqueous solution, and heat on a steam bath for about 30 min. Cool, add 100 mg of hydroxylamine hydrochloride, mix, and add 5 mL of 1 *N* sodium hydroxide. Allow to stand for 5 min, then adjust the pH to within a range of 2.5 to 3.0 with 1 *N* hydrochloric acid, and add 1 drop of ferric chloride TS. A purple-red color develops.

C. The infrared absorption spectrum of a film of the sample exhibits maxima only at the same wavelengths as those of a similar preparation of USP Dexpanthenol Reference Standard.
Assay Not less than 98.0% and not more than 102.0% of $C_9H_{19}NO_4$, calculated on the anhydrous basis.
Aminopropanol Not more than 1%.
Lead Not more than 5 mg/kg.
Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +29.0° and +31.5°.
Refractive Index Between 1.495 and 1.502 at 20°.
Residue on Ignition Not more than 0.1%.
Water Not more than 1%.

TESTS

Assay Transfer about 400 mg of sample, accurately weighed, into a 300-mL reflux flask fitted with a standard-taper glass joint, add 50.0 mL of 0.1 *N* perchloric acid in glacial acetic acid, and reflux for 5 h.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Cool, covering the condenser with foil to prevent contamination by moisture, and rinse the condenser with glacial acetic acid. Add 5 drops of crystal violet TS, and titrate with 0.1 *N* potassium acid phthalate in glacial acetic acid to a blue-green endpoint. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 20.53 mg of $C_9H_{19}NO_4$.
Aminopropanol Transfer about 5 g of sample, accurately weighed, into a 50-mL flask, and dissolve in 10 mL of water. Add bromothymol blue TS, and titrate with 0.1 *N* sulfuric acid from a microburet to a yellow endpoint. Each milliliter of 0.1 *N* sulfuric acid is equivalent to 7.5 mg of aminopropanol.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 500 mg of sample, calculated on the anhydrous basis, in each 10 mL of water.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Dextrin

INS: 1400 CAS: [9004-53-9]

DESCRIPTION

Dextrin occurs as free-flowing white, yellow, or brown powders and consist chiefly of polygonal, rounded, or oblong or truncated granules. Dextrin is partially hydrolyzed starch converted by heat alone, or by heating in the presence of suitable food-grade acids and buffers, from any of several grain- or root-based unmodified native starches (e.g., corn, waxy maize, high-amylose maize, milo, waxy milo, potato, arrowroot, wheat, rice, tapioca, sago, etc.). Dextrin is partially to completely soluble in water.

Function Thickener; colloidal stabilizer; binder; surface-finishing agent.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.
Identification Suspend about 1 g of sample in 20 mL of water, and add a few drops of iodine TS. A dark blue to red-brown color appears.
Chloride Not more than 0.2%.
Crude Fat Not more than 1.0%.
Lead Not more than 1 mg/kg.
Loss on Drying Not more than 13.0%.
Protein Not more than 1.0%.
Reducing Sugars Not more than 18.0% (expressed as D-glucose), calculated on the dried basis.
Residue on Ignition Not more than 0.5%.
Sulfur Dioxide Not more than 0.005%.

TESTS

Chloride Dissolve 1 g of sample in 25 mL of boiling water, cool, dilute to 100 mL with water, and filter. Add 24 mL of water, 2 mL of nitric acid, and 1 mL of silver nitrate TS to 1 mL of the filtrate. Any turbidity produced does not exceed that shown in a control containing 20 µg of chloride ion.
Crude Fat Determine as directed under *Crude Fat*, Appendix X.
Lead Transfer 4.0 g of sample to an evaporating dish, add 4 mL of sulfuric acid solution (1:4), distributing it evenly throughout the sample, and evaporate most of the water on a steam bath. Char and dehydrate the sample by heating on a hot plate, while at the same time, heating with an infrared lamp from above, and then heat in a muffle furnace at 500° until the residue is free from carbon. Remove the dish from the furnace, cool, and cautiously wash down the inside of the dish with water. Add 1 mL of 1 *N* hydrochloric acid, evaporate to dryness on a steam bath, then add 2 mL of 1 *N* hydrochloric acid, and heat briefly, while stirring, on a steam bath. Quantitatively transfer the solution into a separator with the aid of small quantities of water, and neutralize with 1 *N* ammonium hydroxide. This *Sample Solution* meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 4 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 5-g sample in a vacuum oven, not exceeding 100 mm Hg, at 120° for 4 h.
Protein Transfer about 10 g of sample, accurately weighed, into an 800-mL Kjeldahl flask, and add 10 g of anhydrous potassium or sodium sulfate, 300 mg of copper selenite or mercuric oxide, and 60 mL of sulfuric acid. Gently heat the mixture, keeping the flask inclined at about a 45° angle, and after frothing has ceased, boil briskly until the solution has remained clear for about 1 h. Cool, add 30 mL of water, mix, and cool again. Cautiously pour about 75 mL (or enough to make the mixture strongly alkaline) of sodium hydroxide solution (2:5) down the inside of the flask so that it forms a layer under the acid solution, and then add a few pieces of granular zinc. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of an accurately measured excess of 0.1 *N* sulfuric acid contained in a 50-mL flask. Gently rotate the contents of the Kjeldahl flask to mix, and distill until all ammonia has passed into the absorbing acid solution (about 250 mL of distillate). Add 0.25 mL of methyl red–methylene blue TS to the receiving flask, and titrate the excess acid with 0.1 *N* sodium hydroxide. Perform a blank determination, substituting pure sucrose or dextrose for the sample, and make any necessary correction (see *General Provisions*). Each mL of 0.1 *N* sulfuric acid consumed is equivalent to 1.401 mg of nitrogen (N). Calculate the percent N in the sample, and then calculate the percent protein by multiplying the percent N by 6.25, in the case of starches obtained from corn, or by 5.7, in the case of starches obtained from wheat. Other factors may be applied as necessary for starches obtained from other sources.

Reducing Sugars Transfer about 10 g of sample, accurately weighed, into a 200-mL collecting flask, dilute to volume with water, shake for 30 min, and filter through Whatman No. 1 filter paper, or equivalent, collecting the filtrate in a clean, dry flask. Pipet 10 mL each of *Fehling's Solution A* and *Fehling's Solution B* (see *Cupric Tartrate TS, Alkaline*, in the section on *General Tests and Assays, Solutions and Indicators*) into a 250-mL Erlenmeyer flask, add 20.0 mL of the sample filtrate and 10 mL of water, and mix. Add two small glass beads, cover the mouth of the flask with a small glass funnel or glass bulb, and heat on a hot plate adjusted to bring the solution to a boil in 3 min. Continue boiling for exactly 2 min (total heating time, 5 min), and then quickly cool to room temperature in an ice bath or in a cold running-water bath. Add 10 mL each of 30% potassium iodide solution and 28% sulfuric acid, and titrate immediately with 0.1 *N* sodium thiosulfate. Near the endpoint, add 1 mL of starch TS, and continue titrating carefully, while agitating the solution continuously, until the blue color is discharged. Record the volume, in mL, of 0.1 *N* sodium thiosulfate required as *S*. Conduct two reagent blank determinations in the same manner, substituting water for the sample filtrate, and record the average volume, in mL, of the blanks as *B*. Obtain the *Titer Difference*, expressed as mL of 0.1 *N* sodium thiosulfate, by subtracting *S* from *B*. Determine the weight, in mg, of reducing sugars, expressed as D-glucose (dextrose), by reference to the table below entitled *Conversion of Titer Difference to Reducing Sugars Content*, and record this value as *R*. Calculate the percentage of reducing sugars, as D-glucose, on the dried basis, by the formula

$$(R \times 200 \times 100)/(W \times 20 \times 1000),$$

in which *W* is the weight, in g, of sample taken, corrected for *Loss on Drying*.

Conversion of Titer Difference to Reducing Sugars Content[a]

Titer Difference (mL)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Reducing Sugar (as Dextrose) (mg)									
0.0	0.0	0.3	0.7	1.0	1.3	1.6	1.9	2.2	2.5	2.8
1.0	3.2	3.5	3.8	4.1	4.4	4.7	5.0	5.3	5.6	5.9
2.0	6.4	6.6	6.9	7.2	7.5	7.8	8.1	8.5	8.8	9.1
3.0	9.4	9.8	10.1	10.4	10.7	11.0	11.4	11.7	12.0	12.3
4.0	12.6	13.0	13.3	13.6	14.0	14.3	14.6	15.0	15.3	15.6
5.0	15.9	16.3	16.6	16.9	17.2	17.6	17.9	18.2	18.5	18.9
6.0	19.2	19.5	19.8	20.1	20.5	20.8	21.1	21.4	21.8	22.1
7.0	22.4	22.7	23.0	23.3	23.7	24.0	24.3	24.6	24.9	25.2
8.0	25.6	25.9	26.2	26.6	26.9	27.3	27.6	28.0	28.3	28.6
9.0	28.9	29.3	29.6	30.0	30.3	30.6	31.0	31.3	31.6	31.9
10.0	32.3	32.7	33.0	33.3	33.7	34.0	34.3	34.6	35.0	35.3
11.0	35.7	36.0	36.3	36.7	37.0	37.3	37.6	38.0	38.3	38.7
12.0	39.0	39.3	39.6	40.0	40.3	40.6	41.0	41.3	41.7	42.0
13.0	42.4	42.8	43.1	43.4	43.7	44.1	44.4	44.8	45.2	45.5
14.0	45.8	46.2	46.5	46.9	47.2	47.6	47.9	48.3	48.6	48.9

Conversion of Titer Difference to Reducing Sugars Content[a] (Cont.)

Titer Difference (mL)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Reducing Sugar (as Dextrose) (mg)									
15.0	49.3	49.6	49.9	50.3	50.7	51.1	51.4	51.7	52.1	52.4
16.0	52.8	53.2	53.5	53.9	54.2	54.5	54.9	55.3	55.6	56.0
17.0	56.3	56.7	57.0	57.3	57.7	58.1	58.4	58.8	59.1	59.5
18.0	59.8	60.1	60.5	60.9	61.2	61.5	61.9	62.3	62.6	63.0
19.0	63.3	63.6	64.0	64.3	64.7	65.0	65.4	65.8	66.1	66.5
20.0	66.9	67.2	67.6	68.0	68.4	68.8	69.1	69.5	69.9	70.3
21.0	70.7	71.1	71.5	71.9	72.2	72.6	73.0	73.4	73.7	74.1
22.0	74.5	74.9	75.3	75.7	76.1	76.5	76.9	77.3	77.7	78.1
23.0	78.5	78.9	79.3	79.7	80.1	80.5	80.9	81.3	81.7	82.1
24.0	82.6	83.0	83.4	83.8	84.2	84.6	85.0	85.4	85.8	86.2
25.0	86.6	87.0	87.4	87.8	88.2	88.6	89.0	89.4	89.8	90.2
26.0	90.7	91.1	91.5	91.9	92.3	92.7	93.1	93.5	93.9	94.3
27.0	94.8									

[a]Use of this table presumes the ability of the analyst to duplicate exactly the conditions under which the data were developed. The risk of error can be avoided by careful duplicate standardization with known quantities of pure dextrose (five samples, ranging from 10 to 70 mg). A plot of *Titer Difference* versus mg of dextrose is slightly curvilinear, passing through the origin. If use of a standardization curve is adopted, the thiosulfate solution need not be standardized. Some additional increase in accuracy results from use of a 0.065 *N* sodium thiosulfate solution, which increases the blank titer to about 44 to 45 mL.

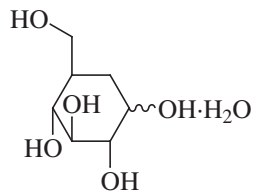
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.

Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X.

Packaging and Storage Store in well-closed containers.

Dextrose

D-Glucose; Glucose; Corn Sugar



$C_6H_{12}O_6$ Formula wt 180.16

CAS: [50-99-7]

DESCRIPTION

Dextrose occurs as white, crystalline granules or as a granular powder. It is purified and crystallized D-glucose. It is anhydrous or contains one molecule of water of crystallization. It

is freely soluble in water, very soluble in boiling water, and slightly soluble in alcohol.

Function Nutritive sweetener; humectant; texturizing agent.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.
Identification Add a few drops of a 1:20 aqueous solution to 5 mL of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide forms.
Assay Not less than 99.5% and not more than 100.5% of reducing sugar content (dextrose equivalent), expressed as D-glucose, calculated on the dried basis.
Arsenic Not more than 1 mg/kg.
Chloride Not more than 0.018%.
Lead Not more than 0.1 mg/kg.
Loss on Drying *Anhydrous*: Not more than 2.0%; *Monohydrate*: Not more than 10.0%.
Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Between +52.6° and +53.2° after drying.
Residue on Ignition Not more than 0.1%.
Starch Passes test.
Sulfur Dioxide Not more than 0.002%.

TESTS

Assay Determine as directed under *Reducing Sugars Assay*, Appendix X.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared using a 1-g sample, and 1 mL of *Standard Arsenic Solution* in the control (1 µg As).
Chloride A 2.0-g sample shows no more chloride than corresponds to 0.50 mL of 0.020 *N* hydrochloric acid.
Lead Determine as directed for *Method I* in *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Loss on Drying Using *Loss on Drying*, Appendix IIC, as a guide, dry 10 g of anhydrous sample or 5 g of monohydrate sample for 2 h at 70° in a vacuum oven not exceeding 50 mm Hg, cool in a desiccator for 30 min, and weigh. Dry for successive 1-h intervals until the weight change is less than 2 mg.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of a previously dried sample and 0.2 mL of 6 *N* ammonium hydroxide in sufficient water to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 10-g sample.
Starch Add 1 drop of iodine TS to 1 g of sample dissolved in 10 mL of water. A yellow color indicates the absence of soluble starch.
Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X, using a 75-g sample.

Packaging and Storage Store in tight containers in a dry place.

Diacetyl Tartaric Acid Esters of Mono- and Diglycerides

DATEM

CAS: [91052-83-4]

INS: 472e CAS: [100085-39-0]

DESCRIPTION

Diacetyl Tartaric Acid Esters of Mono- and Diglycerides occur over a range in appearance from sticky, viscous liquids through a fatlike consistency to a waxy solid, depending on the iodine value of the oils or fats used in their manufacture. They are the reaction product of partial glycerides of edible oils, fats, or fat-forming fatty acids with diacetyl tartaric anhydride. The diacetyl tartaroyl esters are miscible in all proportions with oils and fats. They are soluble in most common fat solvents, in methanol, in acetone, and in ethyl acetate, but are insoluble in other alcohols, in acetic acid, and in water. They are dispersible in water and resistant to hydrolysis for moderate periods of time. The pH of a 3% dispersion in water is between 2 and 3.

Function Emulsifier.

REQUIREMENTS

Identification Add, dropwise, lead acetate TS to a solution of 500 mg of sample in 10 mL of methanol. A white, flocculent, practically insoluble precipitate forms.
Assay for Tartaric Acid Between 17.0 and 20.0 g of tartaric acid ($C_4H_6O_6$) per 100 g of sample after saponification.
Acetic Acid Between 14.0 and 17.0 g of acetic acid (CH_3COOH) per 100 g of sample after hydrolysis.
Acid Value Between 62 and 76.
Fatty Acids (Total) Not less than 56.0 g of total fatty acids per 100 g of sample after hydrolysis.
Glycerin Not less than 12.0 g of glycerin ($C_3H_8O_3$) per 100 g of sample after hydrolysis.
Lead Not more than 2 mg/kg.
Residue on Ignition Not more than 0.01%.
Saponification Value Between 380 and 425.

TESTS

Assay for Tartaric Acid

Standard Reference Curve Transfer 100 mg of reagent-grade tartaric acid, accurately weighed, into a 100-mL volumetric flask, dissolve it in about 90 mL of water, add water to volume, and mix well. Transfer 3.0-, 4.0-, 5.0-, and 6.0-mL portions into separate 19- × 150-mm matched cuvettes, and add sufficient water to make 10.0 mL. Add 4.0 mL of a freshly prepared 1:20 sodium metavanadate solution and 1.0 mL of glacial acetic acid to each cuvette.

> **Note**: Use these solutions within 10 min after color development.

Prepare a blank in the same manner, using 10 mL of water in place of the tartaric acid solutions. Set a suitable spectropho-

tometer or a photoelectric colorimeter equipped with a 520-nm filter at zero with the blank, and then determine the absorbance of the four solutions of tartaric acid at 520 nm. From the data thus obtained, prepare a reference curve by plotting the absorbances on the ordinate against the corresponding quantities, in milligrams, of tartaric acid on the abscissa.

Assay Preparation Transfer about 4 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, and add 80 mL of 0.5 *N* potassium hydroxide and 0.5 mL of phenolphthalein TS. Connect an air condenser at least 65 cm long to the flask, and heat the mixture on a hot plate for about 2.5 h. Remove the air condenser and add approximately 10% phosphoric acid to the hot mixture until it is definitely acid to congo red test paper. Reconnect the air condenser, and heat until the fatty acids are liquified and clear. Cool, and transfer the mixture into a 250-mL separator with the aid of small portions of water and hexane. Extract the liberated fatty acids with three successive 25-mL portions of hexane, and collect the extracts in a second separator. Wash the combined hexane extracts with two 25-mL portions of water, and add the washings to the separator containing the water layer. Retain the combined hexane extracts for the determination of total fatty acids. Transfer the contents of the first separator to a 250-mL beaker, heat on a steam bath to remove traces of hexane, filter through acid-washed, fine-texture filter paper into a 500-mL volumetric flask, and finally dilute to volume with water (*Solution I*). Pipet 25.0 mL of this solution into a 100-mL volumetric flask, and dilute to volume with water (*Solution II*). Retain the rest of *Solution I* for the determination of *Glycerin* (below).

Procedure Transfer 10.0 mL of *Solution II* into a 19- × 150-mm cuvette, and continue as directed under *Standard Reference Curve*, beginning with ''add 4.0 mL of a freshly prepared 1:20 sodium metavanadate solution. . . .'' From the reference curve, determine the weight, in milligrams, of tartaric acid in the final dilution, multiply this by 20, and divide the result by the weight of the original sample to obtain the percentage of tartaric acid.

Acetic Acid Determine as directed under *Volatile Acidity*, Appendix VII, using a 4-g sample, accurately weighed, and 30.03 as the equivalence factor (*e*).

Acid Value Transfer about 1 g of sample, accurately weighed, into a 125-mL Erlenmeyer flask. Prepare a solvent by mixing 1 volume of hexane with 4 volumes of methanol, adding phenol red TS, and neutralizing, if necessary. Dissolve the sample in about 25 mL of this solvent by gently warming, if necessary. Titrate the solution with 0.1 *N* methanolic potassium hydroxide to a light red endpoint. Perform a blank determination (see *General Provisions*) using a 25-mL portion of the solvent, and make any necessary correction. Calculate the acid value by the formula

$$56.1V \times N/W,$$

in which V is the volume, in milliliters, of the methanolic potassium hydroxide and N is the normality; and W is the weight, in grams, of the sample taken.

Fatty Acids (Total) Dry the combined hexane extracts of fatty acids obtained in the *Assay for Tartaric Acid* by shaking with a few grams of anhydrous sodium sulfate. Filter the solution into a tared, 250-mL beaker, evaporate the hexane on a steam bath, cool, and weigh.

Glycerin Prepare periodic acid solution by dissolving 2.7 g of periodic acid (H_5IO_6) in 50 mL of water, adding 950 mL of glacial acetic acid, and mixing thoroughly (protect this solution from light). Transfer 5.0 mL of *Solution I*, prepared in the *Assay for Tartaric Acid* (above), into a 250-mL glass-stoppered Erlenmeyer or iodine flask. Add 15 mL of glacial acetic acid and 25.0 mL of periodic acid solution to the flask, shake the mixture for 1 or 2 min, allow it to stand for 15 min, add 15 mL of a 15:100 potassium iodide solution and 15 mL of water, swirl, let it stand for 1 min, and then titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Perform a *Residual Blank Titration* (see *General Provisions*) using water in place of sample, and make any necessary correction. The corrected volume is the number of milliliters of 0.1 *N* sodium thiosulfate required for the glycerin and the tartaric acid in the sample represented by the 5 mL of *Solution I*. From the percentage determined in the *Assay for Tartaric Acid*, calculate the volume of 0.1 *N* sodium thiosulfate required for the tartaric acid in the titration. The difference between the corrected volume and the calculated volume required for the tartaric acid is the number of milliliters of 0.1 *N* sodium thiosulfate consumed because of the glycerin in the sample. One milliliter of 0.1 *N* sodium thiosulfate is equivalent to 2.303 mg of glycerin and to 7.505 mg of tartaric acid.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 10-g sample.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 2 g of sample, accurately weighed.

> **Note**: Add 5 to 10 mL of water to samples and blanks before saponification; otherwise, sufficient salts precipitate during saponification to cause serious bumping and spattering.

Packaging and Storage Store in well-closed containers.

Diatomaceous Earth

Diatomaceous Silica; Diatomite; D.E.

CAS: natural powder and calcined powder [61790-53-2]

CAS: flux-calcined powder [68855-54-9]

DESCRIPTION

Diatomaceous Earth occurs as a powder of varying colors consisting of processed siliceous skeletons of diatoms. The *natural powder* (gray to off white) is air dried and classified

by particle size; the *calcined powder* (pink to buff-colored) is air dried, classified, calcined at a high temperature (815° to 982°), and again classified; the *flux-calcined powder* (white) is air dried, classified, calcined in the presence of a suitable flux (generally soda ash or other alkaline salt), and again classified; and the *acid-washed powder* is any of the preceding powders having been further purified by washing in acid and rinsing with water. It is insoluble in water, in acids (except hydrofluoric), and in dilute alkalis.

Function Filter aid in food processing.

REQUIREMENTS

Identification When the powder is examined with a 100- to 200-power microscope, typical diatom shapes are observed.
Arsenic Not more than 10 mg/kg.
Lead Not more than 10 mg/kg.
Loss on Drying *Natural and acid-washed powders*: Not more than 10.0%; *Calcined and flux-calcined powders*: Not more than 3.0%.
Loss on Ignition *Natural Powders*: Not more than 7.0%, calculated on the dried basis; *Calcined and Flux-Calcined Powders*: Not more than 0.5%, calculated on the dried basis.
Nonsiliceous Substances Not more than 25.0%, calculated on the dried basis.
pH of Filtrate *Natural, Calcined, or Acid-Washed Powders*: Between 5.0 and 10.0; *Flux-Calcined Powders*: Between 8.0 and 11.0.

TESTS

Arsenic Transfer 10.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat at 70° for 15 min. Cool, and decant through a Whatman No. 3 filter paper, or equivalent, into a 100-mL volumetric flask. Wash the slurry with three 10-mL portions of hot water and wash the filter paper with 15 mL of hot water, dilute to volume with water, and mix. A 3.0-mL portion of this solution meets the requirements of the *Arsenic Limit Test*, Appendix IIIB.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 10.0-mL portion of the solution prepared under *Arsenic Test*, above, and 10 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.
Loss on Ignition Accurately weigh about 1 g of sample, and ignite at 800° to constant weight in a suitable, tared crucible.
Nonsiliceous Substances Transfer about 200 mg of sample, accurately weighed, into a tared platinum crucible, add 5 mL of hydrofluoric acid and 2 drops of 1:2 sulfuric acid, and evaporate gently to dryness. Cool, add 5 mL of hydrofluoric acid, evaporate again to dryness, and then ignite to constant weight.
pH of Filtrate Boil 10 g of sample with 100 mL of water for 30 min, make up to 100 mL with water, and filter through a fine-porosity sintered-glass funnel. Determine the pH of the filtrate as described under *pH Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Dilauryl Thiodipropionate

$(C_{12}H_{25}OOCCH_2CH_2)_2S$

$C_{30}H_{58}O_4S$ Formula wt 514.85
INS: 389 CAS: [123-28-4]

DESCRIPTION

Dilauryl Thiodipropionate occurs as white, crystalline flakes. It is soluble in most organic solvents, but insoluble in water.

Function Antioxidant.

REQUIREMENTS

Identification Dilauryl Thiodipropionate may be identified by its solidification point, as determined under *Solidification Point* (below).
Assay Not less than 99.0% and not more than 100.5% of $C_{30}H_{58}O_4S$.
Acidity (as thiodipropionic acid) Not more than 0.2% of 3,3′-thiodipropionic acid.
Lead Not more than 10 mg/kg.
Solidification Point Not below 40°.

TESTS

Assay Transfer about 700 mg of sample, accurately weighed, into a 250-mL Erlenmeyer flask, and add 100 mL of glacial acetic acid and 50 mL of alcohol. Heat the mixture at a temperature of about 40° until the sample is completely dissolved, then add 3 mL of hydrochloric acid and 4 drops of *p*-ethoxychrysoidin TS, and immediately titrate the solution with 0.1 *N* bromine. When the endpoint is approached (pink color), add 4 more drops of the indicator solution and continue the titration, dropwise, to a color change from red to pale yellow. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* bromine is equivalent to 25.74 mg of $C_{30}H_{58}O_4S$. Multiply the percentage of thiodipropionic acid, determined in the *Acidity* test (below), by 2.89, and subtract this value from the percentage of Dilauryl Thiodipropionate calculated from the titration. The difference is the percent purity of $C_{30}H_{58}O_4S$.
Acidity (as thiodipropionic acid) Transfer about 2 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask. Dissolve the sample in 50 mL of a mixture comprising 1 part methyl alcohol and 3 parts benzene, add 5 drops of phenolphthalein TS, and titrate with 0.1 *N* alcoholic potassium

hydroxide. Each milliliter of 0.1 *N* alcoholic potassium hydroxide is equivalent to 8.91 mg of 3,3′-thiodipropionic acid.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 µg of lead (Pb) ion in the control.
Solidification Point Determine as directed under *Solidification Point*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Dill Seed Oil, European Type

View IR

DESCRIPTION

Dill Seed Oil, European Type, occurs as a pale yellow to light yellow liquid with a caraway odor and flavor. It is the volatile oil obtained by steam distillation from the crushed, dried fruit or seeds of *Anethum graveolens* L. (Fam. Umbelliferae). It is soluble in most fixed oils and in mineral oil. It is soluble, with slight opalescence, in propylene glycol, but it is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 42.0% and not more than 60.0%, by volume, of ketones as carvone.
Angular Rotation Between +70° and +82°.
Refractive Index Between 1.483 and 1.490 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.890 and 0.915.

TESTS

Assay Determine as directed in the *Neutral Sulfite Method* under *Aldehydes and Ketones*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 80% alcohol, with slight opalescence that may not disappear on dilution to as much as 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Dill Seed Oil, Indian Type

Dill Seed Oil, Indian; Dill Oil, Indian Type

View IR

DESCRIPTION

Dill Seed Oil, Indian Type, occurs as a light yellow to light brown liquid with a rather harsh, caraway odor and flavor. It is the volatile oil obtained by steam distillation from the crushed mature fruit of Indian Dill, *Anethum sowa* D.C. (Fam. Umbelliferae). It is soluble in most fixed oils and in mineral oil, occasionally with slight opalescence. It is sparingly soluble in propylene glycol and practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 20.0% and not more than 30.0%, by volume, of ketones as carvone.
Angular Rotation Between +40° and +58°.
Refractive Index Between 1.486 and 1.495 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.925 and 0.980.

TESTS

Assay Determine as directed in the *Neutral Sulfite Method* under *Aldehydes and Ketones*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 0.5 mL of 90% alcohol and remains clear on dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Dillweed Oil, American Type

Dill Oil; Dill Herb Oil, American Type

CAS: [8006-75-5]

View IR

DESCRIPTION

Dillweed Oil, American Type, occurs as a light yellow to yellow liquid. It is the volatile oil obtained by steam distillation from the freshly cut stalks, leaves, and seeds of the plant *Anethum graveolens* L. (Fam. Umbelliferae). It is soluble in most fixed oils and in mineral oil. It is soluble, usually with opalescence or turbidity, in propylene glycol, but it is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 28.0%, except in specific cases (see *Note*, below), and not more than 45.0%, by volume, of ketones as carvone.

> **Note**: Oil obtained from early season distillation may show a carvone content as low as 25.0% and a correspondingly lower specific gravity, lower refractive index, and higher angular rotation.

Angular Rotation Between +84° and +95° (see *Note*, above).
Refractive Index Between 1.480 and 1.485 at 20° (see *Note*, above).
Solubility in Alcohol Passes test.
Specific Gravity Between 0.884 and 0.900 (see *Note*, above).

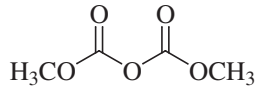
TESTS

Assay Determine as directed in the *Neutral Sulfite Method* under *Aldehydes and Ketones*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 90% alcohol, frequently with opalescence that may not disappear on dilution to as much as 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Dimethyl Dicarbonate

Dicarbonic Acid; Dimethyl Ester; Dimethyl Pyrocarbonate; DMDC



$C_4H_6O_5$ Formula wt 134.09

INS: 242 CAS: [4525-33-1]

View IR

DESCRIPTION

Dimethyl Dicarbonate occurs as a clear, colorless liquid. Its solubility in water is 35 g/L at 20° with decomposition, its melting point is about 17°, and its flash point is 85°. It reacts quantitatively with water, producing carbon dioxide and methanol.

Caution: Dimethyl Dicarbonate is toxic if inhaled.

Function Preservative; antimicrobial.

REQUIREMENTS

Identification The infrared absorption spectrum of a neat dispersion of the sample between two sodium chloride plates exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 99.8% and not more than 101.5% of $C_4H_6O_5$.
Dimethyl Carbonate Not more than 0.2%.
Lead Not more than 1 mg/kg.

TESTS

Assay

1 N *Di*-n-*butylamine* Transfer 12.93 g of di-*n*-butylamine into a 100-mL volumetric flask, dilute to volume with toluene, and mix.

Procedure Transfer about 2 g of sample, accurately weighed, into a 250-mL beaker, and dissolve it in 100 mL of acetone. Add 25 mL of *1* N *Di*-n-*butylamine* by pipet, allow the mixture to stand for 5 min, and titrate with 1 *N* hydrochloric acid, determining the endpoint potentiometrically. Perform a blank titration (see *General Provisions*), and make any necessary correction. Calculate the percent Dimethyl Dicarbonate in the sample taken by the formula

$$100(B - A)0.134/W,$$

in which B and A are the volumes, in milliliters, of hydrochloric acid used for the blank and the sample, respectively; 0.134 is the milliequivalent weight of Dimethyl Dicarbonate; and W is the weight, in milligrams, of sample taken.

Dimethyl Carbonate (**Note**: Conduct this procedure without delays.)

Internal Standard Solution Dissolve about 100 mg of methyl isobutylketone, accurately weighed, in 10 mL of methanol contained in a 100-mL volumetric flask, dilute to volume with methanol, and mix.

Standard Solution Transfer about 20 mg of 99% dimethyl carbonate (Aldrich, or equivalent), accurately weighed, into a 10-mL volumetric flask, dilute to volume with *Internal Standard Solution*, and mix.

Test Solution Transfer about 10 g of sample, accurately weighed, into a 10-mL volumetric flask, dilute to volume with *Internal Standard Solution*, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and containing a 50-m × 0.3-mm (id) capillary column coated with SE 30-D, or equivalent, and maintain the column, initially at 30° for a 5-min hold time, followed by a linear temperature gradient of 40° per minute to a final temperature of 120° held for 5 min. Use helium as the carrier gas at a flow rate of 11 mL/min, and use hydrogen as the fuel gas at a flow rate of 35 mL/min. Chromatograph five replicate injections of the *Standard Solution*. The relative standard deviation is not greater than 2.0%.

Procedure Using a metal-free syringe, separately inject suitable portions (about 5 µL) of the *Standard Solution* and the *Test Solution* into the gas chromatograph, and record the chromatograms. Measure the peak area ratio of dimethyl carbonate to that of the internal standard obtained with the *Standard Solution*. Similarly, measure the same peak area ratio for the *Test Solution*. The ratio is equal to or smaller than that obtained with the *Standard Solution*.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Packaging and Storage Store in the original container in a cool (about 20°), dry, and well-ventilated area. Do not repackage because it is particularly susceptible to contamination by water.

Dimethylpolysiloxane

Dimethyl Silicone; Polydimethylsiloxane

CAS: [9006-65-9]

DESCRIPTION

Dimethylpolysiloxane occurs as a clear, colorless, viscous liquid. It is a mixture of fully methylated linear siloxane polymers containing repeating units of the formula $(CH_3)_2SiO$ that are terminated with trimethylsiloxy end-blocking units of the formula $(CH_3)_3SiO—$. It is soluble in most aliphatic and aromatic hydrocarbon solvents, but it is insoluble in water.

Note: Dimethylpolysiloxane is frequently used in commerce as such, or as a liquid containing silica (usually 4% to 5%), which must be removed by high-speed centrifugation (about 20,000 rpm) before testing the Dimethylpolysiloxane for *Identification, Refractive Index, Specific Gravity,* and *Viscosity*. This monograph does not apply to aqueous emulsions containing emulsifying agents and preservatives in addition to silica.

Function Defoaming agent.

REQUIREMENTS

Identification The infrared absorption spectrum of a neat dispersion of the sample between two sodium chloride plates exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Dimethylpolysiloxane Reference Standard.

Lead Not more than 5 mg/kg.

Loss on Heating Not more than 18.0%.

Refractive Index Between 1.4000 and 1.4050.

Specific Gravity Between 0.96 and 0.98.

Viscosity Between 300 and 1500 centistokes.

TESTS

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Heating Transfer 15 g of sample, accurately weighed, into an open, tared aluminum cup having an internal surface area of about 30 cm^2, weigh the cup and its contents, heat for 4 h at 200° in a circulating air oven, cool, and weigh again.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

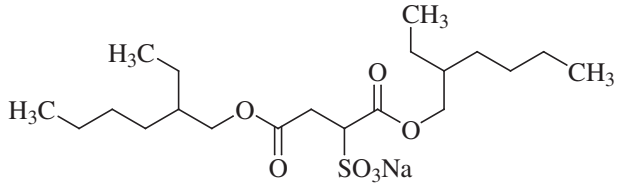
Specific Gravity Determine by any reliable method (see *General Provisions*).

Viscosity Determine as directed under *Viscosity of Dimethylpolysiloxane*, Appendix IIB.

Packaging and Storage Store in tightly closed containers.

Dioctyl Sodium Sulfosuccinate

DOSS; Docusate Sodium



$C_{20}H_{37}NaO_7S$ Formula wt 444.56

INS: 480 CAS: [577-11-7]

DESCRIPTION

Dioctyl Sodium Sulfosuccinate occurs as a white, waxlike, plastic solid. One gram of sample dissolves slowly in about

70 mL of water. It is freely soluble in alcohol and in glycerin, and it is very soluble in solvent hexane.

Function Emulsifier; wetting agent.

REQUIREMENTS

Identification The infrared absorption spectrum of a film of the sample between two salt plates exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Docusate Sodium Reference Standard.
Assay Not less than 98.5% of $C_{20}H_{37}NaO_7S$, calculated on the dried basis.
Bis(2-ethylhexyl)maleate Not more than 0.4%.
Clarity of Solution Passes test.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 2.0%.
Residue on Ignition Between 15.5% and 16.2%.

TESTS

Assay

Sample Solution Transfer about 3.8 g of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in and dilute to volume with chloroform, and mix.

Tetra-n-*butylammonium Iodide Solution* Transfer 1.250 g of tetra-*n*-butylammonium iodide into a 500-mL volumetric flask, dilute to volume with water, and mix.

Salt Solution Dissolve 100 g of anhydrous sodium sulfate and 10 g of sodium carbonate in sufficient water to make 1000.0 mL.

Procedure Pipet 10.0 mL of the *Sample Solution* into a 250-mL flask, and add 40 mL of chloroform, 50 mL of *Salt Solution*, and 10 drops of bromophenol blue TS. Titrate with *Tetra*-n-*butylammonium Iodide Solution* to the first appearance of a blue color in the chloroform layer after vigorous shaking. Calculate the percent $C_{20}H_{37}NaO_7S$ by the formula

$$(V \times 1.250 \times 444.6 \times 10)/(W \times 369.4),$$

in which V is the volume, in milliliters, of *Tetra*-n-*butylammonium Iodide Solution* required; 444.6 is the approximate molecular weight of Dioctyl Sodium Sulfosuccinate; W is the weight, in grams, of the sample taken; and 369.4 is the molecular weight of tetra-*n*-butylammonium iodide.

Bis(2-ethylhexyl)maleate

Supporting Electrolyte Dissolve 21.2 g of anhydrous lithium perchlorate ($LiClO_4$) in 175 mL of water contained in a 250-mL beaker. Adjust the pH of this solution to 3.0 by adding, dropwise, glacial acetic acid (usually 1 or 2 drops is sufficient), using a suitable pH meter. Quantitatively transfer the solution into a 200-mL volumetric flask, dilute to volume with water, and mix.

Standard Solution Transfer 100 to 110 mg of USP Bis(2-ethylhexyl)maleate Reference Standard, accurately weighed, into a 100-mL volumetric flask. Record the exact weight to the nearest 0.1 mg as W_A. Add 60 to 70 mL of isopropyl alcohol, swirl to dissolve, then dilute to volume with water, and mix.

Sample Stock Solution Transfer 12.5 g of sample, accurately weighed, into a 150-mL beaker. Record the exact weight to the nearest 0.1 mg as W_S. Add 80 to 90 mL of isopropyl alcohol, and stir with a glass stirring rod until the sample is dissolved. Quantitatively transfer this solution, with the aid of isopropyl alcohol, into a 250-mL volumetric flask, then dilute to volume with isopropyl alcohol, and mix.

Test Solution A Pipet 50.0 mL of *Sample Stock Solution* and 20.0 mL of *Supporting Electrolyte* into a 100-mL volumetric flask. Dilute with isopropyl alcohol to within 15 mm of the graduated volume line, stopper, shake to facilitate solution, and set aside for 2 min. Dilute to volume with isopropyl alcohol, and mix. A completely clear solution is obtained.

Test Solution B Pipet 50.0 mL of *Sample Stock Solution*, 10.0 mL of *Standard Solution*, and 20.0 mL of *Supporting Electrolyte* into a 100-mL volumetric flask, and complete the preparation as described for *Test Solution A*.

Blank Pipet 20.0 mL of the *Supporting Electrolyte* into a 100-mL volumetric flask, dilute to volume with isopropyl alcohol, and mix.

Procedure Rinse a polarographic H-cell several times with small portions of *Test Solution A*, then fill the cell half-full with the same solution, place a paper tissue in the top of the sample side of the cell, and pass a moderate stream of saturated nitrogen through the solution for 15 min.

> **Note**: First saturate the nitrogen by passing it through a suitable scrubber containing isopropyl alcohol.

After 15 min, divert the nitrogen stream over the surface of the solution, and remove the tissue from the cell.

Set the polarizing voltage of a suitable, previously calibrated polarograph (Metrohm Polarocord E-261, or equivalent) at –1.3 V. Adjust the current sensitivity to the lowest range (most sensitive) at which the current oscillations will remain on scale. Record the polarogram, scanning a voltage range of –0.9 V to –1.5 V at this sensitivity and using a saturated calomel electrode as the reference electrode. Record the average oscillations, in millimeters, at –1.3 V as A, and those at –1.0 V as B.

> **Note**: If a manual polarograph is used, record the average oscillations of the solutions at –1.3 V and –1.0 V, respectively.

Repeat the entire procedure using *Test Solution B*, recording the average oscillations at –1.3 V as D, and those at –1.0 V as E. Similarly, repeat the entire procedure using the *Blank*, recording the average oscillations at –1.3 V as G, and those at –1.0 V as H.

Calculation Make the following preliminary calculations to obtain C (net diffusion current of *Test Solution A*); F (net diffusion current of *Test Solution B*); I (net current introduced by the *Blank*); J (diffusion current due to added maleate); and K (diffusion current due to originally present maleate):

$$C = (A - B) \times S_1,$$
$$F = (D - E) \times S_2,$$
$$I = (G - H) \times S_3,$$
$$J = F - C,$$
$$K = C - I,$$

in which S_1, S_2, and S_3 represent the current sensitivities used for *Test Solution A*, *Test Solution B*, and the *Blank*, respectively.

Finally, calculate the percent bis(2-ethylhexyl)maleate in the original sample taken by the formula

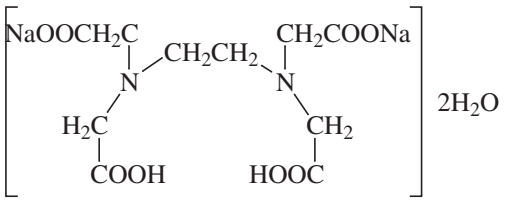
$$(K \times 50W_A)/(J \times W_S).$$

Clarity of Solution Dissolve 25 g of sample in 94 mL of alcohol. The solution does not develop a haze within 24 h.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

Disodium EDTA

Disodium Ethylenediaminetetraacetate; Disodium (Ethylenedinitrilo)tetraacetate; Disodium Edetate



$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ Formula wt 372.24

INS: 386 CAS: [6381-92-6]

DESCRIPTION

Disodium EDTA occurs as a white, crystalline powder. It is soluble in water.

Function Preservative; sequestrant; stabilizer.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution responds to the flame test for *Sodium*, Appendix IIIA.

B. Add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS to 5 mL of water in a test tube. Add about 50 mg of sample to the deep red solution so obtained, and mix. The deep red color disappears.

C. The infrared absorption spectrum of a potassium bromide dispersion of sample exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Edetate Disodium Reference Standard.

Assay Not less than 99.0% and not more than 101.0% of $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$.
Calcium Negative.
Lead Not more than 10 mg/kg.
Nitrilotriacetic Acid Not more than 0.1%.
pH of a 1:100 Solution Between 4.3 and 4.7.

TESTS

Assay

Assay Preparation Transfer about 5 g of sample, accurately weighed, into a 250-mL volumetric flask, dissolve in water, dilute to volume, and mix.

Procedure Place about 200 mg of chelometric standard calcium carbonate, accurately weighed, in a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 mL of 2.7 *N* hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute to about 100 mL with water. While stirring, preferably with a magnetic stirrer, add about 30 mL of the *Assay Preparation* from a 50-mL buret, then add 15 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Calculate the weight, in mg, of $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ in the sample taken by the formula

$$929.8(W/V),$$

in which W is the weight, in mg, of calcium carbonate, and V is the volume, in mL, of the *Assay Preparation* consumed in the titration.

Calcium Add 2 drops of methyl red TS to a 1:20 aqueous solution of the sample, and neutralize with 6 *N* ammonium hydroxide. Add 3 *N* hydrochloric acid dropwise until the solution is just acid, and then add 1 mL of ammonium oxalate TS. No precipitate forms.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 10 µg of lead (Pb) ion in the control.

Nitrilotriacetic Acid

Mobile Phase Add 10 mL of a 1:4 solution of tetrabutylammonium hydroxide in methanol to 200 mL of water, and adjust with 1 *M* phosphoric acid to a pH of 7.5 ± 0.1. Transfer the solution to a 1000-mL volumetric flask, add 90 mL of methanol, dilute with water to volume, mix, filter through a membrane filter (0.5-µm or finer porosity), and de-gas.

Cupric Nitrate Solution Prepare an aqueous solution containing about 10 mg of cupric nitrate per milliliter.

Stock Standard Solution Transfer about 100 mg of nitrilotriacetic acid, accurately weighed, to a 10-mL volumetric flask, add 0.5 mL of ammonium hydroxide, and mix. Dilute to volume, and mix.

Standard Preparation Transfer 1.0 g of sample to a 100-mL volumetric flask, add 100 µL of *Stock Standard Solution*, dilute to volume with *Cupric Nitrate Solution*, and mix. Sonicate, if necessary, to achieve complete solution.

Test Preparation Transfer 1.0 g of sample to a 100-mL volumetric flask, dilute with *Cupric Nitrate Solution* to volume, and mix. Sonicate, if necessary, to achieve complete solution.

Chromatographic System Set up the system with reference to *High-Performance Liquid Chromatography* under *Chromatography*, Appendix IIA. The HPLC chromatograph has a 254-nm detector and a 4.6-mm × 15-cm column that contains 5- to 10-mm porous microparticles of silica bonded to octylsilane (Zorbax 8, or equivalent). The flow rate is about 2 mL/min. Chromatograph three replicate injections of the *Standard Preparation*, and record the peak responses as directed under *Procedure*. The relative standard deviation is not more than 2.0%, and the resolution factor between nitrilotriacetic acid and Disodium EDTA is not less than 4.0.

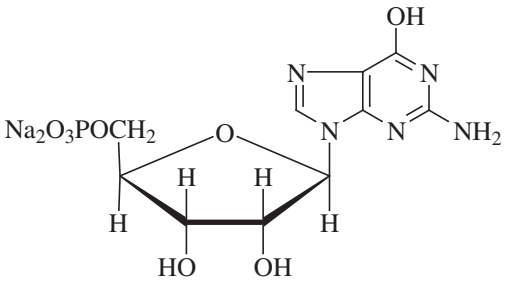
Procedure Separately inject equal volumes (about 50 µL) of the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention times are about 3.5 min for nitrilotriacetic acid and 9 min for Disodium EDTA. The response of the nitrilotriacetic acid peak of the *Test Preparation* does not exceed the difference between the nitrilotriacetic acid peak responses obtained from the *Standard Preparation* and the *Test Preparation*.

pH of a 1:100 Solution Determine as directed under *pH Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Disodium Guanylate

Disodium 5′-Guanylate; Disodium Guanosine-5′-monophosphate



$C_{10}H_{12}N_5Na_2O_8P{\cdot}xH_2O$ Formula wt, anhydrous 407.19

INS: 627 CAS: [5550-12-9]

DESCRIPTION

Disodium Guanylate occurs as colorless or white crystals, or as a white, crystalline powder. It contains approximately seven molecules of water of crystallization. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether.

Function Flavor enhancer.

REQUIREMENTS

Identification The ultraviolet absorption spectrum of a 1:50,000 solution in 0.01 *N* hydrochloric acid exhibits an absorbance maximum at 256 ± 2 nm.

Assay Not less than 97.0% and not more than 102.0% of $C_{10}H_{12}N_5Na_2O_8P$, calculated on the dried basis.

Amino Acids Passes test.

Ammonium Salts Passes test.

Clarity and Color of Solution Passes test.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 25.0%.

Other Nucleotides Passes test.

pH of a 1:20 Solution Between 7.0 and 8.5.

TESTS

Assay Transfer about 500 mg of sample, accurately weighed, into a 1000-mL volumetric flask, dissolve in and dilute to volume with 0.01 *N* hydrochloric acid, and mix. Transfer 10.0 mL of this solution into a 250-mL volumetric flask, dilute to volume with 0.01 *N* hydrochloric acid, and mix. Using a suitable spectrophotometer and 0.01 *N* hydrochloric acid as the blank, determine the absorbance of this solution and of a similarly prepared solution of USP Disodium Guanylate Reference Standard, at a concentration of 20 µg/mL, in 1-cm cells, at the maximum at about 260 nm. Calculate the quantity, in milligrams, of $C_{10}H_{12}N_5Na_2O_8P$ in the sample taken by the formula

$$25C \times A_U/A_S,$$

in which C is the exact concentration, in micrograms per milliliter, of the Reference Standard solution; A_U is the absorbance of the *Sample Solution*; and A_S is the absorbance of the Reference Standard solution.

Amino Acids Add 1 mL of ninhydrin TS to 5 mL of a 1:1000 aqueous solution, and heat for 3 min. No color appears.

Ammonium Salts Transfer about 100 mg of sample into a small test tube, and add 50 mg of magnesium oxide and 1 mL of water. Moisten a piece of red litmus paper with water, suspend it in the tube, cover the mouth of the tube, and heat in a water bath for 5 min. The litmus paper does not change to blue.

Clarity and Color of Solution A 100-mg portion of sample dissolved in 10 mL of water is colorless and shows no more than a trace of turbidity.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 120° for 4 h.

Other Nucleotides Prepare a strip of Whatman No. 2, or equivalent, filter paper about 20 × 40 cm, and draw a line across the narrow dimension about 5 cm from one end. Using

a micropipet, apply 10 µL of a 1:100 aqueous solution on the center of the line, and dry the paper in air.

Fill the trough of an apparatus suitable for descending chromatography (see *Chromatography*, Appendix IIA) with a 160:3:40 solution of saturated ammonium sulfate solution:*tert*-butyl alcohol:0.025 *N* ammonia, and suspend the strip in the chamber, placing the end of the strip in the trough at a distance about 1 cm from the pencil line. Seal the chamber, and allow the chromatogram to develop until the solvent front descends to a distance about 30 cm from the starting line. Remove the strip from the chamber, dry in air, and observe under shortwave (254 nm) ultraviolet light in the dark. Only one spot is visible.

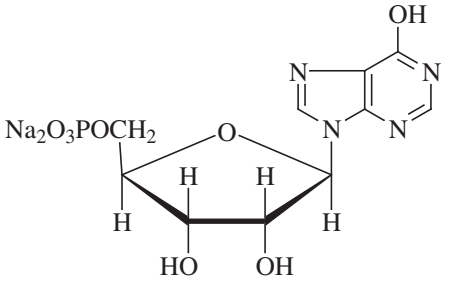
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 120° for 4 h.

pH of a 1:20 Solution Determine as directed under *pH Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Disodium Inosinate

Disodium 5′-Inosinate; Disodium Inosine-5′-monophosphate



$C_{10}H_{11}N_4Na_2O_8P{\cdot}xH_2O$ Formula wt, anhydrous 392.17

INS: 631 CAS: [4691-65-0]

DESCRIPTION

Disodium Inosinate occurs as colorless or white crystals or as a white, crystalline powder. It contains approximately 7.5 molecules of water of crystallization. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether.

Function Flavor enhancer.

REQUIREMENTS

Identification The ultraviolet absorption spectrum of a 1:50,000 solution in 0.01 *N* hydrochloric acid exhibits an absorbance maximum at 250 ± 2 nm. The ratio A_{250}/A_{260} is between 1.55 and 1.65, and the ratio A_{280}/A_{260} is between 0.20 and 0.30.

Assay Not less than 97.0% and not more than 102.0% of $C_{10}H_{11}N_4Na_2O_8P$, calculated on the anhydrous basis.

Amino Acids Passes test.

Ammonium Salts Passes test.

Clarity and Color of Solution Passes test.

Lead Not more than 5 g/kg.

Other Nucleotides Passes test.

pH of a 1:20 Solution Between 7.0 and 8.5.

Water Not more than 28.5%.

TESTS

Assay Transfer about 500 mg of sample, accurately weighed, into a 1000-mL volumetric flask, dissolve in and dilute to volume with 0.01 *N* hydrochloric acid, and mix. Transfer 10.0 mL of this solution into a 250-mL volumetric flask, dilute to volume with 0.01 *N* hydrochloric acid, and mix. Using a suitable spectrophotometer and 0.01 *N* hydrochloric acid as the blank, determine the absorbance of this solution and of a similarly prepared solution of USP Disodium Inosinate Reference Standard in 1-cm cells with the maximum at about 250 nm. Calculate the quantity, in milligrams, of $C_{10}H_{11}N_4Na_2O_8P$ in the sample taken by the formula

$$25C \times A_U/A_S,$$

in which C is the exact concentration, in micrograms per milliliter, of the Reference Standard solution; A_U is the absorbance of the *Sample Solution*; and A_S is the absorbance of the Reference Standard solution.

Amino Acids Add 1 mL of ninhydrin TS to 5 mL of a 1:1000 aqueous solution. No color appears.

Ammonium Salts Transfer about 100 mg of sample into a small test tube, and add 50 mg of magnesium oxide and 1 mL of water. Moisten a piece of red litmus paper with water, suspend it in the tube, cover the mouth of the tube, and heat in a water bath for 5 min. The litmus paper does not change to blue.

Clarity and Color of Solution A 500-mg portion of sample dissolved in 10 mL of water is colorless and shows no more than a trace of turbidity.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.

Other Nucleotides Prepare a strip of Whatman No. 2, or equivalent, filter paper about 20 × 40 cm, and draw a line across the narrow dimension about 5 cm from one end. Using a micropipet, apply 10 µL of a 1:100 aqueous solution on the center of the line, and dry the paper in air.

Fill the trough of an apparatus suitable for descending chromatography (see *Chromatography*, Appendix IIA) with a 160:3:40 mixture of saturated ammonium sulfate solution:*tert*-butyl alcohol:0.025 *N* ammonia, and suspend the strip in the chamber, placing the end of the strip in the trough at a distance about 1 cm from the pencil line. Seal the chamber, and allow the chromatogram to develop until the solvent front moves a distance about 30 cm from the starting line. Remove the strip from the chamber, dry in air, and observe under shortwave (254 nm) ultraviolet light in the dark. Only one spot is visible.

pH of a 1:20 Solution Determine as directed under *pH Determination*, Appendix IIB.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Enzyme-Modified Fats

DESCRIPTION

Enzyme-Modified Fats occur as light to medium tan liquids, pastes, or powders with a strong fatty acid odor and flavor. They are produced by enzyme lipolysis of fats obtained from milk, refined beef fat, or steam-rendered chicken fat, using suitable food-grade enzymes. Enzyme-modified milkfat may be prepared from milk, concentrated milk, dry whole milk, cream, concentrated cream(s), dry cream, butter, butter oil, dried butter, or anhydrous milkfat. For enzyme-modified milkfat, optional dairy ingredients such as skim milk, concentrated skim milk, nonfat dry milk, buttermilk, concentrated buttermilk, dried buttermilk, liquid whey, concentrated whey, and dried whey may be used to adjust the concentration of the flavors. Fat emulsions are reacted with suitable food-grade enzymes under controlled conditions to increase the flavor components. Thermoprocessing is then used to destroy the enzyme activity and provide acceptable microbiological quality. Suitable preservatives, emulsifiers, buffers, stabilizers, and antioxidants as well as sodium chloride may be added. The resulting product is concentrated or dried.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate the *Acid Value.*
Identification A sample has a very strong fatty acid odor.
Acid Value Not less than 98.0% and not more than 102.0% of the labeled value.
Lead Not more than 1 mg/kg.
Loss on Drying Not more than 4.0% for the dry product.
Microbial Limits:

Aerobic Plate Count Not more than 10,000 CFU per gram.

Coliforms Not more than 10 CFU per gram.

Salmonella Negative in 25 g.

Staphylococcal Enterotoxins Negative in 1 g.

Staphylococcus aureus Not more than 100 CFU per gram.

Yeasts and Molds Not more than 10 CFU per gram.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII, using a 5-g sample.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 48 h.
Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

Aerobic Plate Count
Coliforms
Salmonella
Staphylococcal Enterotoxins
Staphylococcus aureus
Yeasts and Molds

Packaging and Storage Store in tight containers in a cool place.

Enzyme Preparations

DESCRIPTION

Enzyme Preparations used in food processing are derived from animal, plant, or microbial sources (see *Classification*, below). They may consist of whole cells, parts of cells, or cell-free extracts of the source used, and they may contain one active component or, more commonly, a mixture of several, as well as food-grade diluents, preservatives, antioxidants, and other substances consistent with good manufacturing practices.

The individual preparations usually are named according to the substance to which they are applied, such as *Protease* or *Amylase*. Traditional names such as *Malt*, *Pepsin*, and *Rennet* also are used, however.

The color of the preparations—which may be liquid, semiliquid, or dry—may vary from virtually colorless to dark brown. The active components consist of the biologically active proteins, which are sometimes conjugated with metals, carbohydrates, and/or lipids. Known molecular weights of the active components range from approximately 12,000 to several hundred thousand.

The activity of enzyme preparations is measured according to the reaction catalyzed by individual enzymes (see below) and is usually expressed in activity units per unit weight of the preparation. In commercial practice (but not for *Food*

Chemicals Codex purposes), the activity of the product is sometimes also given as the quantity of the preparation to be added to a given quantity of food to achieve the desired effect.

Additional information relating to the nomenclature and the sources from which the active components are derived is provided under *Enzyme Assays*, Appendix V.

Function Enzyme (see discussion under *Classification*, below).

CLASSIFICATION

Animal-Derived Preparations

Catalase, Bovine Liver Produced as partially purified liquid or powdered extracts from bovine liver. Major active principle: *catalase*. Typical application: used in the manufacture of certain cheeses.

Chymotrypsin Obtained from purified extracts of bovine or porcine pancreatic tissue. Produced as white to tan, amorphous powders soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *chymotrypsin*. Typical application: used in the hydrolysis of protein.

Lipase, Animal Obtained from the edible forestomach tissue of calves, kids, or lambs; and from animal pancreatic tissue. Produced as purified edible tissue preparations or as aqueous extracts dispersible in water, but insoluble in alcohol. Major active principle: *lipase*. Typical applications: used in the manufacture of cheese and in the modification of lipids.

Lysozyme Obtained from extracts of purified chicken egg whites. Generally prepared and used in the hydrochloride form as a white powder. Major active principle: *lysozyme*. Typical application: used as an antimicrobial in food processing.

Pancreatin Obtained from porcine or bovine (ox) pancreatic tissue. Produced as a white to tan, water-soluble powder. Major active principles: (1) α-*amylase*; (2) *protease*; and (3) *lipase*. Typical applications: used in the preparation of precooked cereals, infant foods, and protein hydrolysates.

Pepsin Obtained from the glandular layer of hog stomach. Produced as a white to light tan, water-soluble powder; amber paste; or clear, amber to brown, aqueous liquids. Major active principle: *pepsin*. Typical applications: used in the preparation of fishmeal and other protein hydrolysates and in the clotting of milk in manufacture of cheese (in combination with rennet).

Phospholipase A_2 Obtained from porcine pancreatic tissue. Produced as a white to tan powder or pale to dark yellow liquid. Major active principle: *phospholipase* A_2. Typical application: used in the hydrolysis of lecithins.

Rennet, Bovine Aqueous extracts made from the fourth stomach of bovines. Produced as a clear, amber to dark brown liquid or a white to tan powder. Major active principle: *protease* (pepsin). Typical application: used in the manufacture of cheese. Similar preparations may be made from the fourth stomach of sheep or goats.

Rennet, Calf Aqueous extracts made from the fourth stomach of calves. Produced as a clear, amber to dark brown liquid or a white to tan powder. Major active principle: *protease* (chymosin). Typical application: used in the manufacture of cheese. Similar preparations may be made from the fourth stomach of lambs or kids.

Trypsin Obtained from purified extracts of porcine or bovine pancreas. Produced as white to tan, amorphous powders soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *trypsin*. Typical applications: used in baking, in the tenderizing of meat, and in the production of protein hydrolysates.

Plant-Derived Preparations

Amylase Obtained from extraction of ungerminated barley. Produced as a clear, amber to dark brown liquid or a white to tan powder. Major active principle: *β-amylase*. Typical applications: used in the production of alcoholic beverages and sugar syrups.

Bromelain The purified proteolytic substance derived from the pineapples *Ananas comosus* and *Ananas bracteatus* L. (Fam. Bromeliaceae). Produced as a white to light tan, amorphous powder soluble in water (the solution is usually colorless to light yellow and somewhat opalescent), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *bromelain*. Typical applications: used in the chillproofing of beer, in the tenderizing of meat, in the preparation of precooked cereals, in the production of protein hydrolysates, and in baking.

Ficin The purified proteolytic substance derived from the latex of *Ficus* sp. (Fam. Moraceae), which include a variety of tropical fig trees. Produced as a white to off white powder completely soluble in water. (Liquid fig latex concentrates are light to dark brown.) Major active principle: *ficin*. Typical applications: used in the chillproofing of beer, in the tenderizing of meat, and in the conditioning of dough in baking.

Malt The product of the controlled germination of barley. Produced as a clear amber to dark brown liquid preparations or as a white to tan powder. Major active principles: (1) *α-amylase* and (2) *β-amylase*. Typical applications: used in baking; used in the manufacture of alcoholic beverages and of syrups.

Papain The purified proteolytic substance derived from the fruit of the papaya *Carica papaya* L. (Fam. Caricaceae). Produced as a white to light tan, amorphous powder or a liquid soluble in water (the solution is usually colorless or light yellow and somewhat opalescent), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *papain* and (2) *chymopapain*. Typical applications: used in the chillproofing of beer, in the tenderizing of meat, in the

preparation of precooked cereals, and in the production of protein hydrolysates.

Microbially Derived Preparations

α-Acetolactatedecarboxylase (*Bacillus subtilis* containing a *Bacillus brevis* gene) Produced as a brown liquid by controlled fermentation using the modified *Bacillus subtilis*. Soluble in water (the solution is usually a light yellow to brown). Major active principle: *decarboxylase*. Typical application: used in the preparation of beer.

Aminopeptidase, Leucine (*Aspergillus niger* var., *Aspergillus oryzae* var., and other microbial species) Produced as a light tan to brown powder or as a brown liquid by controlled fermentation using *Aspergillus niger* var., *Aspergillus oryzae* var., or other microbial species. The powder is soluble in water (the solution is usually light yellow to brown). Major active principles: (1) *aminopeptidase*, (2) *protease*, and (3) *carboxypeptidase* activities in varying amounts. Typical applications: used in the preparation of protein hydrolysates and in the development of flavors in processed foods.

Carbohydrase (*Aspergillus niger* var., including *Aspergillus aculeatus*) Produced as an off white to tan powder or a tan to dark brown liquid by controlled fermentation using *Aspergillus niger* var. (including *Aspergillus aculeatus*). Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase*, (2) *pectinase* (a mixture of enzymes, including *pectin depolymerase*, *pectin methyl esterase*, *pectin lyase*, and *pectate lyase*), (3) *cellulase*, (4) *glucoamylase* (amyloglucosidase), (5) *amylo-1,6-glucosidase*, (6) *hemicellulase* (a mixture of enzymes, including *poly(galacturonate) hydrolase*, *arabinosidase, mannosidase, mannanase*, and *xylanase*), (7) *lactase*, (8) *β-glucanase*, (9) *β-D-glucosidase*, (10) *pentosanase*, and (11) *α-galactosidase*. Typical applications: used in the preparation of starch syrups and dextrose, alcohol, beer, ale, fruit juices, chocolate syrups, bakery products, liquid coffee, wine, dairy products, cereals, and spice and flavor extracts.

Carbohydrase (*Aspergillus oryzae* var.) Produced as an off white to tan, amorphous powder or a liquid by controlled fermentation using *Aspergillus oryzae* var. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase*, (2) *glucoamylase* (amyloglucosidase), and (3) *lactase*. Typical applications: used in the preparation of starch syrups, alcohol, beer, ale, bakery products, and dairy products.

Carbohydrase (*Bacillus acidopullulyticus*) Produced as an off white to brown, amorphous powder or a liquid by controlled fermentation using *Bacillus acidopullulyticus*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *pullulanase*. Typical applications: used in the hydrolysis of amylopectins and other branched polysaccharides.

Carbohydrase (*Bacillus stearothermophilus*) Produced as an off white to tan powder or a light yellow to dark brown liquid by controlled fermentation using *Bacillus stearothermophilus*. Soluble in water, but practically insoluble in alcohol, in ether, and in chloroform. Major active principle: *α-amylase*. Typical applications: used in the preparation of starch syrups, alcohol, beer, dextrose, and bakery products.

Carbohydrase (*Candida pseudotropicalis*) Produced as an off white to tan, amorphous powder or a liquid by controlled fermentation using *Candida pseudotropicalis*. Soluble in water (the solution is usually light yellow to dark brown) but insoluble in alcohol, in chloroform, and in ether. Major active principle: *lactase*. Typical applications: used in the manufacture of candy and ice cream and in the modification of dairy products.

Carbohydrase (*Kluyveromyces marxianus* var. *lactis*) Produced as an off white to tan, amorphous powder or a liquid by controlled fermentation using *Kluyveromyces marxianus* var. *lactis*. Soluble in water (the solution is usually light yellow to dark brown), but insoluble in alcohol, in chloroform, and in ether. Major active principle: *lactase*. Typical applications: used in the manufacture of candy and ice cream and in the modification of dairy products.

Carbohydrase (*Mortierella vinaceae* var. *raffinoseutilizer*) Produced as an off white to tan powder or as pellets by controlled fermentation using *Mortierella vinaceae* var. *raffinoseutilizer*. Soluble in water (pellets may be insoluble in water), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *α-galactosidase*. Typical application: used in the production of sugar from sugar beets.

Carbohydrase (*Rhizopus niveus*) Produced as an off white to brown, amorphous powder or a liquid by controlled fermentation using *Rhizopus niveus*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase* and (2) *glucoamylase*. Typical application: used in the hydrolysis of starch.

Carbohydrase (*Rhizopus oryzae* var.) Produced as a powder or a liquid by controlled fermentation using *Rhizopus oryzae* var. Soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase*, (2) *pectinase*, and (3) *glucoamylase* (amyloglucosidase). Typical applications: used in the preparation of starch syrups and fruit juices, vegetable purees, and juices and in the manufacture of cheese.

Carbohydrase (*Saccharomyces* species) Produced as a white to tan, amorphous powder by controlled fermentation using a number of species of *Saccharomyces* traditionally used in the manufacture of food. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *invertase* and (2) *lactase*. Typical applications: used in the manufacture of candy and ice cream and in the modification of dairy products.

Carbohydrase [(*Trichoderma longibrachiatum* var.) (formerly *reesei*)] Produced as an off white to tan, amorphous powder or as a liquid by controlled fermentation using *Trichoderma longibrachiatum* var. Soluble in water (the solution is usually tan to brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *cellulase,* (2) *β-glucanase*, (3) *β-D-glucosidase*, (4) *hemicellulase*, and (5) *pentosanase*. Typical applications: used in the preparation of fruit juices, wine, vegetable oils, beer, and baked goods.

Carbohydrase (*Bacillus subtilis* containing a *Bacillus megaterium* α-*amylase* gene) Produced as an off white to brown, amorphous powder or liquid by controlled fermentation using the modified *Bacillus subtilis*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: α-*amylase*. Typical applications: used in the preparation of starch syrups, alcohol, beer, and dextrose.

Carbohydrase (*Bacillus subtilis* containing a *Bacillus stearothermophilus* α-amylase gene) Produced as an off white to brown, amorphous powder or a liquid by controlled fermentation using the modified *Bacillus subtilis*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: maltogenic *amylase*. Typical applications: used in the preparation of starch syrups, dextrose, alcohol, beer, and baked goods.

Carbohydrase and Protease, Mixed (*Bacillus licheniformis* var.) Produced as an off white to brown, amorphous powder or as a liquid by controlled fermentation using *Bacillus licheniformis* var. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase* and (2) *protease*. Typical applications: used in the preparation of starch syrups, alcohol, beer, dextrose, fishmeal, and protein hydrolysates.

Carbohydrase and Protease, Mixed (*Bacillus subtilis* var. including *Bacillus amyloliquefaciens*) Produced as an off white to tan, amorphous powder or as a liquid by controlled fermentation using *Bacillus subtilis* var. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase*, (2) *β-glucanase*, (3) *protease*, and (4) *pentosanase*. Typical applications: used in the preparation of starch syrups, alcohol, beer, dextrose, bakery products, and fishmeal; in the tenderizing of meat; and in the preparation of protein hydrolysates.

Catalase (*Aspergillus niger* var.) Produced as an off white to tan, amorphous powder or as a liquid by controlled fermentation using *Aspergillus niger* var. Soluble in water (the solution is usually tan to brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *catalase*. Typical applications: used in the manufacture of cheese, egg products, and soft drinks.

Catalase (*Micrococcus lysodeikticus*) Produced by controlled fermentation using *Micrococcus lysodeikticus*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *catalase*. Typical application: used in the manufacture of cheese, egg products, and soft drinks.

Chymosin (*Aspergillus niger* var. *awamori*, *Escherichia coli K-12*, and *Kluyveromyces marxianus*, each microorganism containing a calf *prochymosin* gene) Produced as a white to tan, amorphous powder or as a light yellow to brown liquid by controlled fermentation using the above-named genetically modified microorganisms. The powder is soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *chymosin*. Typical application: used in the manufacture of cheese and in the preparation of milk-based desserts.

Glucose Isomerase (*Actinoplanes missouriensis, Bacillus coagulans, Streptomyces olivaceus, Streptomyces olivochromogenes, Microbacterium arborescens, Streptomyces rubiginosus* var., or *Streptomyces murinus*) Produced as an off white to tan, brown, or pink, amorphous powder, granules, or liquid by controlled fermentation using any of the above-named organisms. The products may be soluble in water, but practically insoluble in alcohol, in chloroform, and in ether; or if immobilized, may be insoluble in water and partially soluble in alcohol, in chloroform, and in ether. Major active principle: *glucose* (or *xylose*) *isomerase*. Typical applications: used in the manufacture of high-fructose corn syrup and other fructose starch syrups.

Glucose Oxidase (*Aspergillus niger* var.) Produced as a yellow to brown solution or as a yellow to tan or off white powder by controlled fermentation using *Aspergillus niger* var. Soluble in water (the solution is usually light yellow to brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *glucose oxidase* and (2) *catalase*. Typical applications: used in the removal of sugar from liquid eggs and in the deoxygenation of citrus beverages.

Lipase (*Aspergillus niger* var.) Produced as an off white to tan, amorphous powder by controlled fermentation using *Aspergillus niger* var. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical application: used in the hydrolysis of lipids (e.g., fish oil concentrates and cereal-derived lipids).

Lipase (*Aspergillus oryzae* var.) Produced as an off white to tan, amorphous powder or a liquid by controlled fermentation using *Aspergillus oryzae* var. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical applications: used in the hydrolysis of lipids (e.g., fish oil concentrates) and in the manufacture of cheese and cheese flavors.

Lipase (*Candida rugosa*; formerly *Candida cylindracea*) Produced as an off white to tan powder by controlled fermentation using *Candida rugosa*. Soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical applications: used in the hydrolysis of lipids, in the manufacture of dairy products and confectionery goods, and in the development of flavor in processed foods.

Lipase [*Rhizomucor (Mucor) miehei*] Produced as an off white to tan powder or as a liquid by controlled fermentation using *Rhizomucor miehei*. Soluble in water (the solution is usually light yellow to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical applications: used in the hydrolysis of lipids, in the manufacture of cheese, and in the removal of haze in fruit juices.

Phytase (*Aspergillus niger* var.) Produced as an off white to brown powder or as a tan to dark brown liquid by controlled fermentation using *Aspergillus niger* var. Soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *3-phytase* and (2) *acid phosphatase*. Typical applications: used in the production of soy protein isolate and in the removal of phytic acid from plant materials.

Protease (*Aspergillus niger* var.) Produced by controlled fermentation using *Aspergillus niger* var. The purified enzyme occurs as an off white to tan, amorphous powder. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: used in the production of protein hydrolysates.

Protease (*Aspergillus oryzae* var.) Produced by controlled fermentation using *Aspergillus oryzae* var. The purified enzyme occurs as an off white to tan, amorphous powder. Soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical applications: used in the chill-proofing of beer, in the production of bakery products, in the tenderizing of meat, in the production of protein hydrolysates, and in the development of flavor in processed foods.

Rennet, Microbial (nonpathogenic strain of *Bacillus cereus*) Produced as a white to tan, amorphous powder or a light yellow to dark brown liquid by controlled fermentation using *Bacillus cereus*. Soluble in water, but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: used in the manufacture of cheese.

Rennet, Microbial (*Endothia parasitica*) Produced as an off white to tan, amorphous powder or as a liquid by controlled fermentation using nonpathogenic strains of *Endothia parasitica*. The powder is soluble in water (the solution is usually tan to dark brown), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: used in the manufacture of cheese.

Rennet, Microbial [*Rhizomucor (Mucor)* sp.] Produced as a white to tan, amorphous powder by controlled fermentation using *Rhizomucor miehei*, or *pusillus* var. Lindt. The powder is soluble in water (the solution is usually light yellow), but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: used in the manufacture of cheese.

Transglutaminase (*Streptoverticillium mobaraense* var.) Produced as an off white to weak yellow-brown, amorphous powder by controlled fermentation using *Streptoverticillium mobaraense* var. Soluble in water but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *transglutaminase*. Typical applications: used in the processing of meat, poultry, and seafood; production of yogurt, certain cheeses, and frozen desserts; and manufacture of pasta products and noodles, baked goods, meat analogs, ready-to-eat cereals, and other grain-based foods.

REACTIONS CATALYZED

Note: The reactions catalyzed by any given active component are essentially the same, regardless of the source from which that component is derived.

α-Acetolactatedecarboxylase Decarboxylation of α-acetolactate to acetoin.

Aminopeptidase, Leucine Hydrolysis of *N*-terminal amino acid, which is preferably leucine, but may be other amino acids, from proteins and oligopeptides, yielding free amino acids and oligopeptides of lower molecular weight.

α-Amylase Endohydrolysis of α-1,4-glucan bonds in polysaccharides (starch, glycogen, etc.), yielding dextrins and oligo- and monosaccharides.

β-Amylase Hydrolysis of α-1,4-glucan bonds in polysaccharides (starch, glycogen, etc.), yielding maltose and beta-limit dextrins.

Bromelain Hydrolysis of polypeptides, amides, and esters (especially at bonds involving basic amino acids, leucine, or glycine), yielding peptides of lower molecular weight.

Catalase $2H_2O_2 \rightarrow O_2 + 2H_2O$.

Cellulase Hydrolysis of β-1,4-glucan bonds in such polysaccharides as cellulose, yielding β-dextrins.

Chymosin (calf and fermentation derived) Cleaves a single bond in *kappa* casein.

Ficin Hydrolysis of polypeptides, amides, and esters (especially at bonds involving basic amino acids, leucine, or glycine), yielding peptides of lower molecular weight.

α-Galactosidase Hydrolysis of terminal nonreducing α-D-galactose residues in α-D-galactosides.

β-Glucanase Hydrolysis of β-1,3- and β-1,4-linkages in β-D-glucans, yielding oligosaccharides and glucose.

Glucoamylase (*amyloglucosidase*) Hydrolysis of terminal α-1,4- and α-1,6-glucan bonds in polysaccharides (starch, glycogen, etc.), yielding glucose (dextrose).

Glucose Isomerase (*xylose isomerase*) Isomerization of glucose to fructose, and xylose to xylulose.

Glucose Oxidase β-D-glucose + O_2 → D-glucono-δ-lactone + H_2O_2.

β-D-Glucosidase Hydrolysis of terminal, nonreducing β-D-glucose residues with the release of β-D-glucose.

Hemicellulase Hydrolysis of β-1,4-glucans, α-L-arabinosides, β-D-mannosides, 1,3-β-D-xylans, and other polysaccharides, yielding polysaccharides of lower molecular weight.

Invertase (β-fructofuranosidase) Hydrolysis of sucrose to a mixture of glucose and fructose (invert sugar).

Lactase (β-galactosidase) Hydrolysis of lactose to a mixture of glucose and galactose.

Lysozyme Hydrolysis of cell-wall polysaccharides of various bacterial species leading to the breakdown of the cell wall most often in Gram-positive bacteria.

Maltogenic Amylase Hydrolysis of α-1,4-glucan bonds.

Lipase Hydrolysis of triglycerides of simple fatty acids, yielding mono- and diglycerides, glycerol, and free fatty acids.

Pancreatin

α-Amylase Hydrolysis of α-1,4-glucan bonds.

Protease Hydrolysis of proteins and polypepticles.

Lipase Hydrolysis of triglycerides of simple fatty acids.

Pectinase

Pectate lyase Hydrolysis of pectate to oligosaccharides.

Pectin depolymerase Hydrolysis of 1,4-galacturonide bonds.

Pectin lyase Hydrolysis of oligosaccharides formed by pectate lyase.

Pectinesterase Demethylation of pectin.

Pepsin Hydrolysis of polypeptides, including those with bonds adjacent to aromatic or dicarboxylic L-amino acid residues, yielding peptides of lower molecular weight.

Phospholipase A_2 Hydrolysis of lecithins and phosphatidylcholine, producing fatty acid anions.

Phytase

3-Phytase *myo*-Inositol hexakisphosphate + H_2O → 1,2,4,5,6-pentakisphosphate + orthophosphate.

Acid Phosphatase Orthophosphate monoester + H_2O → an alcohol + orthophosphate.

Protease (generic) Hydrolysis of polypeptides, yielding peptides of lower molecular weight.

Pullulanase Hydrolysis of 1,6-α-D-glycosidic bonds on amylopectin and glycogen and in α- and β-limit dextrins, yielding linear polysaccharides.

Rennet (bovine and calf) Hydrolysis of polypeptides; specificity may be similar to *pepsin*.

Transglutaminase Binding of proteins.

Trypsin Hydrolysis of polypeptides, amides, and esters at bonds involving the carboxyl groups of L-arginine and L-lysine, yielding peptides of lower molecular weight.

GENERAL REQUIREMENTS

Enzyme preparations are produced in accordance with good manufacturing practices. Regardless of the source of derivation, they should cause no increase in the total microbial count in the treated food over the level accepted for the respective food.

Animal tissues used to produce enzymes must comply with the applicable U.S. meat inspection requirements and must be handled in accordance with good hygienic practices.

Plant material used to produce enzymes or culture media used to grow microorganisms consist of components that leave no residues harmful to health in the finished food under normal conditions of use.

Preparations derived from microbial sources are produced by methods and under culture conditions that ensure a controlled fermentation, thus preventing the introduction of microorganisms that could be the source of toxic materials and other undesirable substances.

The carriers, diluents, and processing aids used to produce the enzyme preparations shall be substances that are acceptable for general use in foods, including water and substances that are insoluble in foods but removed from the foods after processing.

Although limits have not been established for mycotoxins, appropriate measures should be taken to ensure that the products do not contain such contaminants.

ADDITIONAL REQUIREMENTS

Assay Not less than 85.0% and not more than 115.0% of the declared units of enzyme activity.

Lead Not more than 5 mg/kg.

Microbial Limits:

Coliforms Not more than 30 CFU per gram.

Salmonella Negative in 25 g.

TESTS

Assay The following procedures, which are included under *Enzyme Assays*, Appendix V, are provided for application as necessary in determining compliance with the declared

representations for enzyme activity:[1] Acid Phosphatase Activity, α-Amylase Activity (Nonbacterial); Bacterial α-Amylase Activity (BAU); Catalase Activity; Cellulase Activity; Chymotrypsin Activity; Diastase Activity (Diastatic Power); α-Galactosidase Activity, β-Glucanase Activity; Glucoamylase Activity (Amyloglucosidase Activity); Glucose Isomerase Activity; Glucose Oxidase Activity; β-D-Glucosidase Activity; Hemicellulase Activity; Invertase Activity; Lactase (Neutral) (β-Galactosidase) Activity; Lactase (Acid) (β-Galactosidase) Activity; Lipase Activity; Lipase/Esterase (Forestomach) Activity; Maltogenic Amylase Activity; Milk-Clotting Activity; Pancreatin Activity; Pepsin Activity; Phospholipase Activity; Phytase Activity; Plant Proteolytic Activity; Proteolytic Activity, Bacterial (PC); Proteolytic Activity, Fungal (HUT); Proteolytic Activity, Fungal (SAP); Pullulanase Activity; and Trypsin Activity.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.

Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

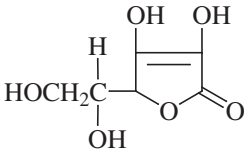
Coliforms

Salmonella

Packaging and Storage Store in tight containers in a cool, dry place.

Erythorbic Acid

D-Araboascorbic Acid



$C_6H_8O_6$ Formula wt 176.13

INS: 315 CAS: [89-65-6]

DESCRIPTION

Erythorbic Acid occurs as white or slightly yellow crystals or powder. It gradually darkens when exposed to light. In the dry state, it is reasonably stable in air, but in solution, it rapidly deteriorates in the presence of air. It melts between 164° and 171° with decomposition. One gram is soluble in about 2.5 mL of water and in about 20 mL of alcohol. It is slightly soluble in glycerin.

Function Preservative; antioxidant.

REQUIREMENTS

Identification

A. Add a few drops of sodium nitroferricyanide TS to 2 mL of a 1:50 aqueous solution, then add 1 mL of approximately 0.1 *N* sodium hydroxide. A transient blue color immediately appears.

B. Dissolve about 15 mg of sample in 15 mL of a 1:20 trichloroacetic acid solution, add about 200 mg of activated charcoal, and shake the mixture vigorously for 1 min. Filter through a small fluted filter, refiltering if necessary to obtain a clear filtrate. Add 1 drop of pyrrole to 5 mL of the clear filtrate, agitate the mixture until the pyrrole is dissolved, then heat in a water bath at 50°. A blue color appears.

Assay Not less than 99.0% and not more than 100.5% of $C_6H_8O_6$, calculated on the dried basis.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 0.4%.

Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Between −16.5° and −18.0°.

Residue on Ignition Not more than 0.3%.

TESTS

Assay Dissolve about 400 mg of sample, accurately weighed, in a 100:25 (v/v) mixture of recently boiled and cooled water:2 *N* sulfuric acid. Titrate the solution immediately with 0.1 *N* iodine, adding starch TS near the endpoint. Each milliliter of 0.1 *N* iodine is equivalent to 8.806 mg of $C_6H_8O_6$.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample under reduced pressure over silica gel for 3 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using the following solution: Transfer about 2.5 g of sample, accurately weighed, into a 25-mL volumetric flask, dissolve it in about 20 mL of water, and dilute to volume.

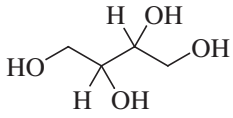
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight, light-resistant containers.

[1] Because of the varied conditions under which pectinases are employed, and because laboratory hydrolysis of a purified pectin substrate does not correlate with results observed with the natural substrates under use conditions, pectinase suppliers and users should develop their own assay procedures that would relate to the specific application under consideration.

Erythritol

1,2,3,4-Butanetetrol; *meso*-Erythritol



$C_4H_{10}O_4$ Formula wt 122.12

CAS: [149-32-6]

DESCRIPTION

Erythritol occurs as white crystals. It is obtained from the fermentation broth of the yeasts *Moniliella pollinis* or *Trichosporonoides megachiliensis*. It is stable to heat and is nonhygroscopic. It is soluble in water and is slightly soluble in alcohol. Erythritol melts between 119° and 123°.

Function Flavor enhancer; humectant; nutritive sweetener; texturizing agent; stabilizer.

REQUIREMENTS

Identification The retention time of the major peak in the chromatogram of the *Assay Preparation* corresponds to that in the chromatogram of the *Standard Preparation* obtained in the *Assay*.

Assay Not less than 99.5% and not more than 100.5% of $C_4H_{10}O_4$, calculated on the dried basis.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 0.2%.

Reducing Sugars (as glucose) Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

Ribitol and Glycerol Not more than 0.1%.

TESTS

Assay

Mobile Phase Use twice-distilled water.

Standard Preparation Transfer 500 mg of Erythritol Standard[1] and 50 mg each of reagent-grade glycerol and ribitol, accurately weighed, into a 100-mL volumetric flask. Dilute to volume with *Mobile Phase*, and mix. Save this preparation for the *Ribitol and Glycerol Test*.

Assay Preparation Transfer 4.0 g of sample, accurately weighed, into a 25-mL volumetric flask. Add *Mobile Phase* to volume, and mix. Filter through a 0.45-μm filter before injecting into the chromatograph. Save this preparation for the *Ribitol and Glycerol Test*.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with a constant-flow, pulseless pump and fitted with a sensitive differential refractive index detector. The column is packed with a strong cation exchange resin in the hydrogen form consisting of a macroreticular sulfonated polystyrene divinylbenzene and an 8% crosslinked copolymer, such as MCI-CKO8SH or Shodex KC811 (Mitsubishi Chemical Corp. and Showa Denko, Ltd.), or equivalent. The flow rate is about 0.6 mL/min, and the maximum pressure of the system is about 1500 psi.

Procedure Separately inject equal volumes of about 10 μL each of the *Standard Preparation*, followed by the *Assay Preparation*, into the chromatograph, and record the peak responses over a period of 60 min. The relative retention times are 1.0 for Erythritol, 1.1 for glycerol, and 0.9 for ribitol. Calculate the percentage of Erythritol in the sample taken by the equation

$$\% \text{ Erythritol} = 100E/T,$$

in which E is the area response of the Erythritol peak, and T is the total area responses in the chromatogram obtained with the *Assay Preparation*.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Reducing Sugars (as glucose) Dissolve about 500 mg of sample, accurately weighed, in 2 mL of water in a 20-mL flask, and mix. Transfer 2 mL of a glucose solution containing 0.75 mg/mL into another flask. Add 1 mL of *Fehling's Solution A* and of *Fehling's Solution B* (see *Cupric Tartrate TS, Alkaline,* under *Solutions and Indicators*) to each flask, heat to boiling, and cool. The sample solution is less turbid than the glucose solution, which forms a red-brown precipitate.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Ribitol and Glycerol Determine as directed in the *Assay*. Identify the peak area responses for glycerol and ribitol in the chromatogram of the *Assay Preparation* by comparison with the chromatogram of the *Standard Preparation*, and calculate the percentage of glycerol and ribitol by the equations

$$\% \text{ glycerol} = 100G/T,$$

$$\% \text{ ribitol} = 100R/T,$$

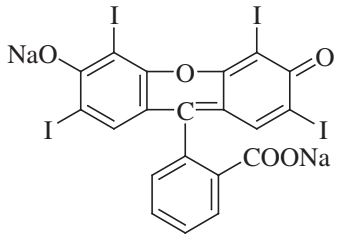
in which G is the area response of the glycerol peak, R is the area response of the ribitol peak, and T is the total area responses in the chromatogram obtained with the *Assay Preparation*. The sum of glycerol and ribitol is not more than 0.1%.

Packaging and Storage Store in well-closed containers.

[1]Available from Cerestar, EBS Vilvoorde R&D Centre, Centre of Expertise Fermentation, Havenstraat 84, 1800 Vilvoorde, Belgium; Mitsubishi Chemical Corporation, Specialty Chemicals Company, Intermediate Chemicals Department, 5-2 Marunouchi 2-chome, Chiyoda-ku, Tokyo 100-0005, Japan; or Nikken Chemicals Co., Ltd., Development Department, Sumitomo-Tsukiji Bldg., No. 4-14, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan.

Erythrosine[1]

CI Food Red 14; CI 45430; Class: Xanthene



$C_{20}H_6O_5I_4Na_2$ Formula wt 879.86

INS: 127 CAS: [16423-68-0]

DESCRIPTION

Erythrosine occurs as a brown powder or granules. It is principally the disodium salt of the monohydrate of 9-(*o*-carboxy-phenyl)-6-hydroxy-2,4,5,7-tetraiodo-3*H*-xanthen-3-one. It dissolves in water to give a solution red at neutrality, with a yellow-brown precipitate in acid, and with a red precipitate in base. When dissolved in concentrated sulfuric acid, it yields a brown-yellow solution that evolves iodine and a precipitate of the free acid when heated. It is insoluble in ethanol.

Function Color.

REQUIREMENTS

Identification An aqueous solution containing 2.8 mg of sample per liter exhibits absorbance intensities (*A*) and wavelength maxima as follows: in neutral (pH = 7) and alkaline (pH = 13) solutions, *A* = 0.32 at 527 nm with a shoulder at 490 nm. In acid solution, a yellow-brown precipitate forms.
Assay Not less than 87.0% total coloring matter.
Arsenic Not more than 3 mg/kg.
Ether Extracts (combined) Not more than 0.2%.
Lead Not more than 10 mg/kg.
Loss on Drying (Volatile Matter) at 135°, **Chlorides**, and **Sulfates** (as sodium salts) Not more than 13.0% in combination.
Subsidiary Colors
Monoiodofluoresceins Not more than 1.0%.
Other Lower Iodinated Fluoresceins Not more than 9.0%.
Uncombined Intermediates and Products of Side Reactions
2-(2′,4′-Dihydroxy-3′,5′-diiodobenzoyl)benzoic Acid Not more than 0.2%.
Sodium Iodide Not more than 0.4%.
Triiodoresorcinol Not more than 0.2%.
Unhalogenated Intermediates Total not more than 0.1%.
Water-Insoluble Matter Not more than 0.2%.

TESTS

Assay Determine the total color strength as the weight percent of the sample using *Methods I and III* in *Total Color* under *Colors*, Appendix IIIC. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 75 to 100 mg of sample, accurately weighed, into a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (*a*) for Erythrosine is 0.110 mg/L/cm at 527 nm.

Method III (Sample Preparation) Proceed as directed for *Method III* in *Total Color* under *Colors*, Appendix IIIC. The gravimetric conversion factor (*F*) for Erythrosine is 1.074.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Chlorides Determine as directed in *Sodium Chloride* under *Colors*, Appendix IIIC.
Ether Extracts Determine as directed in *Ether Extracts* under *Colors*, Appendix IIIC, using a solution with a pH of not less than 7.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 μg of lead (Pb) ion in the control.
Loss on Drying (Volatile Matter) at 135° Determine as directed in *Loss on Drying (Volatile Matter)* under *Colors*, Appendix IIIC.
Subsidiary Colors

Solvent System Use a solvent system composed of 95 mL of acetone, 25 mL of chloroform, 10 mL of butylamine, and 10 mL of water.

Sample Solution Transfer approximately 2 g of sample, accurately weighed, into a 100-mL volumetric flask. Fill the flask about ¾-full with water, incubate in the dark for 1 h; dilute to volume with water, and mix well.

Procedure Spot 0.1 mL of *Sample Solution* in a line across a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G, approximately 3 cm from the bottom edge. Allow the plate to dry for about 20 min in the dark, then develop with the *Solvent System* in an unlined tank equilibrated for at least 20 min before inserting the plate. Allow the solvent front to reach to within about 3 cm of the top of the plate. Dry the developed plate in the dark.

Scrape off each subsidiary color and extract with 3- to 5-mL portions of 50% aqueous ethanol until no color remains on the gel by visual inspection. Dilute each sample to 13 to 15 mL, add a few drops of ammonium hydroxide, and record the final volume. Repeat this procedure for the band of Erythrosine using 10- to 20-mL portions of 50% ethanol, and dilute the eluant to 250 mL in a volumetric flask after adding enough

[1]To be used or sold for use to color food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Red No. 3 can be applied only to FDA-certified batches of this color additive. Erythrosine is a common name given to the uncertified colorant. See the monograph entitled FD&C Red No. 3 for directions for producing an FDA-certified batch.

ammonium hydroxide to make the solution slightly alkaline. The approximate band positions (R_f), wavelengths of maximal absorbance (λ), and absorptivities (a) are as follows:

Color	R_f	λ	a
Unknown	0.84	524	0.110
Red-3	0.84	526	0.110
2,4,7	0.76	521	0.140
2,4,5	0.67	521	0.116
2,4/2,5	0.45	513	0.145
Unknown	0.45	524	0.110

Record the spectrum of each solution between 400 and 600 nm, and calculate the quantity, in percent (P), of each subsidiary color by the equation

$$P = (A \times V \times 100)/(a \times W \times b),$$

in which A is the absorbance at the wavelength maximum; V is the volume, in milliliters, of the solution; a is the absorptivity, in milligrams per liter per centimeter, as given above; W is the weight, in milligrams, of the sample; and b is the pathlength, in centimeters, of the cell.

Sulfates Determine as directed in *Sodium Sulfate* under *Colors*, Appendix IIIC.

Uncombined Intermediates and Products of Side Reactions Determine as directed for *Method* I in *Uncombined Intermediates and Products of Side Reactions* under *Colors*, Appendix IIIC, using the following *Sample Solution*: Transfer 2 g of sample, accurately weighed, into a 100-mL volumetric flask; dissolve in and dilute to volume with water. Calculate the concentrations of 2-(2,4-dihydroxy-3,5-diiodobenzoyl) benzoic acid, iodine, phthalic acid, sodium iodide, and triiodoresorcinol, using the following absorptivities:

2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic Acid: a = 0.047 mg/L/cm at 348 nm (alkaline solution).

Iodine: a = 0.082 mg/L/cm at 245 nm (acidic solution).

Phthalic Acid: a = 0.045 mg/L/cm at 228 nm (acidic solution).

Sodium Iodide: a = 0.091 mg/L/cm at 220 nm (acidic solution).

Triiodoresorcinol: a = 0.079 mg/L/cm at 223 nm (acidic solution).

Water-Insoluble Matter Determine as directed in *Water-Insoluble Matter* under *Colors*, Appendix IIIC.

Packaging and Storage Store in well-closed containers.

Ethoxylated Mono- and Diglycerides

Polyoxyethylene (20) Mono- and Diglycerides of Fatty Acids; Polyglycerate (60)

INS: 488

DESCRIPTION

Ethoxylated Mono- and Diglycerides occur as a pale, slightly yellow-colored, oily liquid or semigel. They are a mixture of stearate, palmitate, and lesser amounts of myristate partial esters of glycerin condensed with approximately 20 moles of ethylene oxide per mole of alpha-monoglyceride reaction mixture, having an average molecular weight of 535 (± 10%). They are soluble in water, in alcohol, and in xylene. They are partially soluble in mineral oil and in vegetable oils.

Note: If the product is manufactured by direct esterification of glycerin with a mixture of primary stearic, palmitic, and myristic acids, then the intermediate product (before reaction with ethylene oxide) has an acid value of not greater than 0.3 and a water content of not greater than 0.2%.

Function Dough conditioner; emulsifier.

REQUIREMENTS

Identification

A. Add 5 mL of 1 *N* sodium hydroxide to 5 mL of a 1:20 aqueous solution, boil for a few min, cool, and acidify with 2.7 *N* hydrochloric acid. The solution is strongly opalescent.

B. Make a 46:54 (v/v) mixture of sample:water at 40° or cooler. A gelatinous mass forms.

Acid Value Not more than 2.

1,4-Dioxane Passes test.

Hydroxyl Value Between 65 and 80.

Lead Not more than 1 mg/kg.

Oxyethylene Content (apparent) Not less than 60.5% and not more than 65.0%, calculated as ethylene oxide (C_2H_4O), on the anhydrous basis.

Saponification Value Between 65 and 75.

Stearic, Palmitic, and Myristic Acids Between 31 and 33 g per 100 g of sample.

Water Not more than 1%.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

1,4-Dioxane Determine as directed under *1,4-Dioxane Limit Test*, Appendix IIIB.

Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Oxyethylene Content (apparent) Determine as directed under *Oxyethylene Determination*, Appendix VII, using 70 mg of sample, accurately weighed.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 6 g of sample, accurately weighed.

Stearic, Palmitic, and Myristic Acids Transfer about 25 g of sample, accurately weighed, into a 500-mL round-bottom boiling flask, add 250 mL of alcohol and 7.5 g of potassium hydroxide, and mix. Connect a suitable condenser to the flask, reflux the mixture for 1 to 2 h, then transfer to an 800-mL beaker, rinsing the flask with about 100 mL of water and

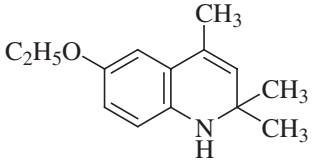
adding the washings to the beaker. Heat on a steam bath to evaporate the alcohol, adding water occasionally to replace the alcohol, and evaporate until the odor of alcohol can no longer be detected. Use hot water to adjust the final volume to about 250 mL. Neutralize the soap solution with 1:2 sulfuric acid; add 10% in excess; and while stirring, heat until the fatty acid layer separates. Transfer the fatty acids into a 500-mL separator, wash with three or four 20-mL portions of hot water, and combine the washings with the original aqueous layer from the saponification. Extract the combined aqueous layer with three 50-mL portions of petroleum ether, add the extracts to the fatty acid layer, evaporate to dryness in a tared dish, cool, and weigh. The product so obtained has an *Acid Value* between 199 and 211 (*Method I*, Appendix VII) and a *Solidification Point* ≥ 50° (Appendix IIB).

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Ethoxyquin

6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline



$C_{14}H_{19}NO$ Formula wt, monomer 217.31

INS: 324 CAS: [91-53-2]

DESCRIPTION

Ethoxyquin occurs as a clear yellow to red liquid that may darken with age without affecting its antioxidant activity. It is a mixture consisting predominantly of the monomer ($C_{14}H_{19}NO$). It also contains dimers and other polymers of $C_{14}H_{19}NO$. Its specific gravity is about 1.02, and its refractive index is about 1.57.

Function Antioxidant.

REQUIREMENTS

Identification A solution of 1 mg of sample in 10 mL of acetonitrile exhibits a strong fluorescence when viewed under short-wavelength ultraviolet light.

Assay Not less than 91.0% of $C_{14}H_{19}NO$.

Lead Not more than 2 mg/kg.

***p*-Phenetidine** Not more than 3.0%.

Ethoxyquin-Related Impurities (low-boiling monomers and high-boiling dimers, trimers, and oligomers of Ethoxyquin) Not more than 8.0%.

TESTS

Assay Transfer about 200 mg of sample, accurately weighed, into a 150-mL beaker containing 50 mL of glacial acetic acid, and immediately titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 21.73 mg of $C_{14}H_{19}NO$.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test,* Appendix IIIB, using a 10-g sample.

***p*-Phenetidine**

Standard Preparation (**Caution**: Perform all steps in a fume hood and away from a source of ignition. Wear appropriate protective equipment, including gloves.) (**Note**: Both the *p*-phenetidine and the diphenyl ether must be of known purity. Unless the reagent supplier's reported purity is certified quantitative and traceable, determine the purity of a reagent standard by conducting an area percent profile by injecting 0.1 µL on the same column and at conditions analogous to those described below. The area percent corresponding to the standard in the chromatograph represents its purity.) Transfer approximately 200 mg of *p*-phenetidine and approximately 200 mg of diphenyl ether, both accurately weighed, into a 4-dram bottle, add 10 mL of toluene and 5 drops of 10% sodium hydroxide, cap, and shake vigorously to dissolve. Prepare in triplicate.

Sample Preparation Transfer about 0.1 g of sample, accurately weighed, into a 4-dram vial. Add between 0.010 to 0.015 g of diphenyl ether, accurately weighed, 10 mL of toluene, and 5 drops of 10% sodium hydroxide solution; cap the vial; and shake well. Allow the vial to stand until the caustic layer settles to the bottom, and filter the neutralized sample through a 0.45-µm polytetrafluoroethylene (PTFE) filter, or equivalent.

Apparatus (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph (HP 6890, or equivalent) equipped with a split injector port, a flame-ionization detector (FID), and a 30-m × 0.25-mm (od) GC capillary column (DB-5MS, or equivalent) having a film thickness of 0.25 µm.

Operating Conditions The operating parameters may vary, depending on the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: initial temperature, 50°; initial hold time, 1 min; program rate, 10°/min; final temperature, 280°; final hold time, 10 min; injector temperature, 250°; detector temperature, 280°. Use helium as the carrier gas, at a column flow rate of 2.3 mL/min and a makeup flow rate of 50 mL/min. Set the hydrogen and air flows to the burner at 45 mL/min and 450 mL/min, respectively. Use a split flow rate of 232 mL/min and a total flow rate of 237 mL/min.

Calibration and Standardization Inject the *Standard Preparation* into the chromatograph. Calculate the *p*-phenetidine factor (*F*) by the formula

$$(A_{DE} \times W_{PF} \times P_{PF})/(A_{PF} \times W_{DE} \times P_{DE}),$$

in which A_{DE} and A_{PF} are the area responses for diphenyl ether and *p*-phenetidine, respectively; W_{PF} and W_{DE} are the weights, in grams, of *p*-phenetidine and diphenyl ether, respectively; and P_{PF} and P_{DE} are the purities of *p*-phenetidine and diphenyl ether, respectively.

Procedure Inject 1.0 µL of sample into the gas chromatograph, and calculate the content of *p*-phenetidine, in percent, by the formula

$$(A_P \times W_D \times F)/(W_S \times A_D),$$

in which A_P is the area of the *p*-phenetidine peak; W_D is the weight, in grams, of diphenyl ether in the *Sample Preparation*; F is the *p*-phenetidine factor; W_S is the weight, in grams, of the sample taken; and A_D is the area of the diphenyl ether peak.

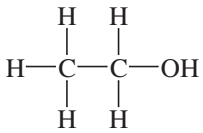
Ethoxyquin-Related Impurities Calculate the quantity, in percent, of related impurities by the formula

$$100 - (\% \text{ Assay} + \% \text{ } p\text{-Phenetidine}).$$

Packaging and Storage Store in tightly closed carbon steel or black iron (not rubber, neoprene, or nylon) containers or in polypropylene or polyethylene drums or lined drums in a cool, dark place. Prolonged exposure to sunlight causes polymerization.

Ethyl Alcohol

Alcohol; Ethanol



C_2H_6O Formula wt 46.07

CAS: [64-17-5]

DESCRIPTION

Ethyl Alcohol occurs as a clear, colorless, mobile liquid. It is miscible with water, with ether, and with chloroform. It boils at about 78° and is flammable. Its refractive index at 20° is about 1.364.

> **Note**: This monograph applies only to undenatured ethyl alcohol.

Function Extraction solvent; carrier solvent.

REQUIREMENTS

Assay Not less than 94.9% by volume (92.3% by weight) of C_2H_6O.

Acidity (as acetic acid) Not more than 0.003%.

Alkalinity (as NH_3) Not more than 3 mg/kg.

Fusel Oil Passes test.

Ketones, Isopropyl Alcohol Passes test.

Lead Not more than 0.5 mg/kg.

Methanol Passes test.

Nonvolatile Residue Not more than 0.003%.

Solubility in Water Passes test.

Substances Darkened by Sulfuric Acid Passes test.

Substances Reducing Permanganate Passes test.

TESTS

Assay The specific gravity of a sample, determined by any reliable method (see *General Provisions*), is not greater than 0.8096 at 25°/25° (equivalent to 0.8161 at 15.56°/15.56°).

Acidity Transfer 10 mL of sample to a glass-stoppered flask containing 25 mL of water, add 0.5 mL of phenolphthalein TS, and then add 0.02 *N* sodium hydroxide to the first appearance of a pink color that persists after shaking for 30 s. Add 25 mL of sample, mix, and titrate with 0.02 *N* sodium hydroxide until the pink color is restored. Not more than 0.5 mL of 0.02 *N* sodium hydroxide is required to restore the pink color.

Alkalinity Add 2 drops of methyl red TS to 25 mL of water, add 0.02 *N* sulfuric acid until a red color just appears, then add 25 mL of sample, and mix. Not more than 0.2 mL of 0.02 *N* sulfuric acid is required to restore the red color.

Fusel Oil Mix 10 mL of sample with 1 mL of glycerin and 1 mL of water, and allow to evaporate from a piece of clean, odorless, absorbent paper. No foreign odor is perceptible when the last traces of alcohol leave the paper.

Ketones, Isopropyl Alcohol Transfer 1 mL of sample, 3 mL of water, and 10 mL of mercuric sulfate TS to a test tube; mix; and heat in a boiling water bath. No precipitate forms within 3 min.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Methanol Transfer 1 drop of sample to a test tube, add 1 drop of 1:20 phosphoric acid and 1 drop of 1:20 potassium permanganate solution; mix; and allow to stand for 1 min. Add, dropwise, 1:10 sodium bisulfite solution until the permanganate color disappears. If a brown color remains, add 1 drop of the phosphoric acid solution. Add 5 mL of freshly prepared chromotropic acid TS to the colorless solution, and heat it in a water bath at 60° for 10 min. No violet color appears.

Nonvolatile Residue Evaporate 125 mL (about 100 g) of sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

Solubility in Water Transfer 50 mL of sample to a 100-mL glass-stoppered graduate, dilute to 100 mL with water, and mix. Place the graduate in a water bath maintained at 10°, and allow it to stand for 30 min. No haze or turbidity develops.

Substances Darkened by Sulfuric Acid Transfer 10 mL of sulfuric acid into a small Erlenmeyer flask, cool to 10°, and with constant agitation, add 10 mL of sample, dropwise.

The mixture is colorless or has no more color than either the acid or the sample before mixing.

Substances Reducing Permanganate Transfer 20 mL of sample, previously cooled to 15°, to a glass-stoppered cylinder, add 0.1 mL of 0.1 *N* potassium permanganate, mix, and allow to stand for 5 min. The pink color does not entirely disappear.

Packaging and Storage Store in tight containers, remote from fire.

Ethyl Cellulose

Modified Cellulose, EC

INS: 462 CAS: [9004-57-3]

DESCRIPTION

Ethyl Cellulose occurs as a free-flowing, white to light tan powder. It is heat-labile, and exposure to high temperatures (240°) causes color degradation and loss of properties. It is practically insoluble in water, in glycerin, and in propylene glycol, but is soluble in varying proportions in certain organic solvents, depending on the ethoxyl content. Ethyl Cellulose containing less than 46% to 48% of ethoxyl groups is freely soluble in tetrahydrofuran, in methyl acetate, in chloroform, and in aromatic hydrocarbon–alcohol mixtures. Ethyl Cellulose containing 46% to 48% or more of ethoxyl groups is freely soluble in alcohol, in methanol, in toluene, in chloroform, and in ethyl acetate. A 1:20 aqueous suspension is neutral to litmus.

Function Protective coating; binder; filler.

REQUIREMENTS

Identification Dissolve 5 g of sample in 95 g of an 80:20 (w/w) mixture of toluene:ethanol. A clear, stable, slightly yellow solution forms. Pour a few milliliters of the solution onto a glass plate, and allow the solvent to evaporate. A thick, tough, clear, flammable film remains.

Assay Not less than 44.0% and not more than 50.0% of ethoxyl groups (—OC_2H_5) after drying (equivalent to not more than 2.6 ethoxyl groups per anhydroglucose unit).

Lead Not more than 3 mg/kg.

Loss on Drying Not more than 3%.

Residue on Ignition Not more than 0.4%.

Viscosity Not less than 90% and not more than 110% of the viscosity stated on the label as 10 centipoises or more; not less than 80% and not more than 120% of the viscosity stated on the label as 10 centipoises or fewer.

TESTS

Assay Place about 50 mg of sample, previously dried at 105° for 2 h, in a tared gelatin capsule, accurately weigh, transfer the capsule and its contents into the boiling flask of a methoxyl determination apparatus, and proceed as directed under *Methoxyl Determination*, Appendix IIIC. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 751 μg of ethoxyl groups (—OC_2H_5).

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared from a 2-g sample as directed for organic compounds, and 6 μg of lead (Pb) ion in the control.

Alternatively, determine as directed for *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Viscosity

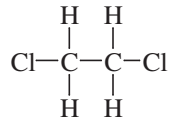
Solvent Systems For Ethyl Cellulose containing less than 46% to 48% of ethoxyl groups, prepare a solvent consisting of a 60:40 (w/w) mixture of toluene:alcohol; for Ethyl Cellulose containing 46% to 48% or more of ethoxyl groups, prepare a solvent consisting of an 80:20 (w/w) mixture of toluene:alcohol.

Procedure Transfer 5.0 g of sample, previously dried at 105° for 2 h and accurately weighed, into a bottle containing 95 ± 0.5 g of the appropriate solvent system. Shake or tumble the bottle until the sample is completely dissolved, and then adjust the temperature of the solution to 25° ± 0.1°. Determine the viscosity as directed under *Viscosity of Methylcellulose*, Appendix IIB, but make all determinations at 25° instead of at 20°.

Packaging and Storage Store in well-closed containers.

Ethylene Dichloride

1,2-Dichloroethane



$C_2H_4Cl_2$ Formula wt 98.96

CAS: [107-06-2]

DESCRIPTION

Ethylene Dichloride occurs as a clear, colorless, flammable, oily liquid. It is slightly soluble in water, and is soluble in alcohol, in ether, and in acetone. Its refractive index at 20° is about 1.445.

Function Extraction solvent.

REQUIREMENTS

Acidity (as HCl) Not more than 10 mg/kg.
Distillation Range Between 82° and 85°.
Free Halogens Passes test.
Lead Not more than 1 mg/kg.
Nonvolatile Residue Not more than 0.002%.
Specific Gravity Between 1.245 and 1.255.
Water Not more than 0.03%.

TESTS

Acidity (as HCl) Transfer 25 mL of alcohol to a 100-mL glass-stoppered flask, add 2 drops of phenolphthalein TS, and titrate with 0.01 *N* sodium hydroxide to the first appearance of a slight pink color. Add 25 mL of sample, mix, and titrate with 0.01 *N* sodium hydroxide until the faint pink color is restored. Not more than 0.85 mL of 0.01 *N* sodium hydroxide is required to restore the pink color.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.
Free Halogens Mix 10 mL of sample with 10 mL of 10% potassium iodide solution and 1 mL of starch TS. Shake the mixture vigorously for 2 min. A blue color does not appear in the water layer.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Nonvolatile Residue Evaporate 80 mL (about 100 g) of sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

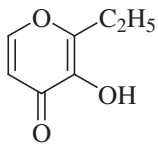
Caution: Use an appropriate fume hood.

Specific Gravity Determine by any reliable method (see *General Provisions*).
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Ethyl Maltol

2-Ethyl-3-hydroxy-4-pyrone



$C_7H_8O_3$ Formula wt 140.14
INS: 637 CAS: [4940-11-8]
FEMA: 3487

DESCRIPTION

Ethyl Maltol occurs as a white, crystalline powder having a cotton-candy odor and a sweet, fruitlike flavor in dilute solution. One gram dissolves in about 55 mL of water, 10 mL of alcohol, 17 mL of propylene glycol, and 5 mL of chloroform. It melts at about 90°.

Function Flavoring agent; flavor enhancer.

REQUIREMENTS

Identification The infrared absorption spectrum of a 1:50 solution in chloroform, determined in a 0.1-mm cell, exhibits relative maxima at the same wavelengths as those of USP Ethyl Maltol Reference Standard, similarly prepared.
Assay Not less than 99.0% of $C_7H_8O_3$, calculated on the anhydrous basis.
Residue on Ignition Not more than 0.2%.
Water Not more than 0.5%.

TESTS

Assay

Standard Solution Dissolve about 50 mg of USP Ethyl Maltol Reference Standard, accurately weighed, in sufficient 0.1 *N* hydrochloric acid to make 250.0 mL, and mix. Transfer 5.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with 0.1 *N* hydrochloric acid, and mix.

Assay Solution Dissolve about 50 mg of sample, accurately weighed, in sufficient 0.1 *N* hydrochloric acid to make 250.0 mL, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute to volume with 0.1 *N* hydrochloric acid, and mix.

Procedure Using a suitable spectrophotometer and 0.1 *N* hydrochloric acid as the blank, determine the absorbance of each solution in a 1-cm cell at the wavelength of maximum absorption (about 276 nm). Calculate the quantity, in milligrams, of $C_7H_8O_3$ in the sample taken by the formula

$$5C(A_U/A_S),$$

in which C is the concentration, in micrograms per milliliter, of USP Ethyl Maltol Reference Standard in the *Standard Solution*; A_U is the absorbance of the *Assay Solution*; and A_S is the absorbance of the *Standard Solution*.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Eucalyptus Oil

CAS: [8000-48-4]

View IR

DESCRIPTION

Eucalyptus Oil occurs as a colorless or pale yellow liquid with a characteristic, aromatic, somewhat camphoraceous odor and

a pungent, spicy, cooling taste. It is the volatile oil obtained by steam distillation from the fresh leaves of *Eucalyptus globulus* Labillardiere and other species of *Eucalyptus* L'Heritier (Fam. Myrtaceae).

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 70.0% of cineole ($C_{10}H_{18}O$).
Phellandrene Passes test.
Refractive Index Between 1.458 and 1.470 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.905 and 0.925.

TESTS

Assay Transfer about 3 g of sample, previously dried with anhydrous sodium sulfate and accurately weighed, into a 25- × 150-mm test tube. Add 2.100 g of melted *o*-cresol that is pure and dry, with a solidification point of 30° or higher, to the sample.

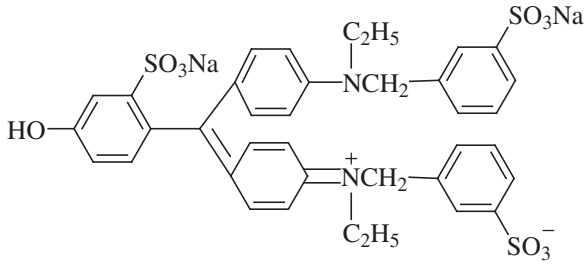
Note: Moisture in the *o*-cresol may cause low results.

Stir the mixture with a thermometer (see *Thermometers*, Appendix I) to induce crystallization, and note the highest temperature reading obtained. Warm the tube gently until the contents are completely melted, then insert the test tube into an apparatus assembled as directed under *Solidification Point*, Appendix IIB. Allow the mixture to cool slowly until crystallization starts, or until the temperature has fallen to the point noted above. Stir the mixture vigorously with the thermometer, rubbing the sides of the test tube with an up and down motion to induce crystallization. Continue the stirring and rubbing until the temperature no longer rises. Record the highest temperature obtained as the solidification point. Repeat the procedure until two results agreeing within 0.1° are obtained. Calculate the percentage of cineole from the *Percentage of Cineole* table, Appendix VI.
Phellandrene Mix 2.5 mL of sample with 5 mL of solvent hexane, add 5 mL of a solution of sodium nitrite (made by dissolving 5 g of sodium nitrite in 8 mL of water), and gradually add 5 mL of glacial acetic acid. No crystals form in the mixture within 10 min.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 5 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in well-filled, tight containers in a cool place protected from light.

Fast Green[1]

Fast Green FCF; CI 42053; Class: Triphenylmethane



$C_{37}H_{34}N_2O_{10}S_3Na_2$ Formula wt 808.86

INS: 143 CAS: [2353-45-9]

DESCRIPTION

Fast Green occurs as a red to brown-violet powder or granules. It is principally the inner disodium salt of *N*-ethyl-*N*-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium hydroxide. It dissolves in water to give a solution blue-green at neutrality, green in acid, and blue-violet in base. When dissolved in sulfuric acid, it yields a brown-orange solution that turns green when diluted with water. When heated to 130° with triacetin and an excess of acetic anhydride, acetylation of its phenolic hydroxyl group causes a color change from green to light blue. It is slightly soluble in ethanol.

Function Color.

REQUIREMENTS

Identification A freshly prepared aqueous solution containing 5 mg of sample per liter exhibits absorbance intensities (*A*) and wavelength maxima as follows: at pH 7, $A = 0.80$ at 624 nm, and $A = 0.08$ at 423 nm; at pH 1, $A = 0.83$ at 625 nm, and $A = 0.09$ at 423 nm; and at pH 13, $A = 0.74$ at 610 nm.
Assay Not less than 85.0% total coloring matter.
Arsenic Not more than 3 mg/kg.
Chromium Not more than 0.005%.
Ether Extracts (combined) Not more than 0.4%.
Lead Not more than 10 mg/kg.
Leuco Base Not more than 5.0%.
Loss on Drying (Volatile Matter) at 135°, Chlorides and Sulfates (as sodium salts) Not more than 15.0% in combination.

[1]To be used or sold for use to color food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Green No. 3 can be applied only to FDA-certified batches of this color additive. Fast Green is a common name given to the uncertified colorant. See the monograph entitled FD&C Green No. 3 for directions for producing an FDA-certified batch.

Subsidiary Colors Not more than 6.0%, total, of isomeric inner salt disodium salt of *N*-ethyl-*N*-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)-methylene]-2,5-cyclohexadien-1-ylidene]-4-sulfobenzenemethanaminium hydroxide, *N*-ethyl-*N*-[4-[[4-[ethyl[(4-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)-methylene]-2,5-cyclohexadien-1-ylidene]-4-sulfobenzenemethanaminium hydroxide, and *N*-ethyl-*N*-[4-[[4-[ethyl[(2-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadiene-1-ylidene]-3-sulfobenzenemethanaminium hydroxide.

Uncombined Intermediates and Products of Side Reactions

Sum of 3- and 4-[[Ethyl(4-sulfophenyl)amino]methyl]benzenesulfonic Acid Disodium Salts Not more than 0.3%.

Sum of 2-, 3-, and 4-Formyl Benzenesulfonic Acids, Sodium Salts Not more than 0.5%.

2-Formyl-5-hydroxybenzenesulfonic Acid, Sodium Salt Not more than 0.5%.

Water-Insoluble Matter Not more than 0.2%.

TESTS

Assay Determine the total color strength as the weight percent of the sample using *Methods I and II* in *Total Color* under *Colors*, Appendix IIIC. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 50 to 75 mg of sample, accurately weighed, into a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (a) for Fast Green is 0.156 mg/L/cm at 625 nm.

Method II (Sample Preparation) Transfer approximately 0.5 g of sample, accurately weighed, into the titration flask. The stoichiometric factor (F_s) for Fast Green is 2.47.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Chloride Determine as directed in *Sodium Chloride* under *Colors*, Appendix IIIC.

Chromium Determine as directed in *Chromium* under *Colors*, Appendix IIIC.

Ether Extracts Determine as directed in *Ether Extracts* under *Colors*, Appendix IIIC.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 µg of lead (Pb) ion in the control.

Leuco Base Determine as directed in *Leuco Base* under *Colors*, Appendix IIIC, using the following *Sample Solution*: Transfer approximately 130 mg of sample, accurately weighed, into a 1-L volumetric flask; dissolve in and dilute to volume with water.

Loss on Drying (Volatile Matter) at 135°, Chlorides and Sulfates (as sodium salts) Determine as directed in *Loss on Drying (Volatile Matter)* under *Colors*, Appendix IIIC.

Subsidiary Colors

Solvent System Use a solvent system composed of 50 mL of acetonitrile, 50 mL of isoamyl alcohol, 15 mL of 2-butanone, 10 mL of water, and 5 mL of ammonium hydroxide.

Sample Solution Transfer approximately 1 g of sample, accurately weighed, into a 100-mL volumetric flask. Fill the flask about ¾ full with water, and incubate in the dark for 1 h; dilute to volume with water, and mix well.

Procedure Spot 0.1 mL of the *Sample Solution* in a line across a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G, approximately 3 cm from the bottom edge. Allow the plate to dry for about 20 min in the dark, then develop with the *Solvent System* in an unlined tank equilibrated for at least 20 min before the plate is inserted. Allow the solvent front to reach within about 3 cm of the top of the plate. Dry the developed plate in the dark.

When the plate has dried, scrape off all the colored bands above the Fast Green, which remains close to the origin, into a 30-mL beaker. Extract the subsidiary colors with three 6-mL portions of 95% ethanol, or until no color remains on the gel by visual inspection. Record the volume of ethanol used and the spectrum of the extract between 400 and 700 nm. Calculate the percent of subsidiary colors (P) by the equation

$$P = (A \times V \times 100)/(a \times W \times b),$$

in which A is the absorbance at the wavelength maximum; V is the volume, in milliliters, of the extract; a is the absorptivity (0.126 mg/L/cm); W is the weight, in milligrams, of the sample; and b is the path-length, in centimeters, of the cell.

Sulfate Determine as directed in *Sodium Sulfate* under *Colors*, Appendix IIIC.

Uncombined Intermediates and Products of Side Reactions Determine as directed for *Method I* under *Uncombined Intermediates and Products of Side Reactions* under *Colors*, Appendix IIIC, except use the following as the *Sample Solution*: Transfer approximately 2 g of sample, accurately weighed, into a 100-mL volumetric flask; dissolve in and dilute to volume with water. Replace the *Calculation* with the following: Calculate the amounts of intermediates and other products present using the following absorptivities after identifying the unknowns by comparing their spectra with standards:

4-Hydroxy-2-sulfobenzaldehyde: $a = 0.080$ mg/L/cm at 335 nm (alkaline solution).

m-*Sulfobenzaldehyde*: $a = 0.495$ mg/L/cm at 246 nm (acidic solution).

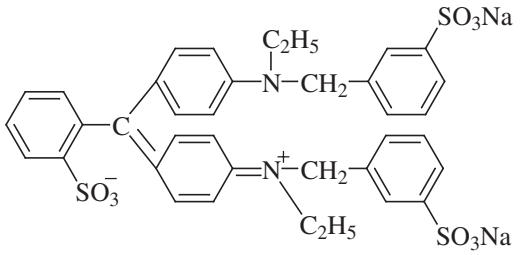
N-*Ethyl*-N-(*3-sulfobenzyl)-sulfanilic Acid*: $a = 0.078$ mg/L/cm at 277 nm (alkaline solution).

Water-Insoluble Matter Determine as directed in *Water-Insoluble Matter* under *Colors*, Appendix IIIC.

Packaging and Storage Store in well-closed containers.

FD&C Blue No. 1[1]

Brilliant Blue FCF;[2] CI 42090;[1] Class: Triphenylmethane



$C_{37}H_{34}N_2O_9S_3Na_2$ Formula wt 792.86

INS: 133 CAS: [3844-45-9]

DESCRIPTION

FD&C Blue No. 1 is principally the disodium salt of ethyl[4-[*p*-[ethyl(*m*-sulfobenzyl)amino]-α-(*o*-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](*m*-sulfobenzyl)ammonium hydroxide inner salt, with smaller amounts of the isomeric disodium salts of ethyl[4-[*p*-[ethyl(*p*-sulfobenzyl)amino]-α-(*o*-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](*p*-sulfobenzyl)ammonium hydroxide inner salt and ethyl[4-[*p*-[ethyl(*o*-sulfobenzyl)amino]-α-(*o*-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](*o*-sulfobenzyl)ammonium hydroxide inner salt.

Function Color.

REQUIREMENTS

Identification The visible absorption spectrum of a sample of FD&C Blue No. 1 dissolved in 0.04 *N* aqueous ammonium acetate has a wavelength maximum of 630 nm, with an absorptivity of 0.164 L/(mg·cm).
Arsenic (as As) Not more than 3 mg/kg.
Chromium (as Cr) Not more than 0.005%.
Manganese (as Mn) Not more than 0.01%.
Ether Extracts[3] (combined) Not more than 0.4%.
Lead (as Pb) Not more than 10 mg/kg.
Leuco Base Not more than 5%.
Subsidiary Colors Not more than 6.0%.
Total Color Not less than 85.0%.
Uncombined Intermediates and Products of Side Reactions

o-, m-, *and* p-*Sulfobenzaldehydes* Not more than 1.5%, combined.

N-*Ethyl*-N-(m-*sulfobenzyl*)*sulfanilic Acid* Not more than 0.3%.
Volatile Matter (at 135°) and Chlorides and Sulfates (as sodium salts) Not more than 15.0% in combination.
Water-Insoluble Matter Not more than 0.2%.

TESTS

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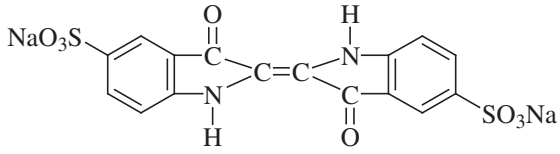
Packaging and Storage Store in well-closed containers.

FD&C Blue No. 2[1]

Indigotine;[2] Indigo Carmine;[2] CI 73015;[2] Class: Indigoid



$C_{16}H_8N_2O_8S_2Na_2$ Formula wt 466.36

INS: 132 CAS: [860-22-0]

DESCRIPTION

FD&C Blue No. 2 is principally the disodium salt of 2-(1,3-dihydro-3-oxo-5-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid, with smaller amounts of the disodium salt of 2-(1,3-dihydro-3-oxo-7-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid and the sodium salt of 2-(1,3-dihydro-3-oxo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid.

Function Color.

REQUIREMENTS

Identification The visible absorption spectrum of a sample of FD&C Blue No. 2 dissolved in 0.04 *N* aqueous ammonium acetate has a wavelength maximum of 610 nm, with an absorptivity of 0.0478 L/(mg·cm).

[1]To be used or sold in the United States, this color additive must be batch certified by the U.S. Food and Drug Administration. The monograph title is the name of the color additive only after batch certification has been completed.

[2]Generic designations; not synonyms for certified batches of color additive.

[3]Not required for certification in the United States.

Arsenic (as As) Not more than 3 mg/kg.
Ether Extracts[3] (combined) Not more than 0.4%.
Lead (as Pb) Not more than 10 mg/kg.
Mercury (as Hg) Not more than 1 mg/kg.
Subsidiary and Isomeric Colors

*Disodium Salt of 2-(1,3-Dihydro-3-oxo-7-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic Acid* Not more than 18%.

*Sodium Salt of 2-(1,3-Dihydro-3-oxo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic Acid* Not more than 2%.

Total Color Not less than 85%.
Decomposition Products

Isatin-5-sulfonic Acid Not more than 0.4%.

5-Sulfoanthranilic Acid Not more than 0.2%.

Volatile Matter (at 135°) and Chlorides and Sulfates (as sodium salts) Not more than 15% in combination.
Water-Insoluble Matter Not more than 0.4%.

TESTS

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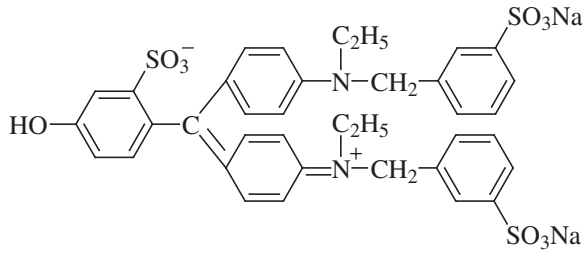
Packaging and Storage Store in well-closed containers.

FD&C Green No. 3[1]

Fast Green FCF;[2] CI 42053;[2] Class: Triphenylmethane



$C_{37}H_{34}N_2O_{10}S_3Na_2$ Formula wt 808.86

INS: 143 CAS: [2353-45-9]

DESCRIPTION

FD&C Green No. 3 is principally the inner salt disodium salt of *N*-ethyl-*N*-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]-phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium hydroxide, with smaller amounts of the isomeric inner salt disodium salt of *N*-ethyl-*N*-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]-phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-4-sulfobenzenemethanaminium hydroxide; of *N*-ethyl-*N*-[4-[[4-[ethyl[(4-sulfophenyl)methyl]amino]-phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-4-sulfobenzenemethanaminium hydroxide; and of *N*-ethyl-*N*-[4-[[4-[ethyl[(2-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium hydroxide.

Function Color.

REQUIREMENTS

Identification The visible absorption spectrum of a sample of FD&C Green No. 3 dissolved in 0.04 *N* aqueous ammonium acetate has a wavelength maximum of 625 nm, with an absorptivity of 0.156 L/(mg·cm).
Arsenic (as As) Not more than 3 mg/kg.
Chromium (as Cr) Not more than 0.005%.
Ether Extracts[3] (combined) Not more than 0.4%.
Lead (as Pb) Not more than 10 mg/kg.
Leuco Base Not more than 5%.
Mercury (as Hg) Not more than 1 mg/kg.
Subsidiary Colors Not more than 6%.
Total Color Not less than 85%.

[1]To be used or sold in the United States, this color additive must be batch certified by the U.S. Food and Drug Administration. The monograph title is the name of the color additive only after batch certification has been completed.

[2]Generic designations; not synonyms for certified batches of color additive.

[3]Not required for certification in the United States.

Uncombined Intermediates and Products of Side Reactions

Sum of 3- and 4-[[Ethyl(4-sulfophenyl)amino]methyl]benzenesulfonic Acid, Disodium Salts Not more than 0.3%.

Sum of 2-, 3-, and 4-Formylbenzenesulfonic Acids, Sodium Salts Not more than 0.5%.

2-Formyl-5-hydroxybenzenesulfonic Acid, Sodium Salt Not more than 0.5%.

Volatile Matter (at 135°) and Chlorides and Sulfates (as sodium salts) Not more than 15.0% in combination.

Water-Insoluble Matter Not more than 0.2%.

TESTS

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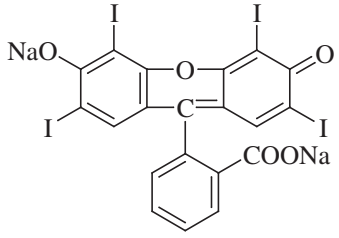
Packaging and Storage Store in well-closed containers.

FD&C Red No. 3[1]

Erythrosine;[2] CI 45430;[2] Class: Xanthene



$C_{20}H_6O_5I_4Na_2$ Formula wt 879.86

INS: 127 CAS: [16423-68-0]

DESCRIPTION

FD&C Red No. 3 is principally the monohydrate of 9-(*o*-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3*H*-xanthen-3-one disodium salt, with smaller amounts of lower iodinated fluoresceins.

Function Color.

REQUIREMENTS

Identification The visible absorption spectrum of a sample of FD&C Red No. 3 dissolved in 0.05% aqueous ammonium hydroxide has a wavelength maximum of 527 nm, with an absorptivity of 0.110 L/(mg·cm).

Arsenic (as As) Not more than 3 mg/kg.

Ether Extracts[3] (combined) Not more than 0.2%.

Lead (as Pb) Not more than 10 mg/kg.

Subsidiary Colors

Monoiodofluoresceins Not more than 1.0%.

Other Lower Iodinated Fluoresceins Not more than 9.0%.

Total Color Not less than 87.0%.

Uncombined Intermediates and Products of Side Reactions

2-(2′,4′-Dihydroxy-3′,5′-diiodobenzoyl)benzoic Acid Not more than 0.2%.

Sodium Iodide Not more than 0.4%.

Triiodoresorcinol Not more than 0.2%.

Unhalogenated Intermediates, Total Not more than 0.1%.

Volatile Matter (at 135°) and Chlorides and Sulfates (as sodium salts) Not more than 13% in combination.

Water-Insoluble Matter Not more than 0.2%.

TESTS

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Packaging and Storage Store in well-closed containers.

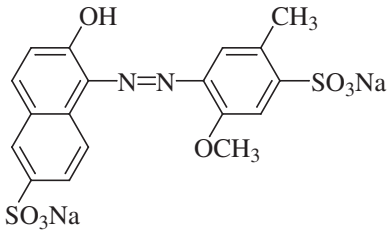
[1]To be used or sold in the United States, this color additive must be batch certified by the U.S. Food and Drug Administration. The monograph title is the name of the color additive only after batch certification has been completed.

[2]Generic designations; not synonyms for certified batches of color additive.

[3]Not required for certification in the United States.

FD&C Red No. 40[1]

Allura Red AC;[2] CI 16035;[2] Class: Monoazo



$C_{18}H_{14}N_2O_8S_2Na_2$ Formula wt 496.43

INS: 129 CAS: [25956-17-6]

DESCRIPTION

FD&C Red No. 40 is principally the disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid.

Function Color.

REQUIREMENTS

Identification The visible absorption spectrum of a sample of FD&C Red No. 40 dissolved in 0.04 *N* aqueous ammonium acetate has a wavelength maximum of 500 nm, with an absorptivity of 0.052 L/(mg·cm).

Arsenic (as As) Not more than 3 mg/kg.

Lead (as Pb) Not more than 10 mg/kg.

Subsidiary Colors

Disodium Salt of 6-Hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-8-(2-methoxy-5-methyl-4-sulfophenoxy)-2-naphthalenesulfonic Acid Not more than 1.0%.

Higher Sulfonated Subsidiary Colors (as sodium salts) Not more than 1.0%.

Lower Sulfonated Subsidiary Colors (as sodium salts) Not more than 1.0%.

Total Color Not less than 85.0%.

Uncombined Intermediates and Products of Side Reactions

4-Amino-5-methoxy-o-*toluenesulfonic Acid* Not more than 0.2%.

Disodium Salt of 6,6′-Oxybis(2-naphthalenesulfonic Acid) Not more than 1.0%.

Sodium Salt of 6-Hydroxy-2-naphthalenesulfonic Acid Not more than 0.3%.

Volatile Matter (at 135°) and Chlorides and Sulfates (as sodium salts) Not more than 14.0% in combination.

Water-Insoluble Matter Not more than 0.2%.

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[2]Generic designations; not synonyms for certified batches of color additive.

TESTS

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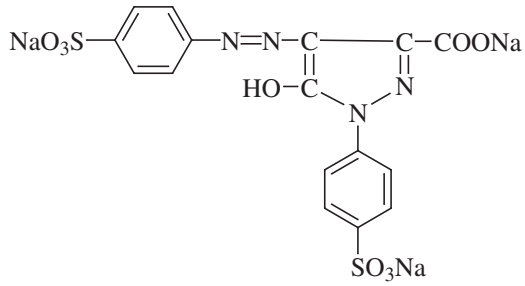
Packaging and Storage Store in well-closed containers.

FD&C Yellow No. 5[1]

Tartrazine;[2] CI 19140;[2] Class: Pyrazolone



$C_{16}H_9N_4O_9S_2Na_3$ Formula wt 534.37

INS: 102 CAS: [1934-21-0]

DESCRIPTION

FD&C Yellow No. 5 is principally the trisodium salt of 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[4-sulfophenyl-azo]-1*H*-pyrazole-3-carboxylic acid.

Function Color.

REQUIREMENTS

Identification The visible absorption spectrum of a sample of FD&C Yellow No. 5 dissolved in 0.04 *N* aqueous ammonium acetate has a wavelength maximum of 428 nm, with an absorptivity of 0.053 L/(mg·cm).

Arsenic (as As) Not more than 3 mg/kg.

Ether Extracts[3] (combined) Not more than 0.2%.

Lead (as Pb) Not more than 10 mg/kg.

[3]Not required for certification in the United States.

Mercury (as Hg) Not more than 1 mg/kg.
Total Color Not less than 87%.
Uncombined Intermediates and Products of Side Reactions

*4,4′-[4,5-Dihydro-5-oxo-4-[(4-sulfophenyl)-hydrazono]-1*H*-pyrazol-1,3-diyl]bis[benzenesulfonic Acid], Trisodium Salt* Not more than 1%.

*4-[(4′,5-Disulfo[1,1′-biphenyl]-2-yl)hydrazono]-4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic Acid, Tetrasodium Salt* Not more than 1%.

*Ethyl or Methyl 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)hydrazono]-1*H*-pyrazole-3-carboxylate, Disodium Salt* Not more than 1%.

*Sum of 4,5-Dihydro-5-oxo-1-phenyl-4-[(4-sulfophenyl)-azo]-1*H*-pyrazole-3-carboxylic Acid, Disodium Salt, and 4,5-Dihydro-5-oxo-4-(phenylazo)-1-(4-sulfophenyl)-1*H*-pyrazole-3-Carboxylic Acid, Disodium Salt* Not more than 0.5%.

4-Aminobenzenesulfonic Acid, Sodium Salt Not more than 0.2%.

*4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic Acid, Disodium Salt* Not more than 0.2%.

*Ethyl or Methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylate, Sodium Salt* Not more than 0.1%.

4,4′-(1-Triazene-1,3-diyl)bis[benzenesulfonic acid], Disodium Salt Not more than 0.05%.

4-Aminoazobenzene Not more than 75 μg/kg.

4-Aminobiphenyl Not more than 5 μg/kg.

Aniline Not more than 100 μg/kg.

Azobenzene Not more than 40 μg/kg.

Benzidine Not more than 1 μg/kg.

1,3-Diphenyltriazene Not more than 40 μg/kg.

Volatile Matter (at 135°) and Chlorides and Sulfates (as sodium salts) Not more than 13% in combination.
Water-Insoluble Matter Not more than 0.2%.

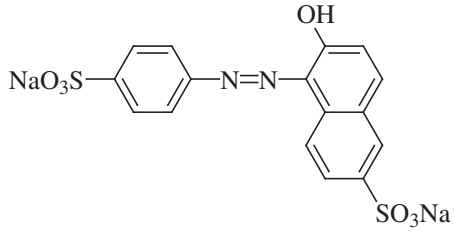
TESTS

FDA-certifiable color additives are batch certified by the United States Food and Drug Administration using analytical chemistry methods developed for this purpose by the FDA. The color additive regulations are described in Title 21, Parts 70 to 82, of the United States *Code of Federal Regulations* (**21** *CFR* Parts 70 to 82). The batch certification process is described in **21** *CFR* Part 80. Current certification analytical methods are available from the Office of Cosmetics and Colors, Colors Certification Branch (HFS-107), U.S. Food and Drug Administration, 5100 Paint Branch Parkway, College Park, Maryland 20740.

Packaging and Storage Store in well-closed containers.

FD&C Yellow No. 6[1]

Sunset Yellow FCF;[2] CI 15985;[2] Class: Monoazo



$C_{16}H_{10}N_2O_7S_2Na_2$ Formula wt 452.37

INS: 110 CAS: [2783-94-0]

DESCRIPTION

FD&C Yellow No. 6 is principally the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid. The trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonic acid may be added in small amounts.

Function Color.

REQUIREMENTS

Identification The visible absorption spectrum of a sample of FD&C Yellow No. 6 dissolved in 0.04 *N* aqueous ammonium acetate has a wavelength maximum of 484 nm, with an absorptivity of 0.054 L/(mg·cm).
Arsenic (as As) Not more than 3 mg/kg.
Ether Extracts[3] (combined) Not more than 0.2%.
Lead (as Pb) Not more than 10 mg/kg.
Mercury (as Hg) Not more than 1 mg/kg.
Total Color Not less than 87%.
Uncombined Intermediates and Products of Side Reactions

4-Aminoazobenzene Not more than 50 μg/kg.

4-Aminobiphenyl Not more than 15 μg/kg.

Aniline Not more than 250 μg/kg.

Azobenzene Not more than 200 μg/kg.

Benzidine Not more than 1 μg/kg.

1,3-Diphenyltriazene Not more than 40 μg/kg.

1-(Phenylazo)-2-naphthalenol Not more than 10 mg/kg.

Sodium Salt of 4-Aminobenzenesulfonic Acid Not more than 0.2%.

Sodium Salt of 6-Hydroxy-2-naphthalenesulfonic Acid Not more than 0.3%.

Disodium Salt of 6,6′-Oxybis[2-naphthalenesulfonic Acid] Not more than 1%.

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[2]Generic designations; not synonyms for certified batches of color additive.

[3]Not required for certification in the United States.

Disodium Salt of 4,4′-(1-Triazene-1,3-diyl)bis[benzenesulfonic Acid] Not more than 0.1%.

Sum of the Sodium Salt of 6-Hydroxy-5-(phenylazo)-2-naphthalenesulfonic Acid and the Sodium Salt of 4-[(2-Hydroxy-1-naphthalenyl)azo]benzenesulfonic Acid Not more than 1%.

Sum of the Trisodium Salt of 3-Hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonic Acid and Other Higher Sulfonated Subsidiaries Not more than 5%.

Volatile Matter (at 135°) and Chlorides and Sulfates (as sodium salts) Not more than 13% in combination.

Water-Insoluble Matter Not more than 0.2%.

TESTS

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Packaging and Storage Store in well-closed containers.

Fennel Oil

CAS: [8006-84-6]

View IR

DESCRIPTION

Fennel Oil occurs as a colorless or pale yellow liquid with the characteristic odor and taste of fennel. It is the volatile oil obtained by steam distillation from the dried ripe fruit of *Foeniculum vulgare* Miller (Fam. Umbelliferae).

> **Note**: If solid material has separated, carefully warm the sample until it is completely liquefied, and mix it before using.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between +12° and +24°.
Refractive Index Between 1.532 and 1.543 at 20°.
Solidification Point Not lower than 3°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.953 and 0.973.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solidification Point Determine as directed under *Solidification Point*, Appendix IIB.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 90% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Ferric Ammonium Citrate, Brown

Iron Ammonium Citrate

INS: 381 CAS: [1185-57-5]

DESCRIPTION

Ferric Ammonium Citrate, Brown, occurs as thin, transparent brown, red-brown, or garnet red scales or granules, or as a brown-yellow powder. It is a complex salt of undetermined structure, composed of iron, ammonia, and citric acid. It is very soluble in water, but is insoluble in alcohol. The pH of a 1:20 aqueous solution is about 5.0 to 8.0. It is deliquescent in air and is affected by light.

Function Nutrient.

REQUIREMENTS

Identification

A. A 500-mg sample, when ignited, chars and leaves a residue of iron oxide.

B. Add 0.3 mL of potassium permanganate TS and 4 mL of mercuric sulfate TS to 5 mL of a 1:10 aqueous solution, and heat the mixture to boiling. A white precipitate forms.

C. Dissolve about 500 mg of sample in 5 mL of water, and add 5 mL of 1 *N* sodium hydroxide. A red-brown precipitate forms, and ammonia is evolved when the mixture is heated.

Assay Not less than 16.5% and not more than 18.5% of iron (Fe).
Ferric Citrate Passes test.
Lead Not more than 2 mg/kg.

Mercury Not more than 1 mg/kg.
Oxalate Passes test.
Sulfate Not more than 0.3%.

TESTS

Assay Transfer about 1 g of sample, accurately weighed, into a 250-mL glass-stoppered Erlenmeyer flask, and dissolve in 25 mL of water and 5 mL of hydrochloric acid. Add 4 g of potassium iodide, stopper, and allow to stand protected from light for 15 min. Add 100 mL of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of iron (Fe).

Ferric Citrate Add potassium ferrocyanide TS to a 1:100 aqueous solution. No blue precipitate forms.

Lead (**Note**: The following method has been found to be satisfactory when the particular atomic absorption spectrophotometer specified is used. The method may be modified as necessary for use with other suitable atomic absorption spectrophotometers capable of determining lead in the sample at the limit specified.)

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL with water, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Preparation Transfer 2.0 mL of *Lead Nitrate Stock Solution* into a 500-mL volumetric flask, dilute to volume with water, and mix. This solution should be prepared on the day of use. Each milliliter contains the equivalent of 0.4 μg of lead ion (Pb).

Sample Preparation Transfer about 20 g of sample, accurately weighed, into a 100-mL volumetric flask (previously rinsed with nitric acid and water), dissolve in a mixture of 50 mL of water and 1 mL of nitric acid, dilute to volume with water, and mix.

Procedure Use a Perkin-Elmer 403 atomic absorption spectrophotometer equipped with a deuterium arc background corrector, a digital readout device, and a burner head capable of handling 20% solids content. Blank the instrument with water following the manufacturer's operating instructions. Aspirate a portion of the *Standard Preparation*, and record the absorbance as A_S; then aspirate a portion of the *Sample Preparation*, and record the absorbance as A_U. Calculate the lead content, in milligrams per kilogram, of the sample taken by the formula

$$100 \times (C/W) \times (A_U/A_S),$$

in which C is the concentration, in micrograms per milliliter, of lead in the *Standard Preparation*, and W is the weight, in grams, of the sample taken.

Mercury

Standard Preparations Prepare a solution containing 1 μg of mercury per milliliter as directed for *Standard Preparation* under *Mercury Limit Test*, Appendix IIIB. Pipet 0.25, 0.50, 1.0, and 3.5 mL of this solution, respectively, into each of four glass-stoppered bottles of about 300-mL capacity, such as BOD (biological oxygen demand) bottles. Dilute the contents of each bottle to 100 mL with water, and mix. These solutions contain the equivalent of 0.25, 0.50, 1.0, and 3.5 mg/kg of mercury, respectively.

Sample Preparation Transfer 1.000 g of sample into a 200-mL screw-cap centrifuge bottle, and add 5 mL of nitric acid and 5 mL of hydrochloric acid. Close the bottle tightly with a Teflon-lined screw-cap, digest on a steam bath for 1 h, and cool. Quantitatively transfer into a suitable glass-stoppered bottle (see *Standard Preparations*), dilute to 100 mL with water, and bubble air through the sample for 2 min. Prepare a reagent blank in the same manner.

10% Stannous Chloride Solution Dissolve 20 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in 40 mL of warm hydrochloric acid, and dilute with 160 mL of water. Prepare fresh each week.

Procedure (**Note**: The *Apparatus* and *Procedure* described under *Mercury Limit Test*, Appendix IIIB, may be suitably modified for this determination.) Use a suitable atomic absorption spectrophotometer assembly designed for mercury analysis, such as the Coleman MAS-50 Mercury Analyzer. Add 5 mL of *10% Stannous Chloride Solution* to the solution to be tested, and immediately insert the bubbler of the mercury analysis apparatus. Obtain the absorbance reading by following the instrument manufacturer's operating instructions. Correct the sample readings for the reagent blank, and determine the mercury concentration of the *Sample Preparation* from a standard curve prepared by plotting the readings obtained with the *Standard Preparations* against mercury concentration, in milligrams per kilogram.

Oxalate Transfer 1 g of sample into a 125-mL separator, dissolve in 10 mL of water, add 2 mL of hydrochloric acid, and extract successively with one 50-mL portion and one 20-mL portion of ether. Transfer the combined ether extracts to a 150-mL beaker, add 10 mL of water, and remove the ether by evaporation on a steam bath. Add 1 drop of glacial acetic acid and 1 mL of a 1:20 calcium acetate solution to the residual aqueous solution. No turbidity develops within 5 min.

Sulfate Dissolve 100 mg of sample in 2.7 *N* hydrochloric acid, and dilute to 30 to 40 mL with water. Proceed as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, beginning with the addition of 3 mL of barium chloride TS. Any turbidity produced does not exceed that shown in a control containing 300 μg of sulfate (SO_4).

Packaging and Storage Store in tight, light-resistant containers in a cool place.

Ferric Ammonium Citrate, Green

Iron Ammonium Citrate

INS: 381 CAS: [1185-57-5]

DESCRIPTION

Ferric Ammonium Citrate, Green, occurs as thin, transparent green scales, as granules, as a powder, or as transparent green crystals. It is a complex salt of undetermined structure, composed of iron, ammonia, and citric acid. It is very soluble in water, but is insoluble in alcohol. Its solutions are acid to litmus. It may deliquesce in air and is affected by light.

Function Nutrient; anticaking agent for sodium chloride.

REQUIREMENTS

Identification A sample responds to the *Identification Tests* in the monograph for *Ferric Ammonium Citrate, Brown*.
Assay Not less than 14.5% and not more than 16.0% of iron (Fe).
Ferric Citrate Passes test.
Lead Not more than 2 mg/kg.
Mercury Not more than 1 mg/kg.
Oxalate Passes test.
Sulfate Not more than 0.3%.

TESTS

Determine as directed for the respective tests in the monograph for *Ferric Ammonium Citrate, Brown*.

Packaging and Storage Store in tight, light-resistant containers in a cool place.

Ferric Citrate

$FeC_6H_5O_7 \cdot xH_2O$ Formula wt, anhydrous 244.95

CAS: anhydrous [2338-05-8]

DESCRIPTION

Ferric Citrate occurs as brown granules or as thin, transparent, garnet red scales. It is more readily soluble in hot water than in cold, but it is insoluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Ferric Iron* and for *Citrate*, Appendix IIIA.
Assay Not less than 16.5% and not more than 18.5% of ferric Fe.
Alkali Citrate Negative.
Ammonia Negative.
Chloride Negative.
Lead Not more than 2 mg/kg.
Sulfate Negative.

TESTS

Assay Dissolve about 1 g of sample, accurately weighed, in a mixture of 5 mL of hydrochloric acid and 25 mL of water contained in a glass-stoppered flask, warming to aid dissolution, if necessary. Cool, add 4 g of potassium iodide, insert the stopper in the flask, and allow the solution to stand for 15 min. Dilute with 100 mL of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of Fe.
Alkali Citrate Ignite about 500 mg of sample until it is thoroughly charred, cool, and add 2 mL of hot water. The water is neutral or shows only a slight alkaline reaction to litmus.
Ammonia Heat 500 mg of sample with 5 mL of 1 *N* sodium hydroxide. The odor of ammonia is not perceptible.
Chloride Heat 1 g of sample with 25 mL of water and 2 mL of nitric acid until the sample dissolves. Cool, dilute with water to 100 mL, and mix. Add 1 mL of silver nitrate TS to 10 mL of the solution. No turbidity immediately develops.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 1-g sample.
Sulfate Add 1 mL of barium chloride TS to 10 mL of the solution obtained in the test for *Chloride* (above). No turbidity develops within 15 s.

Packaging and Storage Store in well-closed containers.

Ferric Phosphate

Iron Phosphate; Ferric Orthophosphate

$FePO_4 \cdot xH_2O$ Formula wt, anhydrous 150.82

CAS: [10045-86-0]

DESCRIPTION

Ferric Phosphate occurs as a yellow-white to buff colored powder. It contains from one to four molecules of water of hydration. It is insoluble in water and in glacial acetic acid, but is soluble in mineral acids.

Function Nutrient.

REQUIREMENTS

Identification Dissolve 1 g of sample in 5 mL of 1:2 hydrochloric acid, and add an excess of 1 *N* sodium hydroxide. A red-brown precipitate forms. Boil the mixture, filter to remove the iron, and strongly acidify a portion of the filtrate with hydrochloric acid. Cool, mix with an equal volume of magnesia mixture TS, and treat with a slight excess of 6 *N* ammonium oxide. An abundant white precipitate forms. This precipitate, after being washed, turns green-yellow when treated with a few drops of silver nitrate TS.
Assay Not less than 26.0% and not more than 32.0% of Fe.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Lead Not more than 4 mg/kg.
Loss on Ignition Not more than 32.5%.
Mercury Not more than 3 mg/kg.

TESTS

Assay Dissolve about 3.5 g of sample, accurately weighed, in 75 mL of 1:2 hydrochloric acid, heat to boiling, and boil for about 5 min. Cool, transfer into a 100-mL volumetric flask, dilute to volume with the dilute hydrochloric acid, and mix. Add 100 mL of the dilute hydrochloric acid to 25.0 mL of this solution, boil again for 5 min, and add, dropwise and while stirring, stannous chloride TS to the boiling solution until the iron is just reduced as indicated by the disappearance of the yellow color. Add 2 drops in excess (but no more) of the stannous chloride TS, dilute with about 50 mL of water, and cool to room temperature. While stirring vigorously, add 15 mL of a saturated solution of mercuric chloride, and then allow to stand for 5 min. Add 15 mL of a sulfuric acid–phosphoric acid mixture, prepared by slowly adding 75 mL of sulfuric acid to 300 mL of water, cooling, adding 75 mL of phosphoric acid, and then diluting to 500 mL with water. Mix, add 0.5 mL of barium diphenylamine sulfonate TS, and titrate with 0.1 *N* potassium dichromate to a red-violet endpoint. Each milliliter of 0.1 *N* potassium dichromate is equivalent to 5.585 mg of Fe.
Arsenic Assemble the special distillation apparatus as shown in Fig. 13 under *Arsenic Limit Test*, Appendix IIIB.

Sample Solution Transfer 2 g of sample, 50 mL of hydrochloric acid, and 5 g of cuprous chloride into the distilling flask (*B*). Reassemble the distillation apparatus and apply gentle suction to flask *F* to produce a continuous stream of bubbles. Heat the solution in flask *B* to boiling and distill until between 30 and 35 mL of distillate has been collected in flask *D*. Quantitatively transfer the distillate to a 100-mL volumetric flask with the aid of water, dilute to volume with water, and mix.

Standard Solution Prepare this solution in the same manner as the *Sample Solution*, but use 6.0 mL of *Standard Arsenic Solution* (see *Arsenic Limit Test*, Appendix IIIB) in place of the sample.

Blank Solution Prepare this solution in the same manner as the *Sample Solution*, but use 6.0 mL of water in place of the sample.

Procedure Transfer 50.0 mL of the *Sample Solution* into the generator flask, add 2 mL of a 15:100 solution of potassium iodide, and continue as directed in the *Procedure* under *Arsenic Limit Test*, Appendix IIIB, beginning with ''[add] 0.5 mL of *Stannous Chloride Solution*, and mix. . . .'' Modify the *Procedure* by using 5.0 g of Devarda's metal in place of the 3.0 g of 20-mesh granular zinc, and maintain the temperature of the reaction mixture in the generator flask between 25° and 27°. Treat 50.0 mL each of the *Standard Solution* and of the *Blank Solution* in the same manner and under the same conditions. Determine the absorbance at 525 nm produced by each solution as directed under *Procedure*.

Calculation Calculate the arsenic content (in milligrams per kilogram) of the sample by the formula

$$3 \times (A_U - A_B)/(A_S - A_B),$$

in which A_U is the absorbance produced by the *Sample Solution*, A_B is the absorbance produced by the *Blank Solution*, and A_S is the absorbance produced by the *Standard Solution*.

> **Note**: If A_B exceeds 0.300, different samples of reagent-grade cuprous chloride and Devarda's metal should be tested for arsenic content by the procedure described herein, and lots of these reagents should be selected that will give blank readings that do not exceed 0.300.

Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB, using a 1.0-g sample, accurately weighed.
Lead (**Note**: In preparing all aqueous solutions and in rinsing glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a lead content as practicable, and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in warm 8 *N* nitric acid for 30 min and by rinsing with deionized water.)

Ascorbic Acid–Sodium Iodide Solution Dissolve 20 g of ascorbic acid and 38.5 g of sodium iodide in water in a 200-mL volumetric flask, dilute with water to volume, and mix.

Trioctylphosphine Oxide Solution (**Caution**: This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.) Dissolve 5.0 g of trioctylphosphine oxide in 4-methyl-2-pentanone in a 100-mL volumetric flask, dilute with the same solvent to volume, and mix.

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute with water to 1000.0 mL, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Preparation and Blank Transfer 5.0 mL of *Lead Nitrate Stock Solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask. Add 10 mL of 9 *N* hydrochloric acid and about 10 mL of water to this volumetric flask and to a second, empty 50-mL volumetric

flask (*Blank*). Add 20 mL of *Ascorbic Acid–Sodium Iodide Solution* and 5.0 mL of *Trioctylphosphine Oxide Solution* to each flask, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of each flask, shake again, and allow the layers to separate. The organic solvent layers are the *Blank* and the *Standard Preparation*, and they contain 0.0 and 2.0 μg of lead per milliliter, respectively.

Test Preparation Add 2.5 g of sample, 10 mL of 9 *N* hydrochloric acid, about 10 mL of water, 20 mL of *Ascorbic Acid–Sodium Iodide Solution*, and 5.0 mL of *Trioctylphosphine Oxide Solution* to a 50-mL volumetric flask, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow the layers to separate. The organic solvent layer is the *Test Preparation.*

Procedure Concomitantly determine the absorbance of the *Blank*, the *Standard Preparation*, and the *Test Preparation* at the lead emission line at 283.3 nm, with a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp and an air–acetylene flame, using 4-methyl-2-pentanone to set the instrument to zero. In a suitable analysis, the absorbance of the *Blank* is not greater than 20% of the difference between the absorbance of the *Standard Preparation* and the absorbance of the *Blank*. The absorbance of the *Test Preparation* does not exceed that of the *Standard Preparation*.

Loss on Ignition Ignite a sample at 800° for 1 h.

Mercury

Standard Preparations Dissolve 338.5 mg of mercuric chloride, in about 200 mL of water in a 250-mL volumetric flask, add 14 mL of 1:2 sulfuric acid, dilute to volume with water, and mix. Pipet 10.0 mL of this solution into a 1000-mL volumetric flask containing about 800 mL of water and 56 mL of 1:2 sulfuric acid, dilute to volume with water, and mix. Pipet 10.0 mL of the second solution into a second 1000-mL volumetric flask containing 800 mL of water and 56 mL of 1:2 sulfuric acid, dilute to volume with water, and mix. Each milliliter of this diluted stock solution contains 0.1 μg of mercury. Pipet 1.25, 2.50, 5.00, 7.50, and 10.00 mL of the last solution (equivalent to 0.125, 0.250, 0.500, 0.750, and 1.00 μg of mercury, respectively) into five separate 150-mL beakers. Add 25 mL of aqua regia to each beaker, cover with watch glasses, heat just to boiling, simmer for about 5 min, and cool to room temperature. Transfer the solutions into separate 250-mL volumetric flasks, dilute to volume with water, and mix. Transfer a 50.0-mL aliquot from each solution into five separate 150-mL beakers, and add 1.0 mL of 1:5 sulfuric acid and 1.0 mL of a filtered solution of 1:25 potassium permanganate solution to each. Heat the solutions just to boiling, simmer for about 5 min, and cool.

Sample Preparation Transfer 5.00 g of sample into a 150-mL beaker, add 25 mL of aqua regia, cover with a watch glass, and allow to stand at room temperature for about 5 min. Heat just to boiling, allow to simmer for about 5 min, and cool. Transfer the solution into a 250-mL volumetric flask, dilute to volume with water, and mix.

Note: Disregard any undissolved material that may be present.

Transfer a 50.0-mL aliquot of this solution into a 150-mL beaker, and add 1.0 mL of 1:5 sulfuric acid and 1.0 mL of a filtered solution of 1:25 potassium permanganate. Heat the solution just to boiling, simmer for about 5 min, and cool. Prepare a reagent blank in the same manner.

Apparatus Use a Mercury Detection Instrument as described and an Aeration Apparatus as shown in Fig. 16 under *Mercury Limit Test*, Appendix IIIB. For the purposes of the test described in this monograph, the Techtron AA-1000 atomic absorption spectrophotometer, equipped with a 10-cm silica absorption cell (Beckman Part No. 75144, or equivalent) and coupled with a strip chart recorder (Varian Series A-25, or equivalent), is satisfactory.

Procedure Assemble the *Aeration Apparatus* as shown in Fig. 16 under *Mercury Limit Test*, Appendix IIIB. Use magnesium perchlorate as the absorbent in the absorption cell (*e*), fill gas washing bottle *c* with 60 mL of water, and place stopcock *b* in the bypass position. Connect the assembly to the 10-cm absorption cell (analogous to *f* in the figure) of the spectrophotometer, and adjust the air or nitrogen flow rate so that, in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a baseline reading at 253.7 nm by following the equipment manufacturer's operating instructions. Using the Techtron AA-1000 spectrophotometer, the following conditions are suitable: slit width: 2 Å; lamp current: 3 mA; and scale expansion: × 1. With the strip chart recorder, set the chart speed at 25 in./h and the span at 2 mV. Precondition the apparatus by an appropriate modification of the procedures described below for treatment of the test solutions.

Note: The fritted bubbler in gas washing bottle *c* should be kept immersed in water between determinations. After each determination, wash the bubbler with a stream of water.

Treat the blank, each of the *Standard Preparations*, and the *Sample Preparation* as follows: Transfer the solution to be tested into a 125-mL gas-washing bottle (*c*), using a few drops of 1:10 hydroxylamine hydrochloride solution to remove any manganese hydroxide from the beaker. Dilute to about 55 mL with water, and add a magnetic stirring bar. Discharge the permanganate color by adding dropwise the hydroxylamine hydrochloride solution, swirling after each drop is added. Add 15.0 mL of 10% stannous chloride solution [prepared by dissolving 20 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in 40 mL of warm hydrochloric acid and diluting with 160 mL of water], and immediately connect gas-washing bottle *c* to the aeration apparatus. Switch on the magnetic stirrer, turn stopcock *b* from the bypass to the aerating position, and obtain the absorbance reading. Disconnect bottle *c* from the aeration apparatus, discard the solution just tested, wash bottle *c* and the fritted bubbler with water, and repeat the procedure with the remaining solutions. Correct the sample readings for the reagent blank, and determine the mercury concentration of the *Sample Preparation* from a standard curve prepared by plotting the readings obtained with

the *Standard Preparations* against mercury concentration, in milligrams per kilogram, suitable adjustments being made for dilution factors.

Packaging and Storage Store in well-closed containers.

Ferric Pyrophosphate

Iron Pyrophosphate

$Fe_4(P_2O_7)_3 \cdot xH_2O$ Formula wt, anhydrous 745.22

CAS: [10058-44-3]

DESCRIPTION

Ferric Pyrophosphate occurs as a tan or yellow-white powder. It is insoluble in water, but is soluble in mineral acids.

Function Nutrient.

REQUIREMENTS

Identification Dissolve 500 mg of sample in 5 mL of 1:2 hydrochloric acid, and add an excess of 1 *N* sodium hydroxide. A red-brown precipitate forms. Allow the solution to stand for several minutes, and then filter, discarding the first few milliliters. Add 1 drop of bromophenol blue TS to 5 mL of the clear filtrate, and titrate with 1 *N* hydrochloric acid to a green color. Add 10 mL of a 1:8 solution of zinc sulfate, and readjust the pH to 3.8 (green color). A white precipitate forms (distinction from *orthophosphates*).
Assay Not less than 24.0% and not more than 26.0% of Fe.
Arsenic Not more than 3 mg/kg.
Lead Not more than 4 mg/kg.
Loss on Ignition Not more than 20.0%.
Mercury Not more than 3 mg/kg.

TESTS

Assay Determine as directed under *Assay* in the monograph for *Ferric Phosphate*.
Arsenic Prepare and test a 2-g sample as directed under *Arsenic* in the monograph for *Ferric Phosphate*.
Lead Determine as directed under *Lead* in the monograph for *Ferrous Gluconate*, using 2.5 g of sample in the *Test Preparation*.
Loss on Ignition Determine as directed under *Loss on Ignition*, Appendix IIC, igniting a sample at 800° for 1 h.
Mercury Determine as directed under *Mercury* in the monograph for *Ferric Phosphate*.

Packaging and Storage Store in well-closed containers.

Ferrous Citrate

$FeC_6H_6O_7$ Formula wt 245.95

CAS: [23383-11-1]

DESCRIPTION

Ferrous Citrate occurs as a slightly gray-green powder or as white crystals.

Function Nutrient.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Ferrous Iron* and for *Citrate*, Appendix IIIA.
Assay Not less than 22.0% of ferrous Fe.
Chloride Not more than 0.2%.
Ferric Iron Not more than 3.0%.
Lead Not more than 2 mg/kg.
Sulfate Not more than 0.06%.

TESTS

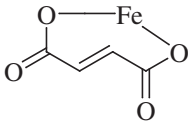
Assay Dissolve about 0.4 g of sample, accurately weighed, in 20 mL of 16:100 sulfuric acid, add 5 mL of 85% phosphoric acid, dilute with approximately 50 mL of water, and immediately titrate with 0.1 *N* ceric sulfate, using orthophenanthroline TS as the indicator. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 5.585 mg of Fe.
Chloride Heat 100 mg of sample, accurately weighed, with 25 mL of water and 2 mL of nitric acid until the sample dissolves. Cool, dilute to 100 mL with water, and mix. Take 10 mL of this solution, and dilute to 30 to 40 mL with water. Proceed as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, beginning with ''add 1 mL of silver nitrate TS. . . .'' Any turbidity produced does not exceed that shown in a control containing 20 μg of chloride (Cl).
Ferric Iron Dissolve about 2 g of sample, accurately weighed, in a mixture of 100 mL of water and 10 mL of hydrochloric acid contained in a 250-mL glass-stoppered flask, add 3 g of potassium iodide, shake well, and allow the mixture to stand in the dark for 5 min. Titrate any liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of ferric iron.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 1-g sample.
Sulfate Dissolve 500 mg of sample, accurately weighed, in 1 mL of 2.7 *N* hydrochloric acid, and dilute to 30 to 40 mL with water. Proceed as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, beginning with ''add 3 mL of barium chloride TS. . . .'' Any turbidity

produced does not exceed that shown in a control containing 300 μg of sulfate (SO_4).

Packaging and Storage Store in well-closed containers.

Ferrous Fumarate

Iron (II) Fumarate



$C_4H_2FeO_4$ Formula wt 169.90

CAS: [141-01-5]

DESCRIPTION

Ferrous Fumarate occurs as a red-orange to red-brown powder. It may contain soft lumps that produce a yellow streak when crushed. It is soluble in water and in alcohol.

Function Nutrient.

REQUIREMENTS

Identification

A. Add 25 mL of 1:2 hydrochloric acid to about 1.5 g of sample, and dilute to 50 mL with water. Heat to effect complete solution; then cool; filter on a fine-porosity, sintered-glass crucible; wash the precipitate with 2:100 hydrochloric acid, saving the filtrate for *Identification Test B*; and dry the precipitate at 105°. Add 3 mL of water and 7 mL of 1 *N* sodium hydroxide to 400 mg of the dried precipitate, and stir until solution is complete. Add, dropwise, 2.7 *N* hydrochloric acid until the solution is just acid to litmus; add 1 g of *p*-nitrobenzyl bromide and 10 mL of alcohol; and reflux the mixture for 2 h. Cool, filter, and wash the precipitate with two small portions of a 2:1 alcohol:water mixture, followed by two small portions of water. The precipitate, recrystallized from hot alcohol and dried at 105°, melts at about 152° (see *Melting Range or Temperature*, Appendix IIB).

B. A portion of the filtrate obtained in *Identification Test A* gives positive tests for *Iron*, Appendix IIIA.

Assay Not less than 97.0% and not more than 101.0% of $C_4H_2FeO_4$, calculated on the dried basis.

Ferric Iron Not more than 2.0%.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 1.5%.

Mercury Not more than 3 mg/kg.

Sulfate Not more than 0.2%.

TESTS

Assay Transfer about 500 mg of sample, accurately weighed, into a 500-mL Erlenmeyer flask, add 25 mL of 2:5 hydrochloric acid, and heat to boiling. Add, dropwise, a solution of 5.6 g of stannous chloride in 50 mL of 3:10 hydrochloric acid until the yellow color disappears, and then add 2 drops in excess. Cool the solution in an ice bath to room temperature, add 8 mL of mercuric chloride TS, and allow to stand for 5 min. Add 200 mL of water, 25 mL of 1:2 sulfuric acid, and 4 mL of phosphoric acid; then add orthophenanthroline TS; and titrate with 0.1 *N* ceric sulfate. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 16.99 mg of $C_4H_2FeO_4$.

Ferric Iron Transfer 2 g of sample into a 250-mL glass-stoppered Erlenmeyer flask, add 25 mL of water and 4 mL of hydrochloric acid, and heat on a hot plate until solution is complete. Stopper the flask, and cool to room temperature. Add 3 g of potassium iodide, stopper, swirl to mix, and allow to stand in the dark for 5 min. Remove the stopper, add 75 mL of water, and titrate with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Not more than 7.16 mL of 0.1 *N* sodium thiosulfate is consumed.

Lead (**Note**: When preparing all aqueous solutions and rinsing glassware before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in warm 8 *N* nitric acid for 30 min and by rinsing with deionized water.)

Ascorbic Acid–Sodium Iodide Solution Transfer 20 g of ascorbic acid and 38.5 g of sodium iodide into a 200-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Trioctylphosphine Oxide Solution (**Caution**: This reagent causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.) Transfer 5.0 g of trioctylphosphine oxide into a 100-mL volumetric flask. Dissolve in and dilute to volume with 4-methyl-2-pentanone, and mix.

Lead Nitrate Stock Solution (100 μg/mL) Dissolve 159.8 mg of ACS reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute with water to 1000.0 mL, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Preparation and *Blank Preparation* Transfer 1.0 mL of *Lead Nitrate Stock Solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution to a 50-mL beaker. Add 6 mL of nitric acid and 10 mL of perchloric acid each both to the beaker and to a second, empty beaker acting as the *Blank*, and evaporate in a fume hood to dryness.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Cool, dissolve the residues in 10 mL of 9 *N* hydrochloric acid, and transfer the solutions, with the aid of about 10 mL

of water, to separate 50-mL volumetric flasks. Add 20 mL of *Ascorbic Acid–Sodium Iodide Solution* and 5.0 mL of *Trioctylphosphine Oxide Solution* to each flask, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of each flask, shake again, and allow the layers to separate. The organic solvent layers are the *Blank Preparation* and the *Standard Preparation*, and they contain 0.0 and 0.4 μg of lead per milliliter, respectively.

Test Preparation Transfer 1.0 g of sample to a 50-mL beaker, and add 6 mL of nitric acid and 10 mL of perchloric acid. Cover the beaker with a ribbed watch glass, and heat in a fume hood until completely dry. Cool, dissolve the residue in 10 mL of 9 *N* hydrochloric acid. Transfer the beaker's contents, with the aid of about 10 mL of water, to a 50-mL volumetric flask. Add 20 mL of *Ascorbic Acid–Sodium Iodide Solution* and 5.0 mL of *Trioctylphosphine Oxide Solution*, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow to separate. The organic solvent layer is the *Test Preparation*.

Procedure Concomitantly determine the absorbance of the *Blank Preparation*, the *Standard Preparation*, and the *Test Preparation* at the lead emission line at 283.3 nm with a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp and an air–acetylene flame, using 4-methyl-2-pentanone to set the instrument to zero. In a suitable analysis, the absorbance of the *Blank Preparation* is not greater than 20% of the difference between the absorbance of the *Standard Preparation* and that of the *Blank Preparation*. The absorbance of the *Test Preparation* does not exceed that of the *Standard Preparation*.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 16 h.

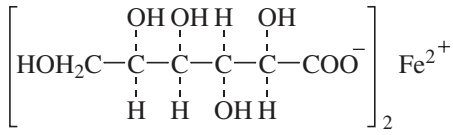
Mercury Determine as directed in *Method II* under *Mercury Limit Test*, Appendix IIIB.

Sulfate Mix 1 g of sample with 100 mL of water in a 250-mL beaker, and heat on a steam bath, adding hydrochloric acid, dropwise, until complete solution is effected (about 2 mL of the acid will be required). Filter the solution, if necessary, and dilute the clear solution or filtrate to 100 mL with water. Heat to boiling, add 10 mL of barium chloride TS, warm on a steam bath for 2 h, cover, and allow to stand overnight. If crystals of ferrous fumarate form, warm on a steam bath to dissolve them, then filter through paper, wash the residue with hot water, and transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents at 600° to constant weight. Each milligram of the residue is equivalent to 0.412 mg (412 μg) of sulfate (SO_4).

Packaging and Storage Store in well-closed containers.

Ferrous Gluconate

Iron (II) Gluconate



$C_{12}H_{22}FeO_{14}\cdot 2H_2O$ Formula wt 482.18

INS: 579 CAS: [299-29-6]

DESCRIPTION

Ferrous Gluconate occurs as a fine, yellow-gray or pale green-yellow powder or granules. One gram dissolves in about 10 mL of water with slight heating. It is practically insoluble in alcohol. A 1:20 aqueous solution is acid to litmus.

Function Nutrient; color adjunct.

REQUIREMENTS

Identification

A. Dissolve a quantity of sample in water, heating in a water bath at 60° if necessary, to obtain a *Test Solution* containing 10 mg/mL. Similarly, prepare a *Standard Solution* of USP Ferrous Gluconate Reference Standard in water, diluting to 10 mg/mL. Apply separate 5-μL portions of the *Test Solution* and the *Standard Solution* on a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent system consisting of a mixture of alcohol, water, ammonium hydroxide, and ethyl acetate (50:30:10:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with a spray reagent prepared as follows: Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 *N* sulfuric acid to volume, and mix. After spraying, heat the plate at 110° for about 10 min. The principal spot obtained from the *Test Solution* corresponds in color, size, and R_f value to that obtained from the *Standard Solution*.

B. A 1:20 aqueous solution gives positive tests for *Ferrous Salts* (Iron), Appendix IIIA.

Assay Not less than 97.0% and not more than 102.0% of $C_{12}H_{22}FeO_{14}$, calculated on the dried basis.

Chloride Not more than 0.07%.

Ferric Iron Not more than 2.0%.

Lead Not more than 2 mg/kg.

Loss on Drying Between 6.5% and 10.0%.

Mercury Not more than 3 mg/kg.

Oxalic Acid Passes test.

Reducing Sugars Passes test.

Sulfate Not more than 0.1%.

TESTS

Assay Dissolve about 1.5 g of sample, accurately weighed, in a mixture of 75 mL of water and 15 mL of 2 *N* sulfuric acid in a 300-mL Erlenmeyer flask, and add 250 mg of zinc dust. Close the flask with a stopper containing a Bunsen valve, allow to stand at room temperature for 20 min, then filter through a sintered-glass filter crucible containing a thin layer of zinc dust, and wash the crucible and contents with 10 mL of 2 *N* sulfuric acid, followed by 10 mL of water. Add orthophenanthroline TS, and titrate the filtrate in the suction flask immediately with 0.1 *N* ceric sulfate. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 44.62 mg of $C_{12}H_{22}FeO_{14}$.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Dissolve 1 g of sample in 100 mL of water. Any turbidity produced by a 10-mL portion of this solution does not exceed that shown in a control containing 70 μg of chloride (Cl) ion.

Ferric Iron Dissolve about 5 g of sample, accurately weighed, in a mixture of 100 mL of water and 10 mL of hydrochloric acid in a 250-mL glass-stoppered flask, add 3 g of potassium iodide, shake well, and allow to stand in the dark for 5 min. Titrate any liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of ferric iron.

Lead (**Note**: When preparing all aqueous solutions and rinsing glassware before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in warm 8 *N* nitric acid for 30 min and by rinsing with deionized water.)

Ascorbic Acid–Sodium Iodide Solution Transfer 20 g of ascorbic acid and 38.5 g of sodium iodide into a 200-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Trioctylphosphine Oxide Solution (**Caution**: This reagent causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.) Transfer 5.0 g of trioctylphosphine oxide to a 100-mL volumetric flask. Dissolve in and dilute to volume with 4-methyl-2-pentanone, and mix.

Lead Nitrate Stock Solution (100 μg/mL) Transfer 159.8 mg of reagent-grade lead nitrate [$Pb(NO_3)_2$] to a 1000-mL volumetric flask, dissolve it in 100 mL of water containing 1 mL of nitric acid, and dilute to volume with water.

Standard Preparation and Blank Preparation Transfer 1.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution into a 50-mL volumetric flask. Add 10 mL of 9 *N* hydrochloric acid and about 10 mL of water to both the volumetric flask and a second, empty, 50-mL volumetric flask (*Blank Preparation*). Add 20 mL of *Ascorbic Acid–Sodium Iodide Solution* and 5.0 mL of *Trioctylphosphine Oxide Solution* to each flask, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of each flask, shake again, and allow the layers to separate. The organic solvent layers are the *Blank Preparation* and the *Standard Preparation*, and they contain 0.0 and 0.4 μg of lead per milliliter, respectively.

Test Preparation Add 1.0 g of sample, 10 mL of 9 *N* hydrochloric acid, about 10 mL of water, 20 mL of *Ascorbic Acid–Sodium Iodide Solution*, and 5.0 mL of *Trioctylphosphine Oxide Solution* to a 50-mL volumetric flask, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow the layers to separate. The organic solvent layer is the *Test Preparation.*

Procedure Concomitantly determine the absorbance of the *Blank Preparation*, the *Standard Preparation*, and the *Test Preparation* at the lead emission line at 283.3 nm, with a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp and an air–acetylene flame, using 4-methyl-2-pentanone to set the instrument to zero. In a suitable analysis, the absorbance of the *Blank Preparation* is not greater than 20% of the difference between the absorbance of the *Standard Preparation* and the absorbance of the *Blank Preparation*. The absorbance of the *Test Preparation* does not exceed that of the *Standard Preparation*.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 16 h.

Mercury Determine as directed in *Method II* under *Mercury Limit Test*, Appendix IIIB.

Oxalic Acid Dissolve 1 g of sample in 10 mL of water, add 2 mL of hydrochloric acid, transfer to a separator, and extract successively with 50 and 20 mL of ether. Combine the ether extracts, add 10 mL of water, and evaporate the ether on a steam bath. Add 1 drop of acetic acid (36%) and 1 mL of a 1:20 calcium acetate solution. No turbidity forms within 5 min.

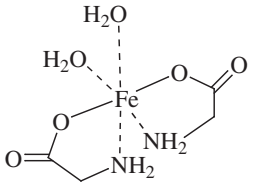
Reducing Sugars Dissolve 500 mg of sample in 10 mL of water, warm, and make the solution alkaline with 1 mL of 6 *N* ammonium hydroxide. Pass hydrogen sulfide gas into the solution to precipitate the iron, and allow the mixture to stand for 30 min to coagulate the precipitate. Filter, and wash the precipitate with two successive 5-mL portions of water. Acidify the combined filtrate and washings with hydrochloric acid, and add 2 mL of 2.7 *N* hydrochloric acid in excess. Boil the solution until the vapors no longer darken lead acetate paper, and continue to boil, if necessary, until the solution has been concentrated to about 10 mL. Cool, add 5 mL of sodium carbonate TS and 20 mL of water, filter, and adjust the volume of the filtrate to 100 mL with water. Add 2 mL of alkaline cupric tartrate TS to 5 mL of filtrate, and boil for 1 min. No red precipitate forms within 1 min.

Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 200-mg sample does not exceed that shown in a control containing 200 μg of sulfate (SO_4).

Packaging and Storage Store in tight containers.

Ferrous Glycinate

Diaquo bis(glycinato) iron (II); Ferrous Bisglycinate



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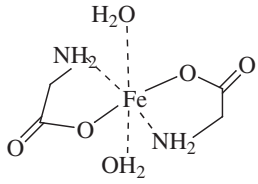


trans

$Fe(OH_2)_2(OOCCH_2NH_2)_2$ Formula wt 239.99

CAS: [20150-34-9]

DESCRIPTION

Ferrous Glycinate occurs as a fine, free-flowing powder. It has an octahedral structure with two water molecules and two chelated glycinate ions coordinated to the central ferrous iron.

Function Source of dietary iron.

REQUIREMENTS

Identification A sample gives a positive test for *Iron* (*Ferrous Salts*), Appendix IIIA.
Assay Not less than 97.0% and not more than 102.0% of $Fe(OH_2)_2(OOCCH_2NH_2)_2$, calculated on the dried basis.
Ferric Iron Not more than 2%.
Lead Not more than 1 mg/kg.
Loss on Drying Not more than 7%.
Nitrogen Between 10% and 12%.
Total Iron Not less than 20% and not more than 22%.

TESTS

Assay Dissolve about 1 g of sample, accurately weighed, in a mixture of 150 mL of water and 10 mL of sulfuric acid in a 300-mL flask. Add 1 drop of orthophenanthroline TS, and immediately titrate with 0.1 *N* ceric sulfate prepared as indicated in *Volumetric Solutions* under *Solutions and Indicators*. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 24.00 mg of $Fe(OH_2)_2(OOCCH_2NH_2)_2$.
Ferric Iron Dissolve about 5 g of sample, accurately weighed, in a mixture of 100 mL of water and 10 mL of hydrochloric acid in a 250-mL glass-stoppered flask. Add 3 g of potassium iodide, shake well, and allow to stand in the dark for 5 min. Titrate any liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of ferric iron.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Nitrogen

Equipment Use a LECO CNS 2000, or equivalent, instrument capable of analyzing for carbon, nitrogen, and sulfur simultaneously. The instrument consists of an autosampler, a combustion furnace, and a computer system for determination and calculations required for operation. Before calibrating the instrument, perform appropriate combustion, helium gas line, and ballast leak checks of the system, and correct any detected leaks. Analyze about ten blanks through the system. Check and monitor that the results for carbon, nitrogen, and sulfur are constant. (Inconsistent blank values indicate problems with the instrument that must be corrected.) Use the results of these blank analyses to zero the instrument, then calibrate it by analyzing at least five 0.2-g samples of sulfamethazine, and verify that the results for carbon (51.7%), nitrogen (20.13%), and sulfur (11.52%) are within ±10% of actual values. Use these values to drift-correct the instrument, thus completing calibration. Analyze at least two more samples of sulfamethazine, and verify that the results for carbon, nitrogen, and sulfur are within ±10% of actual values.

Procedure Place 0.2 g of sample into a ceramic weigh boat. Mix in a small amount of comcat (100 g of tungstic anhydride and 15 g of 97% lithium metaphosphate) to facilitate combustion. Analyze the sample through the system.
Total Iron

Sample Digest Mix the sample thoroughly to achieve homogeneity. Some samples may require additional grinding to attain homogeneity. Do so by placing small amounts of sample into a clean, dry laboratory grinder and grinding until the sample has attained the desired level of homogeneity. Place 0.500 g of sample, accurately weighed, into an appropriate digestion vessel. Add 5 mL of concentrated nitric acid, mix the slurry, and cover the vessel with a watch glass or vapor recovery device. Heat the sample to 95° ± 5° for 30 to 40 min without boiling. If brown fumes evolve after heating for the allotted time, indicating that the nitric acid has incompletely oxidized the sample, add 2 mL of concentrated nitric acid repeatedly, with heating for 15 to 20 min, until no brown fumes evolve. Heat the sample digest until the volume has been reduced to about 3 mL, ensuring that the bottom of the vessel is covered with the sample digest at all times. Remove the vessel from the heating source, and allow its contents to cool thoroughly. Add 2 mL of concentrated hydrochloric acid to the sample digest, and cover with a watch glass. Place the vessel on the heating source, and reflux the sample digest at 95° ± 5° for 15 to 20 min. Before removing the vessel from the heating source, be sure the evolving vapor is clear. Allow the sample digest to cool to room temperature, and dilute it to 50 mL with water. Add 3 g of potassium iodide, shake well, and allow to stand in the dark for 5 min. Titrate any liberated iodine with 0.1 *N* sodium thiosulfate, using starch

TS as the indicator. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of iron.

Packaging and Storage Store in tight containers.

Ferrous Lactate

Iron (II) Lactate; Iron (II) 2-Hydroxypropionate

$$[CH_3CH(OH)COO]_2Fe$$

$C_6H_{10}FeO_6 \cdot xH_2O$ Formula wt, anhydrous 233.99

INS: 585 CAS: [5905-52-2]

DESCRIPTION

Ferrous Lactate occurs as a green-white powder or crystals. The *levo* enantiomer occurs as the dihydrate, and the racemic mixture occurs as the trihydrate. It is sparingly soluble in water and practically insoluble in ethanol. A 1:50 aqueous solution has a pH between 5 and 6.

Function Nutrient.

REQUIREMENTS

Labeling Indicate the state of hydration.
Identification A 1:50 aqueous solution gives positive tests for *Lactate* and for *Iron (Ferrous Salts)*, Appendix IIIA.
Assay Not less than 97.0% and not more than 100.5% of $C_6H_{10}FeO_6$, calculated on the anhydrous basis.
Chloride Not more than 0.1%.
Ferric Iron Not more than 0.2%.
Lead Not more than 1 mg/kg.
Optical (Specific) Rotation *Dihydrate:* $[\alpha]_D^{20°}$: Between +6.0° and +11.0°, calculated on the anhydrous basis.
Oxalic Acid Passes test.
Sulfate Not more than 0.1%.
Water *Dihydrate:* Between 12.0% and 14.0%; *Trihydrate:* Between 18.0% and 20.0%.

TESTS

Assay Dissolve about 800 mg of sample, accurately weighed, in a mixture of 150 mL of water and 10 mL of sulfuric acid contained in a 300-mL flask. Add 5 mL of phosphoric acid, and cool to room temperature if necessary. Add 1 drop of orthophenanthroline TS, and immediately titrate with 0.1 *N* ceric sulfate. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 23.40 mg of $C_6H_{10}FeO_6$.
Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIA, using 1 g of sample, accurately weighed, in 100 mL of water. Any turbidity produced by a 10-mL portion of this solution does not exceed that shown in a control containing 100 µg of chloride (Cl) ion. (Save the remaining *Sample Solution* for the *Sulfate Test*, below.)
Ferric Iron Dissolve about 5 g of sample, accurately weighed, in a mixture of 100 mL of water and 10 mL of hydrochloric acid contained in a 250-mL glass-stoppered flask, add 3 g of potassium iodide, shake well, and allow the mixture to stand in the dark for 5 min. Titrate any liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of ferric iron.
Lead (**Note**: In preparing all aqueous solutions and for rinsing glassware before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking it in warm 8 *N* nitric acid for 30 min and then rinsing it with deionized water.)

Standard Lead Solution Prepare all lead solutions in 0.1% nitric acid. Use a single-element 1000 µg/mL lead stock solution to prepare (weekly) an intermediate stock solution (1 µg/mL). Prepare (daily) a *Standard Lead Solution* (10 ng/mL) by diluting the intermediate stock solution 1:100 with 0.1 *N* nitric acid.

Modifier Working Solution Weigh an amount of palladium nitrate equivalent to 1 g of palladium, and dilute to 100 mL with 15% nitric acid to make a stock solution. Just before use, prepare a *Modifier Working Solution* by diluting the stock solution 1:10 with water.

Blank Solution Use 0.1% nitric acid.

Sample Preparation Dissolve 2 g of sample, accurately weighed, in 5 mL of water and 10 mL of 10% nitric acid. Dilute to 100.0 mL with water.

Procedure Use the following furnace program with a suitable graphite furnace atomic absorption spectrophotometer set at 283.3 nm and equipped with an autosampler:

Temperature (°C)	Time (s)	Gas flow (argon) (L/min)
85	5.0	3.0
95	40.0	3.0
120	10.0	3.0
300	30.0	3.0
900	5.0	3.0
900	1.0	3.0
900	2.0	0.0
2100	0.6	0.0
2100	2.0	0.0
2800	3.0	3.0

Separately inject the following solution mixtures:
Solution A: 30 µL of the *Blank Solution* and 5 µL of the *Modifier Working Solution*;

Solution B: 10 µL of the *Standard Lead Solution*, 10 µL of the *Sample Preparation*, 10 µL of the *Blank Solution*, and 5 µL of the *Modifier Working Solution*;
Solution C: 20 µL of the *Standard Lead Solution*, 10 µL of the *Sample Preparation*, and 5 µL of the *Modifier Working Solution*;
Solution D: 10 µL of the *Sample Preparation*, 20 µL of the *Blank Solution*, and 5 µL of the *Modifier Working Solution*.

Calculate the blank-corrected absorbances of Solutions B, C, and D by subtracting from each the absorbance measured for Solution A. Plot the blank-corrected absorbances of Solutions B, C, and D (y-axis) versus the quantity of lead, in nanograms, added to each solution (x-axis). These are equal to 0.1, 0.2, and 0 ng, respectively. Draw the best straight line through the points. Extrapolate the line to the x-axis intercept to obtain the quantity *C*, in nanograms, of lead in 10 µL of the *Sample Solution*. Calculate the concentration, in milligrams per kilogram, of lead in the sample taken by the formula

$$10C/W,$$

in which *W* is the weight, in grams, of the sample taken.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 2 g of sample in 100 mL of oxygen-free water.

Oxalic Acid Dissolve 1 g of sample in 10 mL of water and 2 mL of hydrochloric acid, transfer to a separator, and extract with two 35-mL portions of ether. Evaporate the combined ether extracts in a rotary evaporator or on a steam bath. Dissolve any residue in 10 mL of water, add 1 mL of glacial acetic acid and 1 mL of a 1:20 solution of calcium acetate. No turbidity develops in 5 min.

Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 20-mL portion of the solution prepared for the *Chloride Test* (above) does not exceed that shown in a control containing 200 µg of sulfate (SO_4).

Water Determine as directed under *Water Determination*, Appendix IIB, at 50°, using 100 mg of sample dissolved in a freshly prepared mixture of 20 mL of methanol and 20 mL of formamide.

Packaging and Storage Store in tight containers.

Ferrous Sulfate

$FeSO_4 \cdot 7H_2O$ Formula wt 278.02

CAS: [7782-63-0]

DESCRIPTION

Ferrous Sulfate occurs as pale, blue-green crystals or granules that are efflorescent in dry air. In moist air, it oxidizes readily to form a brown-yellow, basic ferric sulfate. A 1:10 aqueous solution has a pH of about 3.7. One gram dissolves in 1.5 mL of water at 25° and in 0.5 mL of boiling water. It is insoluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification A sample gives positive tests for *Ferrous Salts* (Iron) and for *Sulfate*, Appendix IIIA.
Assay Not less than 99.5% and not more than 104.5% of $FeSO_4 \cdot 7H_2O$.
Lead Not more than 2 mg/kg.
Mercury Not more than 1 mg/kg.

TESTS

Assay Dissolve about 1 g of sample, accurately weighed, in a mixture of 25 mL of 2 *N* sulfuric acid and 25 mL of recently boiled and cooled water, and immediately titrate with 0.1 *N* ceric sulfate, using orthophenanthroline TS as the indicator. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 27.80 mg of $FeSO_4 \cdot 7H_2O$.
Lead Determine as directed in the monograph for *Ferrous Gluconate*.
Mercury Determine as directed in *Method II* under *Mercury Limit Test*, Appendix IIIB.

Packaging and Storage Store in tight containers.

Ferrous Sulfate, Dried

$FeSO_4 \cdot xH_2O$ Formula wt, anhydrous 151.91

CAS: [7720-78-7]

DESCRIPTION

Ferrous Sulfate, Dried, occurs as a gray-white to buff colored powder consisting primarily of $FeSO_4 \cdot H_2O$, with varying amounts of $FeSO_4 \cdot 4H_2O$. It dissolves slowly in water, but is insoluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification A sample gives positive tests for *Ferrous Salts* (Iron) and for *Sulfate*, Appendix IIIA.
Assay Not less than 86.0% and not more than 89.0% of $FeSO_4$.
Insoluble Substances Not more than 0.05%.
Lead Not more than 2 mg/kg.
Mercury Not more than 1 mg/kg.

TESTS

Assay Determine as directed under *Assay* in the monograph for *Ferrous Sulfate*. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 15.19 mg of $FeSO_4$.
Insoluble Residue Dissolve 2 g of sample in 20 mL of freshly boiled 1:100 sulfuric acid, heat to boiling, and then digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°. The weight of the insoluble residue does not exceed 1 mg.
Lead Determine as directed in the monograph for *Ferrous Gluconate*.
Mercury Determine as directed in *Method II* under *Mercury Limit Test*, Appendix IIIB.

Packaging and Storage Store in tight containers.

Fir Needle Oil, Canadian Type

Balsam Fir Oil

View IR

DESCRIPTION

Fir Needle Oil, Canadian Type, occurs as a colorless to faintly yellow liquid with a pleasant, balsamic odor. It is the volatile oil obtained by steam distillation from needles and twigs of *Abies balsamea* L., Mill (Fam. Pinaceae). It is soluble in most fixed oils and in mineral oil. It is slightly soluble in propylene glycol, but it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 8.0% and not more than 16.0% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −19° and −24°.
Refractive Index Between 1.473 and 1.476 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.872 and 0.878.

TESTS

Assay Measure about 5 g of sample, accurately weighed, and proceed as directed in *Ester Determination* under *Esters*, Appendix VI, using 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 4 mL of 90% alcohol, occasionally with haziness.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Fir Needle Oil, Siberian Type

Pine Needle Oil

View IR

DESCRIPTION

Fir Needle Oil, Siberian Type, occurs as an almost colorless or faintly yellow liquid with a piney, balsamic odor. It is the volatile oil obtained by steam distillation from needles and twigs of *Abies sibirica* Lebed. (Fam. Pinaceae). It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 32.0% and not more than 44.0% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −33° and −45°.
Refractive Index Between 1.468 and 1.473 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.898 and 0.912.

TESTS

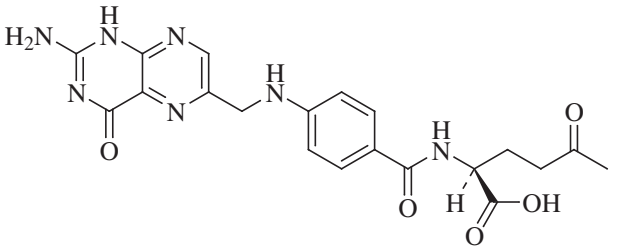
Assay Measure about 2 g of sample, accurately weighed, and proceed as directed in *Ester Determination* under *Esters*, Appendix VI, using 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 90% alcohol. Occasionally the solution may become hazy on further dilution.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Folic Acid

N-[4-[[(2-Amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic Acid; *N*-[*p*-[[(2-Amino-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]glutamic Acid; Pteroylglutamic Acid.



$C_{19}H_{19}N_7O_6$ Formula wt 441.40

CAS: [59-30-3]

DESCRIPTION

Folic Acid occurs as yellow or yellow-orange crystals or crystalline powder. About 1.6 mg dissolves in 1 mL of water. It is insoluble in acetone, in alcohol, in chloroform, and in ether, but dissolves in solutions of alkali hydroxides and carbonates. The pH of a suspension of 1 g in 10 mL of water is between 4.0 and 4.8.

Function Nutrient.

REQUIREMENTS

Identification The ultraviolet absorption spectrum of a 1:100,000 aqueous solution in 1:250 sodium hydroxide solution exhibits maxima and minima at the same wavelengths as those of a similar solution of USP Folic Acid Reference Standard, concomitantly measured. The ratio A_{256}/A_{365} is between 2.80 and 3.00.

Assay Not less than 95.0% and not more than 102.0% of $C_{19}H_{19}N_7O_6$, calculated on the anhydrous basis.

Lead Not more than 2 mg/kg.

Residue on Ignition Not more than 0.3%.

Water Not more than 8.5%.

TESTS

Assay

Standard Solution Accurately weigh about 30 mg of USP Folic Acid Reference Standard, corrected for water content, and dissolve in an aqueous solvent containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL of solvent. Using the same solvent, adjust the volume quantitatively, according to the injection size to be used in the *Procedure*, so that between 5 and 20 μg of Folic Acid is chromatographed.

Sample Solution Prepare as directed for the *Standard Solution*, using an accurately weighed quantity of sample in place of the USP Folic Acid Reference Standard.

Mobile Phase Transfer 35.1 g of sodium perchlorate, 1.40 g of monobasic potassium phosphate, 7.0 mL of 1 *N* potassium hydroxide, and 40 mL of methanol to a 1000-mL volumetric flask, dilute to volume with water, and mix. Adjust the pH to 7.2 with 1 *N* potassium hydroxide.

> **Note**: The methanol concentration may be varied to meet system suitability requirements and to provide a suitable resolution (*R*) for the *System Suitability Solution*.

System Suitability Solution Using an aqueous solvent containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL of solvent, prepare a solution containing about 1 mg/mL each of USP Folic Acid Reference Standard and USP Calcium Formyltetrahydrofolate Authentic Substance.

> **Note**: Before injection, filter all injection solutions through a membrane filter of 1-μm porosity or finer.

Procedure (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with an ultraviolet detector that measures absorption at 254 nm and a 25- to 30-cm × 4-mm (id) stainless-steel column, or equivalent, packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles 5 to 10 μm in diameter, or equivalent. Maintain the mobile phase at a pressure and flow rate capable of giving the required resolution (see below). Inject a volume, up to 25 μL, of the *System Suitability Solution* in a similar manner. Calculate the resolution, R (≥3.6), between calcium formyltetrahydrofolate and Folic Acid by the equation

$$R = 2(t_2 - t_1)/(W_2 + W_1),$$

in which t_2 and t_1 are the retention times of the two components, and W_2 and W_1, are the corresponding widths at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

Chromatograph five injections of equal volume, up to 25 μL, of the *Standard Solution*, and measure the peak response. The relative standard deviation, calculated by the formula

$$100 \times (\text{standard deviation/mean peak response})$$

for the peak response does not exceed 2%.

Introduce volumes of the *Sample Solution* equal to those used for the *Standard Solution* into the chromatograph. Measure the responses for the major peaks obtained with the *Sample Solution* and the *Standard Solution*. Calculate the quantity, in milligrams, of $C_{19}H_{19}N_7O_6$ in the sample taken by the formula

$$VC \times (P_U/P_S)/w \times 100,$$

in which V is the volume, in milliliters, of the *Sample Solution*; C is the concentration, in milligrams per milliliter, of USP Folic Acid Reference Standard in the *Standard Solution*; P_U and P_S are the peak responses of the solutions from the *Sample Solution* and the *Standard Solution*, respectively; w is the weight, in milligrams, of the sample taken; and 100 is the conversion factor for percent.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, using a 1-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB, using a 200-mg sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Food Starch, Modified

Modified Food Starch; Food Starch–Modified

DESCRIPTION

Food Starch, Modified, usually occurs as white or nearly white powders; as intact granules; and if pregelatinized (that is, subjected to heat treatment in the presence of water), as flakes, amorphous powders, or coarse particles. Modified food starches are products of the treatment of any of several grain- or root-based native starches (for example, corn, sorghum, wheat, potato, tapioca, and sago), with small amounts of certain chemical agents, which modify the physical characteristics of the native starches to produce desirable properties.

Starch molecules are polymers of anhydroglucose and occur in both linear and branched form. The degree of polymerization and, accordingly, the molecular weight of the naturally occurring starch molecules vary radically. Furthermore, they vary in the ratio of branched-chain polymers (amylopectin) to linear-chain polymers (amylose), both within a given type of starch and from one type to another. These factors, in addition to any type of chemical modification used, affect the viscosity, texture, and stability of the starch sols significantly.

Starch is chemically modified by mild degradation reactions or by reactions between the hydroxyl groups of the native starch and the reactant selected. One or more of the following processes are used: mild oxidation (bleaching), moderate oxidation, acid and/or enzyme depolymerization, monofunctional esterification, polyfunctional esterification (cross-linking), monofunctional etherification, alkaline gelatinization, and certain combinations of these treatments. These methods of preparation can be used as a basis for classifying the starches thus produced (see *Additional Requirements*, below). Generally, however, the products are called Modified Food Starch, or Food Starch–Modified.

Modified food starches are insoluble in alcohol, in ether, and in chloroform. If not pregelatinized, they are practically insoluble in cold water. Upon heating in water, the granules usually begin to swell at temperatures between 45° and 80°, depending on the botanical origin and the degree of modification. They gelatinize completely at higher temperatures. Pregelatinized starches hydrate in cold water.

Function Thickener; colloidal stabilizer; binder.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.

Identification

A. Suspend about 1 g of sample in 20 mL of water, and add a few drops of iodine TS. A dark blue to red color appears.

B. Place about 2.5 g of sample in a boiling flask, add 10 mL of 3% hydrochloric acid and 70 mL of water, mix, reflux for about 3 h, and cool. Add 0.5 mL of the resulting solution to 5 mL of hot alkaline cupric tartrate TS. A copious red precipitate forms.

C. Examine a portion of the sample with a polarizing microscope in polarized light under crossed Nicol prisms. The typical polarization cross is observed, except in the case of pregelatinized starches.

Crude Fat Not more than 0.15%.

Lead Not more than 1 mg/kg.

Loss on Drying *Cereal Starch*: Not more than 15.0%; *Potato Starch*: Not more than 21.0%; *Sago and Tapioca Starch*: Not more than 18.0%.

pH of Dispersions Between 3.0 and 9.0.

Protein Not more than 0.5%; except in modified high-amylose starches, not more than 1%.

Sulfur Dioxide Not more than 0.005%.

ADDITIONAL REQUIREMENTS

The modified food starches listed below according to method of preparation must meet all of the above *Requirements* in addition to the specified methods of *Treatment* (the reagent that, if not specifically limited, should not exceed the amount reasonably required to accomplish the intended modification) and any requirements for *Residuals Limitation*.

Alkaline Gelatinization (Gelatinized Starch)

Treatment to Produce Gelatinized Starch	*Residuals Limitation*
Sodium hydroxide, not to exceed 1%	—

Depolymerization (Thin-Boiling, or Acid-Modified Starch) This treatment results in partial depolymerization, causing a reduction in viscosity. Any of these treatments may be used in combination with the other treatments that follow.

Treatment to Produce Thin-Boiling Starch	*Residuals Limitation*
Hydrochloric acid and/or sulfuric acid	—
Alpha-amylase enzyme	The enzyme must be generally recognized as safe or approved as a food additive for this purpose. The resulting nonsweet nutritive saccharide polymer has a dextrose equivalent of less than 20

Etherification and Esterification (Starch Ether-Esters)

Treatment to Produce Hydroxypropyl Distarch Phosphate	*Residuals Limitation*
Phosphorus oxychloride, not to exceed 0.1%, and propylene oxide, not to exceed 10%	Not more than 3 mg/kg of residual propylene chlorohydrin

Etherification with Oxidation (Oxidized Starch Ethers)

Treatment to Produce Oxidized Hydroxypropyl Starch	*Residuals Limitation*
Chlorine, as sodium hypochlorite, not to exceed 0.055 lb. (25 g) of chlorine per lb. (454 g) of dry starch; active oxygen obtained from hydrogen peroxide, not to exceed 0.45%; and propylene oxide, not to exceed 25%	Not more than 1 mg/kg of residual propylene chlorohydrin

Mild Oxidation (Bleached Starch) The starches resulting from mild oxidation are not altered chemically; in all cases, extraneous color bodies are oxidized, solubilized, and removed by washing and filtration. These treatments may be used in combination with the other forms of treatment listed in this section.

Treatment to Produce Bleached Starch	*Residuals Limitation*
Active oxygen obtained from hydrogen peroxide, and/or peracetic acid, not to exceed 0.45% of active oxygen	—
Ammonium persulfate, not to exceed 0.075%, and sulfur dioxide, not to exceed 0.05%	—
Chlorine, as sodium hypochlorite, not to exceed 0.0082 lb. (3.72 g) of chlorine per lb. (454 g) of dry starch	—
Chlorine, as calcium hypochlorite, not to exceed 0.036% of dry starch	—
Potassium permanganate, not to exceed 0.2%	Not more than 0.005% of residual manganese (as Mn)
Sodium chlorite, not to exceed 0.5%	—

Moderate Oxidation (Oxidized Starch) The maximum specified treatment introduces about 1 carboxyl group per 28 anhydroglucose units. The starch is whitened, and its molecular weight and viscosity are reduced.

Treatment to Produce Oxidized Starch	*Residuals Limitation*
Chlorine, as sodium hypochlorite, not to exceed 0.055 lb. (25 g) of chlorine per lb. (454 g) of dry starch	—

Monofunctional and/or Polyfunctional Esterification (Starch Esters) The starch esters are named individually, depending on the method of preparation.

Treatment to Produce Starch Acetate	*Residuals Limitation*
Acetic anhydride or vinyl acetate	Not more than 2.5% of acetyl groups introduced into finished product

Treatment to Produce Acetylated Distarch Adipate	*Residuals Limitation*
Adipic anhydride, not to exceed 0.12%, and acetic anhydride	Not more than 2.5% of acetyl groups introduced into finished product

Treatment to Produce Starch Phosphate	*Residuals Limitation*
Monosodium orthophosphate	Not more than 0.4% of residual phosphate (calculated as P)

Treatment to Produce Starch Octenyl Succinate	*Residuals Limitation*
Octenyl succinic anhydride, not to exceed 3%, followed by treatment with alpha-amylase enzyme	—

Treatment to Produce Starch Sodium Octenyl Succinate	*Residuals Limitation*
Octenyl succinic anhydride, not to exceed 3%	—

Treatment to Produce Starch Aluminum Octenyl Succinate	*Residuals Limitation*
Octenyl succinic anhydride, not to exceed 2%, and aluminum sulfate, not to exceed 2%	—

Treatment to Produce Distarch Phosphate	*Residuals Limitation*
Phosphorus oxychloride, not to exceed 0.1%	—
Sodium trimetaphosphate	Not more than 0.04% of residual phosphate (calculated as P)

Treatment to Produce Phosphated Distarch Phosphate	*Residuals Limitation*
Sodium tripolyphosphate and sodium trimetaphosphate	Not more than 0.4% of residual phosphate (calculated as P)

Treatment to Produce Acetylated Distarch Phosphate	*Residuals Limitation*
Phosphorus oxychloride, not to exceed 0.1%, followed by either acetic anhydride, not to exceed 8%, or vinyl acetate, not to exceed 7.5%	Not more than 2.5% of acetyl groups introduced into finished product

Treatment to Produce Starch Sodium Succinate	*Residuals Limitation*
Succinic anhydride, not to exceed 4%	—

Monofunctional Etherification

Treatment to Produce Hydroxypropyl Starch	*Residuals Limitation*
Propylene oxide, not to exceed 25%	Not more than 1 mg/kg of residual propylene chlorohydrin

TESTS

Crude Fat Determine as directed under *Crude Fat*, Appendix X.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 5-g sample in a vacuum oven, not exceeding 100 mm Hg, at 120° for 4 h.

pH of Dispersions Determine as directed under *pH Determination*, Appendix IIB, using the following suspension: Mix 20 g of sample with 80 mL of water, and agitate continuously at a moderate rate for 5 min. (For pregelatinized starches, suspend 3 g of sample in 97 mL of water.)

> **Note**: The water used for sample dispersion should require not more than 0.05 mL of 0.1 *N* acid or alkali per 200 mL of sample to obtain the methyl red or phenolphthalein endpoint, respectively.

Protein Transfer about 10 g of sample, accurately weighed, into an 800-mL Kjeldahl flask, and add 10 g of anhydrous potassium sulfate or anhydrous sodium sulfate, 300 mg of copper selenite or mercuric oxide, and 60 mL of sulfuric acid. Gently heat the mixture, keeping the flask inclined at about a 45° angle, and after frothing has ceased, boil briskly until the solution remains clear for about 1 h. Cool, add 30 mL of water, mix, and cool again. Cautiously pour about 75 mL (or enough to make the mixture strongly alkaline) of a 2:5 aqueous solution of sodium hydroxide down the inside of the flask so that it forms a layer under the acid solution, and then add a few pieces of granular zinc. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of an accurately measured excess of 0.1 *N* sulfuric acid contained in a 50-mL flask. Gently rotate the contents of the Kjeldahl flask to mix, and distill until all ammonia has passed into the absorbing acid solution (about 250 mL of distillate). Titrate the excess acid with 0.1 *N* sodium hydroxide, using 0.25 mL of methyl red–methylene blue TS as the indicator. Perform a blank determination (see *General Provisions*), substituting pure sucrose or dextrose for the sample, and make any necessary correction. Each milliliter of 0.1 *N* sulfuric acid consumed is equivalent to 1.401 mg of nitrogen. Calculate the percent of nitrogen in the sample, and then calculate the percent of protein in starches obtained from corn by multiplying the percent of nitrogen by 6.25, or in starches obtained from wheat, by 5.7. Other factors may be applied as necessary for starches obtained from other sources.

Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X.

TESTS (ADDITIONAL REQUIREMENTS)

Acetyl Groups Determine the content of acetyl groups in *starch acetate*, *acetylated distarch adipate*, and *acetylated distarch phosphate* as directed under *Acetyl Groups*, Appendix X.

Manganese Determine the residual manganese in *bleached starch prepared with potassium permanganate* as directed under *Manganese*, Appendix IIIB.

Phosphate Determine the residual phosphate (calculated as P) in *starch phosphate*, *distarch phosphate*, and *phosphated distarch phosphate* as directed under *Phosphorus*, Appendix IIIB.

Propylene Chlorohydrin Determine the residual propylene chlorohydrin in *hydroxypropyl starch*, *hydroxypropyl starch phosphate*, and *oxidized hydroxypropyl starch* as directed under *Propylene Chlorohydrin*, Appendix X.

Packaging and Storage Store in well-closed containers.

Food Starch, Unmodified

DESCRIPTION

Food Starch, Unmodified, occurs as white or nearly white powders; as intact granules; and if pregelatinized, as flakes, powders, or coarse particles. Food starches are extracted from any of several grain or root crops, including corn (maize), sorghum, wheat, potato, tapioca, sago, and arrowroot and hybrids of these crops such as waxy maize and high-amylose maize. They are chemically composed of either one or a mixture of two glucose polysaccharides (amylose and amylopectin), the composition and relative proportions of which are characteristic of the plant source. Food starches are generally produced by extraction from the plant source using wet milling

processes in which the starch is liberated by grinding aqueous slurries of the raw material. The extracted starch may be subjected to other nonchemical treatments such as purification, extraction, physical treatments, dehydration, heating, and minor pH adjustment during further processing steps. Food starch may be pregelatinized by heat treatment in the presence of water or made cold-water swelling.

Food starches are insoluble in alcohol, in ether, and in chloroform. If they are not treated to be pregelatinized or cold-water swelling, then they are practically insoluble in cold water. Pregelatinized and cold-water swelling starches hydrate in cold water. When heated in water, the granules usually begin to swell at temperatures between 45° and 80°, depending on the botanical origin of the starch. They gelatinize completely at higher temperatures.

Function Thickener; colloidal stabilizer; binder.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.

Identification

A. Suspend about 1 g of sample in 20 mL of water, and add a few drops of iodine TS. A dark blue to red color appears.

B. Place about 2.5 g of sample in a boiling flask, add 10 mL of 3% hydrochloric acid and 70 mL of water, mix, reflux for about 3 h, and cool. Add 0.5 mL of the resulting solution to 5 mL of hot alkaline cupric tartrate TS. A copious, red precipitate forms.

C. Examine a portion of sample with a polarizing microscope in polarized light under crossed Nicol prisms. The typical polarization cross is observed, except in the case of pregelatinized starches.

Crude Fat Not more than 0.15%.

Lead Not more than 1 mg/kg.

Loss on Drying *Cereal Starch*: Not more than 15.0%; *Potato Starch*: Not more than 21.0%; *Sago and Tapioca Starch*: Not more than 18.0%.

pH of Dispersions Between 3.0 and 9.0.

Protein Not more than 0.5%; except in high-amylose and other hybrid starches, not more than 1%.

Sulfur Dioxide Not more than 0.005%.

TESTS

Crude Fat Determine as directed under *Crude Fat*, Appendix X.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 5-g sample in a vacuum oven, not exceeding 100 mm Hg, at 120° for 4 h.

pH of Dispersions Determine as directed under *pH Determination*, Appendix IIB, using the following suspension: Mix 20 g of sample with 80 mL of water, and agitate continuously at a moderate rate for 5 min. (For pregelatinized starches, suspend 3 g of sample in 97 mL of water.)

Note: The water used for sample dispersion should require not more than 0.05 mL of 0.1 *N* acid or alkali per 200 mL of sample to obtain the methyl red or phenolphthalein endpoint, respectively.

Protein Transfer about 10 g of sample, accurately weighed, into an 800-mL Kjeldahl flask, and add 10 g of anhydrous potassium sulfate or anhydrous sodium sulfate, 300 mg of copper selenite or mercuric oxide, and 60 mL of sulfuric acid. Gently heat the mixture, keeping the flask inclined at about a 45° angle, and after frothing has ceased, boil briskly until the solution remains clear for about 1 h. Cool, add 30 mL of water, mix, and cool again. Cautiously pour about 75 mL (or enough to make the mixture strongly alkaline) of a 2:5 aqueous solution of sodium hydroxide down the inside of the flask so that it forms a layer under the acid solution, and then add a few pieces of granular zinc. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of an accurately measured excess of 0.1 *N* sulfuric acid contained in a 50-mL flask. Gently rotate the contents of the Kjeldahl flask to mix, and distill until all ammonia has passed into the absorbing acid solution (about 250 mL of distillate). Titrate the excess acid with 0.1 *N* sodium hydroxide, using 0.25 mL of methyl red–methylene blue TS as the indicator. Perform a blank determination (see *General Provisions*), substituting pure sucrose or dextrose for the sample, and make any necessary correction. Each milliliter of 0.1 *N* sulfuric acid consumed is equivalent to 1.401 mg of nitrogen. Calculate the percent nitrogen in the sample, and then calculate the percent protein in starches obtained from corn by multiplying the percent of nitrogen by 6.25, or in starches obtained from wheat, by 5.7. Other factors may be applied as necessary for starches obtained from other sources.

Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X, using a 25-g sample.

Packaging and Storage Store in well-closed containers.

Formic Acid

HCOOH

CH_2O_2 Formula wt 46.03

INS: 236 CAS: [64-18-6]

FEMA: 2487

DESCRIPTION

Formic Acid occurs as a clear, colorless, *highly corrosive* liquid with a characteristic, pungent odor. It is miscible with

water, with alcohol, with glycerin, and with ether. Its specific gravity is about 1.20.

Function Flavoring adjunct; preservative.

REQUIREMENTS

Identification

A. Add 2 mL of mercuric chloride TS to 5 mL of sample, and warm the mixture. A white precipitate of mercurous chloride forms.

B. Neutralize 1 mL of sample with sodium hydroxide TS, and then add 2 drops in excess and 1 mL of ferric chloride TS. A deep, red-orange color appears that turns to yellow-orange on the addition of mineral acids.

C. Place 2 mL of sample in a test tube, add 5 mL of sulfuric acid, and test the gas evolved with a lighted splinter. A blue flame characteristic of carbon monoxide is produced.

Assay Not less than 85.0% of CH_2O_2.

Acetic Acid Not more than 0.4%.

Dilution Test Passes test.

Sulfate Not more than 0.004%.

TESTS

Assay Tare a small glass-stoppered Erlenmeyer flask containing about 15 mL of water. Transfer about 1.5 mL of sample into the flask, and weigh. Dilute the solution to 50 mL with water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sodium hydroxide is equivalent to 46.03 mg of CH_2O_2.

Acetic Acid Dilute 1 mL of sample to 100 mL with water, transfer 50 mL of this solution into a 250-mL boiling flask, and add 5 g of yellow mercuric oxide. While continuously stirring, boil the mixture under a reflux condenser for 2 h, cool, filter, and wash the residue with about 25 mL of water. Add phenolphthalein TS to the combined filtrate and washings, and titrate with 0.02 *N* sodium hydroxide. Not more than 2.0 mL of 0.02 *N* sodium hydroxide is required to produce a pink color.

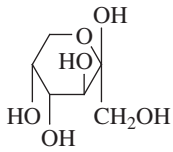
Dilution Test Dilute 1 volume of sample with 3 volumes of water. No turbidity develops within 1 h.

Sulfate Add about 10 mg of sodium carbonate to 2.1 mL (2.5 g) of sample contained in a beaker, and evaporate to dryness on a steam bath. Any turbidity produced by the residue does not exceed that shown in a control containing 100 µg of sulfate (SO_4).

Packaging and Storage Store in tight containers.

Fructose

D-Fructose; Levulose; Fruit Sugar



$C_6H_{12}O_6$ Formula wt 180.16

CAS: [57-48-7]

DESCRIPTION

Fructose occurs as white, hygroscopic, purified crystals or as a purified crystalline powder. It is a natural constituent of fruit, and is obtained from glucose in corn syrup by the use of glucose isomerase. Its density is about 1.6. It is soluble in methanol and in ethanol, freely soluble in water, and insoluble in ether.

Function Nutritive sweetener.

REQUIREMENTS

Identification

A. Add a few drops of a 1:10 aqueous solution to 5 mL of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide is formed.

B. The infrared absorption spectrum of a potassium bromide dispersion of sample, previously dried, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Fructose Reference Standard.

Assay Not less than 98.0% and not more than 102.0% of $C_6H_{12}O_6$ after drying.

Chloride Not more than 0.018%.

Glucose Not more than 0.5%.

Hydroxymethylfurfural Not more than 0.1%, calculated on the dried basis.

Lead Not more than 0.1 mg/kg.

Loss on Drying Not more than 0.5%.

Residue on Ignition Not more than 0.5%.

Sulfate Not more than 0.025%.

TESTS

Assay Transfer about 10 g of sample, previously dried in vacuum at 70° for 4 h and accurately weighed, into a 100-mL volumetric flask, dissolve in 50 mL of water, add 0.2 mL of 15.2 *N* ammonium hydroxide, dilute to volume with water, and mix. After 30 min, determine the angular rotation [see *Optical (Specific) Rotation,* Appendix IIB] in a 100- or 200-mm tube at 25° with the sodium D line. The observed rotation, in degrees (absolute value), multiplied by 1.124 (or 0.562 for the 200-mm tube), represents the weight, in grams, of Fructose in the sample taken.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 2-g sample does not exceed that shown in a control containing 0.5 mL of 0.02 *N* hydrochloric acid.

Glucose

0.1 M *Acetate Buffer* Dissolve 13.608 g of sodium acetate trihydrate in sufficient water to make 1000 mL, add 2.7 mL of acetic acid, and adjust the pH to 5.5 with glacial acetic acid or sodium acetate.

Reagent Solution Dissolve 40 mg of *o*-dianisidine dihydrochloride, 40 mg of horseradish peroxidase (Worthington Biochemical Co., Freehold, NJ, or equivalent), and 0.4 mL of purified glucose oxidase (1000 glucose oxidase units per milliliter, Miles Laboratories, Inc., or equivalent) in *0.1* M *Acetate Buffer*, and dilute to 100 mL with *0.1* M *Acetate Buffer*.

Note: Commercially available preparations containing the reagents in the proper proportions may also be used.

Glucose Standard Solution Transfer about 300 mg, accurately weighed, of USP Dextrose Reference Standard, previously dried in vacuum at 70° for 4 h, into a 1000-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Allow to stand for 2 h to allow mutarotation to occur, then transfer 20.0 mL to a 100-mL volumetric flask, dilute to volume with water, and mix. Prepare fresh on the day of use.

Sample Preparation Transfer 14 g of sample, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 20.0 mL into a second 100-mL volumetric flask, dilute to volume with water, and mix.

Procedure Pipet 2 mL each of the *Sample Preparation*, the *Glucose Standard Solution*, and water into separate 150- × 18-mm test tubes. Heat the tubes for 5 min in a water bath maintained at 30°. At zero time and after 30 and 60 s, add 1.0 mL of the *Reagent Solution* to the first, second, and third tubes, respectively, mix the contents of the tubes, and allow them to react for exactly 30 min from zero time. Immediately stop the reaction in the first tube by adding 10.0 mL of 25% sulfuric acid. Similarly, add 10.0 mL of 25% sulfuric acid to the remaining tubes after they have reacted for exactly 30 min. Mix the contents of each tube, and cool them to room temperature. Using a suitable spectrophotometer, determine the absorbance values of the mixtures obtained from the *Sample Preparation* and from the *Glucose Standard Solution* at 540 nm versus the mixture obtained from the *Reagent Solution* in the reference cell. Calculate the percentage of glucose in the sample by the formula

$$(50C/W) \times A_U/A_S,$$

in which C is the exact concentration, in milligrams per milliliter, of the *Glucose Standard Solution*; W is the weight, in grams, of sample taken; A_U is the absorbance of the mixture obtained from the *Sample Preparation*; and A_S is the absorbance of the mixture obtained from the *Glucose Standard Solution*.

Hydroxymethylfurfural Transfer approximately 1 g of sample, accurately weighed, into a 100-mL volumetric flask, dilute to volume with water, and mix. Read the absorbance of this solution against a water blank at 283 nm in a 1-cm quartz cell in a spectrophotometer. Calculate the percentage of 5-hydroxymethylfurfural (HMF) by the following equation:

$$\% \text{ HMF} = (0.749 \times A)/C,$$

in which A is the absorbance of the sample solution, and C is the concentration, in milligrams per milliliter, of the sample solution corrected for ash and moisture.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in vacuum at 70° for 4 h.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 2-g sample does not exceed that shown in a control containing 0.5 mL of 0.02 *N* sulfuric acid.

Packaging and Storage Store in tight containers protected from humidity.

Fumaric Acid

(*E*)-Butenedioic Acid; *trans*-1,2-Ethylenedicarboxylic Acid

$C_4H_4O_4$ Formula wt 116.07

INS: 297 CAS: [110-17-8]

FEMA: 2488

DESCRIPTION

Fumaric Acid occurs as white granules or as a crystalline powder. A 1:30 aqueous solution has a pH of 2.0 to 2.5. It is soluble in alcohol, slightly soluble in water and in ether, and very slightly soluble in chloroform.

Function Acidifier; flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits maxima only at the same wavelengths as those of a similar preparation of USP Fumaric Acid Reference Standard.

Assay Not less than 99.5% and not more than 100.5% of $C_4H_4O_4$, calculated on the anhydrous basis.

Lead Not more than 2 mg/kg.

Maleic Acid Not more than 0.1%.

Residue on Ignition Not more than 0.1%.
Water Not more than 0.5%.

TESTS

Assay Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, add 50 mL of methanol, and dissolve the sample by warming gently on a steam bath. Cool, add phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a pink color that persists for at least 30 s. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.5 *N* sodium hydroxide is equivalent to 29.02 mg of $C_4H_4O_4$.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Maleic Acid

Mobile Phase Prepare 0.005 *N* sulfuric acid that has been suitably filtered and degassed.

Standard Preparation Transfer about 1 mg of USP Maleic Acid Reference Standard, accurately weighed, into a 1000-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Test Preparation Transfer about 100 mg of sample, accurately weighed, into a 100-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Resolution Solution Transfer 1 mg of USP Fumaric Acid Reference Standard and 0.5 mg of USP Maleic Acid Reference Standard into a 100-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a liquid chromatograph equipped with a 210-nm detector and a 22-cm × 4.6-mm column packed with a strong cation exchange resin consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the hydrogen form (Polypore H from Brownlee Laboratories, Inc., or equivalent). Set the flow rate at about 0.3 mL/min. Chromatograph the *Resolution Solution*, and record the peak responses. The resolution, *R*, between the maleic acid and fumaric acid peaks is not less than 2.5, and the relative standard deviation of the maleic acid peak for replicate injections is not more than 2.0%.

Procedure Separately inject equal volumes (about 5 μL) of the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms, and measure the peak responses. The relative retention times are about 0.5 for maleic acid and 1.0 for fumaric acid. Calculate the quantity, in milligrams, of maleic acid in the total weight of the sample taken by the formula

$$100C(R_U/R_S),$$

in which *C* is the concentration, in milligrams per milliliter, of USP Maleic Acid Reference Standard in the *Standard Preparation*, and R_U and R_S are the responses of the maleic acid peaks obtained from the *Test Preparation* and the *Standard Preparation*, respectively.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Furcelleran

Danish Agar

CAS: [9000-21-9]

DESCRIPTION

Furcelleran occurs as a brown or tan to white, coarse to fine powder. It is soluble in water at a temperature of about 80°, forming a viscous, clear or slightly opalescent solution that flows readily. It disperses in water more readily if first moistened with alcohol, glycerin, or a saturated solution of sucrose in water.

Furcelleran is a hydrocolloid obtained from *Furcellaria fastigiata* of the class Rhodophyceae (red seaweeds) by extraction with water or aqueous alkali. It consists mainly of the potassium, sodium, magnesium, calcium, and ammonium sulfate esters of galactose and 3,6-anhydrogalactose copolymers. These hexoses are alternately linked α-1,3 and β-1,4 in the polymer. The relative proportion of cations existing in Furcelleran may be changed during processing to the extent that one may become predominant.

The ester sulfate content of Furcelleran ranges from 8% to 20% (see *Requirements*, below). In addition, it contains inorganic salts that originate from the seaweed and the process of recovery from the extract. Furcelleran is recovered by alcohol precipitation, by potassium precipitation, or by freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol, and isopropanol.

Function Stabilizer; thickener; gelling agent.

REQUIREMENTS

Identification

A. Add a 4-g sample to 200 mL of water, and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel.

B. Add 200 mg of potassium chloride to 50 mL of the solution or gel obtained in *Identification Test A*, then reheat, mix well, and cool. A short-textured (''brittle'') gel forms.

C. Add 1 drop of a 1:100 solution of methylene blue to 5 mL of the solution obtained in *Identification Test A*. A fibrous precipitate forms.

D. Obtain the infrared absorption spectrum of the sample by the following procedure: Prepare a 0.2% aqueous solution, cast films 0.0005 cm thick (when dry) on a suitable nonstick-

ing surface such as Teflon, and obtain the infrared absorption spectrum. (Alternatively, the spectrum may be obtained on potassium bromide pellets if care is taken to avoid moisture).

Furcelleran has strong, broad absorption bands in the 1000 to 1100 cm^{-1} region. The absorption maximum is 1065. Other characteristic absorption bands and their intensities relative to the absorbance at 1050 cm^{-1} are as follows:

Wave Number (cm^{-1})	Molecular Assignment	Absorbance Relative to 1050 cm^{-1}
1220–1260	ester sulfate	0.2–0.6
928–933	3,6-anhydrogalactose	0.2–0.3
840–850	galactose-4-sulfate	0.1–0.3

Acid-Insoluble Matter Not more than 1.0%.
Arsenic Not more than 3 mg/kg.
Ash (Acid-Insoluble) Not more than 1.0%.
Ash (Total) Not more than 35.0%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 12.0%.
Solubility in Water Not more than 30 mL of water is required to completely dissolve 1 g at 80°.
Sulfate Between 8.0% and 20.0% on the dry weight basis.
Viscosity of a 1.5% Solution Not less than 5 centipoises at 75°.

TESTS

Acid-Insoluble Matter Transfer about 2 g of sample, accurately weighed, to a 250-mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid. Cover with a watch glass, and heat on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod, and replacing any water lost by evaporation. Transfer about 500 mg of a suitable filtering aid, accurately weighed, to the beaker, and filter through a tared filtering crucible containing a 2.4-cm glass fiber filter. Wash the residue several times with hot water, dry at 105° for 3 h, cool in a desiccator, and weigh. The difference between the total weight and the sum of the weights of the filter aid, crucible, and glass fiber filter is the weight of the acid-insoluble matter.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, testing a *Sample Solution* prepared as directed for organic compounds.

Ash (Acid-Insoluble) Determine as directed under *Ash (Acid-Insoluble)*, Appendix IIC.

Ash (Total) Transfer about 2 g of sample, accurately weighed, into a previously ignited, tared, silica or platinum crucible. Heat the sample with a suitable infrared heat lamp, increasing the intensity gradually, until the sample is completely charred, and then continue for an additional 30 min. Transfer the crucible and charred sample into a muffle furnace, and ignite at about 550° for 1 h, then cool in a desiccator, and weigh. Repeat the ignition in the muffle furnace until a constant weight is attained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spots with a 1:10 solution of ammonium nitrate, and dry under an infrared heat lamp before reigniting.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Solubility in Water Add 1 g of sample to 30 mL of cold water, stir well, and heat to a temperature of 80° to completely dissolve the sample. The resulting solution, when maintained at 80°, is uniformly viscous and clear or slightly opalescent.

Sulfate Transfer about 500 mg of sample, previously dried at 105° for 12 h and accurately weighed, into a 100-mL Kjeldahl flask. Add 10 mL of nitric acid, and heat gently for 30 min, adding more of the acid, if necessary, to prevent evaporation to dryness and to yield a volume of about 3 mL at the end of the heating. Cool the mixture to room temperature, and decompose the excess nitric acid by adding formaldehyde TS, dropwise, heating if necessary, until no brown fumes are evolved. Continue heating until the volume of the reaction mixture is reduced to about 5 mL, and then cool. Transfer the residue quantitatively, with the aid of water, into a 400-mL beaker, dilute it to about 100 mL, and filter, if necessary, to produce a clear solution. Dilute the solution to about 200 mL, and add 1 mL of hydrochloric acid. Heat to boiling and while constantly stirring, add, dropwise, an excess (about 6 mL) of hot barium chloride TS. Heat the mixture for 1 h on a steam bath, collect the precipitate of barium sulfate on a filter, wash it until it is free from chloride, dry, ignite, and weigh. The weight of the barium sulfate so obtained, multiplied by 0.4116, gives the equivalent of sulfate (SO_4).

Viscosity of a 1.5% Solution Transfer 7.5 g of sample into a tared, 600-mL tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 mL of deionized water. Add sufficient water to bring the final weight to 500 g, and heat in a water bath, with continuous agitation, until a temperature of 80° is reached (20 to 30 min). Add water to adjust for loss by evaporation, cool to 76° to 77°, and place in a constant-temperature bath at 75°. Preheat the bob and guard of a Brookfield LVF or LVT viscometer, or equivalent, to approximately 75° in water, then dry the bob and guard and attach them to the viscometer, which should be equipped with a No. 1 spindle (19-mm diameter, approximately 65 mm long) capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm, and after six complete revolutions, take the reading on the 0 to 100 scale. Record the results in centipoises by multiplying the reading by 2.

> **Note**: Some samples may be too viscous to be read when a No. 1 spindle is used. Such samples obviously pass the specification, but if a viscosity reading is desired for other reasons, use a No. 2 spindle, take the reading on the 0 to 100 scale, and multiply the reading by 10 to obtain the viscosity in centipoises, or read on the 0 to 500 scale and multiply by 2. If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter, in which case the viscometer reading on the 0 to 100 scale should

be multiplied by 0.2 to obtain the viscosity in centipoises.

Packaging and Storage Store in a well-closed container.

Garlic Oil

CAS: [8000-78-0]

View IR

DESCRIPTION

Garlic Oil occurs as a clear yellow to red-orange liquid with a strong, pungent odor and a flavor characteristic of garlic. It is the volatile oil obtained by steam distillation from the crushed bulbs or cloves of the common garlic plant, *Allium sativum* L. (Fam. Liliaceae). It is soluble in most fixed oils and in mineral oil. It may be incompletely soluble in alcohol. It is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Refractive Index Between 1.550 and 1.580 at 20°.
Specific Gravity Between 1.050 and 1.095.

TESTS

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Gelatin

Food-Grade Gelatin; Edible Gelatin

CAS: [9000-70-8]

DESCRIPTION

Gelatin is the product obtained from the acid, alkaline, or enzymatic hydrolysis of collagen, the chief protein component of the skin, bones, and connective tissues of animals, including fish and poultry. These animal sources shall not have been exposed to pentachlorophenol.

Type A Gelatin is produced by the acid processing of collagenous raw materials and exhibits an isoelectric point between pH 7 and pH 9. *Type B* Gelatin is produced by the alkaline or lime processing of collagenous raw materials and exhibits an isoelectric point between pH 4.6 and pH 5.2. Mixtures of *Types A* and *B* as well as Gelatins produced by modifications of the above mentioned processes may exhibit isoelectric points outside of the stated ranges.

Gelatin is a vitreous, brittle solid that is faintly yellow. When Gelatin granules are immersed in cold water, they hydrate into discrete, swollen particles. On being warmed, Gelatin disperses into the water, resulting in a stable suspension. Water solutions of Gelatin will form a reversible gel if cooled below the specific gel point of Gelatin. The gel point is dependent on the source of the raw material. Gelatin extracted from the tissues of warm-blooded animals will have a gel point in the range of 30° to 35°. Gelatin extracted from the skin of cold-water ocean fish will have a gel point in the range of 5° to 10°. Gelatin is soluble in aqueous solutions of polyhydric alcohols such as glycerin and propylene glycol. It is insoluble in most organic solvents.

Function Firming agent; stabilizer and thickener; surface-active agent; surface-finishing agent.

REQUIREMENTS

Identification

A. Dissolve 10 g of sample in 100 mL of hot water contained in a suitable flask, and cool in a refrigerator at 2° for 24 h. A gel forms. Transfer the flask to a water bath heated to 60°. Within 30 min, when stirred, the gel reverts to the original liquid state.

B. Add trinitrophenol TS or a 1:1.5 solution of potassium dichromate, previously mixed with about one-fourth its volume of 3 *N* hydrochloric acid, to a 1:100 aqueous solution of sample. A yellow precipitate forms.
Ash (Total) Not more than 3.0%.
Chromium Not more than 10 mg/kg.
Lead Not more than 1.5 mg/kg.
Loss on Drying Not more than 15.0%.
Microbial Limits:
E. coli Negative in 25 g.
Salmonella Negative in 25 g.
Pentachlorophenol Limit Not more than 0.3 mg/kg.
Protein The specification conforms to the representations of the vendor.
Sulfur Dioxide Not more than 0.005%.

TESTS

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC, but use a 5-g sample. Before ashing in a muffle furnace at 500° to 550° for 15 to 20 h, add 1.5 to 2.0 g of paraffin to the sample, then heat the crucible on a low-flame hot plate or muffle furnace until the sample is thoroughly charred.

Chromium

Test Solution Transfer 10 g of sample, accurately weighed, into a 100-mL silica dish. Using a very low flame, heat the dish over a Bunsen burner, taking care that the sample does not swell over the lip of the dish or catch fire. Gradually increase the flame until the sample is completely charred, transfer it into a muffle furnace at 550°, and ash overnight. Cool to room temperature, add 10 mL of hydrochloric acid and 10 mL of nitric acid, and heat on a steam bath for 10 min. Cool and transfer into a 25-mL volumetric flask, cautiously dilute to volume with water, and mix.

Chromium Stock Solution Transfer 192.3 mg of chromium trioxide, accurately weighed, into a 1000-mL volumetric flask, dissolve in 100 mL of water and 10 mL of nitric acid, dilute to volume with water, and mix. This solution contains 0.1 mg of chromium per milliliter. Transfer 100.0 mL of the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. This solution contains 10 μg of chromium per milliliter.

Standard Preparations Transfer 10.0, 30.0, 50.0, and 70.0 mL, respectively, of *Chromium Stock Solution* to separate, 100-mL volumetric flasks, dilute to volume with water, and mix. The *Standard Preparations* contain, respectively, 1.0, 3.0, 5.0, and 7.0 μg of chromium per milliliter.

Procedure Concomitantly determine the absorbances of the *Standard Preparations* and the *Test Solution* at the chromium emission line of 357.9 nm, with a suitable atomic absorption spectrophotometer equipped with a chromium hollow-cathode lamp and a slightly reducing air–acetylene flame using water as the blank. Plot the absorbances of the *Standard Preparations* versus concentration, in micrograms per milliliter, of chromium, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, C_S, in micrograms per milliliter, of chromium in the *Test Solution*. Calculate the concentration of chromium, in milligrams per kilogram, in the portion of sample taken, by the formula

$$25C_S/W,$$

in which W is the quantity, in grams, of the sample taken.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 5-g sample at 105° for 16 to 18 h to constant weight.

Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

E. coli

Salmonella

Pentachlorophenol

Pentachlorophenol (PCP) Stock Solution Transfer about 4.0 mg of PCP Reference Standard (Standard No. 5260, Pesticide Reference Standards Section, Environmental Protection Agency, Research Triangle Park, NC 27711, or equivalent, available from Aldrich Chemical Co.), accurately weighed, into a 1000-mL volumetric flask, dissolve in pesticide-grade benzene, dilute to volume with benzene, and mix. Each milliliter of this solution contains 4.0 μg of PCP.

PCP Standard Solutions Prepare a series of *PCP Standard Solutions* by serially diluting the *PCP Stock Solution* in hexane. Pipet 0.0, 1.0, 5.0, 10.0, 25.0, 50.0, and 100.0 mL, respectively, of *PCP Stock Solution*, into separate 1000-mL volumetric flasks, dilute to volume with hexane, and mix. The *PCP Standard Solutions* contain in each milliliter 0.0, 0.004, 0.020, 0.040, 0.100, 0.200, and 0.400 μg of PCP, respectively.

Sample Preparation Transfer about 2 g of sample, accurately weighed, and 2.0 mL of water (to serve as the blank) into separate 25- × 150-mm screw-cap test tubes equipped with Teflon-lined caps. Treat each in the following manner: Add 10 mL of 12 *N* sulfuric acid, close the tube, tighten the cap, and heat for 1 h in a fume hood in a water bath maintained at 100°, removing the tube periodically and mixing the sample by shaking. Remove the tube from the bath, and allow it to cool to room temperature. Add 10 mL of a 4:1 (v/v) solution of hexane:isopropanol to the tube, and shake vigorously. Centrifuge for 2 min at 1000 × g in a suitable centrifuge (International Equipment Co., or equivalent) with a head equipped to accommodate 25- × 150-mm test tubes. Use a Pasteur pipet to transfer the upper hexane layer to a second 25- × 150-mm test tube. Repeat the extraction and centrifugation two additional times, and combine the hexane extracts in the second test tube. Add 5.0 mL of 1.0 *N* potassium hydroxide to the combined extracts, tighten the cap, shake the test tube vigorously, and centrifuge for 2 min at 1000 × g as before. Remove the upper layer with a Pasteur pipet, and discard. Add 10 mL of hexane to the test tube, tighten the cap, shake the test tube vigorously, and centrifuge as before. Remove the upper layer with a Pasteur pipet, and discard. Add 5.0 mL of 12 *N* sulfuric acid to the test tube, tighten the cap, and mix by carefully swirling the tube. Add 5.0 mL of hexane, tighten the cap, shake the test tube vigorously, centrifuge as before, and transfer the upper layer to a 10-mL volumetric flask. Repeat twice, using 2.0 mL of hexane each time, and transfer the upper layer into the 10-mL volumetric flask. Dilute to volume with hexane.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a ^{63}Ni electron capture detector and a 1.8-m × 4-mm (id) glass column, or equivalent, containing 1% SP-1240DA on 100- to 120-mesh Supelcoport (Supelco Inc.), or equivalent. Place a small plug (2 to 3 mm) of phosphoric acid-washed glass wool in the detector end of the column. Use 5% methane in argon as the carrier gas, with a flow rate of 60 mL/min. Condition the column by purging with carrier gas at ambient temperature for 10 to 15 min; program the column oven to increase from 70° to 190° at 1°/min, and hold the temperature at 190° for 8 h while continuing to purge with carrier gas.

> **Caution**: Use only recently prepared and thoroughly conditioned columns; the appearance of ghost PCP peaks may be noted following the injection of samples containing high levels of PCP; repeated injections of solvent may be necessary until ghost PCP peaks disappear.

For sample analyses, maintain the temperatures of the column oven, injector port, and detector at 180°, 250°, and 350°, respectively. Adjust the electrometer to provide about half of the full-scale deflection when 0.1 ng of PCP is injected.

Procedure (**Note**: Inject each *PCP Standard Solution* and *Sample Preparation* twice to ensure that consistent responses are obtained. Following each injection of *PCP Standard Solutions* or *Sample Preparation*, rinse the syringe 10 times with hexane. After each injection of *PCP Standard Solutions* or *Sample Preparation*, inject 5 µL of hexane onto the gas chromatograph, or equivalent, and record the chromatogram. If peaks are observed at the retention time for PCP, repeat the hexane injection until such peaks are no longer encountered.) Inject 5-µL portions of each of the *PCP Standard Solutions* (0.0, 0.02, 0.10, 0.20, 0.50, 1.0, and 2.0 ng, respectively) and the *Reagent Blank* into the gas chromatograph sequentially, and record the chromatograms. Measure the areas under the PCP peaks and the peak heights for each of the *PCP Standard Solutions* (retention time for PCP should be about 10 min), corrected for the *Reagent Blank*. The maximum acceptable *Reagent Blank* for satisfactory performance of the method is 0.01 µg/g. Similarly, inject 5 µL of the *Sample Preparation* into the gas chromatograph, and record the chromatogram. Measure the area under the PCP peak and the peak height, corrected for the *Reagent Blank*. Determine the amount of PCP in the *Sample Preparation* by comparing the peak area and height to the peak area and height obtained from injection of known amounts of *PCP Standard Solutions*; to ensure valid measurement of PCP in the *Sample Preparation*, the size of the PCP peak from the *Sample Preparation* and the standards should be within ±10%. The *Sample Preparation* may require further dilution. Designate as A_S the amount of PCP, expressed in nanograms, in the aliquot of the *Sample Preparation*. Calculate the concentration of PCP, in micrograms per gram, in the sample by the formula

$$5A_S.$$

Protein Determine as directed under *Nitrogen Determination*, Appendix IIIC, transferring 1 g of sample, accurately weighed, into a 500-mL Kjeldahl flask. Percent protein equals percent N × 5.55.

Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X. However, instead of using a 50-g sample, dissolve a 20.0-g sample in 100 mL of a 5% alcohol in water mixture, and proceed as directed under *Sample Introduction and Distillation*.

Packaging and Storage Store in tight containers.

Gellan Gum

INS: 418 CAS: [71010-52-1]

DESCRIPTION

Gellan Gum occurs as an off white powder. It is a high-molecular-weight polysaccharide gum produced by fermentation of a carbohydrate with a pure culture of *Pseudomonas elodea*, purified by recovery with isopropyl alcohol, dried, and milled. It is a heteropolysaccharide comprising a tetrasaccharide repeating unit of one rhamnose, one glucuronic acid, and two glucose units. The glucuronic acid is neutralized to mixed potassium, sodium, calcium, and magnesium salts. It may contain acyl (glyceryl and acetyl) groups as the *O*-glycosidically linked esters. It is soluble in hot or cold deionized water.

Function Stabilizer; thickener.

REQUIREMENTS

Identification

A. Prepare a 1% solution by dissolving 1 g of sample in 99 mL of deionized water. Using a motorized stirrer and a propeller-type stirring blade, stir the mixture for about 2 h. (Save part of this solution for *Identification Test B*). Draw a small amount of the solution into a wide-bore pipet, and transfer it into a solution of 10% calcium chloride. A tough, wormlike gel forms instantly.

B. Add 0.5 g of sodium chloride to the 1% deionized water solution prepared for *Identification Test A*, heat the solution to 80°, stirring constantly, and hold the temperature at 80° for 1 min. Stop heating and stirring the solution, and allow it to cool to room temperature. A firm gel forms.

Assay A sample yields not less than 3.3% and not more than 6.8% of carbon dioxide (CO_2), calculated on the dried basis.

Isopropyl Alcohol Not more than 0.075%.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 15.0%.

TESTS

Assay Determine as directed under *Alginates Assay*, Appendix IIIC, but use about 1.2 g of undried sample, accurately weighed.

Isopropyl Alcohol

IPA Standard Solution Transfer 500.0 mg of chromatographic-quality isopropyl alcohol into a 50-mL volumetric flask, dilute to volume with water, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix.

TBA Standard Solution Transfer 500.0 mg of chromatographic-quality *tert*-butyl alcohol into a 50-mL volumetric flask, dilute to volume with water, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix.

Mixed Standard Solution Pipet 4 mL each of the *IPA Standard Solution* and of the *TBA Standard Solution* into a 125-mL, graduated Erlenmeyer flask, dilute to about 100 mL with water, and mix. This solution contains approximately 40 µg each of isopropyl alcohol and of *tert*-butyl alcohol per milliliter.

Sample Preparation Disperse 1 mL of a suitable antifoam emulsion, such as Dow-Corning G-10, or equivalent, in 200 mL of water contained in a 1000-mL 24/40 round-bottom distilling flask. Add about 5 g of sample, accurately weighed,

and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distill about 100 mL, adjusting the heat so that foam does not enter the column. Add 4.0 mL of *TBA Standard Solution* to the distillate to obtain the *Sample Preparation*.

Procedure (See *Chromatoghaphy*, Appendix IIA.) Inject about 5 µL of the *Mixed Standard Solution* into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m × 3.2-mm stainless steel column, or equivalent, packed with 80- to 100-mesh Porapak QS, or equivalent. Maintain the column at 165°. Set the temperature of both the injection port and the detector to 200°. Use helium as the carrier gas, flowing at 80 mL/min. The retention time of isopropyl alcohol is about 2 min, and that of *tert*-butyl alcohol is about 3 min.

Determine the areas of the *IPA* and *TBA* peaks, and calculate the response factor, *f*, by the formula

$$A_{IPA}/A_{TBA},$$

in which A_{IPA} is the area of the isopropyl alcohol peak, and A_{TBA} is the area of the *tert*-butyl alcohol peak.

Similarly, inject about 5 µL of the *Sample Preparation*, and determine the peak areas, recording the area of the isopropyl alcohol peak as S_{IPA}, and that of the *tert*-butyl alcohol peak as S_{TBA}. Calculate the isopropyl alcohol content, in milligrams per kilogram, in the sample taken by the formula

$$(S_{IPA} \times 4000)/(f \times S_{TBA} \times W),$$

in which *W* is the weight, in grams, of the sample taken.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, using 2 g of sample, and 4 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2.5 h.

Packaging and Storage Store in well-closed containers.

Geranium Oil, Algerian Type

Rose Geranium Oil, Algerian Type

CAS: [8000-46-2]

View IR

DESCRIPTION

Geranium Oil, Algerian Type, occurs as a light to deep yellow liquid with a characteristic odor resembling rose and geraniol. It is the oil obtained by steam distillation from the leaves of *Pelargonium graveolens* L'Her. (Fam. Geraniaceae). It is soluble in most fixed oils, and it is soluble, usually with opalescence, in mineral oil and in propylene glycol. It is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 13.0% and not more than 29.5% of esters, calculated as geranyl tiglate ($C_{15}H_{24}O_2$).
Acid Value Between 1.5 and 9.5.
Angular Rotation Between −7° and −13°.
Ester Value after Acetylation Between 203 and 234.
Refractive Index Between 1.464 and 1.472 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.886 and 0.898.

TESTS

Assay Determine as directed in *Ester Value* under *Esters*, Appendix VI, using about 6 g of sample, accurately weighed. The ester value multiplied by 0.422 equals the percentage of geranyl tiglate ($C_{15}H_{24}O_2$).
Acid Value Determine as directed under *Acid Value*, Appendix VI, using about 5 g of sample, accurately weighed. Modify the procedure by using 15 mL of water, instead of alcohol, as diluent and by agitating the mixture thoroughly during the titration to keep the oil in suspension.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value after Acetylation Determine as directed under *Total Alcohols*, Appendix VI, using about 1.9 g of the acetylated sample oil, accurately weighed, for saponification. Calculate the ester value after acetylation by the formula

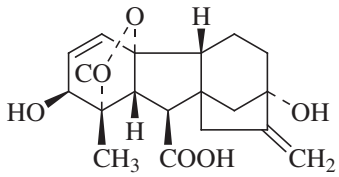
$$A \times 28.05/B,$$

in which *A* is the number of milliliters of 0.5 *N* alcoholic potassium hydroxide consumed in the saponification, and *B* is the weight, in grams, of acetylated sample oil.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 70% alcohol, but on further dilution with the alcohol, opalescence may occur, sometimes followed by separation of paraffin particles.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Gibberellic Acid

2,4α,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic Acid 1,4-α-Lactone



$C_{19}H_{22}O_6$ Formula wt 346.38

CAS: [77-06-5]

DESCRIPTION

Gibberellic Acid occurs as a white to pale yellow, crystalline powder. It melts at about 234°. It is slightly soluble in water and is soluble in alcohol and in acetone.

Function Enzyme activator.

REQUIREMENTS

Identification Dissolve a few milligrams of sample in 2 mL of sulfuric acid. A red solution having a green fluorescence forms.
Assay Not less than 90.0% of $C_{19}H_{22}O_6$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 3.0%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +75.0° and +90.0°, calculated on the dried basis.

TESTS

Assay

Standard Preparation Transfer an accurately weighed quantity of Gibberellic Acid containing not less than 90% of total gibberellins as Gibberellic Acid (USP, or equivalent), equivalent to about 25 mg of pure Gibberellic Acid, into a 50-mL volumetric flask, dissolve in and dilute to volume with methanol, and mix. Transfer 10.0 mL of this solution into a second 50-mL volumetric flask, dilute to volume with methanol, and mix.

Assay Preparation Transfer about 40 mg of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in and dilute to volume with methanol, and mix. Transfer 10.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with methanol, and mix.

Procedure Transfer 5.0 mL of the *Assay Preparation* into a 25- × 200-mm glass-stoppered tube, and transfer 4.0-mL and 5.0-mL portions of the *Standard Preparation* into separate, similar tubes. Place the tubes in a boiling water bath, evaporate to dryness, and then dry in an oven at 90° for 10 min. Remove the tubes from the oven, stopper, and allow to cool to room temperature. Dissolve the residue in each tube in 10.0 mL of 8:10 sulfuric acid, heat in a boiling water bath for 10 min, and then cool in a 10° water bath for 5 min. Determine the absorbance of the solutions in 1-cm cells at 535 nm with a suitable spectrophotometer, using the dilute sulfuric acid as the blank. Record the absorbance of the solution from the *Assay Preparation* as A_U. Note the absorbance of the two solutions prepared from the 4.0-mL and 5.0-mL aliquots of the *Standard Preparation*, and record the absorbance of the final solution giving the value nearest to that of the sample as A_S; record as V the volume of the aliquot used in preparing this solution. Calculate the quantity, in milligrams, of $C_{19}H_{22}O_6$ in the sample taken by the formula

$$500C \times (V/5) \times (A_U/A_S),$$

in which C is the concentration, in milligrams per milliliter, of the *Standard Preparation*.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead ion (Pb) in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 100° in vacuum for 7 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution in alcohol containing 100 mg of sample in each milliliter. Do not use heat in preparing the solution.

Packaging and Storage Store in well-closed containers.

Ginger Oil

CAS: [8007-08-7]

View IR

DESCRIPTION

Ginger Oil occurs as a light yellow to yellow liquid with the aromatic, characteristic odor of ginger. It is the volatile oil obtained by steam distillation of the dried ground rhizome of *Zingiber officianale*, Roscoe (Fam. Zingiberaceae). It is soluble in most fixed oils and in mineral oil. It is soluble, usually with turbidity, in alcohol, but it is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

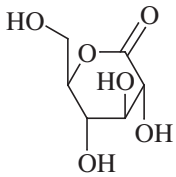
Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown under *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between −28° and −47°.
Refractive Index Between 1.488 and 1.494 at 20°.
Saponification Value Not more than 20.
Specific Gravity Between 0.870 and 0.882.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed under *Saponification Value*, Appendix VI, using about 5 g of sample, accurately weighed.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Glucono Delta-Lactone



$C_6H_{10}O_6$ Formula wt 178.14

INS: 575 CAS: [90-80-2]

DESCRIPTION

Glucono Delta-Lactone occurs as a fine, white, crystalline powder. It is freely soluble in water and is sparingly soluble in alcohol. It decomposes at about 153°.

Function Acidifier; leavening agent; sequestrant.

REQUIREMENTS

Identification Dissolve a portion of sample in water, heating at 60° if necessary, to obtain a test solution containing 10 mg/mL. Similarly prepare a standard solution with the same concentration using USP Potassium Gluconate Reference Standard. Separately apply 5-µL portions of both solutions on a thin-layer chromatographic plate (see *Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent mixture of ethanol, water, ammonium hydroxide, and ethyl acetate (5:3:1:1) until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, dry at 110° for 20 min, and cool. Spray the plate with a reagent prepared as follows: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute to volume with 2 *N* sulfuric acid, and mix. Heat the plate at 110° for about 10 min. The principal spot obtained from the sample solution corresponds in color, size, and R_f value to that obtained from the standard solution.
Assay Not less than 99.0% and not more than 100.5% of $C_6H_{10}O_6$.
Lead Not more than 4 mg/kg.
Reducing Substances (as D-glucose) Not more than 0.5%.

TESTS

Assay Dissolve about 600 mg of sample, accurately weighed, in 100 mL of water in a 300-mL Erlenmeyer flask, add 50.0 mL of 0.1 *N* sodium hydroxide, and allow to stand for 15 min. Add phenolphthalein TS, and titrate the excess alkali with 0.1 *N* hydrochloric acid. Perform a blank determination (see *General Provisions*). Each milliliter of 0.1 *N* hydrochloric acid is equivalent to 17.81 mg of $C_6H_{10}O_6$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 4 µg of lead (Pb) ion in the control.
Reducing Substances Transfer 10.0 g of sample, accurately weighed, into a 400-mL beaker, dissolve the sample in 40 mL of water, add a few drops of phenolphthalein TS, and neutralize with 1:2 sodium hydroxide solution. Dilute to 50.0 mL with water, and add 50 mL of alkaline cupric tartrate TS. Heat the mixture over a Bunsen burner, regulating the flame so that boiling begins in 4 min, and continue the boiling for exactly 2 min. Filter through a sintered-glass filter crucible, wash the filter with 3 or more small portions of water, and place the crucible in an upright position in the original beaker. Add 5 mL of water and 3 mL of nitric acid to the crucible, mix with a stirring rod to ensure complete solution of the cuprous oxide, and wash the solution into a beaker with several mL of water. Add bromine TS (5 to 10 mL) to the beaker until the color becomes yellow, and dilute with water to about 75 mL. Add a few glass beads, boil over a Bunsen burner until the bromine is completely removed, and cool. Slowly add ammonium hydroxide until a deep blue color appears, then adjust the pH to approximately 4 with glacial acetic acid, and dilute to about 100 mL with water. Add 4 g of potassium iodide, and titrate with 0.1 *N* sodium thiosulfate, adding starch TS just before the endpoint is reached. Not more than 16.1 mL of titrant is required.

Packaging and Storage Store in well-closed containers.

Glucose Syrup

Corn Syrup

DESCRIPTION

Glucose Syrup occurs as a clear, white to light yellow, viscous liquid. It is a clarified, concentrated, aqueous solution of sac-

charides obtained by the partial hydrolysis of edible starch by food-grade acids and/or enzymes. Depending on the degree of hydrolysis, it contains varying amounts of D-glucose. When obtained from corn starch, it is commonly designated as corn syrup. It is miscible in all proportions with water.

Function Nutritive sweetener.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.
Identification Add a few drops of a 1:20 aqueous solution to 5 mL of hot alkaline cupric tartrate TS. A red precipitate of cuprous oxide forms.
Assay Not less than 20.0% reducing sugar content (dextrose equivalent) expressed as D-glucose, calculated on the dried basis.
Lead Not more than 0.1 mg/kg.
Residue on Ignition Not more than 0.5%.
Starch Passes test.
Sulfur Dioxide Not more than 0.004%.
Total Solids Not less than 70.0%.

TESTS

Assay Determine as directed under *Reducing Sugars Assay*, Appendix X.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 20-g sample.
Starch Add 1 drop of iodine TS to 1 g of sample dissolved in 10 mL of water. A yellow color indicates the absence of soluble starch.
Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X, using a 35-g sample.
Total Solids Determine the refractive index of a sample at 20° or 45°, and use the appropriate Glucose Syrup table under *Glucose Syrup (Corn Syrup)*, Appendix X.

Packaging and Storage Store in tightly closed containers in a dry place.

Glucose Syrup, Dried

Dried Glucose Syrup; Glucose Syrup Solids

DESCRIPTION

Glucose Syrup, Dried, occurs as a white to light yellow powder or granules. It is a purified, concentrated mixture of nutritive saccharides obtained by the hydrolysis of edible starch and by partially drying the resulting solution (glucose syrup). When obtained from corn starch, it is commonly designated dried corn syrup or corn syrup solids. Depending on the degree of hydrolysis, it contains varying amounts of D-glucose. It is soluble in water.

Function Nutritive sweetener.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.
Identification Add a few drops of a 1:20 aqueous solution to 5 mL of hot alkaline cupric tartrate TS. A red precipitate of cuprous oxide forms.
Assay Not less than 20.0% of reducing sugar content (dextrose equivalent) expressed as D-glucose, calculated on the dried basis.
Lead Not more than 0.1 mg/kg.
Residue on Ignition Not more than 0.5%.
Starch Passes test.
Sulfur Dioxide Not more than 0.004%.
Total Solids Not less than 90.0% when the reducing sugar content is 88.0% or greater; not less than 93.0% when the reducing sugar content is between 20.0% and 88.0%.

TESTS

Assay Determine as directed under *Reducing Sugars Assay*, Appendix X.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample at 525° for 2 h.
Starch Add 1 drop of iodine TS to 1 g of sample dissolved in 10 mL of water. A yellow color indicates the absence of soluble starch.
Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X, using a 25-g sample.
Total Solids Determine the water content of an accurately weighed sample as directed under *Water Determination*, Appendix IIB. Calculate the percent *Total Solids* by the formula

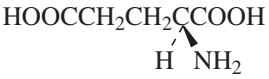
$$100(W_U - W_W)/W_U,$$

in which W_U is the weight, in milligrams, of the sample taken, and W_W is the weight, in milligrams, of water determined.

Packaging and Storage Store in tightly closed containers in a dry environment.

L-Glutamic Acid

Glutamic Acid; L-2-Aminopentanedioic Acid



$C_5H_9NO_4$ Formula wt 147.13

INS: 620 CAS: [56-86-0]

View IR

DESCRIPTION

L-Glutamic Acid occurs as a white, free-flowing, crystalline powder. It is slightly soluble in water, forming acidic solutions. The pH of a saturated solution is about 3.2.

Function Salt substitute; nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_5H_9NO_4$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.1%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +31.5° and +32.5°, calculated on the dried basis.
Residue on Ignition Not more than 0.3%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely.

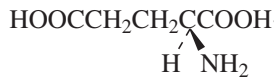
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 14.71 mg of $C_5H_9NO_4$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of a previously dried sample in sufficient 2 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

L-Glutamic Acid Hydrochloride

2-Aminopentanedioic Acid Hydrochloride



$C_5H_9NO_4 \cdot HCl$ Formula wt 183.59

CAS: [138-15-8]

View IR

DESCRIPTION

L-Glutamic Acid Hydrochloride occurs as a white, crystalline powder. One gram dissolves in about 3 mL of water. It is almost insoluble in alcohol and in ether. Its solutions are acid to litmus.

Function Salt substitute; flavoring agent; nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% $C_5H_9NO_4 \cdot HCl$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.5%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +25.2° and +25.8°, calculated on the dried basis.
Residue on Ignition Not more than 0.25%.

TESTS

Assay Dissolve about 100 mg of sample, previously dried at 80° for 4 h and accurately weighed, in 0.5 mL of water, add exactly 15.0 mL of 0.1 *N* perchloric acid, and heat on a water bath for 30 min.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

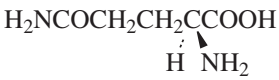
After cooling, add 45 mL of glacial acetic acid, and titrate the excess perchloric acid with 0.1 *N* sodium acetate, determining the endpoint potentiometrically. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 18.36 mg of $C_5H_9NO_4 \cdot HCl$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 80° for 4 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of sample in sufficient 2 *N* hydrochloric acid to make 100 mL.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

L-Glutamine

L-2-Aminoglutaramic Acid



$C_5H_{10}N_2O_3$ Formula wt 146.15

CAS: [56-85-9]

View IR

DESCRIPTION

L-Glutamine occurs as white crystals or a crystalline powder. It is soluble in water and practically insoluble in alcohol and in ether. Its solutions are acid to litmus. It melts with decomposition at about 185°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits maxima only at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_5H_{10}N_2O_3$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +6.3° and +7.3°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 150 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 14.62 mg of $C_5H_{10}N_2O_3$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 4 g of a previously dried sample in sufficient water to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Glutaraldehyde

Glutaral; 1,5-Pentanedial

$C_5H_8O_2$ Formula wt 100.12

CAS: [111-30-8]

DESCRIPTION

Glutaraldehyde occurs as a clear, nearly colorless, aqueous solution. It is miscible with water. The grades of Glutaraldehyde suitable for food use usually have concentrations between 15% and 50%.

Function Fixing agent in the immobilization of enzyme preparations; cross-linking agent for microencapsulating flavoring substances; antimicrobial for sugar milling.

REQUIREMENTS

Labeling Indicate the concentration of Glutaraldehyde.
Identification

2,4-Dinitrophenylhydrazine Reagent Add 4 mL of sulfuric acid to 0.8 g of 2,4-dinitrophenylhydrazine, then while swirling, add 6 mL of water, dropwise. When dissolution is essentially complete, add 20 mL of alcohol, mix, and filter. The filtrate is the reagent.

Procedure Add 0.4 mL of sample to 20 mL of *2,4-Dinitrophenylhydrazine Reagent*. Mix by swirling, and allow the mixture to stand for 5 min. Collect the precipitate on a filter, and rinse thoroughly with alcohol. Dissolve the precipitate in 20 mL of hot ethylene dichloride, filter, and cool the filtrate in an ice bath until crystallization occurs. Collect the precipitate on a filter. Redissolve the precipitate by refluxing with 30 mL of acetone, filter, and cool the filtrate in an ice bath until crystallization occurs. Collect the precipitate on a filter. The 2,4-dinitrophenylhydrazone so obtained melts between 185° and 195° (see *Melting Range or Temperature*, Appendix IIB).
Assay Not less than 100.0% and not more than 105.0% of the labeled amount of $C_5H_8O_2$.
Lead Not more than 2 mg/kg.
pH Between 3.1 and 4.5.

TESTS

Assay

Hydroxylamine Hydrochloride Solution Prepare a 0.5 *N* solution by dissolving 35.0 g of hydroxylamine hydrochloride in water contained in a 1-L volumetric flask, dilute to volume with water, and mix.

Triethanolamine Solution Prepare a 0.5 *N* solution by transferring 65 mL (74 g) of 98% triethanolamine into a 1-L volumetric flask, dilute to volume with water, and mix.

Procedure Adjust to pH 3.60 a volume of *Hydroxylamine Hydrochloride Solution* sufficient for analyzing both the blank and the sample. Using a suitable autotitrator, titrate with *Triethanolamine Solution*.

> **Caution**: The stirring rate is critical throughout the neutralization and analysis. When stirring is required, ensure adequate mixing without whipping air bubbles into the solution. The stirring speed should be consistent for both the sample and the blank.

Transfer 65.0 mL of the pH 3.60 *Hydroxylamine Hydrochloride Solution* into each of two titration cups. Add a Teflon (or equivalent) stirrer to each cup. Using the autotitrator, add 30.8 mL of the neutralized *Triethanolamine Solution* to each titration cup, cover, and mix. Using a weighing pipet, introduce into one of the cups a suitable portion of sample equivalent to about 300 mg of Glutaraldehyde. Mix the solutions thoroughly, and allow the sample and blank to stand at room temperature for at least 60 min but not for more than 90 min.

Titrate the sample and the blank to pH 3.60 with 0.5 *N* hydrochloric acid, determining the endpoint potentiometrically. Calculate the percentage, by weight, of $C_5H_8O_2$ in the sample by the formula

$$[N(B - A)(0.05006)/W]100,$$

in which N is the normality of the hydrochloric acid; B and A are the volumes, in milliliters, of 0.5 *N* hydrochloric acid consumed by the blank and the sample solutions, respectively; 0.05006 is the milliequivalent weight, in grams per milliequivalent, of Glutaraldehyde; and W is the weight, in grams, of sample taken.

Lead Determine as directed in the *Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

pH Determine as directed under *pH Determination*, Appendix IIB.

Packaging and Storage Store in tight, light-resistant containers protected from heat.

Glycerin

Glycerol

$$CH_2OHCHOHCH_2OH$$

$C_3H_8O_3$ Formula wt 92.09

INS: 422 CAS: [56-81-5]

DESCRIPTION

Glycerin occurs as a clear, colorless, viscous liquid. It is hygroscopic, and its solutions are neutral. Glycerin is miscible with water and with alcohol. It is insoluble in chloroform, in ether, and in fixed and volatile oils.

Function Humectant; solvent; bodying agent; plasticizer.

REQUIREMENTS

Identification The infrared absorption spectrum of a thin film of sample exhibits a very strong, broad band at 2.7 μm to 3.3 μm; a strong doublet at about 3.4 μm; a maximum at about 6.1 μm; a strong region of absorption between 6.7 μm and 8.3 μm, having maxima at about 7.1 μm, 7.6 μm, and 8.2 μm, and a very strong region of bands at about 9.0 μm, 9.6 μm, 10.1 μm, 10.9 μm, and 11.8 μm.

> **Note**: Glycerin having a low water content may not exhibit a maximum at about 6.1 μm.

Assay Not less than 95.0% and not more than 100.5% of $C_3H_8O_3$.

Chlorinated Compounds (as Cl) Not more than 0.003%.

Color Passes test.

Fatty Acids and Esters Passes test (limit about 0.1%, calculated as butyric acid).

Lead Not more than 1 mg/kg.

Readily Carbonizable Substances Passes test.

Residue on Ignition Not more than 0.01%.

Specific Gravity Not less than 1.249.

TESTS

Assay

Sodium Periodate Solution Dissolve 60 g of sodium metaperiodate ($NaIO_4$) in sufficient water containing 120 mL of 0.1 *N* sulfuric acid to make 1000 mL. Do not heat to dissolve the periodate. If the solution is not clear, filter through a sintered-glass filter. Store the solution in a glass-stoppered, light-resistant container. Test the suitability of this solution as follows: Pipet 10 mL into a 250-mL volumetric flask, dilute to volume with water, and mix. Dissolve about 550 mg of sample in 50 mL of water, and add 50 mL of the diluted periodate solution by pipet. For a blank, pipet 50 mL of the diluted periodate solution into a flask containing 50 mL of water. Allow the solutions to stand for 30 min, then add 5 mL of hydrochloric acid and 10 mL of potassium iodide TS

to each, and rotate to mix. Allow to stand for 5 min, add 100 mL of water, and titrate with 0.1 *N* sodium thiosulfate, shaking continuously and adding starch TS near the endpoint. The ratio of the volume of 0.1 *N* sodium thiosulfate required for the Glycerin:periodate mixture to that required for the blank should be between 0.750 and 0.765.

Procedure Transfer about 400 mg of sample, accurately weighed, into a 600-mL beaker, dilute with 50 mL of water, add bromothymol blue TS, and acidify with 0.2 *N* sulfuric acid to a definite green or green-yellow color. Neutralize with 0.05 *N* sodium hydroxide to a definite blue endpoint free of green color. Prepare a blank containing 50 mL of water, and neutralize in the same manner. Pipet 50 mL of the *Sodium Periodate Solution* into each beaker, mix by swirling gently, cover with a watch glass, and allow to stand for 30 min at room temperature (not above 35°) in the dark or in subdued light. Add 10 mL of a mixture consisting of equal volumes of ethylene glycol and water, and allow to stand for 20 min. Dilute each solution to about 300 mL with water, and titrate with 0.1 *N* sodium hydroxide to a pH of 8.1 ± 0.1 for the sample and 6.5 ± 0.1 for the blank, using a pH meter previously calibrated with pH 4.0 Acid Phthalate Standard Buffer Solution (see *Solutions and Indicators*). Each milliliter of 0.1 *N* sodium hydroxide, after correction for the blank, is equivalent to 9.210 mg of Glycerin ($C_3H_8O_3$).

Chlorinated Compounds Transfer 5.0 g of sample into a dry, 100-mL round-bottom, ground-joint flask, and add 15 mL of morpholine to it. Connect the flask with a ground joint reflux condenser, and reflux the mixture gently for 3 h. Rinse the condenser with 10 mL of water, receiving the washing into the flask, and cautiously acidify with nitric acid. Transfer the solution to a suitable comparison tube, add 0.5 mL of silver nitrate TS, dilute with water to 50.0 mL, and mix thoroughly. Any turbidity does not exceed that produced by 150 µg of chloride (Cl) in an equal volume of solution containing the quantities of reagents used in the test, omitting the refluxing.

Color The color of sample, when viewed downward against a white surface in a 50-mL Nessler tube, is not darker than the color of a standard made by diluting 0.40 mL of ferric chloride CS with water to 50 mL and similarly viewed in a Nessler tube of the same diameter and color as that containing the sample.

Fatty Acids and Esters Mix a 40.0-mL (50-g) sample with 50 mL of recently boiled water and 5.0 mL of 0.5 *N* sodium hydroxide. Boil the mixture for 5 min, cool, add phenolphthalein TS, and titrate the excess alkali with 0.5 *N* hydrochloric acid. More than 4 mL of 0.5 *N* hydrochloric acid is consumed.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Readily Carbonizable Substances Rinse a glass-stoppered 25-mL cylinder with 95% sulfuric acid, and allow it to drain for 10 min. Add 5 mL of sample and 5 mL of 95% sulfuric acid, shake vigorously for 1 min, and allow to stand for 1 h. The mixture is no darker than *Matching Fluid H* as described under *Readily Carbonizable Substances*, Appendix IIB.

Residue on Ignition Heat 50 g of sample in a tared, open dish, and ignite the vapors, allowing them to burn until the sample has been completely consumed. After cooling, moisten the residue with 0.5 mL of sulfuric acid, and complete the ignition by heating for 15-min periods at 800° ± 25° to constant weight.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in tight containers.

Glycerol Ester of Gum Rosin

View IR

DESCRIPTION

Glycerol Ester of Gum Rosin occurs as a hard, pale amber-colored resin (color N or paler as determined by ASTM Designation D 509) produced by the esterification of pale gum rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone and in toluene, but is insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Acid Number Between 3 and 9.

Lead Not more than 1 mg/kg.

Ring-and-Ball Softening Point 82° or higher.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX.

Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 µg of lead ion (Pb) in the control.

Ring-and-Ball Softening Point Determine as directed in the *Ring-and-Ball Method* under *Softening Point*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Glycerol Ester of Partially Dimerized Rosin

View IR

DESCRIPTION

Glycerol Ester of Partially Dimerized Rosin occurs as a hard, pale, amber-colored resin (color M or paler as determined by ASTM Designation D 509) produced by the esterification of partially dimerized rosin with food-grade glycerin, and purified by steam stripping. It is soluble in acetone, but is insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Number Between 3 and 8.
Lead Not more than 1 mg/kg.
Ring-and-Ball Softening Point 103° or higher.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 μg of lead ion (Pb) in the control.
Ring-and-Ball Softening Point Determine as directed in the *Ring-and-Ball Method* under *Softening Point*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Glycerol Ester of Partially Hydrogenated Gum Rosin

View IR

DESCRIPTION

Glycerol Ester of Partially Hydrogenated Gum Rosin occurs as a medium-hard, pale amber-colored resin (color N or paler as determined by ASTM Designation D 509). It is produced by the esterification of partially hydrogenated gum rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone and in toluene, but is insoluble in water and in alcohol.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Number Between 3 and 10.
Drop Softening Point 79° or higher.
Lead Not more than 1 mg/kg.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX.
Drop Softening Point Determine as directed in the *Drop Method* under *Softening Point*, Appendix IX, using a bath temperature of 100°.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV, using 5 g of sample. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 μg of lead ion (Pb) in the control.

Packaging and Storage Store in well-closed containers.

Glycerol Ester of Partially Hydrogenated Wood Rosin

View IR

DESCRIPTION

Glycerol Ester of Partially Hydrogenated Wood Rosin occurs as a medium-hard, pale amber-colored resin (color N or paler as determined by ASTM Designation D 509). It is produced by the esterification of partially hydrogenated wood rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone, but is insoluble in water and in alcohol.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a dispersion of the melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Number Between 3 and 10.
Lead Not more than 1 mg/kg.
Ring-and-Ball Softening Point 68° or higher.

TESTS

Acid Number Determine as directed under Acid Number, Appendix IX.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 μg of lead ion (Pb) in the control.

Ring-and-Ball Softening Point Determine as directed in *Ring-and-Ball Method* under *Softening Point*, Appendix IX, using a water bath.

Packaging and Storage Store in well-closed containers.

Glycerol Ester of Polymerized Rosin

View IR

DESCRIPTION

Glycerol Ester of Polymerized Rosin occurs as a hard, pale amber-colored resin (color M or paler as determined by ASTM Designation D 509). It is produced by the esterification of polymerized rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone, but is insoluble in water and in alcohol.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a dispersion of the melted sample on a potassium bromide plate exhibits its relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Number Between 3 and 12.
Lead Not more than 1 mg/kg.
Ring-and-Ball Softening Point 80° or higher.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 µg of lead ion (Pb) in the control.
Ring-and-Ball Softening Point Determine as directed for *Ring-and-Ball Method* under *Softening Point*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Glycerol Ester of Tall Oil Rosin

View IR

DESCRIPTION

Glycerol Ester of Tall Oil Rosin occurs as a pale amber-colored resin (color N or paler as determined by ASTM Designation D 509). It is produced by the esterification of tall oil rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone, but is insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Number Between 2 and 12.
Lead Not more than 1 mg/kg.
Ring-and-Ball Softening Point 80° or higher.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 µg of lead (Pb) ion in the control.
Ring-and-Ball Softening Point Determine as directed in the *Ring-and-Ball Method* under *Softening Point*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Glycerol Ester of Wood Rosin

Ester Gum

INS: 445 CAS: [8050-30-4]

View IR

DESCRIPTION

Glycerol Ester of Wood Rosin occurs as a hard, pale amber-colored resin (color N or paler as determined by ASTM Designation D 509). It is produced by the esterification of pale wood rosin with food-grade glycerin. The rosin is obtained by solvent extraction of aged pine stumps, followed by a liquid–liquid solvent refining process. When intended for use in chewing gum base, the product is usually purified by steam stripping, but when intended for use in adjusting the density of citrus oils for beverages, it is purified by countercurrent steam distillation. It is soluble in acetone, but it is insoluble in water.

Function Masticatory substance in chewing gum base; beverage stabilizer.

REQUIREMENTS

Identification The infrared spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the

same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Number Between 3 and 9.
Lead Not more than 1 mg/kg.
Ring-and-Ball Softening Point 82° or higher.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV, using 3.3 μg of lead (Pb) ion in the control.
Ring-and-Ball Softening Point Determine as directed in the *Ring-and-Ball Method* under *Softening Point*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Glyceryl Behenate

Glyceryl Tribehenate; Glyceryl Tridocosanoate; Tribehenoyl-*sn*-glycerol; Tridocosanoyl-*sn*-glycerol

$C_{69}H_{134}O_6$ Formula wt 1059.83

CAS: [30233-64-8]

DESCRIPTION

Glyceryl Behenate occurs as a fine powder. It is a mixture of fatty acid glycerides, primarily glyceryl esters of behenic acid, that melts at about 70°. It is soluble in chloroform and practically insoluble in water and in alcohol.

Function Emulsifier; texturizer.

REQUIREMENTS

Identification

A. (**Caution**: Ether is highly volatile and flammable. Its vapor, when mixed with air and ignited, may explode.)

Solvent Mixture Prepare a 96:4 chloroform:acetone mixture.

Standard Solution Prepare a 6% solution of USP Glyceryl Behenate Reference Standard in chloroform.

Test Solution Prepare a 6% solution of sample in chloroform.

Chromatographic Plates Use suitable thin-layer chromatographic plates (see *Chromatography,* Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel. Pretreat the plates by placing them in a chromatographic chamber saturated with ether. Remove the plates from the chamber, allow the ether to evaporate, and immerse them in a 2.5% solution of boric acid in alcohol. After about 1 min, withdraw the plates, and allow them to dry at ambient temperature. Heat to 110° for 30 min to activate the plates, and then keep them in a desiccator.

Procedure Apply 10 μL of *Test Solution* and 10 μL of *Standard Solution* on one of the chromatographic plates. Develop the chromatogram in the *Solvent Mixture* until the solvent front has moved about 12 cm. Remove the plate from the developing chamber and allow the solvent to evaporate. Spray the chromatogram with a 0.02% solution of dichlorofluorescein in alcohol. Examine the spots under short-wavelength ultraviolet light. The R_f values of the spots obtained from the *Test Solution* correspond to those obtained from the *Standard Solution*.

B. (See *Chromatography*, Appendix IIA.) Dissolve about 22 mg of sample in 1 mL of toluene in a screw-cap vial with a Teflon-lined septum. Add about 0.4 mL of 0.2 *N* methanolic (*m*-trifluoromethylphenyl) trimethylammonium hydroxide, attach the cap, and mix. Allow the vial to stand at room temperature for at least 30 min. Introduce a suitable volume of the mixture into a gas chromatograph equipped with a flame-ionization detector and a 1.8-m × 4-mm (id) column packed with a 10% coating of 50% 3-cyanopropyl–50% phenylmethylsilicone (SP 2300, or equivalent) on a silanized siliceous earth support (Supelcoport, or equivalent) maintained at a temperature of about 225°. Repeat the procedure using USP Glyceryl Behenate Reference Standard in lieu of a sample. The retention time of the main peak in the chromatogram of a sample corresponds to that of the main peak in the chromatogram of the preparation of USP Glyceryl Behenate Reference Standard. The ratio of the response of the main peak to the sum of all the responses is not less than 0.83.
Acid Value Not more than 4.
Iodine Value Not more than 3.
Free Glycerin Not more than 1.0%.
Lead Not more than 1 mg/kg.
1-Monoglycerides Content Not less than 12.0% and not more than 18.0%.
Residue on Ignition Not more than 0.1%.
Saponification Value Not less than 145 and not more than 165.

TESTS

Acid Value Suspend about 10 g of sample, accurately weighed, in a flask containing 50 mL of a 1:1 mixture of alcohol and ether that has been neutralized to phenolphthalein with 0.1 *N* sodium hydroxide. Connect the flask with a suitable condenser, and while frequently shaking, warm for about 10 min. Add 1 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 30 s. Calculate the acid value by the formula

$$56.1V \times N/W,$$

in which V is the volume, in milliliters, of the 0.1 *N* sodium hydroxide solution; N is the normality of the sodium hydroxide solution; and W is the weight, in grams, of sample taken.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Free Glycerin Determine as directed under *Free Glycerin or Propylene Glycol*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

1-Monoglycerides Content Determine as directed under *1-Monoglycerides*, Appendix VII, using about 1 g of a sample that has been melted at a temperature not higher than 80°, stirred, and accurately weighed.

> **Note**: If the sample titration is less than 0.8 volumes of the blank titration, discard and repeat, using a smaller weight of sample.

Calculate the percentage of 1-monoglycerides, as glyceryl monobehenate, by the formula

$$20.73N \times (B \times S)/W,$$

in which 20.73 is one-twentieth of the molecular weight of glyceryl monobehenate; N is the normality of the sodium thiosulfate; B and S are the volumes, in milliliters, of 0.1 N sodium thiosulfate consumed by the blank and the sample, respectively; and W is the weight, in grams, of sample taken.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Packaging and Storage Store in tight containers at a temperature not higher than 35°.

Glyceryl-Lacto Esters of Fatty Acids

Lactated Mono-Diglycerides; Lactic and Fatty Acid Esters of Glycerol

INS: 472b

DESCRIPTION

Glyceryl-Lacto Esters of Fatty Acids occur as a waxy solid that varies in consistency from soft to hard. They are a mixture of partial lactic and fatty acid esters of glycerin. They are dispersible in hot water and are moderately soluble in hot isopropanol, in xylene, and in cottonseed oil.

Function Emulsifier; stabilizer.

REQUIREMENTS

Identification Transfer 1 mL of the *Sample Solution* remaining at the end of the test for *Total Lactic Acid* (below) into a 25-mL glass-stoppered test tube, add 0.1 mL of cupric sulfate solution (1 g of $CuSO_4 \cdot 5H_2O$ in 25 mL of water) and 6 mL of sulfuric acid, and mix. Stopper loosely, heat in a boiling water bath for 5 min, and then cool in an ice bath for 5 min. Remove from the ice bath, add 0.1 mL of *p*-phenylphenol solution (75 mg dissolved in 5 mL of 1 *N* sodium hydroxide), and mix. Allow to stand at room temperature for 1 min, then heat in a boiling water bath for 1 min. A deep, blue-violet color indicates the presence of lactic acid.

Lead Not more than 0.5 mg/kg.

Residue on Ignition Not more than 0.1%.

Unsaponifiable Matter Not more than 2.0%.

The following specifications should conform to the representations of the vendor: *Acid Value*, *Free Glycerin*, *1-Monoglyceride Content*, *Total Lactic Acid*, and *Water*.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

Free Glycerin Determine as directed under *Free Glycerin or Propylene Glycol*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

1-Monoglyceride Content Determine as directed under *1-Monoglycerides*, Appendix VII.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Total Lactic Acid Transfer an accurately weighed portion of melted sample, equivalent to between 140 and 170 mg of lactic acid, into a 250-mL Erlenmeyer flask. Pipet 20 mL of 0.5 *N* alcoholic potassium hydroxide into the flask, connect an air condenser at least 65 cm long, and reflux for 30 min. Run a blank determination (see *General Provisions*) using the same volume of 0.5 *N* alcoholic potassium hydroxide. Add 20 mL of water to each flask, then disconnect the condensers, evaporate to a volume of 20 mL, and cool to about 40°. Add methyl red TS to each flask, and titrate the blank with 0.5 *N* hydrochloric acid. While swirling the sample flask, add exactly the same volume of 0.5 *N* hydrochloric acid as with the blank. Add 50 mL of hexane to each flask. Swirl the sample flask vigorously to dissolve the fatty acids, then quantitatively transfer the contents of each flask into separate 250-mL separators, and shake for 30 s. Collect the aqueous phases in 300-mL Erlenmeyer flasks, wash the hexane solutions with 50 mL of water, and combine the wash solutions with the original aqueous phases in the Erlenmeyer flasks, discarding the hexane solutions. Titrate with 0.1 *N* potassium hydroxide, using phenolphthalein TS as the indicator, to a pink color that persists for at least 30 s. Calculate the percent of total lactic acid by the formula

$$[9.008(S - B)(N)]/W,$$

in which 9.008 is the equivalence factor for lactic acid; $(S - B)$ is the difference, in milliliters, between the volumes of 0.1 N potassium hydroxide required for the sample and for the blank, respectively; N is the exact normality of the potassium hydroxide solution; and W is the weight, in grams, of the sample taken.

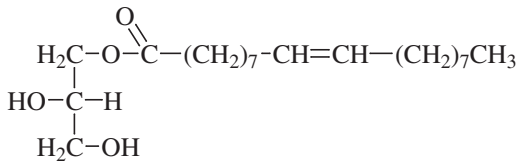
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Glyceryl Monooleate

Monoolein; 1,2,3-Propanetriol



$C_{21}H_{40}O_4$ Formula wt 356.54

INS: 471 CAS: [25496-72-4]

FEMA: 2526

DESCRIPTION

Glyceryl Monooleate occurs as a clear liquid at room temperature. It has a mild, fatty taste. It is prepared by esterifying glycerin with food-grade oleic acid in the presence of a suitable catalyst such as aluminum oxide. It also occurs in many animal and vegetable fats such as tallow and cocoa butter. It is soluble in hot alcohol and in chloroform; very slightly soluble in cold alcohol, in ether, and in petroleum ether; and insoluble in water. It melts at around 15°. It may also contain tri- and diesters.

Function Emulsifier; flavoring agent.

REQUIREMENTS

Identification Glyceryl Monooleate exhibits the following typical composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	≤12	12:0	14:0	16:0	16:1
Weight % (Range):	0	0	<4	1–5	<9
Fatty Acid:	18:0	18:1	18:2	≥20	
Weight % (Range):	<3.0	≥82	3–7	<1.5	

Assay Not less than 35.0% monoglycerides, calculated on the anhydrous basis.
Acid Value Not more than 6.
Free Glycerin Not more than 6.0%.
Hydroxyl Value Between 300 and 330.
Iodine Value Between 58 and 80.
Lead Not more than 1 mg/kg.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 160 and 176.
Water Not more than 1.0%.

TESTS

Assay

Propionating Reagent Mix 10 mL of pyridine and 20 mL of propionic anhydride.

Internal Standard Solution Transfer about 400 mg of hexadecyl hexadecanoate, accurately weighed, into a 100-mL volumetric flask, dilute with chloroform to volume, and mix.

Standard Preparation Transfer about 50 mg of USP Monoglycerides Reference Standard, accurately weighed, into a 25-mL flask, add 5 mL of *Internal Standard Solution* by pipet, and mix. When solution is complete, immerse the flask in a water bath maintained at a temperature between 45° and 50°, and volatilize the chloroform with the aid of a stream of nitrogen. Add 3.0 mL of *Propionating Reagent*, and heat on a hot plate at 75° for 30 min. Evaporate the reagents with the aid of a stream of nitrogen and gentle steam heat. Add 15 mL of chloroform, and swirl to dissolve the residue.

Assay Preparation Transfer about 50 mg of sample, accurately weighed, into a 25-mL conical flask, and proceed as directed for *Standard Preparation*, beginning with ''add 5 mL of *Internal Standard Solution*. . . .''

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector, and containing a 2.4-m × 4-mm (id) borosilicate glass column, or equivalent, packed with 2% liquid phase, 5% phenyl methyl silicone on 80- to 100-mesh support (Supelcoport, or equivalent). Maintain the column isothermally at a temperature between 270° and 280°, and the injection port and detector block at about 310°. Use helium as the carrier gas at a flow rate of about 70 mL/min.

System Suitability Chromatograph 6 to 10 injections of the *Standard Preparation* as directed under *Procedure*. The resolution factor, R, between the peaks for the derivatized glyceryl hexadecanoate and glyceryl octadecanoate is not less than 2.0, and the relative standard deviation of the ratio of the peak area of the derivatized glyceryl *cis*-9-octadecanoate to that of the hexadecyl hexadecanoate is not more than 2.0%.

Procedure Inject a suitable portion of the *Standard Preparation* into the gas chromatograph, and record the chromatogram. Measure the areas under the peaks, and record the values of the sum of the areas under the derivatized monoglyceride peaks and of the area under the hexadecyl hexadecanoate peak as A_S and A_D, respectively. Calculate the response factor, F, taken by the formula

$$(A_S/A_D)(W_D/W_S),$$

in which W_D and W_S are the weights, in milligrams, of hexadecyl hexadecanoate and USP Monoglycerides Reference Standard, respectively, in the *Standard Preparation*. Similarly inject a suitable portion of the *Assay Preparation*, and record the chromatogram. Measure the areas under the peaks, and record the values of the sum of the areas under the derivatized monoglyceride peaks and of the area under the hexadecyl hexadecanoate peak as a_U and a_D, respectively. Calculate the quantity, in milligrams, of monoglycerides in the amount of sample taken by the formula

$$(W_D/F)(a_U/a_D).$$

Acid Value Determine as directed under *Acid Value*, Appendix VII.

Free Glycerin

Propionating Reagent Mix 10 mL of pyridine with 20 mL of propionic anhydride.

Internal Standard Solution Dissolve a suitable quantity of tributyrin, accurately weighed, in chloroform and dilute quantitatively with chloroform to obtain a solution having a concentration of about 0.2 mg/mL.

Standard Preparation Transfer about 15 mg of glycerin and about 50 mg of tributyrin, both accurately weighed, into a glass-stoppered, 25-mL conical flask, add 3 mL of *Propionating Reagent*, and heat at 75° for 30 min. Volatilize the reagents with the aid of a stream of nitrogen at room temperature, add about 12 mL of chloroform, and mix. Dilute about 1 mL of this mixture with chloroform to about 20 mL, and mix.

Test Preparation Transfer about 50 mg of sample, accurately weighed, into a glass-stoppered, 25-mL conical flask; add 5 mL of *Internal Standard Solution* by pipet; and mix to dissolve. Immerse the flask in a water bath maintained at a temperature between 45° and 50°, and volatilize the chloroform with the aid of a stream of nitrogen. Add 3 mL of *Propionating Reagent*, and heat at 75° for 30 min. Volatilize the reagents with the aid of a stream of nitrogen at room temperature, add about 5 mL of chloroform, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and containing a 2.4-m × 4-mm borosilicate glass column, or equivalent, packed with 2% liquid phase consisting of a high-molecular-weight compound of polyethylene glycol and a diepoxide (Carbowax 20 M, or equivalent) on an 80- to 100-mesh siliceous earth support (Chromosorb W AW DMCS, or equivalent). Maintain the column isothermally at a temperature between 190° and 200° and the injection port and detector block at about 300° and 310°, respectively. Use helium as the carrier gas at a flow rate of about 70 mL/min.

System Suitability Chromatograph 6 to 10 injections of the *Standard Preparation* as directed under *Procedure*. The resolution factor, R, between the peaks for the derivatized glycerin and tributyrin is not less than 4.0, and the relative standard deviation of the ratio of their peak areas is not more than 2.0%.

Procedure Inject a suitable portion of the *Standard Preparation* into the gas chromatograph, or equivalent, and record the chromatogram. Measure the areas under the peaks and record the values of the areas under the tripropionin and tributyrin peaks as A_S and A_D, respectively. Calculate the response factor, F, taken by the formula

$$(A_D/A_S)(W_S/W_D),$$

in which W_S and W_D are the weights, in milligrams, of glycerin and tributyrin, respectively, in the *Standard Preparation*. Similarly inject a suitable portion of the *Test Preparation*, and record the chromatogram. Measure the areas under the peaks and record the values of the areas under the tripropionin and tributyrin peaks as a_U and a_D, respectively. Calculate the percentage of glycerin by the formula

$$100F(a_U/a_D)(w_D/w_U),$$

in which w_D is the weight, in milligrams, of tributyrin in 5 mL of *Internal Standard Solution*, and w_U is the weight, in milligrams, of Glyceryl Monooleate in the *Test Preparation*.

Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Water Determine as directed under *Water Determination*, Appendix IIB, using 0.5 g of sample and 20 mL of a 1:1 methanol:chloroform mixture.

Packaging and Storage Store in tight, light-resistant containers.

Glyceryl Monostearate

Monostearin; 1,2,3-Propanetriol Octadecanoate

CAS: [31566-31-1]

DESCRIPTION

Glyceryl Monostearate occurs as a white, waxlike solid, as flakes, or as beads. It is a mixture of Glyceryl Monostearate and glyceryl monopalmitate. It may contain a suitable antioxidant. It is soluble in hot organic solvents such as acetone, alcohol, and ether and in mineral or fixed oils. It is dispersible in hot water with the aid of soap or suitable surfactants.

Function Emulsifier.

REQUIREMENTS

Identification Heat the sample with 3 parts water to 2° to 5° above its melting point. An irreversible gel forms when the sample is held at this temperature.

Assay Not less than 90.0% monoglycerides of saturated fatty acids.

Acid Value Not more than 6.

Free Glycerin Not more than 1.2%.

Hydroxyl Value Between 300 and 330.

Iodine Value Not more than 3.

Lead Not more than 1 mg/kg.

Melting Range Not below 65°.

Residue on Ignition Not more than 0.1%.

Saponification Value Not less than 150 and not more than 165.

TESTS

Assay

Propionating Reagent Mix 10 mL of pyridine and 20 mL of propionic anhydride.

Internal Standard Solution Transfer about 400 mg of hexadecyl hexadecanoate, accurately weighed, into a 100-mL volumetric flask, dissolve in chloroform, dilute with chloroform to volume, and mix.

Standard Preparation Transfer about 50 mg of USP Monoglycerides Reference Standard, accurately weighed, to a 25-mL conical flask, add 5 mL of *Internal Standard Solution* by pipet, and mix. When solution is complete, immerse the flask in a water bath maintained at a temperature between 45° and 50°, and volatilize the chloroform with the aid of a stream of nitrogen. Add 3.0 mL of *Propionating Reagent*, and heat the flask on a hot plate at 75° for 30 min. Evaporate the reagents with the aid of a stream of nitrogen and gentle steam heat. Add 15 mL of chloroform, and swirl to dissolve the residue.

Assay Preparation Transfer about 50 mg of sample, accurately weighed, into a 25-mL conical flask, and proceed as directed for *Standard Preparation*, beginning with ''add 5 mL of *Internal Standard Solution*. . . .''

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and containing a 2.4-m × 4-mm borosilicate glass column, or equivalent, packed with 2% liquid phase 5% phenyl methyl silicone (SE 52, or equivalent) on an 80- to 100-mesh siliceous earth support (Diatoport S, or equivalent). Maintain the column isothermally at a temperature between 270° and 280°, and the injection port and detector block at about 310°. Use helium as the carrier gas at a flow rate of about 70 mL/min.

System Suitability Chromatograph 6 to 10 injections of the *Standard Preparation* as directed under *Procedure*. The resolution factor, R, between the peaks for the derivatized glyceryl hexadecanoate and glyceryl octadecanoate is not less than 2.0, and the relative standard deviation of the ratio of the peak area of the derivatized glyceryl octadecanoate to that of the hexadecyl hexadecanoate is not more than 2.0%.

Procedure Inject a suitable portion of the *Standard Preparation* into the gas chromatograph, or equivalent, and record the chromatogram. Measure the areas under the peaks, and record the values of the sum of the areas under the derivatized monoglyceride peaks and of the area under the hexadecyl hexadecanoate peak as A_S and A_D, respectively. Calculate the response factor, F, taken by the formula

$$(A_S/A_D)(W_D/W_S),$$

in which W_D and W_S are the weights, in milligrams, of hexadecyl hexadecanoate and USP Monoglycerides Reference Standard, respectively, in the *Standard Preparation*. Similarly, inject a suitable portion of the *Assay Preparation* and record the chromatogram. Measure the areas under the peaks, and record the values of the sum of the areas under the derivatized monoglyceride peaks and of the area under the hexadecyl hexadecanoate peak as a_U and a_D, respectively. Calculate the quantity, in milligrams, of monoglycerides in the amount of sample taken by the formula

$$(W_D/F)(a_U/a_D).$$

Acid Value Determine as directed under *Acid Value*, Appendix VII.

Free Glycerin

Propionating Reagent Mix 10 mL of pyridine with 20 mL of propionic anhydride.

Internal Standard Solution Dissolve a suitable quantity of tributyrin, accurately weighed, in chloroform, and dilute quantitatively with chloroform to obtain a solution with a concentration of about 0.2 mg/mL.

Standard Preparation Transfer about 15 mg of glycerin and about 50 mg of tributyrin, both accurately weighed, into a glass-stoppered, 25-mL conical flask, add 3 mL of *Propionating Reagent*, and heat at 75° for 30 min. Volatilize the reagents with the aid of a stream of nitrogen at room temperature, add about 12 mL of chloroform, and mix. Dilute about 1 mL of this mixture with chloroform to about 20 mL, and mix.

Test Preparation Transfer about 50 mg of sample, accurately weighed, into a glass-stoppered, 25-mL conical flask, add 5 mL of *Internal Standard Solution* by pipet, and mix to dissolve. Immerse the flask in a water bath maintained at a temperature between 45° and 50°, and volatilize the chloroform with the aid of a stream of nitrogen. Add 3 mL of *Propionating Reagent*, and heat at 75° for 30 min. Volatilize the reagents with the aid of a stream of nitrogen at room temperature, add about 5 mL of chloroform, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and containing a 2.4-m × 4-mm borosilicate glass column, or equivalent, packed with 2% liquid phase consisting of a high-molecular-weight compound of polyethylene glycol and a diepoxide (Carbowax 20 M, or equivalent) on an 80- to 100-mesh siliceous earth support (Chromosorb W AW DMCS, or equivalent). Maintain the column isothermally at a temperature between 190° and 200° and the injection port and detector block at about 300° and 310°, respectively. Use helium as the carrier gas at a flow rate of about 70 mL/min.

System Suitability Chromatograph 6 to 10 injections of the *Standard Preparation* as directed under *Procedure*. The resolution factor, R, between the peaks for the derivatized glycerin and tributyrin is not less than 4.0, and the relative standard deviation of the ratio of their peak areas is not more than 2.0%.

Procedure Inject a suitable portion of the *Standard Preparation* into the gas chromatograph, and record the chromatogram. Measure the areas under the peaks, and record the values of the areas under the tripropionin and tributyrin peaks as A_S and A_D, respectively. Calculate the response factor, F, taken by the formula

$$(A_D/A_S)(W_S/W_D),$$

in which W_S and W_D are the weights, in milligrams, of glycerin and tributyrin, respectively, in the *Standard Preparation*. Similarly, inject a suitable portion of the *Test Preparation*, and record the chromatogram. Measure the areas under the peaks, and record the values of the areas under the tripropionin and

tributyrin peaks as a_U and a_D, respectively. Calculate the percentage of glycerin by the formula

$$100F(a_U/a_D)(w_D/w_U),$$

in which w_D is the weight, in milligrams, of tributyrin in 5 mL of *Internal Standard Solution*, and w_U is the weight, in milligrams, of sample in the *Test Preparation*.

Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Melting Range Determine as directed under *Melting Range*, Appendix VII.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Packaging and Storage Store in tight, light-resistant containers.

Glyceryl Palmitostearate

DESCRIPTION

Glyceryl Palmitostearate occurs as a fine powder or waxy solid. It is a mixture of fatty acid glycerides, primarily glyceryl esters of palmitic and stearic acids. The waxy solid melts at about 55°. Glyceryl Palmitostearate is soluble in chloroform, but practically insoluble in water and in alcohol.

Function Emulsifier

REQUIREMENTS

Identification Transfer about 100 mg of sample into a small, conical flask fitted with a suitable reflux condenser. Transfer 50 mg each of USP Palmitic Acid Reference Standard and USP Stearic Acid Reference Standard into a similar flask to serve as the *Standard Solution*. Treat the contents of each flask as follows: Add 5.0 mL of a solution prepared by dissolving 14 g of boron trifluoride in methanol to make 100 mL [commercial reagent, 14% w/v, may be used (Applied Science, or equivalent)]. Swirl to mix, and reflux for 15 min. Cool, transfer the reaction mixture with the aid of 10 mL of chromatographic-grade hexane to a 60-mL separator, and add 10 mL of water and 10 mL of saturated sodium chloride solution. Shake, allow the mixture to separate, then drain and discard the lower, aqueous layer. Pass the hexane layer through 6 g of anhydrous sodium sulfate into a suitable flask.

Concomitantly introduce a 1-µL to 2-µL portion of the filtered hexane solution from each of the two flasks into a gas chromatograph, or equivalent, and obtain the chromatograms. Use a gas chromatograph, or equivalent, equipped with a flame-ionization detector and a 1.5-m × 3-mm (id) column packed with 15% diethylene glycol succinate polyester on flux-calcined, acid-washed siliceous earth. Maintain the column at 165°. Set the temperatures of the inlet port and the detector to 210°. Use helium as the carrier gas. The retention times of the main peaks of methyl palmitate and methyl stearate obtained in the *Sample Solution* chromatogram correspond to those of the main peaks obtained from the *Standard Solution*. The ratio of the response of the main peaks to the sum of all the responses is between 0.42 and 0.55 for methyl palmitate, and between 0.43 and 0.55 for methyl stearate.

Acid Value Not more than 6.

Free Glycerin Not more than 1%.

Iodine Value Not more than 3.

Lead Not more than 1 mg/kg.

1-Monoglycerides Content Not more than 18.0%.

Residue on Ignition Not more than 0.1%.

Saponification Value Between 170 and 200.

TESTS

Acid Value Transfer about 10 g of sample, accurately weighed, into a flask, and add 50 mL of a 1:1 ethanol:diethyl ether mixture that has been neutralized to phenolphthalein with 0.1 *N* sodium hydroxide. Connect the flask to a suitable condenser, and warm it slowly while frequently shaking it. Add 1 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking it for 30 s. Calculate the acid value by the formula

$$56.11V \times (N/W),$$

in which V is the volume, in milliliters, of the 0.1 *N* sodium hydroxide solution used; N is the normality of the sodium hydroxide solution; and W is the weight, in grams, of the sample taken.

Free Glycerin Determine as directed under *Free Glycerin or Propylene Glycol*, Appendix VII.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method*, under *Lead Limit Test*, Appendix IIIB.

1-Monoglycerides Content Determine as directed under *1-Monoglycerides*, Appendix VII, using about 1 g of sample, accurately weighed, that has been melted at a temperature not higher than 80° and mixed.

Calculate the percentage of 1-monoglycerides as a normalized content of monopalmitin and monostearin by the formula

$$[17.2N \times (B - S)]/W,$$

in which 17.2 is one-twentieth of the formula weight of glyceryl monopalmitostearate; N is the normality of the sodium thiosulfate solution; B and S are the volumes, in milliliters, of 0.1 *N* sodium thiosulfate solution consumed by the blank and by the sample, respectively; and W is the weight, in grams, of the sample taken.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Packaging and Storage Store in tight containers at a temperature no higher than 25°.

Glyceryl Tristearate

Tristearin; Stearin; Octadecanoic Acid; 1,2,3-Propane Tristearoyl Ester

$C_{57}H_{110}O_6$ Formula wt 891.49

CAS: [555-43-1]

DESCRIPTION

Glyceryl Tristearate occurs as a white, microfine, crystalline powder. It is prepared by reacting glycerin with stearic acid in the presence of a suitable catalyst such as aluminum oxide. It is also found in many animal and vegetable fats such as tallow and cocoa butter. It is soluble in hot alcohol and in chloroform; very slightly soluble in cold alcohol, in ether, and in petroleum ether; but insoluble in water.

Function Crystallization accelerator; lubricant; surface-finishing agent.

REQUIREMENTS

Identification Glyceryl Tristearate exhibits the following typical composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	≤12	12:0	14:0	16:0	16:1
Weight % (Range):	0.0–0.3	0.0–0.5	0.0–1.0	0.0–0.1	0.0–0.1
Fatty Acid:	18:0	18:1	18:2	≥20	
Weight % (Range):	>95	0.0–0.5	0.0–0.5	0.0–0.5	

Acid Value Not more than 1.0.
Free Glycerin Not more than 0.5%.
Hydroxyl Value Not more than 5.0.
Iodine Number Not more than 1.0.
Lead Not more than 1 mg/kg.
Melting Range Between 69° and 73°.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 186 and 192.
Unsaponifiable Matter Not more than 0.5%.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VII.
Free Glycerin Determine as directed under *Free Glycerin and Propylene Glycol*, Appendix VII.
Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Melting Range Determine as directed in *Procedure for Class II* under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.

Packaging and Storage Store in tight, light-resistant containers.

Glycine

Aminoacetic Acid; Glycocoll

H_2NCH_2COOH

$C_2H_5NO_2$ Formula wt 75.07

INS: 640 CAS: [56-40-6]

View IR

DESCRIPTION

Glycine occurs as a white, crystalline powder. One gram dissolves in about 4 mL of water. It is very slightly soluble in alcohol and in ether. Its solution is acid to litmus.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_2H_5NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.2%.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 175 mg of sample, previously dried at 105° for 3 h and accurately weighed, into a 250-mL flask. Dissolve the sample in 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint.

Caution: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*) and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 7.507 mg of $C_2H_5NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed containers.

Grapefruit Oil, Coldpressed

Grapefruit Oil, Expressed; Oil of Shaddock

CAS: [8016-20-4]

View IR

DESCRIPTION

Grapefruit Oil, Coldpressed, occurs as a yellow, sometimes red, liquid that often shows a flocculent separation of waxy material. It is the oil obtained by expression from the fresh peel of the grapefruit *Citrus paradisi* Macfayden (*Citrus decumana* L.) (Fam. Rutaceae). It is soluble in most fixed oils and in mineral oil, often with opalescence or cloudiness. It is slightly soluble in propylene glycol and insoluble in glycerin. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown under *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between +91° and +96°.
Refractive Index Between 1.475 and 1.478 at 20°.
Residue on Evaporation Between 5.0% and 10.0%.
Specific Gravity Between 0.848 and 0.856.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Residue on Evaporation Determine as directed under *Residue on Evaporation*, Appendix VI, heating a sample for 5 h.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Grape Skin Extract

Enocianina

INS: 163(ii) CAS: [11029-12-2]

DESCRIPTION

Grape Skin Extract occurs as a red to purple powder or liquid concentrate. It is prepared by aqueous extraction of grape marc remaining from the pressing of grapes to obtain juice. Extraction is effected with water containing sulfur dioxide. During the steeping process, sulfur dioxide is added, and the sugar content is reduced by fermentation; further concentration removes most of the alcohol. The primary color components are anthocyanins such as the glucosides of malvidin, peonidin, petunidin, delphinidin, or cyanidin. Other components naturally present are sugars, tartrates, malates, tannins, and minerals. The powder may contain an added carrier such as maltodextrin, modified starch, or gum. In acid solution, Grape Skin Extract is red; in neutral to alkaline solution, it is unstable and violet to blue.

Function Color.

REQUIREMENTS

Identification Transfer 1 g of sample and 1 g of potassium metabisulfite to a 100-mL volumetric flask, dissolve in about 50 mL of pH 3.0 *Citrate–Citric Acid Buffer* (see *Assay*, below), and dilute to volume with the same buffer. The red color caused by anthocyanins is bleached.
Assay Not less than 90% of the color strength as represented by the vendor.
Arsenic Not more than 1 mg/kg.
Lead Not more than 5 mg/kg.

TESTS

Assay

pH 3.0 Citrate–Citric Acid Buffer Add, dropwise, 0.1 *M* sodium citrate to 0.1 *M* citric acid until a pH of 3.0 is reached, as determined by a glass electrode.

Procedure Transfer about 0.2 g of sample, accurately weighed, to a 100-mL volumetric flask, dissolve it in about 25 mL of pH 3.0 *Citrate–Citric Acid Buffer*, and dilute to volume with the same buffer. Remove any undissolved material by filtration or centrifugation. Adjust the pH to 3.0, and determine the absorbance of the clarified solution at the maximum, near 525 nm, in a cell with a 1-cm pathlength. The

color strength, expressed as the absorbance of a 1% solution in a cell of 1-cm pathlength, is calculated as

$$A_{525}/S,$$

in which A_{525} is the absorbance at 525 nm and S is the weight, in grams, of sample.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.

Packaging and Storage Store liquid Grape Skin Extract with aseptic packaging or in high-density polyethylene containers at 4° to 14°. Store powdered Grape Skin Extract in fiber drums at room temperature.

Guar Gum

INS: 412 CAS: [9000-30-0]

DESCRIPTION

Guar Gum occurs as a white to yellow-white powder. It is a gum obtained from the ground endosperms of *Cyamopsis tetragonolobus* (L.) Taub (Fam. Leguminosae) (synonym *Cyamopsis psoraloides* [Lam.] D.C.). It consists chiefly of a high-molecular-weight polysaccharide composed of galactose and mannose units and may be described chemically as a galactomannan. It is dispersible in either hot or cold water, forming a sol, having a pH between 5.4 and 7.0, that may be converted to a gel by the addition of small amounts of sodium borate.

Function Stabilizer; thickener; emulsifier.

REQUIREMENTS

Identification

A. Transfer a 2-g sample into a 400-mL beaker, moisten it thoroughly with about 4 mL of isopropyl alcohol, add, with vigorous stirring, 200 mL of cold water, and continue to stir until the gum is completely and uniformly dispersed. An opalescent, viscous dispersion forms.

B. Transfer 100 mL of the dispersion prepared in *Identification Test A* into another 400-mL beaker, heat the mixture in a boiling water bath for about 10 min, and then cool to room temperature. No appreciable increase in viscosity develops.

Acid-Insoluble Matter Not more than 7.0%.

Ash (Total) Not more than 1.5%.

Galactomannans Not less than 70.0%.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 15.0%.

Protein Not more than 10.0%.

Starch Passes test.

TESTS

Acid-Insoluble Matter Transfer 1.5 g of sample, accurately weighed, into a 250-mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid. Cover the beaker with a watch glass, and heat the mixture on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. At the end of the 6-h heating period, add about 500 mg of a suitable filter aid, previously dried for 3 h at 105° and accurately weighed, and filter through a tared, sintered-glass filter crucible. Wash the residue several times with hot water, dry the crucible and its contents at 105° for 3 h, cool in a desiccator, and weigh. The difference between the weight of the filter aid and that of the residue is the weight of *Acid-Insoluble Matter*.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.

Galactomannans The difference between 100 and the sum of the percent *Acid-Insoluble Matter*, *Total Ash*, *Loss on Drying*, and *Protein* represents the percent *Galactomannans*.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 5 h.

Protein Determine as directed in *Method I* under *Nitrogen Determination*, Appendix IIIC, using about 3.5 g of sample, accurately weighed, transferred into a 500-mL Kjeldahl flask. The percentage of nitrogen determined, multiplied by 6.25, gives the percentage of protein in the sample.

Starch Add a few drops of iodine TS to a 1:10 aqueous solution. No blue color appears.

Packaging and Storage Store in well-closed containers.

Gum Arabic

Acacia

INS: 414 CAS: [9000-01-5]

DESCRIPTION

Gum Arabic occurs as a dried, gummy exudation obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or of related species of *Acacia* (Fam. Leguminosae). The unground product occurs as white or yellow-white, spheroidal tears of varying size or in angular fragments. It is also available commercially as white to yellow-white flakes, granules, or powder. One gram dissolves in 2 mL of water, forming a solution that flows readily and is acid to litmus. It is insoluble in alcohol.

Function Stabilizer; emulsifier.

REQUIREMENTS

Identification Add 0.2 mL of diluted lead subacetate TS to 10 mL of a cold 1:50 aqueous solution. A flocculent or curdy, white precipitate forms immediately.
Arsenic Not more than 3 mg/kg.
Ash (Acid-Insoluble) Not more than 0.5%.
Ash (Total) Not more than 4.0%.
Insoluble Matter Not more than 1.0%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 15.0%.
Starch or Dextrin Passes test.
Tannin-Bearing Gums Passes test.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Ash (Acid-Insoluble) Determine as directed under *Ash (Acid Insoluble)*, Appendix IIC.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Insoluble Matter Dissolve 5 g of sample in about 100 mL of water contained in a 250-mL Erlenmeyer flask, add 10 mL of 2.7 *N* hydrochloric acid, and boil gently for 15 min. Filter the hot solution by suction through a tared filtering crucible, wash the residue thoroughly with hot water, dry at 105° for 2 h, and weigh.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying (**Note**: Powder unground samples sufficiently to pass through a No. 40 sieve, and mix well before weighing and drying.) Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 5 h.
Starch or Dextrin Boil a 1:50 aqueous solution, cool, and add a few drops of iodine TS. No blue or red color appears.
Tannin-Bearing Gums Add about 0.1 mL of ferric chloride TS to 10 mL of a 1:50 aqueous solution. No black coloration or precipitate forms.

Packaging and Storage Store in well-closed containers.

Gum Ghatti

Indian Gum

CAS: [9000-28-6]

DESCRIPTION

Gum Ghatti occurs as colorless or light to dark tan tears. It is also available as a gray to red-gray powder. It is the dried gummy exudate from the stems of *Anogeissus latifolia* Wall (Fam. Combretaceae). It is a complex, water-soluble, acidic polysaccharide composed of the calcium and magnesium salts of L-arabinose, D-galactose, D-mannose, D-xylose, and D-glucuronic acids in the approximate molar ratio of 10:6:2:1:2. It is slightly soluble in water, but it is insoluble in 90% alcohol.

Function Emulsifier.

REQUIREMENTS

Labeling Indicate the viscosity in centipoises.
Identification

Lead Acetate Solution (**Caution**: Use gloves and goggles to avoid contact with skin and eyes. Use an effective fume-removal device or other respiratory protection.) Activate 50 to 60 g of lead (II) oxide by heating it for 2.5 to 3 h in a furnace at 650° to 670° (cooled product should have a lemon color). Boil 80 g of lead acetate trihydrate and 40 g of the freshly activated lead (II) oxide with 250 g of water in a 500-mL Erlenmeyer flask provided with a reflux condenser for 45 min. Cool, filter off any residue, and dilute with recently boiled water to a density of 1.25 at 20°. Add 4 mL of water to 1 mL of the lead acetate solution, and filter.

Procedure Add 0.2 mL of the *Lead Acetate Solution* to 5 mL of a cold 1:100 aqueous solution. A slight precipitate or clear solution results in which an opaque flocculent precipitate forms on the addition of 1 mL of 3 *N* ammonium hydroxide.
Arsenic Not more than 3 mg/kg.
Ash (Acid-Insoluble) Not more than 1.75%.
Ash (Total) Not more than 6.0%.
Insoluble Matter Not more than 1.0%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 14.0%.
Viscosity A 5% solution exhibits a viscosity, measured in centipoises, within the range stated on the label.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Ash (Acid-Insoluble) Determine as directed under *Ash (Acid-Insoluble)*, Appendix IIC.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Insoluble Matter Dissolve 5 g of sample, accurately weighed, in about 100 mL of water contained in a 250-mL Erlenmeyer flask, add 10 mL of 2.7 *N* hydrochloric acid, and boil gently for 15 min. Filter the hot solution, using suction through a tared filtering crucible, wash thoroughly with hot water, dry at 105° for 2 h, and weigh.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a ground sample at 105° for 5 h. Powder unground samples to pass through a No. 40 sieve, and mix well before weighing.

Viscosity Determine as directed under *Viscosity of Cellulose Gum*, Appendix IIB, at 75°, using spindle No. 2 at 60 rpm.

Packaging and Storage Store in well-closed containers.

Gum Guaiac

Guaiac Resin

INS: 314 CAS: [9000-29-7]

DESCRIPTION

Gum Guaiac occurs as irregular masses enclosing fragments of vegetable tissues; as large, nearly homogeneous masses; and occasionally, as more-or-less rounded or ovoid tears. It is externally brown-black to dusky brown, acquiring a green color on long exposure to air, the fractured surface having a glassy luster, the thin pieces being transparent and varying in color from brown to yellow-orange. The powder is moderate yellow-brown, becoming olive brown on exposure to air. It is the resin of the wood of *Guajacum officinale* L. or *Guajacum sanctum* L. (Fam. Zygophyllaceae). Gum Guaiac dissolves incompletely, but readily, in alcohol, in ether, in chloroform, and in solutions of alkalies. It is slightly soluble in carbon disulfide.

Function Antioxidant.

REQUIREMENTS

Identification

A. Add 1 drop of ferric chloride TS to 5 mL of a 1:100 alcoholic solution. A blue color appears that gradually changes to green, finally becoming green-yellow.

B. A mixture of 5 mL of a 1:100 alcoholic solution and 5 mL of water becomes blue when shaken with 20 mg of lead peroxide. Filter the solution, and boil a portion of the filtrate. The color disappears but may be restored by adding lead peroxide and shaking the filtrate. Add a few drops of 2.7 *N* hydrochloric acid to a second portion of the filtrate. The color immediately disappears.

Alcohol-Insoluble Residue Not more than 15.0%.
Ash (Acid-Insoluble) Not more than 2.0%.
Ash (Total) Not more than 5.0%.
Lead Not more than 2 mg/kg.
Melting Range Between 85° and 90°.
Rosin Passes test.

TESTS

Alcohol-Insoluble Residue Place 2 g of sample, finely powdered and accurately weighed, in a dry, tared extraction thimble, and extract it with alcohol in a suitable continuous extraction apparatus for 3 h or until completely extracted. Dry the insoluble residue remaining in the thimble for 4 h at 105°, and weigh.

Ash (Acid-Insoluble) Determine as directed under *Ash (Acid-Insoluble)*, Appendix IIC.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 4 µg of lead (Pb) ion in the control.

Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.

Rosin A 1:10 solution of sample in petroleum ether is colorless, and when mixed and shaken with an equal quantity of a fresh, 1:200 aqueous solution of cupric acetate, is not more green than a similar solution of cupric acetate in petroleum ether.

Packaging and Storage Store in well-closed containers.

Helium

He Formula wt 4.00

INS: 939 CAS: [7440-59-7]

DESCRIPTION

Helium occurs as a colorless gas that is not combustible and does not support combustion. It is very slightly soluble in water. One liter of the gas weighs about 180 mg at 0° and 760 mm Hg.

Function Processing aid.

REQUIREMENTS

Note: Reduce the sample gas cylinder pressure with a regulator. Measure the sample gas with a gas volume meter downstream from the detector tube to minimize contamination of or change to the gas samples.

The detector tube called for in one test is described under *Solutions and Indicators*.

Identification

A. Insert a burning wood splinter into an inverted test tube filled with sample gas. The flame is extinguished.

Note: Use caution.

B. A small balloon filled with sample gas shows buoyancy.

Assay Not less than 99.0% of He, by volume.
Air Not more than 1.0%, by volume.
Carbon Monoxide Not more than 10 ppm, by volume.
Odor Passes test.

TESTS

Assay (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a thermal-conductivity detector

and a 6-m × 4-mm (id) column, or equivalent, packed with porous polymer beads (PoraPak Q, or equivalent), which permit complete separation of nitrogen and oxygen from Helium, although nitrogen and oxygen may not be separated from each other. Maintain the column at 60°. Use industrial-grade helium (99.99%) as the carrier gas. Introduce a gas sample into the gas-sampling valve. Select the operating conditions of the gas chromatograph, or equivalent, so that the peak signal of an air–helium certified standard (a mixture of 1.0% air in industrial-grade helium is available from most suppliers) corresponds to not less than 70% of the full-scale reading. The peak response produced by the sample gas exhibits a retention time corresponding to that produced by the air–helium standard and, when compared with the peak response of that standard, indicates not more than 1.0% air, by volume, and not less than 99.0% of He, by volume.

Air Determine as directed under *Assay* (above).

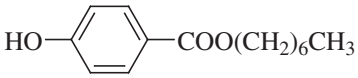
Carbon Monoxide Pass 1050 ± 50 mL of sample gas through a carbon monoxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 10 ppm, by volume.

Odor Carefully open the sample gas cylinder valve to produce a moderate flow of sample gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose. No appreciable odor is discernible.

Packaging and Storage Store in appropriate gas cylinders.

Heptylparaben

n-Heptyl-*p*-hydroxybenzoate



$C_{14}H_{20}O_3$ Formula wt 236.31

CAS: [1085-12-7]

DESCRIPTION

Heptylparaben occurs as small, colorless crystals or as a white, crystalline powder. It is very slightly soluble in water, but is freely soluble in alcohol and in ether.

Function Preservative; antimicrobial agent.

REQUIREMENTS

Identification Dissolve 500 mg of sample in 10 mL of 1 *N* sodium hydroxide, boil for 30 min, allow the solution to evaporate to a volume of about 5 mL, and cool. Acidify the solution with 2 *N* sulfuric acid, collect the crystals on a filter, wash several times with small portions of water, and dry in a desiccator over silica gel. The *p*-hydroxybenzoic acid so obtained melts between 212° and 217° (see *Melting Range or Temperature*, Appendix IIB).

Assay Not less than 99.0% and not more than 100.5% of $C_{14}H_{20}O_3$, calculated on the dried basis.

Acidity Passes test.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 0.5%.

Melting Range Between 48° and 51°.

Residue on Ignition Not more than 0.05%.

TESTS

Assay Transfer 3.5 g of sample, accurately weighed, into a flask, add 40.0 mL of 1 *N* sodium hydroxide, and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h, cool, and titrate the excess sodium hydroxide with 1 *N* sulfuric acid to pH 6.5. Perform a blank determination (see *General Provisions*) with the same quantities of the same reagents in the same manner, and make any necessary correction. Each milliliter of 1 *N* sodium hydroxide is equivalent to 236.3 mg of $C_{14}H_{2O}O_3$, calculated on the dried basis.

Acidity Mix 750 mg of sample with 15 mL of water, heat at 80° for 1 min, cool, and filter. The filtrate is acid or neutral to litmus. Add 0.2 mL of 0.1 *N* sodium hydroxide and 2 drops of methyl red TS to 10 mL of the filtrate. The solution is yellow, without even a light cast of pink.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in a desiccator over silica gel for 5 h.

Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in tight containers.

Hexanes

Mixed Paraffinic Hydrocarbons

C_6H_{14} Formula wt 86.18

CAS: [110-54-3]

DESCRIPTION

Hexanes occur as a clear, colorless, flammable liquid. It is composed predominantly of C_6, with some C_5 and C_7, isomeric paraffins. The relative proportion of isomers varies with the producer and the production lot. It is soluble in alcohol, in acetone, and in ether and is insoluble in water.

Function Extraction solvent.

REQUIREMENTS

Benzene Not more than 0.05%.
Color (APHA) Not more than 10.
Distillation Range Between 56° and 71°.
Lead Not more than 1 mg/kg.
Nonvolatile Residue Not more than 10 mg/kg.
Specific Gravity Between 0.655 and 0.675.
Sulfur Not more than 5 mg/kg.

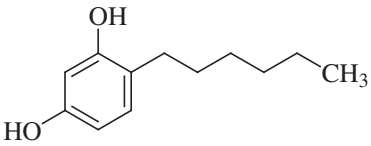
TESTS

Benzene Determine as directed under *Benzene*, Appendix IIIC.
Color (APHA) Dilute 2.0 mL of platinum–cobalt stock solution (APHA No. 500) with water in a 100-mL volumetric flask. Compare this solution (APHA No. 10) with 100 mL of sample in 100-mL Nessler tubes, viewed vertically over a white background.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Nonvolatile Residue Evaporate 150 mL (about 100 g) of sample to dryness in a tared dish on a steam bath. Dry the residue at 105° for 30 min, cool, and weigh.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Sulfur Determine as directed under *Sulfur*, Appendix IIIC.

Packaging and Storage Store in tight containers, protected from fire.

4-Hexylresorcinol

Hexylresorcinol; 4-Hexyl-1,3-benzenediol



$C_{12}H_{18}O_2$ Formula wt 194.27

CAS: [136-77-6]

View IR

DESCRIPTION

4-Hexylresorcinol occurs as a white powder. It is very slightly soluble in water and freely soluble in ether and in acetone.

Caution: 4-Hexylresorcinol is irritating to the oral mucosa and respiratory tract and to the skin, and its solution in alcohol has vesicant properties.

Function Color stabilizer; enzymatic browning inhibitor.

REQUIREMENTS

Identification

A. Add 1 mL of nitric acid to 1 mL of a saturated solution of sample. A light red color appears.

B. Add 1 mL of bromine TS to 1 mL of a saturated solution of the sample. A yellow flocculent precipitate forms. Add 2 mL of 6 *N* ammonium hydroxide, and the precipitate dissolves, producing a yellow solution.

C. The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits maxima only at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.0% and not more than 100.5% of $C_{12}H_{18}O_2$ after drying.
Acidity Passes test.
Lead Not more than 2 mg/kg.
Melting Range Between 62° and 67°.
Mercury Not more than 3 mg/kg.
Nickel Not more than 2 mg/kg.
Residue on Ignition Not more than 0.1%.
Resorcinol and Other Phenols Negative by test.

TESTS

Assay Dissolve 70 to 100 mg of sample, previously dried over silica gel for 4 h and accurately weighed, in 10 mL of methanol in a 250-mL iodine flask. Add 30.0 mL of 0.1 *N* bromine, then quickly add 5 mL of hydrochloric acid, and insert the stopper in the flask immediately. Cool the flask under running water to room temperature, shake vigorously for 5 min, then set aside for 5 min. Add 6 mL of potassium iodide TS around the stopper, cautiously loosen the stopper, again insert the stopper tightly, and swirl gently. Add 1 mL of chloroform, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the milligrams of $C_{12}H_{18}O_2$ in the sample taken by the formula

$$4.857 \times (B - S),$$

in which 4.857 is the milliequivalent factor, B is the number of milliliters of 0.1 *N* sodium thiosulfate required for the blank, and S is the number of milliliters of 0.1 *N* sodium thiosulfate required for the sample.

Acidity Dissolve 250 mg of sample in 500 mL of water, add a few drops of methyl red TS, and titrate with 0.02 *N* sodium hydroxide. Not more than 1.0 mL is required for neutralization (0.05%).
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 2-g sample.

Melting Range Determine as directed in *Procedure for Class I* under *Melting Range or Temperature*, Appendix IIB.

Mercury (**Note**: Select all reagents for this test to have as low a content of mercury as practicable, and store all reagent solutions in containers of borosilicate glass. Specially clean all glassware used in this test by soaking it in warm 8 *N* nitric acid for 30 min and rinsing with water. Keep flasks for this determination separate from other flasks, and use them only for mercury determinations.)

Standard Solution Transfer 34.0 mg of mercuric chloride to a 250-mL volumetric flask. Add 1 drop of hydrochloric acid, and dissolve in and dilute to volume with water. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 1 drop of hydrochloric acid, and dilute with water to volume. Transfer 1.0 mL of this solution to a 500-mL volumetric flask, add 1 drop of hydrochloric acid, and dilute with water to volume.

Test Solution Transfer 134 mg of sample to a 250-mL beaker, and cautiously add 10 mL of 11 *N* nitric acid and 10 mL of 18 *N* sulfuric acid. Digest with the aid of heat in a well-ventilated hood until brown fumes cease to evolve. Cautiously add an additional 10 mL of 11 *N* nitric acid, and continue heating until no more fumes evolve. Cool, transfer to a 200-mL volumetric flask, and dilute to volume with water.

Procedure Transfer 100 mL of *Standard Solution* to a 300-mL mercury analysis reaction vessel, add 2 drops of a 1:20 potassium permanganate solution, and mix (the solution should be purple; add additional permanganate solution, dropwise, if necessary). Add 5 mL of 11 *N* nitric acid, stir, and allow to stand for not less than 15 s. Add 5 mL of 18 *N* sulfuric acid, stir, and allow to stand for not less than 45 s. Add 5 mL of a 3:200 hydroxylamine hydrochloride solution, stir, and allow to stand until the solution turns light yellow or colorless. Add 5 mL of a 1:10 stannous chloride solution, immediately insert the aerator connected to an air pump, and determine the maximum absorbance of the treated *Standard Solution* at the mercury resonance line of 253.65 nm, with a suitable atomic absorption spectrophotometer equipped with a mercury hollow-cathode lamp and an absorption cell that permits the flameless detection of mercury.

> **Note**: Disregard the presence of insoluble matter in this solution; mix before use.

In a closed system with a circulating air pump, connect a calcium chloride drying tube and an aerator inserted in a 300-mL reaction vessel so that air passed through the treated preparation contained in the reaction vessel evaporates any metallic mercury present. In a similar manner, treat 100 mL of the *Test Solution* and 100 mL of water (reagent blank), and determine the maximum absorbances at the same wavelength. The absorbance of the solution from the *Test Solution* does not exceed that of the solution from the *Standard Solution*.

> **Note**: Check the zero setting of the instrument frequently.

Nickel

Standard Nickel Solution Transfer 40.1 mg of nickel chloride hexahydrate, accurately weighed, into a 1-L volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute to volume, and mix. Each milliliter of this solution contains 1 μg of nickel ion.

Dissolve 2 g of sample in methanol to yield 20 mL. Add 3 mL of bromine TS and 2 mL of a 1:5 citric acid solution, and mix. Add 10 mL of 6 *N* ammonium hydroxide and 1 mL of a 1:100 dimethylglyoxime:ethanol solution. Mix, dilute with water to 50 mL, and allow to stand for 5 min. Any color the solution produces is not more intense than that of a solution containing 4 mL of *Standard Nickel Solution* and treated in the same manner.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Resorcinol and Other Phenols Shake about 1 g of sample with 50 mL of water for a few minutes, filter, and add 3 drops of ferric chloride TS to the filtrate. No red or blue color appears.

Packaging and Storage Store in tight, light-resistant containers.

High-Fructose Corn Syrup

DESCRIPTION

High-Fructose Corn Syrup (HFCS) occurs as a water white to light yellow, somewhat viscous liquid that darkens at high temperatures. It is a saccharide mixture prepared as a clear, aqueous solution from high-dextrose-equivalent corn starch hydrolysate by the partial enzymatic conversion of glucose (dextrose) to fructose, using an insoluble glucose isomerase preparation that complies with **21** *CFR* 184.1372 and that has been obtained from a pure culture fermentation that produces no antibiotics. It is miscible in all proportions with water.

Function Nutritive sweetener.

REQUIREMENTS

Labeling Indicate the color range and presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.

Identification Add a few drops of a 1:10 aqueous solution to 5 mL of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide forms.

Assay *42% HFCS*: Not less than 97.0% total saccharides, expressed as a percentage of solids, of which not less than 42.0% consists of fructose, not less than 92.0% consists of monosaccharides, and not more than 8.0% consists of other saccharides. *55% HFCS*: Not less than 95.0% total saccharides, expressed as a percentage of solids, of which not less than 55.0% consists of fructose, not less than 95.0% consists of monosaccharides, and not more than 5.0% consists of other saccharides.

Arsenic Not more than 1 mg/kg.
Color Within the range specified by the vendor.
Lead Not more than 0.1 mg/kg.
Residue on Ignition Not more than 0.05%.
Sulfur Dioxide Not more than 0.003%.
Total Solids *42% HFCS*: Not less than 70.5%; *55% HFCS*: Not less than 76.5%.

TESTS

Assay

Apparatus (See *Chromatography*, Appendix IIA.) Use a suitable high-performance liquid chromatography system such as described in *Standard Analytical Methods of the Corn Refiners Association*, equipped with a 22- to 31-cm stainless-steel column, or equivalent, a strip-chart recorder, and a differential refractometer detector maintained at 45° ± 0.005°.

Stationary Phase Use prepacked macroreticular polystyrene sulfonate divinylbenzene cation-exchange resin (2% to 8% cross-linked, 8- to 25-μm particle size), preferably in the calcium or silver form. Examples of acceptable resins are Bio-Rad Aminex HPX-87C, or equivalent, for separating DP_1–DP_4 saccharides, and Aminex HPX-42C and HPX-42A, or equivalent, for separating DP_1–DP_7 saccharides. Maintain the column at 85° during operation.

Mobile Phase Use degassed, purified water passed through a 0.22-μm filter before use; maintain the water at 85° during operation of the chromatograph.

Standardization Prepare a *Standard Solution* containing a total of about 10% solids, using sugars of known purity (e.g., USP Fructose Reference Standard; USP Dextrose Reference Standard, or NIST Standard Reference Material; maltose, Aldrich Chemical Company; or equivalent) that approximates, on the dry basis, the composition of the sample to be analyzed. Dissolve each standard sugar, accurately weighed, in 20 mL of purified water contained in a 50-mL beaker. Heat on a steam bath until all sugars are dissolved, then cool, and transfer to a 100-mL volumetric flask. Dilute to volume with water and mix. Freeze the solution if it is to be reused.

If a corn syrup or maltodextrin is used to supply a DP_{4+} fraction, take care to include all saccharides in the standard composition calculation.

Compute the dry-basis concentration, in percent, of each individual component in the *Standard Solution* by the formula

$$(W_C/\Sigma W_I) \times 100,$$

in which W_C is the weight of the sugar of interest and ΣW_i is the sum of the weights of all sugar components. Standardize by injecting 10 to 20 μL (about 1.0 to 2.0 mg of solids) of the standard sugar solution into the chromatograph. Integrate the peaks and normalize. Sum the individual DP_{4+} responses from the normalized printout to obtain the total DP_{4+} normalized response. Calculate the response factors as follows (see *Chromatography*, Appendix IIA):

R_I = (known concentration, dry basis %)/(measured concentration, normalized %),

in which R_I is the response factor for component *i*.

Compute the response factor for each component relative to glucose (R'_I) using the following equation:

$$R'_I = R_I/R_{G'},$$

in which R_G is the response factor for glucose. The R'_I for DP_{4+} should be programmed as a default value (if automated equipment is used) and used to compute the concentration of higher saccharides.

Sample Analysis Determine the solids content (below) of the sample, and dilute to approximately 10% solids with water. Inject a volume (10 to 50 μL) appropriate for the specific solids content into the chromatograph.

Calculation Calculate the concentration of each component as follows:

$$C_I = (A_I \times R_I \times 100)/(\Sigma A_N R_N),$$

in which A_I is the area recorded for that component and $\Sigma A_N R_N$ is the sum of the product of the areas (A) and response factors (R) for all components detected.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds and 1 mL of *Standard Arsenic Solution* (1 μg As).

Color

Apparatus Use a suitable variable-wavelength spectrophotometer capable of measuring percent transmittance throughout the visible spectrum and designed to permit the use of sample and reference cells with pathlengths of 2 to 4 cm. The transmittance of all paired cells should agree within 0.5%.

Standard Solution Dissolve 0.10 g of reagent-grade potassium dichromate ($K_2Cr_2O_7$) in 1 L of water, and mix thoroughly.

Procedure Using water in the sample and reference cells of 2-cm pathlength, normalize the percent transmittance scale of the spectrophotometer to 100%. Leave the reference cell in place and replace the water in the sample cell with the *Standard Solution*. Determine the wavelength at which the solution exhibits exactly 54.5% transmittance. This wavelength is defined as λ_c, the corrected 450-nm wavelength. Remove the 2-cm cells from the spectrophotometer, and with water in the sample and reference cells of 4-cm pathlength, adjust the percent transmittance scale to 100% with the spectrophotometer set at λ_c. Leave the reference cell in place, and replace the water in the sample cell with sample. Measure the percent transmittance (T_{450}). Remove the sample cell, set the wavelength at 600 nm, replace the sample with water, and adjust the percent transmittance scale to 100%. Determine the percent transmittance at 600 nm (T_{600}) with the same sample in the sample cell. Calculate the *Color* of the sample taken using the following formula:

$$(\log T_{600} - \log T_{450})/4,$$

in which T_{600} is the percent transmittance at 600 nm and T_{450} is the percent transmittance at 450 nm.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

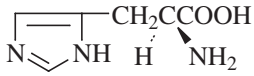
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIIB, igniting a 10-g sample.

Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X, using a 50-g sample.
Total Solids Determine the refractive index of a sample at 20° or 45°, and use the tables in *High-Fructose Corn Syrup Solids*, under *Total Solids*, Appendix X, to obtain the percent *Total Solids*.

Packaging and Storage Store in tight containers.

L-Histidine

L-α-Amino-4(or 5)-imidazolepropionic Acid



$C_6H_9N_3O_2$ Formula wt 155.16

CAS: [71-00-1]

View IR

DESCRIPTION

L-Histidine occurs as white crystals or as a crystalline powder. It is soluble in water, very slightly soluble in alcohol, and insoluble in ether. It melts with decomposition between about 277° and 288°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_9N_3O_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.2%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +11.5° and +13.5°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between +12.0° and +14.0°, calculated on the dried basis.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Dissolve about 150 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

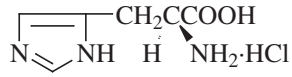
Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 15.52 mg of $C_6H_9N_3O_2$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 11 g of previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

L-Histidine Monohydrochloride

L-α-Amino-4(or 5)-imidazolepropionic Acid Monohydrochloride



$C_6H_9N_3O_2 \cdot HCl \cdot H_2O$ Formula wt 209.63

CAS: monohydrate [5934-29-2]

View IR

DESCRIPTION

L-Histidine Monohydrochloride occurs as white crystals or as a crystalline powder. It is soluble in water, and insoluble in alcohol and in ether. It melts with decomposition at about 250° (after drying).

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +8.5° and +10.5°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 100 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 3 mL of formic

acid, add exactly 15.0 mL of 0.1 *N* perchloric acid, and heat on a water bath for 30 min.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

After cooling, add 45 mL of glacial acetic acid, and titrate the excess perchloric acid with 0.1 *N* sodium acetate, determining the endpoint potentiometrically. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 10.48 mg of $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 11 g of previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Hops Oil

CAS: [8007-04-3]

View IR

DESCRIPTION

Hops Oil occurs as a light yellow to green-yellow liquid with a characteristic, aromatic odor. Age darkens the color, and the oil tends to become viscous. It is the volatile oil obtained by steam distillation of the freshly dried membranous cones of the female plants of *Humulus lupulus* L. or *Humulus americanus* Nutt. (Fam. Moraceae). It is soluble in most fixed oils and, sometimes with opalescence, in mineral oil. It is practically insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Acid Value Not more than 11.0.

Angular Rotation Between −2° and +2°5′.

Refractive Index Between 1.470 and 1.494 at 20°.

Saponification Value Between 14 and 69.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.825 and 0.926.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI, using about 5 g of sample, accurately weighed.

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample usually is not soluble in 95% alcohol. Older oils are less soluble than fresh oils.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Hydrochloric Acid

HCl Formula wt 36.46

INS: 507 CAS: [7647-01-0]

DESCRIPTION

Hydrochloric Acid occurs as a clear, colorless or slightly yellow, corrosive liquid. It is a water solution of hydrogen chloride of varied concentrations. It is miscible with water and with alcohol. Concentrations of Hydrochloric Acid are expressed in percent by weight or may be expressed in degrees Baumé (°Bé) from which percents of Hydrochloric Acid and specific gravities may readily be derived (see *Hydrochloric Acid Table*, Appendix IIC). The usually available concentrations are 18°, 20°, 22°, and 23°Bé. Concentrations above 13°Bé (19.6%) fume in moist air, lose hydrogen chloride, and create a corrosive atmosphere. Because of these characteristics, observe suitable precautions during sampling and analysis to prevent losses.

> **Note**: Hydrochloric Acid is produced by various methods that might impart trace amounts of organic compounds as impurities. The manufacturer, vendor, or user is responsible for identifying the specific organic compounds that are present and for meeting the *Requirements* for organic compounds (below). Methods are provided for their determination under *Tests*. In applying the procedures, use any necessary standards

to quantitate the organic compounds present in each specific product.

The variety of organic impurities that might conceivably be found in Hydrochloric Acid is such that it is impossible to provide a comprehensive and accurate list here. Therefore, the manufacturer, vendor, or user is responsible for establishing the suitability of such Hydrochloric Acid for its intended application in foods or food processing in accordance with the provision of *Trace Impurities* (see *General Provisions*).

Function Acidifier.

REQUIREMENTS

Labeling Indicate the content, by weight, of Hydrochloric Acid (HCl). Alternatively, indicate the range of Hydrochloric Acid content, the range of degrees Baumé, and/or the specific gravity range.

Identification A sample gives positive tests for *Chloride*, Appendix IIIA.

Assay Not less than 97.0% and not more than 103.0% of the labeled amount of HCl, or within the range specified on the label.

Color Passes test.

Degrees Baumé Within the range shown on the label or claimed by the vendor.

Iron Not more than 5 mg/kg.

Lead Not more than 1 mg/kg.

Nonvolatile Residue Not more than 0.5%.

Organic Compounds

Total Organic Compounds (*Non-Fluorine-Containing*) Not more than 5 mg/kg, including

Benzene Not more than 0.05 mg/kg.

Fluorinated Organic Compounds (*total*) Not more than 0.0025%.

Oxidizing Substances (as Cl_2) Not more than 0.003%.

Reducing Substances (as SO_3) Not more than 0.007%.

Specific Gravity Within the range specified or implied by the vendor.

Sulfate Not more than 0.5%.

TESTS

Assay Accurately tare a 125-mL glass-stoppered Erlenmeyer flask containing 35.0 mL of 1 *N* sodium hydroxide. Without the use of vacuum, partially fill a 10-mL serological pipet from near the bottom of a flask containing the sample, remove any acid adhering to the outside, and discard the first milliliter flowing from the pipet. Hold the tip of the pipet just above the surface of the sodium hydroxide solution, and transfer between 2.5 and 3 mL of the sample into the flask, leaving at least 1 mL in the pipet. Stopper the flask, gently swirl to mix the contents, and accurately weigh to obtain the sample weight. Add methyl orange TS, and titrate the excess sodium hydroxide with 1 *N* Hydrochloric Acid. Each milliliter of 1 *N* sodium hydroxide is equivalent to 36.46 mg of HCl.

Color A sample shows no more color than does *Matching Fluid A* under *Readily Carbonizable Substances*, Appendix IIB.

Degrees Baumé Transfer about 200 mL of sample, previously cooled to a temperature below 15° into a 250-mL hydrometer cylinder. Insert a suitable Baumé hydrometer graduated at 0.1 °Bé intervals, adjust the temperature to 15.6°, and note the reading at the bottom of the meniscus.

Iron Dilute 4.3 mL (5 g) of sample to 40 mL with water, and add about 40 mg of ammonium persulfate and 10 mL of ammonium thiocyanate TS. Any red color produced does not exceed that produced by 2.5 mL of *Iron Standard Solution* (25 μg Fe) (see *Solutions and Indicators*) in an equal volume of solution containing the same quantities of ACS Reagent-Grade Hydrochloric Acid and the reagents used in the test.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Nonvolatile Residue Transfer 1 g of sample into a tared glass dish, evaporate to dryness on a steam bath, and then dry at 110° for 1 h, cool in a desiccator, and weigh. The weight of the residue does not exceed 5 mg.

Organic Compounds (**Note**: Use either of the methods presented below for analysis of all listed elements, except for benzene, which requires the *Vapor Partitioning Method*.)

VAPOR PARTITIONING METHOD

This method is suitable for the determination of extractable organic compounds at 0.05 to 100 mg/kg but is most appropriate for organic compounds with a vapor pressure greater than 10 mm Hg at 25°.

Preparation of Standard Solutions Prepare a standard solution of each of the organic compounds to be quantitated in Hydrochloric Acid (known to be free of interfering impurities) at approximate concentrations of 5 mg/kg, or within ±50% of the concentrations in the samples to be analyzed.

Place a stirring bar in a 1-L volumetric flask equipped with a ground-glass stopper, and tare the combination. Fill the flask with reagent-grade Hydrochloric Acid so that no air space is present when the flask is stoppered, and determine the weight of the Hydrochloric Acid. Calculate the volume (*V*), in microliters, of each organic component to be added using the equation

$$V = (C \times W)/(D \times 1000),$$

in which *C* is the desired concentration, in milligrams per kilogram, of the organic compound; *W* is weight, in grams, of the Hydrochloric Acid; *D* is the density, in milligrams per microliter, of the organic compound; and 1000 is a conversion factor with the units of grams per kilogram. Add the calculated amount of each component to the Hydrochloric Acid with a syringe (ensure that the syringe tip is under the solution surface), stopper the flask, and stir the solution for at least 2 h using a magnetic stirrer.

Calibration Treat the standards in the same way as described for the sample under *Procedure* (below). Determine a blank for each lot of reagent-grade Hydrochloric Acid, and calculate a response factor (*R*) by dividing the concentration (*C*), in milligrams per kilogram, for each component by the

peak area (A) for that component (subtract any area obtained from the blank sample):

$$R = C/(A - \text{area of blank}).$$

Gaseous compounds present special problems in the preparation of standards. Therefore, to determine response factors for gaseous compounds use the following *Method of Multiple Extractions*. Dilute a sample of Hydrochloric Acid known to contain the gaseous compound of interest with an equal volume of water. Draw 20 mL of this solution into a 50-mL glass syringe; then draw 20 mL of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Withdraw 1 mL of the vapor through the septum, and inject it into the chromatograph. Expel the vapor phase from the 50-mL syringe, draw in another 20 mL of air, repeat the extraction, and inject another 1-mL vapor sample into the chromatograph. Carry out the extraction and analysis on the same sample of acid six times. For each impurity, plot the area (A_N) determined for extraction (n) against the difference between A_N and the area determined for extraction ($N + 1$); that is, plot A_N against ($A_N - A_{N+1}$). The slope of this line is the extraction efficiency (E) for that impurity into the air.

Inject 1 mL of a 0.1% (by volume) standard gas sample of each impurity in air into the chromatograph, and determine the absolute factor (F_A), in grams, per peak area (A) by the following formula:

$$F_A = (M \times 4.0816 \times 10^{-8})/A,$$

in which M is the molecular weight of the compound.

The concentration (C), in milligrams per kilogram, of the component in the original sample is calculated by the formula

$$C = (A \times F_A \times 1.6949 \times 10^6)/E,$$

in which A is the peak area corresponding to the compound (as above), F_A is the absolute factor, and E is extraction efficiency. The response factor is then calculated as

$$R = C/A.$$

Procedure (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and a 4-m × 2-mm (id) stainless-steel column, or equivalent, packed with 15%, by weight, methyl trifluoropropyl silicone (DCFS 1265, or QF-1, or OV-210, or SP-2401) stationary phase on 80- to 100-mesh Gas Chrom R, or the equivalent. Condition a newly packed column at 120° and with a 30-mL/min helium flow for at least 2 h (preferably overnight) before it is attached to the detector. For analysis, maintain the column isothermally at 105°; the injection port and detector at 250°; the carrier gas flow rate at 11 mL/min; with fuel gas flows optimized for the gas chromatograph and detector in use. Change the experimental conditions as necessary for optimal resolution and sensitivity. The signal-to-noise ratio should be at least 10:1.

Dilute a 10-mL sample with an equal volume of water. Draw this solution into a 50-mL glass syringe. Then draw 20 mL of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Draw 1 mL of the vapor through the septum, and inject it into the gas chromatograph. Approximate elution times, in minutes, for some specific organic compounds are as follows:

Methane and acetylene	1.70
Methyl chloride	2.21
Vinyl chloride	2.29
1,1,1-Trichlorofluoromethane	2.62
Ethyl chloride	2.90
Vinylidene chloride	3.20
Methylene chloride	3.64
Chloroform	4.49
1,1-Dichloroethane	4.53
Carbon tetrachloride	4.86
1,1,1-Trichloroethane	5.50
Benzene	6.00
Trichloroethylene	6.22
Ethylene dichloride	6.61
Propylene dichloride	8.41
Perchloroethylene	9.73

Alternative columns may be required to resolve some combinations of components. Methyl chloride and vinyl chloride are resolved by a 3.7-m × 3-mm (id) squalane column, or equivalent, at 45° and a helium flow of 10 mL/min. Chloroform and 1,1-dichloroethane are resolved by a 4-m × 3-mm (id) DC 550R column, or equivalent, at 110° and a helium flow of 12 mL/min.

Calculation Calculate the concentration (C) in milligrams per kilogram of each compound by multiplying its corresponding peak area (A) by the appropriate response factor (R) determined in the *Calibration* protocol:

$$C = R \times A.$$

Precision The relative standard deviation at 5 mg/kg should not exceed 15% for five analyses.

SOLVENT EXTRACTION METHOD

This method is suitable for the determination of extractable organic compounds at 0.3 to 100 mg/kg, but is most appropriate for organic compounds with vapor pressures less than 10 mm Hg at 25°.

Preparation of Standards Prepare the *Standard Solution* as described under the *Vapor Partitioning Method.*

Calibration Extract a sample of the *Standard Solution* as directed under *Procedure* (below) and inject it into the gas chromatograph, or equivalent. Determine a blank for each lot of reagent-grade Hydrochloric Acid and perchloroethylene by extracting the Hydrochloric Acid in the same way as for the standard. Calculate a response factor (R) by dividing the concentration (C), in milligrams per kilogram, for each component by the peak area (A) for that component (subtract any area obtained from the blank sample):

$$R = C/(A - \text{area of blank}).$$

Procedure The conditions for the gas chromatograph, or equivalent, are the same as for the *Vapor Partitioning Method*, except that the column temperature is set at 120°, and the carrier-gas flow is set to 21 mL/min. Accurately transfer 90 mL of sample and 10 mL of perchloroethylene (free of interfer-

ing impurities) into a narrow-mouth, 4-oz bottle. Place the bottle in a mechanical shaker for 30 min. Separate the two phases (perchloroethylene on the bottom) and inject 3 μL of the perchloroethylene extract into the gas chromatograph, or equivalent. Approximate elution times, in minutes, for some chlorinated organic compounds are as follows:

Vinylidene chloride	2.94
Methylene chloride	3.27
Chloroform	3.83
Carbon tetrachloride	4.07
1,1,1-Trichloroethane	4.50
Trichloroethylene	4.97
Ethylene dichloride	5.26
Propylene dichloride	6.36
Perchloroethylene	6.95
1,1,1,2-Tetrachloroethane	10.12
1,1,2,2-Tetrachloroethane	13.70
Pentachloroethane	16.19

To determine perchloroethylene and higher-boiling impurities, substitute methylene chloride (free of interfering impurities) for perchloroethylene in the extraction step. For higher-boiling impurities such as monochlorobenzene and the three dichlorobenzenes, use a 2.74-m × 2.1-mm (id) stainless-steel column packed with 10% carbowax 20M/2% KOH on 80- to 100-mesh chromasorb W (acid washed), set at 150° and with a nitrogen flow of 35 mL/min.

Calculation Calculate the concentration (*C*), in milligrams per kilogram, of each compound by multiplying the corresponding peak area (*A*) (subtract any area obtained from a blank sample) by the appropriate response factor (*R*) determined in the *Calibration* protocol:

$$C = R \times (A - \text{area of blank}).$$

Precision The relative standard deviation at 5 mg/kg should not exceed 15% for five analyses.

Oxidizing Substances (as Cl_2) Transfer 1 mL of sample into a 30-mL test tube, dilute to 20 mL with freshly boiled and cooled water, and add 1 mL of potassium iodide TS and 1 mL of starch TS. Stopper the test tube, and mix thoroughly. Any blue color does not exceed that produced in a control consisting of 1.0 mL of 0.001 *N* iodine in an equal volume of water containing the same quantities of the same reagents and 1 mL of ACS Reagent-Grade Hydrochloric Acid.

Reducing Substances (as SO_3) Transfer 1 mL of ACS Reagent-Grade Hydrochloric Acid into a 30-mL test tube, dilute to 20 mL with recently boiled and cooled water, and add 1 mL of potassium iodide TS, 1 mL of starch TS, and 2.0 mL of 0.001 *N* iodine. Stopper the test tube, and mix thoroughly. The blue color that appears does not disappear when 1 mL of sample is added.

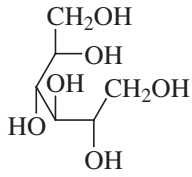
Specific Gravity Determine at 15.6° with a hydrometer, or calculate it from the degrees Baumé observed in the *Degrees Baumé Test*.

Sulfate Dilute 1 g of sample to 100.0 mL with water, transfer 5.0 mL of this dilution into a 50-mL tall-form Nessler tube, and dilute to 20 mL with water. Add a drop of phenolphthalein TS, neutralize the solution with 6 *N* ammonium hydroxide, and then add 1 mL of 2.7 *N* Hydrochloric Acid. Add 3 mL of barium chloride TS to the resulting clear solution, previously filtered, if necessary, dilute to 50 mL with water, and mix. Prepare a control consisting of 1 mL of ACS Reagent-Grade Hydrochloric Acid and 250 μg of sulfate (SO_4) and the same quantities of the reagents used for the sample. Any turbidity shown in the sample does not exceed that shown in the control.

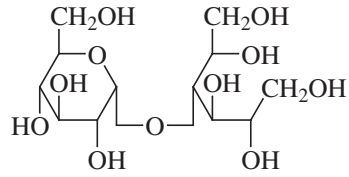
Packaging and Storage Store in tight containers.

Hydrogenated Starch Hydrolysate

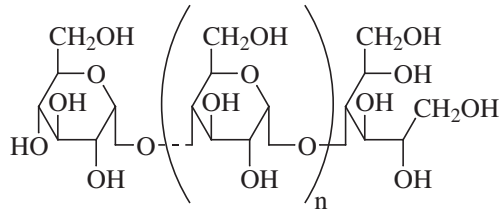
Polyglucitol



Sorbitol



Maltitol



Hydrogenated Polysaccharides

$C_6H_{14}O_6$	Formula wt, Sorbitol 182.17
$C_{12}H_{24}O_{11}$	Formula wt, Maltitol 344.31
$C_{12}H_{24}O_{11}$ plus $C_6H_{10}O_5$ for each additional glucose moiety in the chain	Formula wt, Dextrose Monomer 162.14

CAS: [68425-17-2]

DESCRIPTION

Hydrogenated Starch Hydrolysate occurs as a concentrated, aqueous solution or spray-dried or dried powder. It is a mixture of sorbitol, maltitol, maltitriol, and hydrogenated polysaccharides containing greater than three D-glucopyranosyl units joined by α-1,4-linkages and terminated with a D-glucityl unit. It is soluble in water.

Function Humectant; texturizing agent; stabilizer; thickener; crystal modification agent.

REQUIREMENTS

Assay Passes test.
Chloride Not more than 50 mg/kg, on the dry basis.
Lead Not more than 1 mg/kg.
Loss on Drying *Dry Samples*: Not more than 15%; *Liquid Samples*: Not more than 50%.
Nickel Not more than 2 mg/kg.
Reducing Sugars Not more than 1%, on the dry basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay
Mobile Phase Use degassed, deionized water.

Standard Solutions Dissolve accurately weighed quantities of USP Sorbitol, USP Dextrose, USP Maltitol, and high-purity maltose monohydrate (Sigma, or equivalent, and previously dried at 105° to constant weight) in sufficient *Mobile Phase* to obtain solutions having concentrations of 0.1% (w/v) for each.

Assay Solution Transfer 0.1 g of sample, accurately weighed, into a 100-mL volumetric flask, and dilute to volume with deionized water. Transfer approximately 10 mL of this solution into a separate container, and add approximately 0.2 g of an MB-1 mixed-bed resin. Shake this mixture for 30 s, and filter through a 0.45-micron nylon disc filter.

Procedure Use a liquid chromatograph equipped with a differential refractive index detector and a 20-cm × 10-mm (id) column (Phenomenex ''Rezex'' 4% silver oligosaccharide, or equivalent). Maintain the column at 80° and the *Mobile Phase* flow rate at 0.3 mL/min.

Separately inject about 50-µL portions of the *Assay Solution* and *Standard Solutions* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The elution order for the standards is maltose, maltitol, dextrose, and sorbitol. The differential refractive index detector should show similar response factors.

Calculate the response factors of the standards by dividing their concentrations by their peak areas. Calculate the concentration of each component in the sample by multiplying the individual peak areas corresponding to each component by the appropriate response factor calculated for its standard, giving the concentration in w/w percent. A comparison of sorbitol and dextrose peak areas shows that at least 95% of the sum of these peak areas is sorbitol. A similar comparison of maltitol and maltose peak areas shows that at least 95% is maltitol. Neither sorbitol nor maltitol fractions comprise more than 50% of the sample.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 10.0-g sample does not exceed that shown in a control containing 5 mL of the standard solution.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Loss on Drying Transfer 5 g of dry sample or 1 g of liquid sample, accurately weighed, into an oven, and dry at 105° for 4 h. Place the sample in a cool desiccator until it has cooled to room temperature, and weigh.

Nickel Determine as directed under *Nickel Limit Test*, Appendix IIIB, using a 20-g sample.

Reducing Sugars

Cupri–Citric Solution Transfer 25 g of copper sulfate, 50 g of citric acid, and 144 g of anhydrous sodium carbonate into a 1000-mL beaker, and dissolve in and dilute to volume with deionized water.

Caution: Add the anhydrous sodium carbonate slowly.

Procedure Dissolve 1.0 g of sample in 6 mL of deionized water with the aid of gentle heat, if necessary. Cool, and add 20-mL of *Cupri–Citric Solution* and a few glass beads. Heat so that boiling begins after 4 min, and continue boiling for 3 min. Cool rapidly, and add 100 mL of a 2.4% (v/v) solution of glacial acetic acid and 20.0 mL of 0.025 *M* iodine. While shaking continuously, add 25 mL of a 6:94 (v/v) mixture of hydrochloric acid:deionized water. After the precipitate dissolves, titrate the excess iodine with 0.05 *M* sodium thiosulfate, using 1 mL of starch solution as the indicator, added towards the end of the titration. Not less than 12.8 mL (1%) of 0.05 *M* sodium thiosulfate is required.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC.

Packaging and Storage Store in well-closed containers.

Hydrogen Peroxide

H_2O_2 Formula wt 34.01

CAS: [7722-84-1]

DESCRIPTION

Hydrogen Peroxide occurs as a clear, colorless liquid. The grades of Hydrogen Peroxide suitable for food use usually have a concentration between 30% and 50%. It is miscible with water.

> **Note**: Although Hydrogen Peroxide undergoes exothermic decomposition in the presence of dirt and other foreign materials, it is safe and stable under recommended conditions of handling and storage. Information on safe handling and use may be obtained from the supplier.

Function Bleaching, oxidizing agent; starch modifier; antimicrobial agent.

REQUIREMENTS

Identification Shake 1 mL of sample with 10 mL of water containing 1 drop of 2 *N* sulfuric acid, and add 2 mL of ether. The subsequent addition of a drop of potassium dichromate

TS produces an evanescent blue color in the water layer that upon agitation and standing passes into the ether layer.

Assay Not less than the labeled concentration or within the range stated on the label.

Acidity (as H_2SO_4) Not more than 0.03%.

Iron Not more than 0.5 mg/kg.

Lead Not more than 4 mg/kg.

Phosphate Not more than 0.005%.

Residue on Evaporation Not more than 0.006%.

Tin Not more than 10 mg/kg.

TESTS

Assay Transfer a volume of sample, equivalent to about 300 mg of H_2O_2 and accurately weighed, into a 100-mL volumetric flask, dilute to volume with water, and mix thoroughly. Add 25 mL of 2 *N* sulfuric acid to a 20.0-mL portion of this solution, and titrate with 0.1 *N* potassium permanganate. Each milliliter of 0.1 *N* potassium permanganate is equivalent to 1.701 mg of H_2O_2.

Acidity (as H_2SO_4) Dilute 9 mL (10 g) of sample in 90 mL of carbon dioxide-free water, add methyl red TS, and titrate with 0.02 *N* sodium hydroxide. The volume of sodium hydroxide solution should not be more than 3 mL greater than the volume required for a blank test on 90 mL of the water used for dilution.

Iron Evaporate 18 mL (20 g) of sample to dryness with 10 mg of sodium chloride on a steam bath, dissolve the residue in 2 mL of hydrochloric acid, and dilute to 50 mL with water. Add about 40 mg of ammonium persulfate crystals and 10 mL of ammonium thiocyanate TS, and mix. Any red or pink color does not exceed that produced by 1.0 mL of *Iron Standard Solution* (10 μg Fe) in an equal volume of solution containing the quantities of the reagents used in the test.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, with the following modifications: (1) Prepare only one *Diluted Standard Lead Solution* by transferring 40 mL of *Lead Nitrate Stock Solution* into a 1000-mL volumetric flask and diluting to volume with water to obtain a solution containing 4 μg/mL of lead (Pb) ion; (2) Replace the first paragraph under *Sample Preparation* with the following: Transfer 10 g of sample, accurately weighed, into an evaporation dish; (3) Under *Procedure,* determine the absorbances of the *Sample Solution* and *Diluted Standard Lead Solution* only—the absorbance of the *Sample Solution* is less than or equal to that of the *Diluted Standard Lead Solution*.

Phosphate Evaporate 400 mg of sample to dryness on a steam bath. Dissolve the residue in 25 mL of approximately 0.5 *N* sulfuric acid, add 1 mL of ammonium molybdate solution [500 mg of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in each 10 mL of water] and 1 mL of *p*-methylaminophenol sulfate TS, and allow it to stand for 2 h. Any blue color does not exceed that produced by 2.0 mL of *Phosphate Standard Solution* (20 μg PO_4) (see *Solutions and Indictors*) in an equal volume of solution containing the quantities of the reagents used in the test.

Residue on Evaporation Evaporate 25 g of sample to dryness in a tared porcelain or silica dish on a steam bath, and continue drying to constant weight at 105°. The weight of the residue does not exceed 1.5 mg.

Tin

Aluminum Chloride Solution Dissolve 8.93 g of aluminum chloride ($AlCl_3\cdot 6H_2O$) in sufficient water to make 1000 mL.

Gelatin Solution On the day of use, dissolve 100 mg of gelatin in 50 mL of boiled water that has been cooled to between 50° and 60°.

Tin Stock Solution Dissolve 250.0 mg of lead-free tin foil in 10 to 15 mL of hydrochloric acid, and dilute to 250.0 mL with 1:2 hydrochloric acid.

Standard Solution On the day of use, transfer 5.0 mL of *Tin Stock Solution* into a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer 2.0 mL of this solution (100 μg Sn) into a 250-mL Erlenmeyer flask, and add 15 mL of water, 5 mL of nitric acid, and 2 mL of sulfuric acid. Place a small, stemless funnel in the mouth of the flask, and heat until strong fumes of sulfuric acid evolve. Cool, add 5 mL of water, evaporate again to strong fumes, and cool. Repeat the addition of water and heating to strong fumes, then add 15 mL of water, heat to boiling, and cool. Dilute to about 35 mL with water, add 1 drop of methyl red TS and 2.0 mL of the *Aluminum Chloride Solution*, and mix. Make the solution just alkaline by adding, dropwise, ammonium hydroxide and stirring gently, then add 0.1 mL in excess.

> **Caution**: To avoid dissolving the aluminum hydroxide precipitate, do not add more ammonium hydroxide than 0.1 mL in excess.

Centrifuge for about 15 min at 4000 rpm, and then decant the supernatant liquid as completely as possible without disturbing the precipitate. Dissolve the precipitate in 5 mL of 1:2 hydrochloric acid, add 1.0 mL of the *Gelatin Solution*, and dilute to 20.0 mL with a saturated solution of aluminum chloride.

Sample Solution Transfer 9 mL (10 g) of sample into a 250-mL Erlenmeyer flask, and add 15 mL of water, 5 mL of nitric acid, and 2 mL of sulfuric acid. Mix, and heat gently on a hot plate to initiate and maintain a vigorous decomposition. When decomposition is complete, place a small, stemless funnel in the mouth of the flask, and continue as directed for the *Standard Solution*, beginning with ''and heat until strong fumes of sulfuric acid evolve.''

Procedure Rinse a polarographic cell or other vessel with a portion of the *Standard Solution*, then add a suitable volume to the cell, immerse it in a constant-temperature bath maintained at 35° ± 0.2°, and deaerate by bubbling oxygen-free nitrogen or hydrogen through the solution for at least 10 min. Insert the dropping mercury electrode of a suitable polarograph, and record the polarogram from −0.2 to −0.7 V at a sensitivity of 0.0003 μA/mm, using a saturated calomel reference electrode. In the same manner, record a polarogram of a portion of the *Sample Solution* at the same current sensitivity. The height of the wave produced by the *Sample Solution* is not greater than that produced by the *Standard Solution* at the same half-wave potential.

Packaging and Storage Store in a cool place in containers with a vent in the stopper.

Hydroxylated Lecithin

CAS: [8029-76-3]

DESCRIPTION

Hydroxylated Lecithin occurs as a light yellow substance that may vary in consistency from fluid to plastic, depending on the content of free fatty acid and oil and on whether it contains diluents. It is derived from a complex mixture of acetone-insoluble phosphatides from soybean and other plant lecithins, consisting chiefly of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol as well as other minor phospholipids and glycolipids mixed with varying amounts of triglycerides, fatty acids, sterols, and carbohydrates. The mixture is treated with hydrogen peroxide, benzoyl peroxide, lactic acid, and sodium hydroxide or with hydrogen peroxide, acetic acid, and sodium hydroxide to produce a hydroxylated product having an iodine value approximately 10% lower than that of the starting material. It is partially soluble in water but hydrates readily to form emulsions; it is more dispersible and hydrates more readily than crude lecithin.

Function Emulsifier; clouding agent.

REQUIREMENTS

Acetone-Insoluble Matter (as phosphatides) Not less than 50.0%.
Acid Value Not more than 70.
Hexane-Insoluble Matter Not more than 0.3%.
Iodine Value Between 85 and 95.
Lead Not more than 1 mg/kg.
Peroxide Value Not more than 100.
Water Not more than 1.5%.

TESTS

Acetone-Insoluble Matter (as phosphatides)

Purification of Phosphatides Dissolve 10 g of sample in 20 mL of petroleum ether, add 50 mL of acetone to the solution, chill, and decant. Dry the solids under flowing nitrogen under a hood. Dissolve 5 g of the solids in 10 mL of petroleum ether, and add 25 mL of acetone to the solution. Transfer approximately equal portions of the precipitate to each of two 40-mL centrifuge tubes, using additional portions of acetone to facilitate the transfer. Stir thoroughly, dilute to 40 mL with acetone, stir again, chill for 15 min in an ice bath, stir again, and then centrifuge for 5 min. Decant the acetone, crush the solids with a stirring rod, refill the tube with acetone, stir, chill, centrifuge, and decant as before. The solids after the second centrifugation require no further purification and may be used for preparing the *Phosphatide–Acetone Solution*. Five grams of the purified phosphatides are required to saturate about 16 L of acetone.

Phosphatide–Acetone Solution Add a quantity of purified phosphatides to sufficient acetone, previously cooled to about 5°, to form a saturated solution, and maintain the mixture at this temperature for 2 h, shaking it vigorously at 15-min intervals. Decant the solution through a rapid filter paper, avoiding the transfer of any undissolved solids to the paper and conducting the filtration under refrigerated conditions (not above 5°).

Procedure If the sample is plastic or semisolid, soften a portion of it by warming it in a water bath at a temperature not exceeding 60°, and then mix it thoroughly. Transfer about 2 g of a well-mixed sample, accurately weighed, into a 40-mL centrifuge tube, previously tared with a glass stirring rod, and add 15 mL of *Phosphatide–Acetone Solution* from a buret. Warm the mixture in a water bath until the sample melts, but avoid evaporation of the acetone. Stir until the sample is completely disintegrated and dispersed, transfer the tube into an ice bath, chill for 5 min, remove from the ice bath, and add about 10 mL of *Phosphatide–Acetone Solution*, previously chilled for 5 min in an ice bath. Stir the mixture to complete dispersion of the sample, dilute to 40 mL with chilled (5°) *Phosphatide–Acetone Solution*, stir to complete dispersion of the sample, and return the tube and contents to the ice bath for 15 min. Subsequently stir again while still in the ice bath, remove the stirring rod, and centrifuge the mixture immediately for 5 min. Decant the supernatant liquid from the centrifuge tube; crush the centrifuged solids with the stirring rod; refill the tube to the 40-mL mark with chilled (5°) *Phosphatide–Acetone Solution*; and repeat the chilling, stirring, centrifugation, and decantation procedure. After the second centrifugation and decantation of the supernatant acetone, again crush the solids with the stirring rod, and place the tube and its contents in a horizontal position at room temperature until the excess acetone has evaporated. Mix the residue again, dry the centrifuge tube and its contents at 105° for 45 min in a forced-draft oven, cool, and weigh. Calculate the percentage of acetone-insoluble matter by the formula

$$(100R/S) - B,$$

in which R is the weight, in grams, of residue, S is the weight, in grams, of the sample taken, and B is the percentage of hexane-insoluble matter determined as directed under *Hexane-Insoluble Matter* (below).

Acid Value If the sample is plastic or semisolid, soften a portion by warming it in a water bath at a temperature not exceeding 60°, and then mix it thoroughly. Transfer about 2 g of a well-mixed sample, accurately weighed, into a 250-mL Erlenmeyer flask, and dissolve it in 50 mL of petroleum ether. Add 50 mL of ethanol, previously neutralized to phenolphthalein with 0.1 *N* sodium hydroxide, to this solution, and mix well. Using phenolphthalein TS as the indicator, titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for 5 s. Calculate the *Acid Value* by the formula

$$5.6 \times A/W,$$

in which *A* is the volume, in milliliters, of 0.1 *N* sodium hydroxide consumed, and *W* is the weight, in grams, of the sample taken.

Hexane-Insoluble Matter Determine as directed under *Hexane-Insoluble Matter*, Appendix VII.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed for *Method II* in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Peroxide Value Transfer about 10 g of sample, accurately weighed, into a suitable container, add 30 mL of a 3:2 solution of glacial acetic acid:chloroform, and mix. Add 1 mL of a saturated solution of potassium iodide, mix, and allow to stand for 10 min. Add 100 mL of water, begin titrating with 0.05 *N* sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value, as milliequivalents of peroxide per kilogram of sample, by the formula

$$[S \times N \times 1000]/W,$$

in which *S* is the net volume, in milliliters, of sodium thiosulfate solution required for the sample; *N* is the exact normality of the sodium thiosulfate solution; and *W* is the weight, in grams, of the sample taken.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Hydroxypropyl Cellulose

Modified Cellulose

INS: 463 CAS: [9004-64-2]

DESCRIPTION

Hydroxypropyl Cellulose occurs as a white powder. It is a cellulose ether containing hydroxypropyl substitution. It may contain a suitable anticaking agent. It is soluble in water and in certain organic solvents.

Function Emulsifier; film coating; protective colloid; stabilizer; suspending agent; thickener.

REQUIREMENTS

Identification

A. Shake a 0.1% solution of sample. A layer of foam appears (distinction from cellulose gum).

B. Add 5 mL of a 5.0% solution of copper sulfate (or aluminum sulfate) to 5 mL of a 0.5% solution of sample. No precipitate forms (distinction from cellulose gum).

Assay Not more than 80.5% of hydroxypropoxyl groups (—$OCH_2CHOHCH_3$) after drying, equivalent to not more than 4.6 hydroxypropyl groups per anhydroglucose unit.

Lead Not more than 3 mg/kg.

Loss on Drying Not more than 5.0%.

pH of a 1% Solution Between 5.0 and 8.0.

Residue on Ignition Not more than 0.5%.

Viscosity of a 10% Solution Not less than 145 centipoises.

TESTS

Assay Determine as directed under *Hydroxypropoxyl Determination*, Appendix IIIC, using 85 mg of sample, previously dried at 105° for 3 h and accurately weighed.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds but with a 2-g sample, and 6 μg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.

pH of a 1% Solution Determine as directed under *pH Determination*, Appendix IIB.

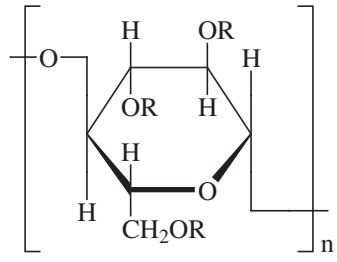
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Viscosity of a 10% Solution Determine as directed under *Viscosity of Cellulose Gum*, Appendix IIB, using an accurately weighed sample, equivalent to 40 g of Hydroxypropyl Cellulose on the dried basis, in a tared sample container.

Packaging and Storage Store in well-closed containers.

Hydroxypropyl Methylcellulose

Propylene Glycol Ether of Methylcellulose; Modified Cellulose, HPMC



in which
R = H or CH_3 or $CH_2CHOHCH_3$

INS: 464 CAS: [9004-65-3]

DESCRIPTION

Hydroxypropyl Methylcellulose occurs as a white to off-white, fibrous powder or as granules. It is the propylene glycol

ether of methylcellulose in which both the hydroxypropyl and the methyl groups are attached to the anhydroglucose rings of cellulose by ether linkages. Several product types are available that are defined by varying combinations of methoxyl and hydroxypropoxyl content. It is soluble in water and in certain organic solvent systems. Aqueous solutions are surface active, form films upon drying, and undergo a reversible transformation from sol to gel upon heating and cooling, respectively.

Function Thickening agent; stabilizer; emulsifier.

REQUIREMENTS

Identification

A. Add 1 g of sample to 100 mL of water. It swells and disperses to form a clear to opalescent, mucilaginous solution, depending on the intrinsic viscosity, which is stable in the presence of most electrolytes.

B. Add 1 g of sample to 100 mL of boiling water, and stir the mixture. A slurry forms that when cooled to 20°, dissolves to form a clear or opalescent, mucilaginous solution.

C. Pour a few milliliters of the solution prepared for *Identification Test B* onto a glass plate, and allow the water to evaporate. A thin, self-sustaining film forms.

Assay for Hydroxypropoxyl Groups Between a minimum of 3.0% and a maximum of 12.0% of hydroxypropoxyl groups (—$OCH_2CHOHCH_3$), within the range claimed by the vendor for any product type.

Assay for Methoxyl Groups Between a minimum of 19.0% and a maximum of 30.0% of methoxyl groups (—OCH_3), within the range claimed by the vendor for any product type.

Lead Not more than 3 mg/kg.

Loss on Drying Not more than 5.0%.

Residue on Ignition Not more than 1.5% for products with viscosities of 50 centipoises or above; not more than 3.0% for products with viscosities below 50 centipoises.

Viscosity The viscosity of an aqueous solution containing 2 g of sample per 100 g of solution is not less than 80.0% and not more than 120.0% of that stated on the label for viscosity types of 100 centipoises or less, and not less than 75.0% and not more than 140.0% of that stated on the label for viscosity types higher than 100 centipoises.

TESTS

Assay (**Caution**: Perform all steps involving hydriodic acid carefully in a well-ventilated hood. Use goggles, acid-resistant gloves, and other appropriate safety equipment. Be extremely careful when handling the hot vials because they are under pressure. In the event of hydriodic acid exposure, wash with copious amounts of water and seek medical attention at once.)

Internal Standard Solution Transfer about 2.5 g of toluene, accurately weighed, into a 1000-mL volumetric flask containing 10 mL of *o*-xylene, dilute with *o*-xylene to volume, and mix.

Standard Preparation Transfer about 135 mg of adipic acid into a suitable serum vial, add 4.0 mL of hydriodic acid followed by 4.0 mL of the *Internal Standard Solution*, and close the vial securely with a septum stopper. Accurately weigh the vial and its contents, add 30 µL of isopropyl iodide with a syringe through the septum, reweigh, and calculate the weight of isopropyl iodide added. Similarly, add 90 µL of methyl iodide, and calculate the weight added. Shake well, and allow the layers to separate.

Assay Preparation Transfer about 0.065 g of sample, accurately weighed, into a 5-mL vial equipped with a pressure-tight septum closure, add an amount of adipic acid equal to the weight of the sample, and pipet 2 mL of the *Internal Standard Solution* into the vial. Cautiously pipet 2 mL of hydriodic acid into the mixture, immediately secure the closure, and accurately weigh. Shake the vial for 30 s, heat at 150° for 20 min, remove from the heat, shake again, using extreme caution, and heat at 150° for 40 min. Allow the vial to cool for about 45 min, and weigh. If the weight loss is greater than 10 mg, discard the mixture and prepare another *Assay Preparation*.

Chromatographic System (See *Chromatography*, Appendix IIA) Use a gas chromatograph equipped with a thermal conductivity detector and a 1.8-m × 4-mm glass column, or equivalent, packed with 10% methylsilicone oil (UCW 982 or equivalent) on 100- to 120-mesh flux-calcined chromatographic siliceous earth (Chromosorb WHP, or equivalent). Maintain the column at 100° and the injection port and detector at 200°. Use helium as the carrier gas with a flow rate of 20 mL/min.

Calibration Inject about 2 µL of the upper layer of the *Standard Preparation* into the chromatograph, and record the chromatogram. The retention times for methyl iodide, isopropyl iodide, toluene, and *o*-xylene are approximately 3, 5, 7, and 13 min, respectively. Calculate the relative response factor, F, of equal weights of toluene and methyl iodide by the formula

$$Q/A,$$

in which Q is the quantity ratio of methyl iodide to toluene in the *Standard Preparation*, and A is the peak area ratio of methyl iodide to toluene obtained from the *Standard Preparation*. Similarly, calculate the relative response factor, F', of equal weights of toluene and isopropyl iodide by the formula

$$Q'/A',$$

in which Q' is the quantity ratio of isopropyl iodide to toluene in the *Standard Preparation*, and A' is the peak area ratio of isopropyl iodide to toluene obtained from the *Standard Preparation*.

Procedure Inject about 2 µL of the upper layer of the *Assay Preparation* into the chromatograph, and record the chromatogram. Calculate the percentage of methoxyl groups (—OCH_3) in the sample by the formula

$$2 \times (31/142) \times F \times a \times (W/w),$$

in which 31/142 is the ratio of the formula weights of methoxyl to methyl iodide; a is the ratio of the area of the methyl iodide peak to that of the toluene peak obtained from the *Assay Preparation*; W is the weight, in grams, of toluene in the *Internal Standard Solution*; and w is the weight, in grams, of sample taken. Similarly, calculate the percentage of hydroxy-

propoxyl groups (—$OCH_2CHOHCH_3$) in the sample by the formula

$$2 \times (75/170) \times F' \times a' \times (W/w),$$

in which a' is the ratio of the area of the isopropyl iodide peak to that of the toluene peak obtained from the *Assay Preparation*.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* containing 2 g of sample prepared as directed for organic compounds, and 6 μg of lead ion (Pb) in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 3-g sample at 105° for 2 h.

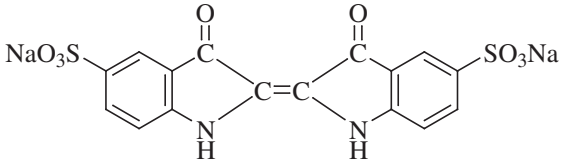
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Viscosity Accurately weigh a sample, equivalent to 2 g of solids on the dried basis, transfer to a wide-mouth 250-mL centrifuge bottle, and add 98 g of water previously heated to between 80° and 90°. Stir with a mechanical stirrer for 10 min, then place the bottle in an ice bath until solution is complete, adjust the weight of the solution with water to 100 g if necessary, and centrifuge it to expel any entrapped air. Adjust the temperature of the solution to 20° ± 0.1°, and determine the viscosity as directed under *Viscosity of Methylcellulose*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Indigotine[1]

CI Food Blue 1; Indigotine Disulfonate; Indigo Carmine; CI 73015; Class: Indigoid



$C_{16}H_8N_2O_8S_2Na_2$ Formula wt 466.36

INS: 132 CAS: [860-22-0]

DESCRIPTION

Indigotine occurs as a blue-brown to red-brown powder or granules. It is principally the disodium salt of 2-(1,3-dihydro-3-oxo-5-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid. It dissolves in water to give a solution that is blue at neutrality, blue-violet in acid, and green to yellow-green in base. When dissolved in concentrated sulfuric acid, it yields a blue-violet solution that turns blue when diluted with water. It is insoluble in ethanol.

Function Color.

REQUIREMENTS

Identification A freshly prepared aqueous solution containing 20 mg of sample per liter exhibits absorbance intensities (A) and wavelength maxima as follows: at pH 7, $A = 0.82$ at 610 nm; at pH 1, $A = 0.81$ at 610 nm; and at pH 13, $A = 0.2$ at 610 nm, and $A = 0.31$ at 442 nm.

Assay Not less than 85.0% total coloring matter.

Arsenic Not more than 3 mg/kg.

Ether Extracts (combined) Not more than 0.2%.

Lead Not more than 10 mg/kg.

Loss on Drying (Volatile Matter) at 135°, Chlorides, and **Sulfates** (as sodium salts) Not more than 15.0% in combination.

Mercury Not more than 1 mg/kg.

Subsidiary and Isomeric Colors

*Disodium Salt of 2-(1,3-Dihydro-3-oxo-7-sulfo-*2H*-indole-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic Acid* Not more than 18.0%.

*Sodium Salt of 2-(1,3-Dihydro-3-oxo-2*H*-indole-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic Acid* Not more than 2.0%.

Uncombined Intermediates and Products of Side Reactions

Isatin-5-sulfonic Acid Not more than 0.4%.

5-Sulfoanthranilic Acid Not more than 0.2%.

Water-Insoluble Matter Not more than 0.4%.

TESTS

Assay Determine the total color strength as the weight percent of the sample using *Methods I and II* in *Total Color* under *Colors*, Appendix IIIC. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 175 to 225 mg of sample, accurately weighed, into a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (a) for Indigotine is 0.0478 mg/L/cm at 610 nm.

Method II (Sample Preparation) Transfer approximately 0.3 g of sample, accurately weighed, into the titration flask. The stoichiometric factor (F_S) for Indigotine is 4.29.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Chloride Determine as directed in *Sodium Chloride* under *Colors*, Appendix IIIC.

Ether Extracts Determine as directed in *Ether Extracts* under *Colors*, Appendix IIIC.

[1]To be used or sold for use to color food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Blue No. 2 can be applied only to FDA-certified batches of this color additive. Indigotine is a common name given to the uncertified colorant. See the monograph entitled FD&C Blue No. 2 for directions for producing an FDA-certified batch.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 µg of lead (Pb) ion in the control.
Loss on Drying (Volatile Matter) at 135° Determine as directed in *Loss on Drying (Volatile Matter)* under *Colors*, Appendix IIIC.
Mercury Determine as directed in *Mercury* under *Colors*, Appendix IIIC.
Subsidiary and Isomeric Colors

Apparatus Use a 40- × 2.5-cm (id) glass column packed with Celite (Johns Mansville No. 595, or equivalent) prepared as described under *Procedure*. Dissolve 20 g of hydroxylamine hydrochloride in 500 mL of water, place the solution into a 2-L separatory funnel, and add 450 mL of butanol, 450 mL of chloroform, 300 mL of water, and 100 mL of hydrochloric acid. Agitate the mixture well, periodically venting the funnel. After settling, separate and store the bottom layer (organic), the *Mobile Phase*, and the top layer (aqueous), the *Stationary Phase*.

Sample Solution Dissolve approximately 100 mg of sample, accurately weighed, in 100 mL of *Stationary Phase*; warm on a steam bath, if necessary, to dissolve the sample.

Procedure Slurry 12 g of Celite with 7 mL of *Stationary Phase*, and pour the slurry into the column. Mix 5 mL of *Sample Solution* with 10 g of Celite, and pour the mixture into the column over the slurry, ensuring that the sample is quantitatively transferred to the column.

Elute the column with the *Mobile Phase*. Collect the monosulfonated derivative, the first band eluting, in a 25-mL graduated cylinder, and note the volume. Collect the next band, the isomeric (unsulfonated) derivative, in a similar manner.

Mix each aliquot collected with an equal volume of hexane, and transfer to a separatory funnel. Extract this mixture with three 15-mL aliquots of water; combine the water extracts, and calculate the percent concentration (P) of the monosulfonated derivative (a = 0.0513 mg/L/cm at 615 nm) and the isomeric derivative (a = 0.0478 mg/L/cm at 610 nm) by the equation

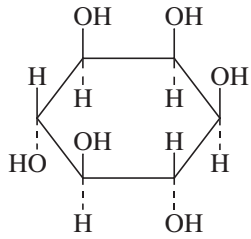
$$P = (A \times V)/(a \times W \times 10),$$

in which A is the absorbance; V is the volume of extract; a is the absorptivity, in milligrams per liter per centimeter; and W is the weight, in milligrams, of the sample.
Sulfate Determine as directed in *Sodium Sulfate* under *Colors*, Appendix IIIC.
Uncombined Intermediates and Products of Side Reactions Determine as directed for *Method I* in *Uncombined Intermediates and Products of Side Reactions* under *Colors*, Appendix IIIC. Calculate the concentration of isatin-5-sulfonic acid using an absorptivity of 0.089 mg/L/cm at 245 nm.
Water-Insoluble Matter Determine as directed in *Water-Insoluble Matter* under *Colors*, Appendix IIIC.

Packaging and Storage Store in well-closed containers.

Inositol

1,2,3,5/4,6-Cyclohexanehexol; *i*-Inositol; *meso*-Inositol; *myo*-Inositol



$C_6H_{12}O_6$ Formula wt 180.16

CAS: [87-89-8]

DESCRIPTION

Inositol occurs as fine, white crystals or as a white, crystalline powder. Its solutions are neutral to litmus. It is optically inactive. It is stable in air. One gram is soluble in 6 mL of water. It is slightly soluble in alcohol, and is insoluble in ether and in chloroform.

Function Nutrient.

REQUIREMENTS

Identification

A. Add 6 mL of nitric acid to 1 mL of a 1:50 aqueous solution in a porcelain evaporating dish, and evaporate to dryness on a water bath. Dissolve the residue in 1 mL of water, add 0.5 mL of a 1:10 aqueous solution of strontium acetate, and again evaporate to dryness on a steam bath. A violet color appears.

B. The inositol hexaacetate obtained in the *Assay* melts between 212° and 216° (see *Melting Range or Temperature*, Appendix IIB).

Assay Not less than 97.0% of $C_6H_{12}O_6$ after drying.
Calcium Passes test.
Chloride Not more than 0.005%.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 0.5%.
Melting Range Between 224° and 227°.
Residue on Ignition Not more than 0.1%.
Sulfate Not more than 0.006%.

TESTS

Assay Transfer about 200 mg of sample, previously dried at 105° for 4 h and accurately weighed, to a 250-mL beaker, add 5 mL of a 1:50 mixture of 2 *N* sulfuric acid:acetic anhydride, and cover the beaker with a watch glass. Heat on a steam bath for 20 min, then chill in an ice bath, and add 100 mL of water. Boil for 20 min, allow to cool, and transfer quantitatively, with the aid of a little water, to a 250-mL separator. Extract the solution with six successive 30-, 25-,

20-, 15-, 10-, and 10-mL portions of chloroform, using the solvent to rinse the original flask. Collect the chloroform extracts in a second 250-mL separator, and wash the combined extracts with 10 mL of water. Transfer the chloroform extracts through a funnel containing a pledget of cotton into a 150-mL tared Soxhlet flask. Wash the separator and funnel with 10 mL of chloroform, and add to the combined extracts. Evaporate to dryness on a steam bath, dry in an oven at 105° for 1 h, cool in a desiccator, and weigh. The weight of the inositol hexaacetate obtained, multiplied by 0.4167, represents the equivalent of $C_6H_{12}O_6$.

Calcium Add 1 mL of ammonium oxalate TS to 10 mL of a 1:10 aqueous solution of sample. The solution remains clear for at least 1 min.

Chloride Determine as directed in *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 400-mg sample does not exceed that produced in a control containing 20 μg of chloride (Cl) ion.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 4 μg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Sulfate Determine as directed in *Sulfate Limit Test*, under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 5-g sample does not exceed that produced in a control containing 300 μg of sulfate (SO_4).

Packaging and Storage Store in well-closed containers.

Invert Sugar

Invert Sugar Syrup

CAS: [8013-17-0]

DESCRIPTION

Invert Sugar occurs as a hygroscopic liquid. It is a mixture of glucose and fructose that results from the hydrolysis of sucrose. Invert Sugar is marketed as Invert Sugar Syrup and contains dextrose (glucose), fructose, and sucrose in various amounts as represented by the manufacturer. It is very soluble in water, in glycerin, and in glycols and is very sparingly soluble in acetone and in ethanol.

Function Nutritive sweetener.

REQUIREMENTS

Labeling Indicate the percentages of sucrose and Invert Sugar.

Identification (See *Chromatography*, Appendix IIA.) Prepare a 10% solution of sample in purified water and inject 7.5 μL of the solution into a high-performance liquid chromatograph equipped with a differential refractometer detector and a column packed with a cation exchange resin maintained at 85°. Use purified water as the liquid phase, at a flow rate of 0.7 mL/min. The chromatogram of the sample gives appropriate elution times for fructose, glucose, and sucrose when compared with a standard solution containing 1 g of each saccharide in 100 mL of water.

Assay Not less than 90.0% and not more than 110.0% of the labeled amount of sucrose and of Invert Sugar.

Lead Not more than 0.5 mg/kg.

pH Not less than 3.0 and not more than 5.5.

Residue on Ignition Not more than 0.2%.

Total Solids As represented by the vendor.

Total Sugars Not less than 99.5% of the total solids content.

TESTS

Assay Determine as directed in *Invert Sugar* under *Total Solids*, Appendix X.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

pH Determine as directed under *pH Determination*, Appendix IIB.

Residue on Ignition Determine as directed in *Method II* under *Residue on Ignition*, Appendix IIC.

Total Solids Determine as directed in *Invert Sugar* under *Total Solids*, Appendix X, using the table provided.

Total Sugars Calculate the percent *Total Sugars* (T_S) by the equation

$$T_S = P_I + P_S,$$

in which P_I is the percentage of Invert Sugar and P_S is the percentage of sucrose as determined under *Assay* (above).

Packaging and Storage Store in tight containers.

Iron, Carbonyl

Fe At wt 55.85

CAS: [37220-42-1]

DESCRIPTION

Iron, Carbonyl, occurs as a dark gray powder. It is elemental iron produced by the decomposition of iron pentacarbonyl. When viewed under a microscope having a magnifying power of 500 diameters or greater, it appears as spheres built up with concentric shells. It is stable in dry air.

Function Nutrient.

REQUIREMENTS

Identification A sample dissolves in dilute mineral acids with the evolution of hydrogen and the formation of solutions of the corresponding salts, which give positive tests for *Ferrous Salts* (Iron), Appendix IIIA.
Assay Not less than 98.0% of Fe.
Acid-Insoluble Substances Not more than 0.2%.
Arsenic Not more than 3 mg/kg.
Lead Not more than 4 mg/kg.
Mercury Not more than 2 mg/kg.
Sieve Analysis Not less than 100% passes through a 200-mesh sieve; not less than 95% passes through a 325-mesh sieve.

TESTS

Assay Determine as directed in the monograph for *Iron, Reduced*.
Acid-Insoluble Substances Determine as directed in the monograph for *Iron, Electrolytic*.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using the following solution: Dissolve 1.0 g of sample in 25 mL of 2 *N* sulfuric acid, heat on a steam bath until the evolution of hydrogen ceases, cool, and dilute to 35 mL with water.
Lead (**Note**: When preparing all aqueous solutions and rinsing glassware before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in warm 8 *N* nitric acid for 30 min and by rinsing with deionized water.)

Ascorbic Acid–Sodium Iodide Solution Transfer 20 g of ascorbic acid and 38.5 g of sodium iodide to a 200-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Trioctylphosphine Oxide Solution (**Caution**: This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.) Transfer 5.0 g of trioctylphosphine oxide to a 100-mL volumetric flask. Dissolve in and dilute to volume with 4-methyl-2-pentanone, and mix.

Lead Nitrate Stock Solution (100 μg/mL) Transfer 159.8 mg of reagent-grade lead nitrate [$Pb(NO_3)_2$] into a 1000-mL volumetric flask, dissolve it in 100 mL of water containing 1 mL of nitric acid, and dilute to volume with water.

Standard Preparation and Blank Preparation Transfer 5.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer 2.0 mL of the resulting solution into a 50-mL beaker. Add 8 mL of hydrochloric acid and 2 mL of nitric acid to this beaker, and to a separate 50-mL beaker (blank). Place a ribbed watch glass over each beaker, and evaporate to dryness on a steam bath. Add 10 mL of 9 *N* hydrochloric acid to each beaker, and transfer the resulting solutions, with the aid of about 10 mL of water, to separate 50-mL volumetric flasks. Add 20 mL of *Ascorbic Acid–Sodium Iodide Solution* and 5.0 mL of *Trioctylphosphine Oxide Solution* to each flask, shake for 30 s, and allow to separate. Add water to bring the organic solvent layer into the neck of each flask, shake again, and allow the layers to separate. The organic solvent layers are the *Blank Preparation* and the *Standard Preparation*, and they contain 0.0 and 2.0 μg of lead per milliliter, respectively.

Test Preparation Transfer 1.0 g of sample to a 50-mL beaker, and cover it with a ribbed watch glass. Slowly add 8 mL of hydrochloric acid and 2 mL of nitric acid, keeping the beaker covered as much as possible. After the initial reaction subsides, evaporate to dryness on a steam bath, cool, and dissolve the residue in 10 mL of 9 *N* hydrochloric acid, warming if necessary to effect solution. Cool, and transfer the resultant solution, with the aid of about 10 mL of water, into a 50-mL volumetric flask. Add 20 mL of *Ascorbic Acid–Sodium Iodide Solution* and 5 mL of *Trioctylphosphine Oxide Solution*, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow the layers to separate. The organic solvent layer is the *Test Preparation*.

Procedure Concomitantly determine the absorbance of the *Blank Preparation*, the *Standard Preparation*, and the *Test Preparation* at the lead emission line at 283.3 nm, with a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp and an air–acetylene flame, using 4-methyl-2-pentanone to set the instrument to zero. In a suitable analysis, the absorbance of the *Blank Preparation* is not greater than 20% of the difference between the absorbance of the *Standard Preparation* and the absorbance of the *Blank Preparation*. The absorbance of the *Test Preparation* does not exceed that of the *Standard Preparation*.
Mercury Determine as directed in the monograph for *Iron, Reduced*, but use 2 g of sample and 40 mL of *Sodium Citrate Solution* in preparing the *Sample Solution*, and prepare the *Diluted Standard Mercury Solution* as follows: Transfer 4.0 mL of *Mercury Stock Solution* into a 250-mL volumetric flask, dilute to volume with 1 *N* hydrochloric acid, and mix (1 mL = 4 μg Hg). Modify the first sentence of the *Procedure* to read: ''Prepare a control by treating 1.0 mL of *Diluted Standard Mercury Solution* (4 μg Hg) in the same manner. . . .''
Sieve Analysis Determine as directed under *Sieve Analysis of Granular Metal Powders*, Appendix IIC.

Packaging and Storage Store in well-closed containers.

Iron, Electrolytic

Fe At wt 55.85

CAS: [7439-89-6]

DESCRIPTION

Iron, Electrolytic, occurs as an amorphous, lusterless, gray-black powder. It is elemental iron obtained by electrode position. It is stable in dry air.

Function Nutrient.

REQUIREMENTS

Identification A sample dissolves in dilute mineral acids with the evolution of hydrogen and the formation of solutions of the corresponding salts, which give positive tests for *Ferrous Salts* (Iron), Appendix IIIA.
Assay Not less than 97.0% of Fe.
Acid-Insoluble Substances Not more than 0.2%.
Arsenic Not more than 3 mg/kg.
Lead Not more than 4 mg/kg.
Mercury Not more than 2 mg/kg.
Sieve Analysis Not less than 100% passes through a 100-mesh sieve; not less than 95% passes through a 325-mesh sieve.

TESTS

Assay Determine as directed in the monograph for *Iron, Reduced*.
Acid-Insoluble Substances Dissolve 1 g of sample in 25 mL of 2 *N* sulfuric acid, and heat on a steam bath until the evolution of hydrogen ceases. Filter through a tared filter crucible, wash with water until free from sulfate, dry at 105° for 1 h, cool, and weigh.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using the following solution: Dissolve 1 g of sample in 25 mL of 2 *N* sulfuric acid, heat on a steam bath until the evolution of hydrogen ceases, cool, and dilute to 35 mL with water.
Lead Determine as directed in the monograph for *Iron, Carbonyl*.
Mercury Determine as directed in the monograph for *Iron, Reduced*, but use 2 g of sample and 40 mL of *Sodium Citrate Solution* in preparing the *Sample Solution*, and prepare the *Diluted Standard Mercury Solution* as follows: Transfer 4.0 mL of *Mercury Stock Solution* into a 250-mL volumetric flask, dilute to volume with 1 *N* hydrochloric acid, and mix (1 mL = 4 μg of Hg). Modify the first sentence of the *Procedure* to read: ''Prepare a control by treating 1.0 mL of *Diluted Standard Mercury Solution* (4 μg Hg) in the same manner. . . .''
Sieve Analysis Determine as directed under *Sieve Analysis of Granular Metal Powders*, Appendix IIC.

Packaging and Storage Store in well-closed containers.

Iron, Reduced

Fe At wt 55.85

CAS: [7439-89-6]

DESCRIPTION

Iron, Reduced, occurs as a gray-black powder. It is elemental iron obtained by a chemical process. It is lusterless or has not more than a slight luster. When viewed under a microscope having a magnifying power of 100 diameters, it appears as an amorphous powder, free from particles having a crystalline structure. It is stable in dry air.

Function Nutrient.

REQUIREMENTS

Identification A sample dissolves in dilute mineral acids with the evolution of hydrogen and the formation of solutions of the corresponding salts, which give positive tests for *Ferrous Salts* (Iron), Appendix IIIA.
Assay Not less than 96.0% of Fe.
Acid-Insoluble Substances Not more than 1.25%.
Arsenic Not more than 8 mg/kg.
Lead Not more than 10 mg/kg.
Mercury Not more than 5 mg/kg.
Sieve Analysis Not less than 100% passes through a 100-mesh sieve.

TESTS

Assay Transfer about 200 mg of sample, accurately weighed, into a 300-mL Erlenmeyer flask, add 50 mL of 2 *N* sulfuric acid, and close the flask with a stopper containing a Bunsen valve (made by inserting a glass tube connected to a short piece of rubber tubing with a slit on the side and a glass rod inserted in the other end and arranged so that gases can escape but air cannot enter). Heat on a steam bath until the iron is dissolved, cool the solution, dilute it with 50 mL of recently boiled and cooled water, add 2 drops of orthophenanthroline TS, and titrate with 0.1 *N* ceric sulfate until the red color changes to a weak blue. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 5.585 mg of Fe.
Acid-Insoluble Substances Dissolve 1.0 g of sample in 25 mL of 2 *N* sulfuric acid, and heat on a steam bath until the evolution of hydrogen ceases. Filter through a tared filter crucible, wash with water until free from sulfate, and dry at 105° for 1 h. The weight of the residue does not exceed 12.5 mg.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using the following solution: Dissolve 1.0 g of sample in 25 mL of 2 *N* sulfuric acid, heat on a steam bath until the evolution of hydrogen ceases, cool, and dilute to 35 mL with water.
Lead Determine as directed in the monograph for *Iron, Carbonyl*.
Mercury

Dithizone Stock Solution Dissolve 30 mg of dithizone in 1000 mL of chloroform, add 5 mL of alcohol, and mix. Store in a refrigerator in a dark bottle. Prepare fresh each month.

Dithizone Extraction Solution On the day of use, dilute 30 mL of *Dithizone Stock Solution* to 100 mL with chloroform.

Hydroxylamine Hydrochloride Solution Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 mL, transfer the solution into a separator, add a few drops of thymol blue TS, and then add ammonium hydroxide until a yellow color appears. Add 10 mL of 1:25 sodium

diethyldithiocarbamate solution, mix, and allow to stand for 5 min. Extract the solution with successive 10- to 15-mL portions of chloroform until a 5-mL test portion of the chloroform extract does not develop a yellow color when shaken with a dilute cupric sulfate solution. Add 2.7 *N* hydrochloric acid until the extracted solution is pink, adding one or two more drops of thymol blue TS, if necessary, then dilute to 100 mL with water, and mix.

Mercury Stock Solution Transfer 33.8 mg of mercuric chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in 1 *N* hydrochloric acid, dilute to volume with the acid, and mix. Each milliliter contains the equivalent of 250 mg of mercury.

Diluted Standard Mercury Solution Transfer 2.0 mL of *Mercury Stock Solution* into a 100-mL volumetric flask, dilute to volume with 1 *N* hydrochloric acid, and mix. Each milliliter contains the equivalent of 5 mg of mercury.

Sodium Citrate Solution Dissolve 250 g of sodium citrate dihydrate in 1000 mL of water.

Sample Solution Transfer 1 g of sample into a 250-mL beaker, add 20 mL of 1:2 nitric acid, and digest on a steam bath for about 45 min. Add 5 mL of 1:3 hydrochloric acid, and continue heating on the steam bath until the sample is dissolved. Cool to room temperature, and filter, if necessary, through a medium-porosity filter paper. Wash the paper with a few milliliters of water, add 20 mL of *Sodium Citrate Solution* and 1 mL of *Hydroxylamine Hydrochloride Solution* to the filtrate, and adjust the pH to 1.8 with ammonium hydroxide.

Procedure (**Note**: Because mercuric dithizonate is light sensitive, this procedure should be performed in subdued light.) Prepare a control by treating 1.0 mL of *Diluted Standard Mercury Solution* (5 mg Hg) in the same manner and with the same reagents as directed for the preparation of the *Sample Solution*. Transfer the control and the *Sample Solution* into separate 250-mL separators, and treat both solutions as follows: Extract with 5 mL of *Dithizone Extraction Solution*, shaking the mixtures vigorously for 1 min. Drain carefully, collecting the chloroform in another separator. If the chloroform does not show a pronounced green color caused by excess reagent, add another 5 mL of the extraction solution, shake again, and drain into the separator. Continue the extraction with 5-mL portions, if necessary, collecting each successive extract in the second separator, until the final chloroform layer contains dithizone in marked excess. Add 15 mL of 1:3 hydrochloric acid, to the combined chloroform extracts, shake the mixture vigorously for 1 min, and discard the chloroform. Extract with 2 mL of chloroform, drain carefully, and discard the chloroform. Add 1 mL of 0.05 *M* disodium EDTA and 2 mL of 6 *N* acetic acid to the aqueous layer. Slowly add 5 mL of 6 *N* ammonium hydroxide, and cool the separator. Transfer the solution into a 150-mL beaker, adjust the pH to 1.8 with 6 *N* ammonium hydroxide or 1:10 nitric acid, using a pH meter, and return the solution to the separator. Add 5.0 mL of *Dithizone Extraction Solution*, and shake vigorously for 1 min. Allow the layers to separate, insert a plug of cotton into the stem of the separator, and collect the dithizone extract in a test tube. Determine the absorbance of each solution in 1-cm cells at 490 nm with a suitable spectrophotometer, using chloroform as the blank. The absorbance of the *Sample Solution* does not exceed that of the control.

Sieve Analysis Determine as directed under *Sieve Analysis of Granular Metal Powders*, Appendix IIC.

Packaging and Storage Store in well-closed containers.

Isobutane

$CH_3CH(CH_3)_2$

C_4H_{10} Formula wt 58.12

CAS: [75-28-5]

DESCRIPTION

Isobutane occurs as a colorless, flammable gas. Its boiling temperature is about −11°.

Function Propellant; aerating agent.

REQUIREMENTS

Caution: Isobutane is highly flammable and explosive. Perform sampling and analytical operations in a well-ventilated fume hood.

Identification

A. The infrared absorption spectrum exhibits maxima, among others, at about the following wavelengths, in μm: 3.4 (vs), 6.8 (s), 7.2 (m), and 10.9 (m).

B. The vapor pressure of a test sample, obtained as directed in the *Sampling Procedure* (below) and determined at 21° by means of a suitable pressure gauge, is approximately 1600 mm Hg (17 psi).

Assay Not less than 95.0% of C_4H_{10}.

Acidity of Residue Passes test.

High-Boiling Residue Not more than 5 mg/kg.

Sulfur Compounds Passes test.

Water Not more than 10 mg/kg.

TESTS

Sampling Procedure Use a stainless steel sampling cylinder equipped with a stainless steel valve and having a capacity of not less than 200 mL and a pressure rating of 240 psi or more. Dry the cylinder with the valve open at 110° for 2 h, and evacuate the hot cylinder to less than 1 mm Hg. Close the valve, and cool and weigh the cylinder. Tightly connect one end of a charging line to the Isobutane container, and loosely connect the other end to the sampling cylinder. Carefully open the Isobutane container, and allow the Isobu-

tane to flush out the charging line through the loose connection. Avoid excessive flushing that causes moisture to freeze in the charging line and connections. Tighten the fitting on the sampling cylinder, and open the sampling cylinder valve, allowing the Isobutane to flow into the evacuated cylinder. Continue sampling until the desired amount of sample is obtained, then close the Isobutane container valve, and finally, close the sampling cylinder valve.

Caution: Do not overload the sampling cylinder.

Weigh the charged sampling cylinder again, and calculate the sample weight.

Assay

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a thermal-conductivity detector and a 6-m × 3-mm (id) aluminum column, or equivalent, packed with 10 weight percent tetraethylene glycol dimethyl ether liquid phase, on a support of crushed firebrick (GasChrom R, or equivalent), which has been calcined or burned with a clay binder above 900° and silanized, or equivalent. Use helium as the carrier gas at a rate of 50 mL/min. Maintain the column at 33°.

System Suitability The peak responses obtained for Isobutane in the chromatograms from duplicate determinations agree within 1%.

Procedure Connect one Isobutane cylinder to the chromatograph through a suitable sampling valve and a flow control valve downstream from the sampling valve. Flush the liquid sample through the sampling valve, taking care to avoid trapping gas or air in the valve. Inject a suitable volume, typically 2 µL, of Isobutane into the chromatograph, and record the chromatogram.

Calculation Calculate the purity of the sample using the following formula:

$$(100 \times B)/(x + y + z + \ldots),$$

in which B is the sample response and x, y, z,. . . represent the sum of all the responses in the chromatogram.

Acidity of Residue Add 10 mL of water to the residue obtained in *High-Boiling Residue* (below), mix by swirling for about 30 s, add 2 drops of methyl orange TS, insert the stopper in the tube, and shake vigorously. No pink or red color appears in the aqueous layer.

High-Boiling Residue Prepare a cooling coil from copper tubing [about 6.1 m × 6 mm (od)] to fit into a suitable vacuum-jacketed flask. Immerse the cooling coil in a mixture of dry ice and acetone in a vacuum-jacketed flask, and connect one end of the tubing to a sampling cylinder (see *Sampling Procedure*, above). Carefully open the sampling cylinder valve, flush the cooling coil with about 50 mL of the liquified Isobutane, and discard this portion of liquid. Continue delivering liquid from the cooling coil, and collect it in a previously chilled 1000-mL sedimentation cone until the cone is filled to the 1000-mL mark (approximately 600 g). Allow the liquid to evaporate, using a warm water bath maintained at about 40° to reduce evaporating time. When all of the liquid has evaporated, rinse the sedimentation cone with two 50-mL portions of pentane, and combine the rinsings in a tared, 150-mL evaporating dish. Transfer 100 mL of the pentane solvent to a second tared, 150-mL evaporating dish, place both evaporating dishes on a water bath, evaporate to dryness, and heat the dishes in an oven at 100° for 60 min. Cool the dishes in a desiccator, and weigh. Repeat the heating for 15-min periods until successive weighings are within 0.1 mg. The weight of the residue obtained from the sample is the difference between the weights of the residues in the two evaporating dishes. Calculate the milligrams per kilogram of high-boiling residue based on a sample weight of 600 g.

Sulfur Compounds Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose. The gas is free from the characteristic odor of sulfur compounds.

Water Determine as directed under *Water Determination*, Appendix IIB, using the following modifications: (a) Provide the closed-system titrating vessel with an opening, and pass through it a coarse-porosity gas dispersion tube connected to a sampling cylinder. (b) Dilute the reagent with anhydrous methanol to give a water equivalence factor of between 0.2 and 1.0 mg/mL; age this diluted solution for at least 16 h before standardization. (c) Obtain a 100-g sample as directed in the *Sampling Procedure* (above), and introduce the sample into the titration vessel through the gas dispersion tube at a rate of about 100 mL of gas per minute; if necessary, heat the sampling cylinder gently to maintain this flow rate.

Packaging and Storage Store in tight cylinders protected from excessive heat.

Isobutylene–Isoprene Copolymer

Butyl Rubber

CAS: [9010-85-9]

View IR

DESCRIPTION

Isobutylene–Isoprene Copolymer is a synthetic copolymer containing from 0.5 to 3.0 molar percent of isoprene, the remainder consisting of isobutylene. It is prepared by copolymerization of isobutylene and isoprene in methyl chloride solution, using aluminum chloride as the catalyst. After completion of polymerization, the rubber particles are treated with hot water containing a suitable food-grade deagglomerating agent, such as stearic acid. Finally, the coagulum is dried to remove residual volatiles.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample dissolved in hot toluene and evaporated on a potassium

bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the test conditions as specified therein.
Lead Not more than 3 mg/kg.
Total Unsaturation Not more than 3.0 molar percent, as isoprene.

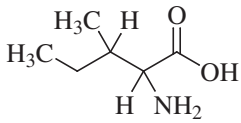
TESTS

Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV.
Total Unsaturation Determine as directed under *Total Unsaturation*, Appendix IV.

Packaging and Storage Store in well-closed containers.

DL-Isoleucine

DL-2-Amino-3-methylvaleric Acid



$C_6H_{13}NO_2$ Formula wt 131.17

CAS: [443-79-8]

View IR

DESCRIPTION

DL-Isoleucine occurs as a white, crystalline powder. It is soluble in water, and practically insoluble in alcohol and in ether. It melts with decomposition at about 292°. The pH of a 1:100 aqueous solution is between 5.5 and 7.0. It is optically inactive.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_{13}NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to the first appearance of a pure green color or until the blue color disappears completely.

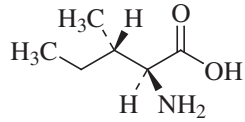
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 13.12 mg of $C_6H_{13}NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

L-Isoleucine

L-2-Amino-3-methylvaleric Acid



$C_6H_{13}NO_2$ Formula wt 131.17

CAS: [73-32-5]

View IR

DESCRIPTION

L-Isoleucine occurs as crystalline leaflets or as a white, crystalline powder. It is soluble in 25 parts of water, slightly soluble in hot alcohol, and soluble in diluted mineral acids and in alkaline solutions. It sublimes at between 168° and 170°, and melts with decomposition at about 284°. The pH of a 1:100 aqueous solution is between 5.5 and 7.0.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_{13}NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +38.6° and +41.5°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between +38.2° and +41.1°, calculated on the dried basis.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to the first appearance of a pure green color or until the blue color disappears completely.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 13.12 mg of $C_6H_{13}NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 4 g of previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

Isopropyl Alcohol

2-Propanol; Isopropanol

$$CH_3CHOHCH_3$$

C_3H_8O Formula wt 60.10

CAS: [67-63-0]

DESCRIPTION

Isopropyl Alcohol occurs as a clear, colorless, flammable liquid. It is miscible with water, with ethyl alcohol, with ether, and with many other organic solvents. Its refractive index at 20° is about 1.377.

Function Extraction solvent.

REQUIREMENTS

Identification The refractive index at 20°, determined as directed under *Refractive Index*, Appendix IIB, is about 1.377.
Assay Not less than 99.7% of C_3H_8O, by weight.
Acidity (as acetic acid) Not more than 10 mg/kg.
Distillation Range Within a range of 1°, including 82.3°.
Lead Not more than 1 mg/kg.
Nonvolatile Residue Not more than 10 mg/kg.
Solubility in Water Passes test.
Specific Gravity Not more than 0.7840.
Substances Reducing Permanganate Passes test.
Water Not more than 0.2%.

TESTS

Assay (See *Chromatography*, Appendix IIA.) Determine the content of propan-2-ol and volatile impurities using a suitable gas chromatograph equipped with flame-ionization detector and a 1.8-m × 6-mm (id) steel column, or equivalent, packed with 10% P.E.G. 400 on 60- to 80-mesh Chromosorb W (or equivalent). Maintain the column at 90°, and set both the injection port temperature and the detector temperature to 150°. Use helium as the carrier gas, with a flow rate of 45 mL/min. Inject between 1-µL and 5-µL samples, and from the chromatograms so obtained, determine the content of each constituent by the method of area normalization.
Acidity (as acetic acid) Add 2 drops of phenolphthalein TS to 100 mL of water, add 0.01 *N* sodium hydroxide to the first pink color that persists for at least 30 s, then add 50 mL (about 39 g) of sample, and mix. Not more than 0.7 mL of 0.01 *N* sodium hydroxide is required to restore the pink color.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Nonvolatile Residue Evaporate 125 mL (about 100 g) of sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.
Solubility in Water Mix 10 mL of sample with 40 mL of water. After 1 h, the solution is as clear as an equal volume of water.
Specific Gravity The specific gravity of a sample, determined by any reliable method (see *General Provisions*), is not greater than 0.7840 at 25°/25° (equivalent to 0.7870 at 20°/20°).
Substances Reducing Permanganate Transfer 50 mL of sample into a 50-mL glass-stoppered cylinder, add 0.25 mL of 0.1 *N* potassium permanganate, mix, and allow to stand for 10 min: The pink color is not entirely discharged.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers, remote from fire.

Juniper Berries Oil

CAS: [8012-91-7]

View IR

DESCRIPTION

Juniper Berries Oil occurs as a colorless, faintly green or yellow liquid with a characteristic odor and an aromatic, bitter

taste. It is the volatile oil obtained by steam distillation from the dried ripe fruit of the plant *Juniperus communis* L. var. *erecta* Pursh (Fam. Cupressaceae). It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol. The oil tends to polymerize during long storage.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between −15° and 0°.
Refractive Index Between 1.474 and 1.484 at 20°.
Specific Gravity Between 0.854 and 0.879.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Kaolin

China Clay

CAS: [1332-58-7]

DESCRIPTION

Kaolin occurs as a fine, white to yellow-white or gray powder that becomes darker when moistened. It is a purified clay consisting mainly of alumina, silica, and water. It is insoluble in water, in alcohol, in dilute acids, and in alkali solutions.

Function Anticaking agent.

REQUIREMENTS

Identification Mix 1 g of sample with 10 mL of water and 5 mL of sulfuric acid in a porcelain dish, and evaporate until the water is removed. Continue heating until dense, white fumes of sulfur trioxide evolve, then cool, and cautiously add 20 mL of water. Boil for a few minutes, and filter. A gray residue of silica remains on the filter. Add 6 *N* ammonium hydroxide to a portion of the filtrate. A gelatinous, white precipitate of aluminum hydroxide forms that is insoluble in an excess of 6 *N* ammonium hydroxide.
Acid-Soluble Substances Not more than 2.0%.
Arsenic Not more than 3 mg/kg.
Carbonate Passes test.
Iron Passes test.
Lead Not more than 10 mg/kg.
Loss on Ignition Not more than 15.0%.
Sulfide Passes test.

TESTS

Acid-Soluble Substances Mix 1 g of sample with 20 mL of 2.7 *N* hydrochloric acid for 15 min, and filter. Evaporate 10 mL of the filtrate to dryness in a tared dish, ignite gently, cool, and weigh the residue (*R*). Calculate the percent Acid-Soluble Substances by the formula

$$(2R \times 100)/w,$$

in which *w* is the sample weight.

Sample Solution for the Determination of Arsenic and Lead Transfer 10.0 g of sample into a 250-mL flask, and add 50 mL of 0.5 *N* hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 3 filter paper, or equivalent, into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using 10 mL of the *Sample Solution* (above).
Carbonate Mix 1 g of sample with 10 mL of water, cool, and keep the mixture cool while adding 5 mL of sulfuric acid. No effervescence occurs during the addition of the acid.
Iron Mix 2 g of sample with 10 mL of water in a mortar, and add 500 mg of sodium salicylate. No more than a light red tint appears.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 10 mL of the *Sample Solution* (above), and 10 μg of lead (Pb) ion in the control.
Loss on Ignition Ignite 2 g of sample, accurately weighed, in a tared crucible at 575° ± 25° to constant weight, cool, and weigh.
Sulfide Add 1 g of sample to 25 mL of water in a 250-mL flask, then add 15 mL of 2.7 *N* hydrochloric acid, and immediately cover the top of the flask with filter paper moistened with lead acetate TS. Heat to boiling, and boil for several minutes. The paper does not show any brown coloration.

Packaging and Storage Store in well-closed containers.

Karaya Gum

Sterculia Gum

INS: 416 CAS: [9000-36-6]

DESCRIPTION

Karaya Gum occurs in tears of variable size or in broken, irregular pieces having a somewhat crystalline appearance. In this form, it is a pale yellow to pink-brown, translucent substance that is compact and homogeneous with a dull luster. It is sometimes admixed with a few darker fragments and occasional pieces of bark. In the powdered form, it is light to pink-gray. It is a dried, gummy exudation from *Sterculia urens* Roxburgh and other species of *Sterculia* (Fam. Sterculiaceae), or from *Cochlospermum gossypium* A. P. De Condolle or other species of *Cochlospermum* Kunth (Fam. Bixaceae). Karaya Gum is insoluble in alcohol, but it swells in water to form a gel.

Function Stabilizer; thickener; emulsifier.

REQUIREMENTS

Identification

A. Add 2 g of sample to 50 mL of water. The sample swells to form a stiff, granular, slightly opalescent mucilage.

B. Add a few drops of Millon's Reagent to a 1:100 aqueous solution. A white, curdy precipitate forms.

C. The sample swells in 60% alcohol.

Ash (Acid-Insoluble) Not more than 1.0%.
Insoluble Matter Not more than 3.0%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 20.0%.
Starch Passes test.
Viscosity of a 1% Solution Not less than the minimum or within the range claimed by the vendor.

TESTS

Ash (Acid-Insoluble) Determine as directed under *Ash (Acid-Insoluble)*, Appendix IIC.
Insoluble Matter Transfer about 5 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, add a 1:1 mixture of 2.7 *N* hydrochloric acid:water, cover the flask with a watch glass, and boil the solution gently until it loses its viscosity. Filter the solution through a tared filtering crucible, wash the residue with water until the washings are free from acid, dry at 105° for 1 h, and weigh.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 4 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, using an unground sample that has been powdered until it passes through a No. 40 sieve. Mix well before weighing, and dry at 105° for 5 h.
Starch Add a few drops of iodine TS to a 1:10 aqueous solution. No blue color appears.
Viscosity Transfer a 4-g sample, finely powdered, into the container of a stirring apparatus equipped with blades capable of being adjusted to about 1000 rpm. Add 10 mL of alcohol to the sample, swirl to wet it uniformly, and then add 390 mL of water, avoiding the formation of lumps. Stir the mixture for 7 min, pour the resulting dispersion into a 500-mL bottle, insert a stopper, and allow to stand for about 12 h in a water bath at 25°. Determine the apparent viscosity at this temperature with a model LVF Brookfield, or equivalent, viscometer (see *Viscosity of Cellulose Gum*, Appendix IIB) using a suitable spindle, speed, and factor.

Packaging and Storage Store in well-closed containers.

Kelp

DESCRIPTION

Kelp occurs as a dark green to olive brown, dry substance. It is the dehydrated seaweed obtained from the class Phaeophyceae (brown algae) of the genera *Macrocystis* (including *M. pyrifera* and related species) and *Laminaria* (including *L. digitata*, *L. cloustoni*, and *L. saccharina*). The seaweed may be chopped to provide coarse particles and/or it may be ground to provide a fine powder.

Function Nutrient (source of iodine).

REQUIREMENTS

Arsenic Not more than 1 mg/kg.
Ash (Total) Not more than 45.0%.
Iodine Content Between 0.1% and 0.5%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 13.0%.

TESTS

Arsenic

Distillation-Reducing Solution Dissolve 36 g of ACS low-arsenic ferrous chloride ($FeCl_2 \cdot 4H_2O$) in 500 mL of 6.6 *N* hydrochloric acid. Prepare fresh on the day of use.

Apparatus Refer to the figure *Special Apparatus for the Determination of Inorganic Arsenic* (*Arsenic Limit Test*, Appendix IIIB). Have all parts available for assembly during the *Procedure*.

Procedure Accurately weigh 2.00 g of sample that has previously been ground to pass through a 60-mesh screen, and transfer it to the distillation flask (*A*). Add 50 mL of *Distillation-Reducing Solution*, connect the flask to the receiver chamber (*B*), complete the assembly of the apparatus, and begin circulating tap water through the condenser (*C*). Half-fill the lower two bulbs of the splash head (*D*) with water.

Maneuver the stopcock to cause the contents of the receiver chamber to drain into the distillation flask, heat the flask until the temperature above the solution reaches 106° to 108°, and continue refluxing at this temperature for 45 min. Close the stopcock, continue heating at 108° to 110°, and collect 30 to 33 mL of distillate in the receiver chamber. Remove the heating source and allow the temperature to drop to about 80°.

Drain the distillate from the receiver chamber into a 250-mL beaker that is contained in an ice-water bath. Close the stopcock, and add a second 50-mL portion of the *Distillation-Reducing Solution* through the thermometer opening to the distillation flask. Replace the thermometer, increase the temperature to 108° to 110°, and collect a second 30- to 33-mL portion of distillate in the receiver chamber.

Drain the second distillate into the beaker containing the first portion, and continue cooling in the ice-water bath until the combined distillate cools to room temperature. Remove the splash head, and wash its contents into the beaker. Also, wash down the insides of the condenser and receiver chamber with water, collecting the washings in the beaker. Filter the beaker contents through a Whatman No. 40, or equivalent, filter paper, collecting the filtrate in a 300-mL Erlenmeyer flask having a 24/40 standard-taper joint, to be used later as an arsine generator flask. Wash the filter three times with water so that the final volume of filtrate measures 200 mL.

Refer to the *Arsenic Limit Test*, Appendix IIIB. Add 2 mL of potassium iodide TS and 0.5 mL of *Stannous Chloride Solution* to the Erlenmeyer flask, and continue as directed in the *Procedure*, beginning with, ''Allow the mixture to stand for 30 min at room temperature . . .'' but use 6.0 mL, rather than 3.0 mL, of *Standard Arsenic Solution* in the preparation of the standard.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.

Iodine Content Transfer about 2 g of sample, accurately weighed, into a large porcelain crucible, and mix thoroughly with 10 g of potassium carbonate. Place the sample in a muffle furnace, starting with low heat, and then ignite at 500° to 600° for 20 min or until combustion is complete. Dissolve the ash in about 200 mL of boiling water, filter, and wash the filter paper with two 15-mL portions of boiling water, adding the washings to the filtrate. Cool to room temperature, neutralize to methyl red TS with approximately 20 mL of 85% phosphoric acid diluted with 20 mL of water, and then add 5 mL in excess. Cool the reaction mixture on an ice bath, and add bromine TS (about 5 mL) until a permanent yellow color appears. Gently boil the solution to remove all free bromine, adding water if necessary to maintain a volume of 200 mL or more. Boil for an additional 5 min after the bromine color has completely disappeared. Add a few milligrams of salicylic acid, stir, and cool to about 20°. Add 1 mL of the diluted phosphoric acid solution and 5 mL of potassium iodide TS, and titrate immediately with 0.01 *N* sodium thiosulfate, using starch TS as the indicator. Each milliliter of 0.01 *N* sodium thiosulfate is equivalent to 211.5 μg of iodine (I).

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit* Test, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Packaging and Storage Store in well-closed containers.

Konjac Flour

Konjac; Konnyaku; Konjac Gum; Yam Flour

CAS: [37220-17-0]

DESCRIPTION

Konjac Flour occurs as a cream to light tan powder. It is a hydrocolloidal polysaccharide obtained from the tubers of various species of *Amorphophallus*. Konjac Flour is a high-molecular-weight, nonionic glucomannan primarily consisting of mannose and glucose at a respective molar ratio of approximately 1.6:1.0. It is a slightly branched polysaccharide connected by β-1,4 linkages and has an average molecular weight of 200 to 2000 kDa. Acetyl groups along the glucomannan backbone contribute to solubility properties and are located, on average, every 9 to 19 sugar units. Konjac Flour is dispersible in hot or cold water and forms a highly viscous solution with a pH between 4.0 and 7.0. Solubility is increased by heat and mechanical agitation. Addition of mild alkali to the solution results in the formation of a heat-stable gel that resists melting, even under extended heating conditions.

Function Gelling agent; thickener; film former; stabilizer.

REQUIREMENTS

Identification

A. *Microscopic Test* Stain about 0.1 g of sample with 0.01% methylene blue powder in 50% isopropyl alcohol, and observe microscopically. The sample should have flattened elliptical particles that are generally 100 to 500 μm in length along the long axis. Unground Konjac Flour is clearly distinguished from other hydrocolloids by the presence of saclike cells that contain glucomannan. The surface of these cells has a reticulated structure. Particles of Konjac Flour are also birefringent under polarized light. These visual characteristics may remain even if the sample is finely ground, but they are less pronounced.

B. *Gel Test* At room temperature, add 5 mL of a 4% sodium borate solution to a 1% solution of sample in a test tube, and shake vigorously. A gel forms. (Konjac Flour solutions gel in the presence of sodium borate, similar in reaction to that of galactomannans such as guar gum and locust bean gum.)

C. *Heat-Stable Gel Test* Prepare a 2% solution of sample by heating it in a boiling water bath for 30 min with continuous agitation and then cooling the solution to room temperature.

For each gram of sample used to prepare the 2% solution, add 1 mL of 10% potassium carbonate solution to the fully hydrated sample at ambient temperature. Heat the mixture in a water bath to 85°, and hold quiescently for 2 h without agitation. The sample forms a thermally stable gel under these conditions. (Related hydrocolloids such as guar gum and locust bean gum do not form thermally stable gels and are negative by this test.)
Arsenic Not more than 3 mg/kg.
Ash (Total) Not more than 5.0%.
Carbohydrate (Total) Not less than 75.0%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 15.0%.
Protein Not more than 8.0%.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Carbohydrate (Total) The remainder, after subtracting from 100% the sum of the percentages of *Ash (Total)*, *Loss on Drying*, and *Protein*, represents the percentage of carbohydrates (glucomannans) in the sample.
Lead Determine as directed for *Flame Atomic Absorption Spectrophotometric Method* under the *Lead Limit Test*, Appendix IIIB, using a 5-g sample and the *Diluted Standard Lead Solutions* specified for a *1 mg/kg Lead Limit*.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 5 h.
Protein Determine as directed under *Nitrogen Determination*, Appendix IIIC, using about 3.5 g of sample, accurately weighed, transferred into a 500-mL Kjeldahl flask. Determine the percent of protein by the formula

$$\%N \times 5.7,$$

in which N is nitrogen and 5.7 is the conversion factor to protein.

Packaging and Storage Store cool and dry in a closed container away from direct heat and sunlight.

Labdanum Oil

CAS: [8016-26-0]

View IR

DESCRIPTION

Labdanum Oil occurs as a golden yellow, viscous liquid with a powerful, balsamic odor, which on dilution, is reminiscent of ambergris. It turns dark brown on standing. It is the volatile oil obtained by steam distillation from crude labdanum gum extracted from the perennial shrub *Cistus ladaniferus* L. (Fam. Cistaceae). It is soluble in most fixed oils and in mineral oil, but it is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Between 18 and 86.
Angular Rotation Between +0°15' and +7°.
Ester Value Between 31 and 86.
Refractive Index Between 1.492 and 1.507 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.905 and 0.993.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed in *Ester Value* under *Esters*, Appendix VI, using about 1 g of sample, accurately weighed.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 0.5 mL of 90% alcohol, but the solution usually becomes opalescent or turbid on further dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Lactic Acid

α-Hydroxypropionic Acid; 2-Hydroxypropionic Acid

$C_3H_6O_3$ Formula wt 90.08

INS: 270 CAS: L(+)-Lactic Acid [79-33-4]
CAS: DL-Lactic Acid [598-82-3]

DESCRIPTION

Lactic Acid occurs as a colorless or yellow, syrupy liquid consisting of a mixture of lactic acid ($C_3H_6O_3$) and lactic acid lactate ($C_6H_{10}O_5$). It is obtained by the lactic fermentation of

sugars or is prepared synthetically. It is usually available in solutions containing the equivalent of from 50% to 90% lactic acid. It is hygroscopic, and when concentrated by boiling, the acid condenses to form lactic acid lactate, 2-(lactoyloxy)propanoic acid, that on dilution and heating, hydrolyzes to Lactic Acid. It is miscible with water and with alcohol.

Function Acidifier.

REQUIREMENTS

Labeling Indicate the concentration of Lactic Acid.
Identification A sample gives positive tests for *Lactate*, Appendix IIIA.
Assay Not less than 95.0% and not more than 105.0% of the labeled concentration of $C_3H_6O_3$.
Chloride Not more than 0.1%.
Citric, Oxalic, Phosphoric, or Tartaric Acid Passes test.
Cyanide Not more than 5 mg/kg.
Iron Not more than 10 mg/kg.
Lead Not more than 0.5 mg/kg.
Residue on Ignition Not more than 0.1%.
Sugars Passes test.
Sulfate Not more than 0.25%.

TESTS

Assay Transfer an accurately weighed quantity of sample, equivalent to 3 g of Lactic Acid, into a 250-mL flask, add 50.0 mL of 1 *N* sodium hydroxide, mix, and boil for 20 min. Add phenolphthalein TS, titrate the excess alkali in the hot solution with 1 *N* sulfuric acid, perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 1 *N* sodium hydroxide is equivalent to 90.08 mg of $C_3H_6O_3$.
Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, diluting 20 g of sample to 1000 mL with water and mixing thoroughly. Any turbidity a 1.0-mL (20 mg of Lactic Acid) portion of this solution produces does not exceed that shown in a control containing 20 μg of chloride (Cl) ion.
Citric, Oxalic, Phosphoric, or Tartaric Acid Dilute 1 g of sample to 10 mL with water, add 40 mL of calcium hydroxide TS, and boil for 2 min. The solution does not become turbid.
Cyanide

p-*Phenylenediamine–Pyridine Mixed Reagent* Dissolve 200 mg of *p*-phenylenediamine hydrochloride in 100 mL of water, warming to effect solution. Cool, allow the solids to settle, and use the supernatant liquid to make the mixed reagent. Dissolve 128 mL of pyridine in 365 mL of water, add 10 mL of hydrochloric acid, and mix. To prepare the mixed reagent, mix 30 mL of the *p*-phenylenediamine solution with all of the pyridine solution, and allow to stand for 24 h before using. The mixed reagent is stable for about 3 weeks when stored in an amber bottle.

Sample Solution Transfer an accurately weighed quantity of sample, equivalent to 20.0 g of Lactic Acid, into a 100-mL volumetric flask, dilute to volume with water, and mix.

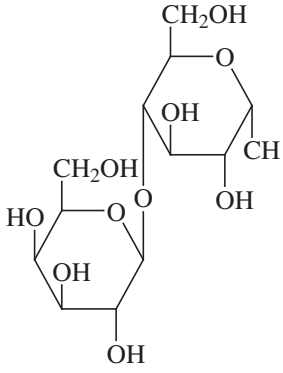
Cyanide Standard Solution Dissolve 0.25 g of potassium cyanide in 100 mL of 0.1 *N* sodium hydroxide. Transfer a 1-mL aliquot into a 100-mL volumetric flask, dilute to volume with 0.1 *N* sodium hydroxide, and mix. Each milliliter of this solution contains 10 μg of cyanide (CN).

Procedure Pipet a 10-mL aliquot of the *Sample Solution* into a 50-mL beaker. Pipet 1.0 mL of the *Cyanide Standard Solution* into a second 50-mL beaker, and add 10 mL of water. Place the beakers in an ice bath, and adjust the pH to between 9 and 10 with 20% sodium hydroxide, stirring slowly and adding the reagent slowly to avoid overheating. Allow the solutions to stand for 3 min, and then slowly add 10% phosphoric acid to a pH between 5 and 6. Transfer the solutions into 100-mL separators containing 25 mL of cold water, and rinse the beakers and pH meter electrodes with a few milliliters of cold water, collecting the washings in the respective separators. Add 2 mL of bromine TS, stopper, and mix. Add 2 mL of 2% sodium arsenite solution, stopper, and mix. Add 10 mL of *n*-butanol to the clear solutions, stopper, and mix. Finally, add 5 mL of p-*Phenylenediamine–Pyridine Mixed Reagent*, mix, and allow to stand for 15 min. Remove and discard the aqueous phases, and filter the alcohol phases into 10-mm cells. The absorbance of the *Sample Solution*, determined at 480 nm with a suitable spectrophotometer, is no greater than that of the *Cyanide Standard Solution*.
Iron Add 2 mL of 1:20 hydrochloric acid to the ash obtained in the test for *Residue on Ignition* (below), and evaporate to dryness on a steam bath. Dissolve the residue in 1 mL of hydrochloric acid, dilute to 40 mL with water, and add about 40 mg of ammonium persulfate crystals and 10 mL of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 2.0 mL of *Iron Standard Solution* (20 μg Fe) in an equal volume of solution containing the quantities of reagents used in the test.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample. Save the ash for the test for *Iron* (above).
Sugars Add 5 drops of sample to 10 mL of hot alkaline cupric tartrate TS. No red precipitate forms.
Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, diluting 10 g of sample to 100 mL with water, and mixing thoroughly. Any turbidity produced by a 1.6-mL (160 mg of Lactic Acid) portion of this solution does not exceed that shown in a control containing 400 μg of sulfate (SO_4) ion.

Packaging and Storage Store in tight containers.

Lactose

4-*O*-β-Galactopyranosyl-D-glucose



(α-Lactose)

$C_{12}H_{22}O_{11}$ Formula wt, anhydrous 342.30
$C_{12}H_{22}O_{11}·H_2O$ Formula wt, monohydrate 360.32

CAS: anhydrous [63-42-3]
CAS: monohydrate [5989-81-1]

DESCRIPTION

Lactose occurs as a white to creamy white, crystalline powder. It is normally obtained from whey. It may be anhydrous, contain one molecule of water of hydration, or contain a mixture of both forms if it has been prepared by a spray-drying process. It is soluble in water, very slightly soluble in alcohol, and insoluble in chloroform and in ether.

Function Nutritive sweetener; processing aid; humectant (anhydrous form); texturizer.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or the monohydrate or a mixture of both forms if it has been prepared by a spray-drying process.
Identification Add 5 mL of 1 *N* sodium hydroxide to 5 mL of a hot, saturated solution of sample, and gently warm the mixture. The liquid turns yellow and, finally, brown-red. Cool to room temperature, and add a few drops of alkaline cupric tartrate TS. A red precipitate of cuprous oxide forms.
Assay Not less than 98.0% and not more than 100.5% of $C_{12}H_{22}O_{11}$, calculated on the dried basis.
Arsenic Not more than 0.5 mg/kg.
Lead Not more than 0.5 mg/kg.
Loss on Drying *Monohydrate and spray-dried mixture*: Not less than 4.5% and not more than 5.5%; *Anhydrous*: Not more than 1.0%.
pH Not less than 4.5 and not more than 7.5, in a 10% aqueous solution.
Residue on Ignition Not more than 0.3%.

TESTS

Assay Determine as directed under *Lactose*, Appendix X. Transfer about 2 g of sample, accurately weighed, to a 100-mL volumetric flask. Add 10 mL of fructose internal standard solution, dilute to volume with water, and mix. Perform the analysis within 24 h.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 4 g of sample in 35 mL of water, and 2.0 mL of *Standard Arsenic Solution* in the control (2 μg As).
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 2-g sample at 120° for 16 h.
pH

Sample Preparation Transfer 10 g of sample, accurately weighed, into a clean, dry 100-mL Erlenmeyer flask, and add 90 mL of recently boiled water set at 25°. Shake until the particles are evenly suspended and the mixture is free of lumps. Heat the sample to boiling, and shake frequently to aid dissolution. Let the suspension stand for 10 min, decant the supernate into the hydrogen-ion vessel, and quickly cool to 25°.

Procedure Determine as directed under *pH Determination*, Appendix IIB, using pH 4.01 and 9.18 buffer solutions to standardize the pH meter.
Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed containers protected from humidity.

Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol

Propylene Glycol Lactostearate

INS: 478

DESCRIPTION

Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol occur as a substance that varies in consistency from a soft solid to a hard, waxy solid. They are a mixture of partial lactic and fatty acid esters of propylene glycol and glycerin produced by the lactylation of a product obtained by reacting edible fats or oils with propylene glycol. They are dispersible in hot water, and are moderately soluble in hot isopropanol, in chloroform, and in soybean oil.

Function Emulsifier; stabilizer; whipping agent; plasticizer.

REQUIREMENTS

Identification Place about 150 mg of melted sample into a 16- × 125-mm tube equipped with a screw cap having a Teflon liner, and add 4 mL of absolute methanol, 4 drops of a 25% sodium methoxide solution in absolute methanol, and a boiling chip. Cap the tube, reflux for 15 min, and cool to room temperature. Extract as follows: Add 8 drops of a 15% potassium acid sulfate solution, 4 mL of water, and 4 mL of *n*-hexane; cap the tube; shake for 1 min; and centrifuge for 30 to 60 s. Decant and discard the *n*-hexane layer, and repeat the extraction with three additional 4-mL portions of *n*-hexane, discarding each extract. Transfer the aqueous alcoholic phase from the tube into a 50-mL round-bottom, glass-stoppered flask; place the flask in a water bath at 50° to 55°; and evaporate to near dryness (about 0.5 mL of residue) in a rotary film evaporator under full water aspirator vacuum.

Caution: Do not heat above 55°.

Remove the flask from the evaporator, add 1 mL of a 1:1 solution of 0.5 *N* hydrochloric acid:methanol, swirl for several minutes, and decant the clear solution into a small flask. Inject a portion of this solution into a suitable gas chromatograph, or equivalent, and obtain the chromatogram. (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and a 1.8-m × 3-mm (id) column packed with 80- to 100-mesh Porapak Q (ethylvinylbenzene–divinylbenzene polymer porous beads), or equivalent. Maintain the column at 175° to 210°, heated at a rate of 4°/min, and held at 210° until the glycerin is eluted. Set the inlet port temperature to 310° and the detector to 385°. Use helium as the carrier gas, with a flow rate of 50 mL/min. Use a recorder with a range of 0 to 1 mV and a 1-s full-scale deflection at a chart speed of 6.5 mm/min. From the chromatogram so obtained, identify the peaks by their relative positions on the chart. The major peaks, representing propylene glycol, methyl lactate, lactic acid, and glycerin, in the order listed, may be identified with suitable reference substances. Major peaks may also be identified by their relative retention times using a suitable internal standard.

Acid Value Not more than 12.0.

Lead Not more than 2 mg/kg.

Water-Insoluble Combined Lactic Acid Between 14.0% and 18.0%.

The following specifications should conform to the representations of the vendor: *Free Glycerin*, *Free Lactic Acid*, *1-Monoglyceride Content*, *Total Lactic Acid*, and *Water*.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

Free Glycerin Determine as directed under *Free Glycerin or Propylene Glycol*, Appendix VII.

Free Lactic Acid Transfer about 15 g of sample, accurately weighed, into a beaker, dissolve it in about 75 mL of benzene, and transfer the solution into a 500-mL glass-stoppered graduate. Wash the beaker with about 125 mL of benzene in divided portions, adding the washings to the graduate. Add 200 mL of water to the graduate, and shake vigorously for 1 min. After 125 mL or more of the aqueous phase has separated, pipet 100.0 mL of the aqueous phase into an Erlenmeyer flask, add 1 mL of phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a slight pink color. Calculate the percentage of free lactic acid in the sample by the formula

$$[45.04 \times V \times N]/(0.5 \times W),$$

in which 45.04 is the equivalence factor for lactic acid; V is the volume, in milliliters, of 0.5 *N* sodium hydroxide required; N is the exact normality of the sodium hydroxide solution; and W is the weight, in grams, of the sample.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

1-Monoglyceride Content Determine as directed under *1-Monoglycerides*, Appendix VII.

Total Lactic Acid Transfer about 3 g of sample, accurately weighed, into a 250-mL glass-stoppered flask, pipet 50.0 mL of 0.7 *N* alcoholic potassium hydroxide into the flask, attach an air condenser, and boil gently on a steam bath for 30 min or until the sample is completely saponified. Remove the flask from the steam bath, immediately remove the air condenser, and allow the solution to cool until it begins to gel. Add 75.0 mL of 0.5 *N* hydrochloric acid, mix, and transfer the solution into a 500-mL separator, washing the flask with two 15-mL portions of water and adding them to the solution in the separator. Cool to 35° or lower, and extract with 100 mL of diethyl ether. Transfer the aqueous layer into a second 500-mL separator, and wash the ether layer with two 20-mL portions of water, adding the wash water to the original aqueous phase in the second separator. Retain the ether solution. Extract the aqueous phase with a second 100-mL portion of diethyl ether, and transfer the aqueous phase into a 500-mL Erlenmeyer flask. Combine and wash the ether extracts with five 20-mL portions of water, and add the wash water to the flask. Add 1 mL of phenolphthalein TS to the combined aqueous phases in the Erlenmeyer flask, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a slight pink color. Perform a blank determination (see *General Provisions*), make any necessary correction, and calculate the percent of total lactic acid in the sample taken by the formula

$$[45.04 \times (S - B) \times N]/W,$$

in which 45.04 is the equivalence factor for lactic acid; $S - B$ represents the difference, in milliliters, between the volumes of 0.5 *N* sodium hydroxide required for the sample and for the blank, respectively; N is the exact normality of the sodium hydroxide solution; and W is the weight, in grams, of the sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Water-Insoluble Combined Lactic Acid Transfer about 3 g of sample, accurately weighed, into a 250-mL separator with the aid of 100 mL of benzene, and wash with three 30-mL portions of water, discarding the washings. Transfer the benzene layer into a 250-mL glass-stoppered Erlenmeyer flask, wash the separator with a few milliliters of benzene,

and completely evaporate the combined benzene solution to dryness. Pipet 50.0 mL of 0.7 *N* alcoholic potassium hydroxide into the flask, attach an air condenser, boil gently on a steam bath for 30 min or until the sample is completely saponified, and remove the flask from the steam bath. Immediately remove the air condenser, and allow the solution to cool until it begins to jell. Add 75.0 mL of 0.5 *N* hydrochloric acid, mix, and transfer the solution into a 500-mL separator, washing the flask with two 15-mL portions of water. Cool to 35° or lower, and extract with 100 mL of diethyl ether. Transfer the water layer to a second 500-mL separator, and wash the diethyl ether with two 20-mL portions of water, adding the wash water to the original aqueous phase in the second separator. Retain the ether solution. Extract the aqueous phase with a second 100-mL portion of diethyl ether, and transfer the aqueous phase to a 500-mL Erlenmeyer flask. Combine and wash the ether extracts with five 20-mL portions of water, and add the wash water to the flask. Add 1 mL of phenolphthalein TS to the combined aqueous phases in the flask, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a slight pink color. Perform a blank determination (see *General Provisions*), make any necessary correction, and calculate the percent of water-insoluble combined lactic acid in the sample taken by the formula

$$[45.04(S - B)(N)]/W,$$

in which 45.04 is the equivalence factor for lactic acid; $S - B$ represents the difference, in milliliters, between the volumes of 0.5 *N* sodium hydroxide required for the sample and for the blank, respectively; *N* is the exact normality of the sodium hydroxide solution; and *W* is the weight, in grams, of the sample.

Packaging and Storage Store in well-closed containers.

Lactylic Esters of Fatty Acids

DESCRIPTION

Lactylic Esters of Fatty Acids occur as liquids to hard, waxy solids. They are mixed fatty acid esters of lactic acid and its polymers, with minor quantities of free lactic acid, polylactic acid, and fatty acids. They are dispersible in hot water and are soluble in organic solvents and in vegetable oils.

Function Emulsifier; surface-active agent.

REQUIREMENTS

Identification

A. Take 1 mL of the solution obtained in the test for *Total Lactic Acid* (below) after titrating with 0.1 *N* potassium hydroxide, and transfer it into a 25-mL glass-stoppered test tube. Add 0.1 mL of cupric sulfate solution (1 g of $CuSO_4 \cdot 5H_2O$ in 25 mL of water) and 6 mL of sulfuric acid, and mix. Stopper loosely, heat in a boiling water bath for 5 min, then cool in an ice bath for 5 min, and remove from the bath. Add 0.1 mL of *p*-phenylphenol TS, mix, allow to stand at room temperature for 1 min, and then heat in a boiling water bath for 1 min. A deep, blue-violet color indicates the presence of lactic acid.

B. *Apparatus* (See *Chromatography*, Appendix IIA.) Assemble a suitable apparatus for ascending thin-layer chromatography. Prepare a slurry of chromatographic silica gel containing about 13% of calcium sulfate (1 g to each 2 mL of water) as the binder, apply a uniformly thin layer to glass plates of convenient size, dry in air for 10 min, and activate by drying at 100° for 1 h. Store the cool plates in a clean, dry place until ready for use.

Sample Solution Transfer 1 g of sample into a 10-mL volumetric flask and dissolve in and dilute to volume with hexane.

Stearic Acid Solution Transfer 250 mg of stearic acid into another 10-mL volumetric flask, and dissolve in and dilute to volume with hexane.

Procedure Spot 2 µL of the *Sample Solution* and 1 µL of the *Stearic Acid Solution* approximately 1.5 cm from the bottom of the plate, allow the spots to dry, and then place the plate in a suitable chromatographic chamber containing a 4:4:92 (v/v) mixture of acetone:glacial acetic acid:hexane. Develop by ascending chromatography until the solvent front travels 15 cm beyond the sample spot. Remove the plate from the chamber, dry thoroughly in air, and spray evenly with a saturated solution of chromium trioxide in sulfuric acid. Immediately place the sprayed plate on a hot plate in a hood, heat it to about 200°, char until white fumes of sulfur trioxide cease to evolve, and cool to room temperature. The spots from the *Sample Solution* are located according to the following R_f values: *Stearic Acid*: 1.00; *Fatty Acid*: 1.00; *Acylated Monolactic Acid*: 0.84; *Acetylated Dilactic Acid*: 0.76; *Aceylated Trilactic Acid*: 0.68; and *Tetralactic Acid*: 0.62.

Lead Not more than 2 mg/kg.

The following specifications should conform to the representations of the vendor: *Acid Value*, *Acylated Monolactic Acid*, *Acylated Polylactic Acid*, *Free Fatty Acids*, *Total Lactic Acid*, *Saponification Value*, and *Water*.

TESTS

Assay for Acylated Monolactic Acid, **Acylated Polylactic Acid**, and **Free Fatty Acids** Transfer about 100 mg of sample into a small, conical flask fitted with a suitable reflux condenser. Add 5.0 mL of a solution prepared by dissolving 14 g of boron trifluoride in methanol to make 100 mL (a commercial reagent, 14% w/v, may be used; Applied Science, or equivalent). Swirl to mix, and reflux for 15 min. Cool, transfer the reaction mixture with the aid of 10 mL of chromatographic-grade hexane to a 60-mL separator, and add 10 mL of water and 10 mL of saturated sodium chloride solution. Shake, allow the mixture to separate, then drain and discard the lower, aqueous layer. Pass the hexane layer through 6 g of anhydrous sodium sulfate into a suitable flask. Inject 0.5

to 2.0 μL of the hexane solution obtained into a suitable gas chromatographic apparatus, or equivalent, using a 10-μL capacity Hamilton fixed needle, or equivalent. (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and a 1.2-m × 6.3-mm (id) column packed with 20% SE-30 or SE-52, or equivalent grades of silicone rubber gums, on Chromosorb P or W or Diatoport S, or equivalent grades of diatomaceous material. Maintain the column between 150° and 310°, heated at a rate of 4°/min. Set the inlet port temperature to 335° and the detector to 315°. Use helium as the carrier gas at a flow rate of about 54 mL/min throughout the determination. Use a recorder that has an attenuator switch, a range of 0- to 1-mV, and a 1-s full-scale deflection at a chart speed of 12.7 mm/min. Adjust the sample size so that the major peak is not attenuated more than ×8. From the chromatogram so obtained, identify the peaks by their relative position on the chart. The esters, appearing in the order of increasing number of carbon atoms in the fatty acid and in order of increasing length of the polymer, are eluted as follows:

- myristate
- palmitate
- stearate
- palmitoyl lactylate (2-palmitoyloxypropionate)
- stearoyl lactylate (2-stearoyloxypropionate)
- palmitoyl lactoyl lactylate
- stearoyl lactoyl lactylate
- palmitoyl dilactoyl lactylate
- stearoyl dilactoyl lactylate
- palmitoyl trilactoyl lactylate
- stearoyl trilactoyl lactylate
- palmitoyl tetralactoyl lactylate

Other esters may be determined by interpolation of a conventional carbon number-retention plot.

Determine the composition of the sample, using the area normalization method, by the formula

$$\%_i = 100A_i/\sum(A_i + \ldots + A_n),$$

in which i represents the component of interest, A_i is the equalized area for the component of interest, and $\sum(A_i + \ldots + A_n)$ is the sum of the equalized areas.

If free and polylactic acids are present, as determined below, the results should be corrected by multiplying $\%_i$ by [(100 − % free and polylactic acid)/100].

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIB, using a 10-g sample.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Total Free and Polylactic Acids Transfer about 500 mg of sample, previously melted and accurately weighed, into a 50-mL glass-stoppered separator with the aid of 15 mL of hexane, and add 10 mL of water. Invert the separator ten times, and allow it to stand until the layers have separated. Filter the aqueous layer through a plug of glass wool into a 125-mL flask, wash the hexane with two 10-mL portions of water, and combine the aqueous layers. Add 5.0 mL of 0.1 *N* sodium hydroxide to the flask, and then heat the flask on a steam bath for 15 min under a nitrogen atmosphere. Titrate with 0.1 *N* hydrochloric acid, using phenolphthalein TS as the indicator, to the disappearance of the pink color. Conduct a blank determination (see *General Provisions*), using 30 mL of water and 5.0 mL of 0.1 *N* sodium hydroxide, and make any necessary correction. Calculate the percent of free and polylactic acids in the sample by the formula

$$[(B - S) \times 9.008]/W,$$

in which $B - S$ represents the difference, in milliliters, between the volumes of 0.1 *N* hydrochloric acid required for the blank and for the sample, respectively; 9.008 is an equivalence factor for the lactic acid; and W is the weight, in grams, of the sample.

Total Lactic Acid Transfer an accurately weighed portion of melted sample, equivalent to between 140 mg and 170 mg of lactic acid, into a 250-mL Erlenmeyer flask. Pipet 20 mL of 0.5 *N* alcoholic potassium hydroxide into the flask, connect an air condenser, at least 65 cm long, and reflux for 30 min. Run a blank determination (see *General Provisions*) using the same volume of 0.5 *N* alcoholic potassium hydroxide. Add 20 mL of water to each flask, then disconnect the condensers, evaporate to a volume of about 20 mL, and cool to about 40°. Add methyl red TS to each flask, and titrate the blank with 0.5 *N* hydrochloric acid. While swirling the sample flask, add exactly the same volume of 0.5 *N* hydrochloric acid. Add 50 mL of hexane to each flask. Swirl the sample flask vigorously to dissolve the fatty acids, then quantitatively transfer the contents of each flask into separate 250-mL separators, and shake for 30 s. Collect the aqueous phases in 300-mL Erlenmeyer flasks, wash the hexane solutions with 50 mL of water, and combine the wash solution with the original aqueous phases in the Erlenmeyer flasks, discarding the hexane solution. Titrate with 0.1 *N* potassium hydroxide, using phenolphthalein TS as the indicator, to a pink color that persists for at least 30 s. Calculate the percent of total lactic acid by the formula

$$[9.008(S - B)(N)]/W,$$

in which 9.008 is the equivalence factor for lactic acid; $(S - B)$ is the difference, in milliliters, between the volumes of 0.1 *N* potassium hydroxide required for the sample and for the blank, respectively; N is the exact normality of the potassium hydroxide solution; and W is the weight, in grams, of the sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight, plastic-lined containers in a cool, dry place.

Lanolin, Anhydrous

Wool Fat

INS: 913 CAS: [8006-54-0]

DESCRIPTION

Lanolin, Anhydrous, occurs as a purified, yellow-white, semisolid, fatlike substance. It is extracted from the wool of sheep. It is insoluble in water, but mixes with about twice its weight of water without separation. It is soluble in chloroform and in ether.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Acid Value Not more than 1.12.
Iodine Value Between 18 and 36.
Lead Not more than 3 mg/kg.
Loss on Heating Not more than 0.5%.
Melting Range Between 36° and 42°.

TESTS

Acid Value Determine as directed for *Method I* under *Acid Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Proceed as directed under *Sample Solution for Lead Limit Test*, Appendix IV.
Loss on Heating Heat a 5-g sample on a steam bath, with frequent stirring, to constant weight.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.

Packaging and Storage Store in well-closed containers, preferably at a temperature not exceeding 30°.

Lard (Unhydrogenated)

DESCRIPTION

Lard (Unhydrogenated) is an off white fat obtained by dry or wet (steam) rendering of fresh fatty porcine tissues (cuttings and trimmings) shortly after slaughtering. *Rendered Lard* may be bleached, or bleached and deodorized. It is soft to semisolid at 27° and melts completely at 42°.

Rendered, *Bleached*, and *Bleached-Deodorized lards* are off white semisolids at 21° to 27°. *Bleached*, and *Bleached-Deodorized lards*, which are pale yellow and clear at 54°, differ from *Rendered Lard*, which is pale yellow, clear to hazy, and may contain extraneous matter.

Function Coating agent; texturizer.

SPECIFIC REQUIREMENTS

	Rendered Lard	*Bleached Lard*	*Bleached-Deodorized Lard*
Color (AOCS-Wesson)	Not more than 3.0 red	Not more than 1.5 red	Not more than 1.5 red
Free Fatty Acids (as oleic acid)	Not more than 1.0%	Not more than 1.0%	Not more than 0.1%
Hexane-Insoluble Matter	Not more than 0.1%	Not more than 0.05%	Not more than 0.05%
Iodine Value	Between 46 and 70	Between 46 and 70	Between 46 and 70
Unsaponifiable Matter	Not more than 1.5%	Not more than 1.5%	Not more than 1.5%
Water	Not more than 0.5%	Not more than 0.1%	Not more than 0.1%

GENERAL REQUIREMENTS

Identification Unhydrogenated Lard exhibits the following composition profile of fatty acids, determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<14:0	14:0	14:1	15:0	16:0
Weight % (Range):	<0.5	0.5–2.5	0.2	<0.1	20–32
Fatty Acid:	16:1	17:0	17:1	18:0	18:1
Weight % (Range):	1.7–5	<1.0	<0.7	5.0–24	35–62
Fatty Acid:	18:2	18:3	20:0	20:1	
Weight % (Range):	3.0–16	<2.0	<1.0	<1.0	

Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.

TESTS

Color (AOCS-Wesson) Determine as directed under *Color* (AOCS-Wesson), Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Hexane-Insoluble Matter If the sample is plastic or semisolid, soften a portion by warming it at a temperature not exceeding 60°, and then mix it thoroughly. Transfer 100 g of well-mixed sample into a 1500-mL wide-mouth Erlenmeyer flask, add 1000 mL of solvent hexane, and shake until the sample is dissolved. Filter the resulting solution through a 600-mL Corning ''C'' porosity, or equivalent, filtering funnel that previously has been dried at 105° for 1 h, cooled in a desiccator, and weighed. Wash the flask with two successive 250-mL portions of solvent hexane, and pass the washings through the filter. Dry the funnel at 105° for 1 h, cool to room

temperature in a desiccator, and weigh. From the gain in weight of the funnel, calculate the percentage of the hexane-insoluble matter in the sample.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 3-g sample.
Peroxide Value Accurately weigh about 10 g of sample, add 30 mL of a 3:2 mixture of glacial acetic acid:chloroform, and mix. Add 1 mL of a saturated solution of potassium iodide, mix the solution for 1 min, add 100 mL of water, and immediately begin titrating with 0.05 *N* sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value, as milliequivalents of peroxide per kilogram of sample, by the formula

$$[S \times N \times 1000]/W,$$

in which S is the net volume, in milliliters, of sodium thiosulfate solution required for the sample; N is the exact normality of the sodium thiosulfate solution; and W is the weight, in grams, of the sample taken.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB; however, in the *Procedure*, use 50 mL of chloroform in place of 35 to 40 mL of methanol to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Laurel Leaf Oil

Bay Leaf Oil

CAS: [8006-78-8]

View IR

DESCRIPTION

Laurel Leaf Oil occurs as a light yellow to yellow liquid with an aromatic, spicy odor. It is the oil obtained by steam distillation from the leaves of *Laurus nobilis* L. (Fam. Lauraceae). It is soluble in most fixed oils, and it is soluble with cloudiness in mineral oil and in propylene glycol. It is insoluble in glycerin.

> **Note**: The oil from *Laurus nobilis* L. should not be confused with that of the West Indian bay tree or the California bay laurel.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 3.0.
Angular Rotation Between −10° and −19°.
Refractive Index Between 1.465 and 1.470 at 20°.
Saponification Value Between 15 and 45.
Saponification Value after Acetylation Between 36 and 85.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.905 and 0.929.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Saponification Value after Acetylation Proceed as directed under *Total Alcohols*, Appendix VI, using about 2.5 g of acetylated oil, accurately weighed. Calculate the saponification value by the formula

$$28.05 \times A/B,$$

in which A is the number of milliliters of 0.5 N alcoholic potassium hydroxide consumed in the titration and B is the weight, in grams, of the acetylated oil.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 80% alcohol and remains in solution upon dilution to 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Lauric Acid

Dodecanoic Acid

$$CH_3(CH_2)_{10}COOH$$

$C_{12}H_{24}O_2$ Formula wt 200.32

CAS: [143-07-7]

DESCRIPTION

Lauric Acid occurs as a white or faintly yellow, somewhat glossy, crystalline solid or powder. It is obtained from coconut

oil and other plant fats. It is practically insoluble in water, but is soluble in alcohol, in chloroform, and in ether.

Function Component in the manufacture of other food-grade additives; defoaming agent.

REQUIREMENTS
Acid Value Between 252 and 287.
Iodine Value Not more than 3.0.
Lead Not more than 0.1 mg/kg.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 253 and 287.
Titer (Solidification Point) Between 26° and 44°.
Unsaponifiable Matter Not more than 0.3%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 10-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 3 g of sample, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, Appendix IIB.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Lavandin Oil, Abrial Type

CAS: [8022-15-9]

View IR

DESCRIPTION

Lavandin Oil, Abrial Type, occurs as a pale yellow to yellow liquid with a slight, camphoraceous odor that is strongly suggestive of lavender. It is obtained by steam distillation of the fresh flowering tops of a hybrid, *Lavandula abrialis* unofficial (Fam. Labiatae), of true lavender, *Lavandula officinalis*, or of spike lavender, *Lavandula latifolia*. It is soluble in most fixed oils and in propylene glycol. It is soluble with opalescence in mineral oil, but it is relatively insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 28.0% and not more than 35.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −2° and −5°.
Refractive Index Between 1.460 and 1.464 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.885 and 0.893.

TESTS

Assay Determine as directed in *Ester Determination* under *Esters*, Appendix VI, using about 3 g of sample, accurately weighed, and 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol. A slight opalescence sometimes develops on further dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Lavender Oil

CAS: [8000-28-0]

FEMA: 2622

View IR

DESCRIPTION

Lavender Oil occurs as a colorless or yellow liquid with the characteristic odor and taste of lavender flowers. It is the volatile oil obtained by steam distillation from the fresh flowering tops of *Lavandula officinalis* Chaix ex Villars (*Lavan-*

dula vera De Candolle) (Fam. Labiatae). It is soluble in alcohol and in most vegetable oils, but is insoluble in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 35.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Alcohol Passes test.
Angular Rotation Between −3° and −10°.
Refractive Index Between 1.459 and 1.470 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.875 and 0.888.

TESTS

Assay Determine as directed for *Ester Determination* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed, and 98.15 as the equivalence factor (*e*) in the calculation.
Alcohol Transfer 5 mL of sample into a narrow, graduated, glass-stoppered, 10-mL cylinder; add 5 mL of water; and shake. The volume of the oil does not diminish.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 4 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers.

Lecithin

INS: 322 CAS: [8002-43-5]

DESCRIPTION

Lecithin, both natural and refined grades, occurs as a substance varying in consistency from plastic to fluid depending on free fatty acid and oil content and on the presence or absence of other diluents. Its color varies from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached. Lecithin is obtained from soybeans and other plant sources. It is a complex mixture of acetone-insoluble phosphatides that consists chiefly of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates. Refined grades of Lecithin may contain any of these components in varying proportions and combinations depending on the type of fractionation used. In its oil-free form, the preponderance of triglycerides and fatty acids is removed and the product contains 90% or more of phosphatides representing all or certain fractions of the total phosphatide complex. Edible diluents, such as cocoa butter and vegetable oils, often replace soybean oil to improve functional and flavor characteristics. Lecithin is only partially soluble in water, but it readily hydrates to form emulsions. The oil-free phosphatides are soluble in fatty acids, but they are practically insoluble in fixed oils. When all phosphatide fractions are present, Lecithin is partially soluble in alcohol and practically insoluble in acetone.

Function Antioxidant; emulsifier.

REQUIREMENTS

Acetone-Insoluble Matter (as phosphatides) Not less than 50.0%.
Acid Value Not more than 36.
Hexane-Insoluble Matter Not more than 0.3%.
Lead Not more than 1 mg/kg.
Peroxide Value Not more than 100.
Water Not more than 1.5%.

TESTS

Acetone-Insoluble Matter (as phosphatides)

Purification of Phosphatides Dissolve 10 g of sample in 20 mL of petroleum ether, add 50 mL of acetone to the solution, chill, and decant. Dry the solids under flowing nitrogen under a hood. Dissolve 5 g of the solids in 10 mL of petroleum ether, and add 25 mL of acetone to the solution. Transfer approximately equal portions of the precipitate to each of two 40-mL centrifuge tubes, using additional portions of acetone to facilitate the transfer. Stir thoroughly, dilute to 40 mL with acetone, stir again, chill for 15 min in an ice bath, stir again, and then centrifuge for 5 min. Decant the acetone, crush the solids with a stirring rod, refill the tube with acetone, stir, chill, centrifuge, and decant as before. The solids after the second centrifugation require no further purification and may be used for preparing the *Phosphatide–Acetone Solution*. Five grams of the purified phosphatides are required to saturate about 16 L of acetone.

Phosphatide–Acetone Solution Add a quantity of purified phosphatides to sufficient acetone, previously cooled to about 5°, to form a saturated solution, and maintain the mixture at this temperature for 2 h, shaking it vigorously at 15-min intervals. Decant the solution through a rapid filter paper, avoiding the transfer of any undissolved solids to the paper and conducting the filtration under refrigerated conditions (not above 5°).

Procedure If the sample is plastic or semisolid, soften a portion of it by warming it in a water bath at a temperature not exceeding 60°, and then mix it thoroughly. Transfer about 2 g of a well-mixed sample, accurately weighed, into a 40-mL centrifuge tube, previously tared with a glass stirring rod, and add 15 mL of *Phosphatide–Acetone Solution* from a buret. Warm the mixture in a water bath until the sample melts, but avoid evaporation of the acetone. Stir until the sample is completely disintegrated and dispersed, transfer the tube into an ice bath, chill for 5 min, remove from the ice bath, and add about 10 mL of *Phosphatide–Acetone Solution*, previously chilled for 5 min in an ice bath. Stir the mixture to complete dispersion of the sample, dilute to 40 mL with chilled (5°) *Phosphatide–Acetone Solution*, stir to complete dispersion of the sample, and return the tube and contents to the ice bath for 15 min. Subsequently stir again while still in the ice bath, remove the stirring rod, and centrifuge the mixture immediately for 5 min. Decant the supernatant liquid from the centrifuge tube; crush the centrifuged solids with the stirring rod; refill the tube to the 40-mL mark with chilled (5°) *Phosphatide–Acetone Solution*; and repeat the chilling, stirring, centrifugation, and decantation procedure. After the second centrifugation and decantation of the supernatant acetone, again crush the solids with the stirring rod, and place the tube and its contents in a horizontal position at room temperature until the excess acetone has evaporated. Mix the residue again, dry the centrifuge tube and its contents at 105° for 45 min in a forced-draft oven, cool, and weigh. Calculate the percentage of acetone-insoluble substances by the formula

$$(100R/S) - B,$$

in which R is the weight, in grams, of residue; S is the weight, in grams, of the sample taken; and B is the percentage of hexane-insoluble matter determined as directed under *Hexane-Insoluble Matter* (below).

Acid Value If the sample is plastic or semisolid, soften a portion by warming it in a water bath at a temperature not exceeding 60°, and then mix it thoroughly. Transfer about 2 g of a well-mixed sample, accurately weighed, into a 250-mL Erlenmeyer flask, and dissolve it in 50 mL of petroleum ether. Add 50 mL of ethanol, previously neutralized to phenolphthalein with 0.1 *N* sodium hydroxide, to this solution, and mix well. Using phenolphthalein TS as the indicator, titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for 5 s. Calculate the *Acid Value* by the formula

$$5.6 \times A/W,$$

in which A is the volume, in milliliters, of 0.1 *N* sodium hydroxide consumed, and W is the weight, in grams, of the sample taken.

Hexane-Insoluble Matter If the sample is plastic or semisolid, soften a portion by warming it at a temperature not exceeding 60°, and then mix it thoroughly. Transfer 10 g of well-mixed sample into a 250-mL wide-mouth Erlenmeyer flask, add 100 mL of solvent hexane, and shake until the sample is dissolved. Filter the resulting solution through a 30-mL Corning ''C'' porosity, or equivalent, filtering funnel that previously has been dried at 105° for 1 h, cooled in a desiccator, and weighed. Wash the flask with two successive 25-mL portions of solvent hexane, and pass the washings through the filter. Dry the funnel at 105° for 1 h, cool to room temperature in a desiccator, and weigh. From the gain in weight of the funnel, calculate the percentage of the hexane-insoluble matter in the sample.

Lead Determine as directed for *Method II* in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Peroxide Value Transfer about 10 g of sample, accurately weighed, into a suitable container, add 30 mL of a 3:2 solution of glacial acetic acid:chloroform, and mix. Add 1 mL of a saturated solution of potassium iodide, mix, and allow to stand for 10 min. Add 100 mL of water, begin titrating with 0.05 *N* sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value, as milliequivalents of peroxide per kilograms of sample, by the formula

$$[S \times N \times 1000]/W,$$

in which S is the net volume, in milliliters, of sodium thiosulfate solution required for the sample; N is the exact normality of the sodium thiosulfate solution; and W is the weight, in grams, of the sample taken.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Lemongrass Oil

CAS: [8007-02-1]

FEMA: 2624

View IR

DESCRIPTION

Lemongrass Oil is a volatile oil prepared by steam distillation of freshly cut and partially dried cymbopogon grasses indigenous to tropical and subtropical areas. Two types of Lemongrass Oil are commercially available. The *East Indian* type, also known as Cochin, Native, and British Indian Lemongrass Oil, usually occurs as a dark yellow to light brown-red liquid with a pronounced heavy lemon odor. The *West Indian* type, also known as Madagascar, Guatemala, or other country of origin Lemongrass Oil, occurs as a light yellow to light brown liquid with a lemon odor of a lighter character than the East Indian type oil. Lemongrass Oils are soluble in mineral oil, freely soluble in propylene glycol, but practically insoluble

in water and in glycerin. The East Indian type dissolves readily in alcohol, but the West Indian type yields cloudy solutions.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is the East Indian or West Indian type.
Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown under *Infrared Spectra* using the same test conditions as specified therein.
Assay Not less than 75.0%, by volume, of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between −10° and 0°.
Refractive Index Between 1.483 and 1.489.
Solubility in Alcohol Passes test.
Specific Gravity *East Indian Type*: Between 0.894 and 0.904; *West Indian Type*: Between 0.869 and 0.894.

TESTS

Assay Mix 50.0 mL of sample with 500 mg of tartaric acid, shake for 5 min, and filter. Dry the filtered oil over anhydrous sodium sulfate, and then pipet 10.0 mL of the clear, treated oil into a 150-mL cassia flask. Add 75 mL of a 30% solution of sodium bisulfite, stopper the flask, and shake until a semisolid to solid sodium bisulfite addition product has formed. Allow the mixture to stand at room temperature for 5 min, then loosen the stopper, and immerse the flask in a water bath heated to between 85° and 90°. Maintain the water bath at this temperature, shaking the flask occasionally, until the addition product dissolves, and then continue heating and intermittently shaking for another 30 min. When the liquids have separated completely, add enough 30% sodium bisulfite solution to raise the lower level of the oily layer within the graduated portion of the flask's neck. Calculate the percentage, by volume, of the citral by the formula

$$100 - (V \times 10),$$

in which V is the number of milliliters of separated oil in the graduated neck of the cassia flask.
Angular Rotation Determine as directed under *Optical Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. *East Indian Type*: One milliliter dissolves in 3 mL of 70% alcohol, usually with slight turbidity; *West Indian Type*: Yields a cloudy solution with 70%, 80%, 90%, and 95% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.

Lemon Oil, Coldpressed

Lemon Oil, Expressed

CAS: [8008-56-8]

FEMA: 2625

View IR

DESCRIPTION

Lemon Oil, Coldpressed, occurs as a pale to deep yellow or green-yellow liquid with the characteristic odor and taste of the outer part of fresh lemon peel. It is the volatile oil obtained by expression, without the aid of heat, from the fresh peel of the fruit of *Citrus limon* L. Burmann filius (Fam. Rutaceae) with or without the previous separation of the pulp and the peel. It is miscible with dehydrated alcohol and with glacial acetic acid. It may contain a suitable antioxidant.

Note: Do not use Lemon Oil that has a terebinthine odor.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is the California type or the Italian type.
Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay *California Type*: Not less than 2.2% and not more than 3.8% of aldehydes, calculated as citral ($C_{10}H_{16}O$); *Italian Type*: Not less than 3.0% and not more than 5.5% of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between +57° and +65.6°.
Refractive Index Between 1.473 and 1.476 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.849 and 0.855.
Ultraviolet Absorbance *California Type*: Not less than 0.2; *Italian Type*: Not less than 0.49.

TESTS

Assay Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones,* Appendix VI, using about 5 mL of sample, accurately weighed. Allow the mixture to stand for 15 min, occasionally shaking, before titrating, and use 76.12 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 95% alcohol, sometimes with a slight haze.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Ultraviolet Absorbance Determine as directed under *Ultraviolet Absorbance of Citrus Oils*, Appendix VI, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 315 ± 3 nm.

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.

Lemon Oil, Desert Type, Coldpressed

Lemon Oil Arizona

View IR

DESCRIPTION

Lemon Oil, Desert Type, Coldpressed, occurs as a pale to deep yellow or green-yellow liquid with the characteristic odor and taste of the outer part of fresh lemon peel. It is the volatile oil obtained by expression, without the aid of heat, from the fresh peel of the fruit of *Citrus limon* L. Burmann filius (Fam. Rutaceae), with or without the previous separation of the pulp and peel. It is miscible with dehydrated alcohol and with glacial acetic acid. It may contain a suitable antioxidant.

Note: Do not use if it has a terebinthine odor.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 1.7% of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between +67° and +78°.
Refractive Index Between 1.473 and 1.476.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.846 and 0.851.
Ultraviolet Absorbance Not less than 0.20.

TESTS

Assay Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 5 mL of sample, accurately weighed. Allow the mixture to stand for 15 min, shaking occasionally, before titrating, and use 76.12 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of alcohol, sometimes with a slight haze.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Ultraviolet Absorbance Determine as directed under *Ultraviolet Absorbance of Citrus Oils*, Appendix VI, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 315 ± 3 nm.

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.

Lemon Oil, Distilled

View IR

DESCRIPTION

Lemon Oil, Distilled, occurs as a colorless to pale yellow liquid with the characteristic odor of fresh lemon peel. It is the volatile oil obtained by distillation from the fresh peel or juice of the fruit of *Citrus limon* L. Burmann filius (Fam. Rutaceae), with or without the previous separation of the juice, pulp, and peel. It is soluble in most fixed oils, in mineral oil, and in alcohol (with haze). It is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those shown in a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Aldehydes Between 1.0% and 3.5% of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between +55° and +75°.
Refractive Index Between 1.470 and 1.475 at 20°.
Specific Gravity Between 0.842 and 0.856.
Ultraviolet Absorbance Not more than 0.01.

TESTS

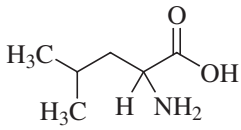
Aldehydes Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 5 mL of sample, accurately weighed, and 76.12 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand at room temperature for 1 h before titrating.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see *General Provisions*).
Ultraviolet Absorbance Determine as directed under *Ultraviolet Absorbance of Citrus Oils*, Appendix VI, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 315 ± 5 nm.

Packaging and Storage Store in a cool place protected from light in full, tight containers.

DL-Leucine

DL-2-Amino-4-methylvaleric Acid



$C_6H_{13}NO_2$ Formula wt 131.17

CAS: [328-39-2]

View IR

DESCRIPTION

DL-Leucine occurs as small, white crystals or as a crystalline powder. It is freely soluble in water, slightly soluble in alcohol, and insoluble in ether. It melts with decomposition at about 290°. The pH of a 1:100 aqueous solution is between 5.5 and 7.0. It is optically inactive.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_{13}NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 400 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to the first appearance of a pure-green color or until the blue color disappears completely.

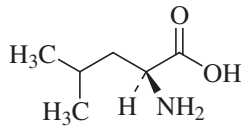
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 13.12 mg of $C_6H_{13}NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

L-Leucine

L-2-Amino-4-methylvaleric Acid



$C_6H_{13}NO_2$ Formula wt 131.17

INS: 641 CAS: [61-90-5]

View IR

DESCRIPTION

L-Leucine occurs as small, white, lustrous plates, or as a white, crystalline powder. One gram dissolves in about 40 mL of water and in about 100 mL of glacial acetic acid. It is sparingly soluble in alcohol, and soluble in dilute hydrochloric acid and in solutions of alkali hydroxides and carbonates.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_{13}NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.2%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +14.5° and +16.5°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between +14.8° and +16.8°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 400 mg of sample, previously dried at 105° for 3 h and accurately weighed, into a 250-mL flask.

Dissolve the sample in 3 mL of formic acid and about 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 4 g of sample in sufficient 6 *N* hydrochloric acid to make 100 mL.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

Lime Oil, Coldpressed

Lime Oil, Expressed

CAS: [8008-26-2]

FEMA: 2631

View IR

DESCRIPTION

Lime Oil, Coldpressed, occurs as a yellow to brown-green to green liquid that often shows a waxy separation and has a fresh lime-peel odor. It is the volatile oil obtained by expression from the fresh peel or crushed whole fruit of *Citrus aurantifolia* Swingle (Mexican type) or *Citrus latifolia* (Tahitian type) (Fam. Rutaceae). It is soluble in most fixed oils and in mineral oil, but is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is the Mexican or Tahitian type.

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay *Mexican Type*: Not less than 4.5% and not more than 8.5% of aldehydes (as citral); *Tahitian Type*: Not less than 3.2% and not more than 7.5% of aldehydes (as citral).

Angular Rotation *Mexican Type*: Between +35° and +41°; *Tahitian Type*: Between +38° and +53°.

Refractive Index *Mexican Type*: Between 1.482 and 1.486; *Tahitian Type*: Between 1.476 and 1.486.

Residue on Evaporation *Mexican Type*: Between 10.0% and 14.5%; *Tahitian Type*: Between 5.0% and 12.0%.

Specific Gravity *Mexican Type*: Between 0.872 and 0.881; *Tahitian Type*: Between 0.858 and 0.876.

Ultraviolet Absorbance *Mexican Type*: Not less than 0.45; *Tahitian Type*: Not less than 0.24.

TESTS

Assay Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 5 mL of sample, accurately weighed. Allow the mixture to stand for 1 h, occasionally shaking, before titrating, and use 76.12 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, using an Abbé or other refractometer of equal or greater accuracy.

Residue on Evaporation Determine as directed under *Residue on Evaporation*, Appendix VI, using a 3-g sample and heating for 6 h.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Ultraviolet Absorbance Determine as directed under *Ultraviolet Absorbance of Citrus Oils*, Appendix VI, using about 20 mg of sample, accurately weighed. The maximum absorbance occurs at 315 ± 3 nm.

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.

Lime Oil, Distilled

View IR

DESCRIPTION

Lime Oil, Distilled, occurs as a colorless to green-yellow liquid with a mild citrus, floral odor. It is the volatile oil obtained by distillation from the juice or the whole crushed fruit of *Citrus aurantifolia* Swingle (Fam. Rutaceae). It is soluble in most fixed oils and in mineral oil, but it is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as

those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Aldehydes Between 0.5% and 2.5% of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between +34° and +47°.
Refractive Index Between 1.474 and 1.477 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.855 and 0.863.

TESTS

Aldehydes Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 5 g of sample, accurately weighed, and 76.12 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand at room temperature for 15 min before titrating.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 5 mL of 90% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Limestone, Ground

DESCRIPTION

Limestone, Ground, is produced as a fine, white to off white, microcrystalline powder mainly consisting of calcium carbonate. It is obtained by crushing, grinding, and classifying naturally occurring limestone benefited by flotation and/or air classification. It is stable in air. It is practically insoluble in water and in alcohol. The presence of any ammonium salt or carbon dioxide increases its solubility in water, but the presence of any alkali hydroxide reduces its solubility.

Function Texturizing and release agent and modifier for chewing gum base and chewing gum.

REQUIREMENTS

Identification A sample dissolves with effervescence in 1 *N* acetic acid, in 2.7 *N* hydrochloric acid, and in 1.7 *N* nitric acid, and the resulting solutions, after boiling, give positive tests for *Calcium*, Appendix IIIA.
Assay Not less than 94.0% and not more than 100.5% of $CaCO_3$ after drying.
Acid-Insoluble Substances Not more than 2.5%.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Lead Not more than 3 mg/kg.
Loss on Drying Not more than 2.0%.
Magnesium and Alkali Salts Not more than 3.5%.

TESTS

Assay Transfer about 200 mg of sample, previously dried at 200° for 4 h and accurately weighed, into a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 mL of 2.7 *N* hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the sample. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute to about 100 mL with water. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, add 15 mL of 1 *N* potassium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 5.004 mg of $CaCO_3$.
Acid-Insoluble Substances Suspend 5 g of sample in 25 mL of water, agitate while cautiously adding 25 mL of 1:2 hydrochloric acid, and add water to make a volume of about 200 mL. Heat the solution to boiling, cover, digest on a steam bath for 1 h, cool, and filter. Wash the precipitate with water until the last washing shows no chloride with silver nitrate TS, and then ignite it. The weight of the residue does not exceed 125 mg.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of 2.7 *N* hydrochloric acid.
Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB.
Lead

Sample Solution Cautiously dissolve 5 g of sample in 25 mL of 1:2 hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in about 15 mL of water, and dilute to 25 mL (1 mL = 200 mg).

Procedure Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 5-mL portion of the *Sample Solution*, and 3 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 200° for 4 h.
Magnesium and Alkali Salts Mix 1 g of sample with 40 mL of water, carefully add 5 mL of hydrochloric acid, mix, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add 6 *N* ammonium hydroxide, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-mL cylinder, dilute to 100 mL with water, and let it stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper, and place 50 mL of the clear filtrate in a platinum dish. Add

0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 17.5 mg.

Packaging and Storage Store in well-closed containers.

Linaloe Wood Oil

CAS: [8006-86-8]

View IR

DESCRIPTION

Linaloe Wood Oil occurs as a colorless to yellow liquid with a pleasant, flowery odor. It is the volatile oil obtained by steam distillation from the wood of *Bursera delpechiana* Poiss. (Fam. Burseraceae) and other *Bursera* species. It is soluble in most fixed oils and in propylene glycol. It is soluble in mineral oil, but it becomes opalescent or turbid on dilution. It is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 85.0% of alcohols, calculated as linalool ($C_{10}H_{18}O$).
Acid Value Not more than 3.0.
Angular Rotation Between −5° and −13°.
Ester Value Between 40 and 75.
Refractive Index Between 1.459 and 1.463 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.876 and 0.883.

TESTS

Assay Determine as directed under *Linalool Determination*, Appendix VI, using about 1.5 g of acetylated oil, accurately weighed, for the saponification.
Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed in *Ester Value* under *Esters*, Appendix VI, using about 2.5 g of sample, accurately weighed.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 5 mL of 60% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Linoleic Acid

(*Z*),(*Z*)-9,12-Octadecadienoic Acid

$$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$$

$C_{18}H_{32}O_2$ Formula wt 280.45

CAS: [60-33-3]

DESCRIPTION

Linoleic Acid occurs as a colorless to pale yellow, oily liquid that is easily oxidized by air. It is an essential fatty acid and the major constituent of many vegetable oils, including cottonseed, soybean, peanut, corn, sunflower seed, safflower, poppy seed, and linseed. Its specific gravity is about 0.901, and its refractive index is about 1.469. It has a boiling point ranging from 225° to 230° and a melting point around −5°. One milliliter dissolves in 10 mL of petroleum ether. It is freely soluble in ether; soluble in absolute alcohol and in chloroform; and miscible with dimethylformamide, fat solvents, and oils. It is insoluble in water.

Function Flavoring adjuvant; nutrient.

REQUIREMENTS

Identification Linoleic Acid exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII.

Fatty Acid:	14:0	16:0	18:0	18:1	18:2	18:3
Weight % (Range):	<1.0	3–5	<1.0	<25.0	>60.0	<9.0

Assay Not less than 60.0% of fatty acid C18:2, equivalent to $C_{18}H_{32}O_2$, calculated on the anhydrous basis.
Acid Value Between 196 and 202.
Iodine Value Between 145 and 160.
Lead Not more than 2 mg/kg.
Residue on Ignition Not more than 0.01%.
Unsaponifiable Matter Not more than 2.0%.
Water Not more than 0.5%.

TESTS

Assay Determine as directed under *Fatty Acid Composition*, Appendix VII.

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 10-g sample.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII, using a 10-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Locust (Carob) Bean Gum

Locust Bean Gum; Carob Bean Gum

INS: 410 CAS: [9000-40-2]

DESCRIPTION

Locust (Carob) Bean Gum occurs as a white to yellow-white powder. It is obtained from the ground endosperms of *Ceratonia siliqua* (L.) Taub. (Fam. Leguminosae). It consists chiefly of a high-molecular-weight hydrocolloidal polysaccharide, composed of galactose and mannose units combined through glycosidic linkages, which may be described chemically as a galactomannan. It is dispersible in either hot or cold water, forming a sol having a pH between 5.4 and 7.0, which may be converted to a gel by the addition of small amounts of sodium borate.

Function Stabilizer; thickener.

REQUIREMENTS

Identification

A. Transfer a 2-g sample into a 400-mL beaker, moisten it with about 4 mL of isopropyl alcohol, while vigorously stirring add 200 mL of cold water, and continue stirring until the gum is uniformly dispersed. An opalescent, slightly viscous solution forms. Save this solution for *Identification Test B*.

B. Transfer 100 mL of the solution prepared in *Identification Test A* into another 400-mL beaker, heat the mixture in a boiling water bath for about 10 min, and then cool to room temperature. The viscosity of the solution increases appreciably (*distinction from guar gum*).

Acid-Insoluble Matter Not more than 4.0%.
Arsenic Not more than 3 mg/kg.
Ash (Total) Not more than 1.2%.
Galactomannans Not less than 75.0%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 14.0%.
Protein Not more than 7.0%.
Starch Passes test.

TESTS

Acid-Insoluble Matter Transfer 1.5 g of sample, accurately weighed, into a 250-mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid. Cover the beaker with a watch glass, and heat the mixture on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. Subsequently add about 500 mg of a suitable filter aid, previously dried for 3 h at 105° and accurately weighed, and filter through a tared, sintered-glass filter crucible. Wash the residue several times with hot water, dry the crucible and its contents at 105° for 3 h, cool in a desiccator, and weigh. The difference between the weight of the filter aid and that of the residue is the weight of the *Acid-Insoluble Matter*.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Galactomannans Add the percentages of *Acid-Insoluble Matter*, *Total Ash*, *Loss on Drying*, and *Protein*, and subtract the total from 100%. The difference represents the percentage of galactomannans in the sample.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 5 h.
Protein Determine as directed under *Nitrogen Determination*, Appendix IIIC, using about 3.5 g of sample, accurately weighed, transferred into a 500-mL Kjeldahl flask. The percent of nitrogen determined, multiplied by 6.25, gives the percent of protein in the sample.
Starch Add a few drops of iodine TS to a 1:10 aqueous solution of the sample. No blue color appears.

Packaging and Storage Store in well-closed containers.

Lovage Oil

CAS: [8016-31-7]

View IR

DESCRIPTION

Lovage Oil occurs as a yellow-green-brown to deep brown liquid with a strong, characteristic aromatic odor and taste. It is the volatile oil obtained by steam distillation of the fresh root of the plant *Levisticum officinale* L. Koch syn. *Angelica levisticum*, Baillon (Fam. Umbelliferae). It is soluble in most fixed oils and slightly soluble, with opalescence, in mineral oil, but it is relatively insoluble in glycerin and in propylene glycol.

Note: This oil becomes darker and more viscous under the influence of air and light.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Between 2.0 and 16.0.
Angular Rotation Between −1° and +5°.
Refractive Index Between 1.536 and 1.554 at 20°.
Saponification Value Between 238 and 258.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.030 and 1.057.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using 1.5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 4 mL of 95% ethanol, sometimes with slight turbidity. The age of the oil has an adverse effect upon solubility.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

L-Lysine Monohydrochloride

2,6-Diaminohexanoic Acid Hydrochloride

$C_6H_{14}N_2O_2 \cdot HCl$ Formula wt 182.65

CAS: [657-27-2]

View IR

DESCRIPTION

L-Lysine Monohydrochloride occurs as a white or nearly white, free-flowing, crystalline powder. It is freely soluble in water, but it is almost insoluble in alcohol and in ether. It melts at about 260° with decomposition.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_6H_{14}N_2O_2 \cdot HCl$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 1.0%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +20.3° and +21.5°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between +20.4° and +21.4°, calculated on the dried basis.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Dissolve about 100 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 2 mL of formic acid, add exactly 15.0 mL of 0.1 *N* perchloric acid, and heat on a water bath for 30 min.

Caution: Handle perchloric acid in an appropriate fume hood.

After cooling, add 45 mL of glacial acetic acid, and titrate the excess perchloric acid with 0.1 *N* sodium acetate, determining the endpoint potentiometrically. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 9.133 mg of $C_6H_{14}N_2O_2 \cdot HCl$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed containers.

Mace Oil

CAS: [8007-12-3]

View IR

DESCRIPTION

Mace Oil occurs as a colorless to pale yellow liquid with the characteristic odor and taste of nutmeg. It is the volatile oil

obtained by steam distillation from the ground, dried arillode of the ripe seed of *Myristica fragrans* Houtt. (Fam. Myristicaceae). Two types of oil, the East Indian and the West Indian, are commercially available. It is soluble in most fixed oils and in mineral oil, but it is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is the East Indian or West Indian type.
Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation *East Indian Type*: Between +2° and +30°; *West Indian Type*: Between +20° and +45°.
Refractive Index *East Indian Type*: Between 1.474 and 1.488; *West Indian Type*: Between 1.469 and 1.480 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity *East Indian Type*: Between 0.880 and 0.930; *West Indian Type*: Between 0.854 and 0.880.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 4 mL of 90% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Magnesium Carbonate

$MgCO_3$ Formula wt, anhydrous 84.31
$4MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$ Formula wt, basic 485.65
$MgCO_3 \cdot H_2O$ Formula wt, monohydrate 102.33

INS: 504(i) CAS: anhydrous [546-93-0]
CAS: basic [39409-82-0]
CAS: monohydrate [23389-33-5]

DESCRIPTION

Magnesium Carbonate occurs as light, white, friable masses, or as a bulky, white powder. It is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate. It is stable in air. It is practically insoluble in water, to which, however, it imparts a slightly alkaline reaction. It is insoluble in alcohol, but dissolves, with effervescence, in dilute acids.

Function pH control; drying agent; color-retention agent; anticaking agent; carrier.

REQUIREMENTS

Identification When treated with 2.7 *N* hydrochloric acid, a sample dissolves with effervescence, and the resulting solution gives positive tests for *Magnesium*, Appendix IIIA.
Assay The equivalent of not less than 40.0% and not more than 43.5% of MgO.
Acid-Insoluble Substances Not more than 0.05%.
Calcium Oxide Not more than 0.6%.
Lead Not more than 2 mg/kg.
Soluble Salts Not more than 1%.

TESTS

Assay Dissolve about 1 g of sample, accurately weighed, in 30.0 mL of 1 *N* sulfuric acid, add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed, deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the weight of the sample taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the magnesium oxide present. Each milliliter of 1 *N* sulfuric acid is equivalent to 20.16 mg of MgO and to 28.04 mg of CaO.
Acid-Insoluble Substances Mix 5.0 g of sample with 75 mL of water; while agitating, add hydrochloric acid in small portions until no more of the sample dissolves; and boil for 5 min. If an insoluble residue remains, filter through a suitable tared porous bottom porcelain crucible, wash well with water until the last washing is free from chloride, ignite at 800° ± 25° for 45 min, cool, and weigh.

> **Note**: Avoid exposing the crucible to sudden temperature changes.

Calcium Oxide Dissolve about 1 g of sample, accurately weighed, in a mixture of 3 mL of sulfuric acid and 22 mL of water. Add 50 mL of alcohol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve them. Filter through a suitable tared porous bottom porcelain crucible, previously washed with 2 *N* sulfuric acid, water, and alcohol. Wash the crystals on the porous disk several times with a 2:1 (v/v) mixture of alcohol:2 *N* sulfuric acid. Ignite the crucible and contents at 450° ± 25° to constant weight. The weight of calcium sulfate so obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

> **Note**: Avoid exposing the crucible to sudden temperature changes.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Soluble Salts Mix 2.0 g of sample with 100 mL of a 1:1 (v/v) mixture of *n*-propyl alcohol:water. Heat the mixture to the

boiling point with constant stirring, cool to room temperature, add water to make 100 mL, and filter. Evaporate 50 mL of the filtrate on a steam bath to dryness, and dry in an oven at 105° for 1 h. The weight of the residue does not exceed 10 mg.

Packaging and Storage Store in well-closed containers.

Magnesium Chloride

$MgCl_2 \cdot 6H_2O$ Formula wt 203.30

INS: 511 CAS: [7791-18-6]

DESCRIPTION

Magnesium Chloride occurs as colorless flakes or crystals. It contains six molecules of water of hydration. It is hygroscopic, very soluble in water, and freely soluble in alcohol.

Function Color-retention agent; firming agent.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Magnesium* and for *Chloride*, Appendix IIIA.
Assay Not less than 99.0% and not more than 105.0% of $MgCl_2 \cdot 6H_2O$.
Ammonium Not more than 0.005%.
Lead Not more than 4 mg/kg.
Sulfate Not more than 0.03%.

TESTS

Assay Dissolve about 450 mg of sample, accurately weighed, in 25 mL of water, add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with 0.05 *M* disodium EDTA until the solution turns blue. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 10.16 mg of $MgCl_2 \cdot 6H_2O$.
Ammonium Dissolve 1 g of sample in 90 mL of water, and slowly add 10 mL of a freshly boiled and cooled solution of 1:10 sodium hydroxide. Allow the mixture to settle, then decant 20 mL of the supernatant liquid into a color comparison tube, dilute to 50 mL with water, and add 2 mL of Nessler's reagent. Any color does not exceed that produced by 10 μg of ammonium (NH_4) ion in 48 mL of water and 2 mL of the sodium hydroxide solution.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample, accurately weighed, in 10 mL of water; and 4 μg of lead (Pb) ion in the control.
Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 1-g sample does not exceed that shown in a control containing 300 μg of sulfate (SO_4).

Packaging and Storage Store in tight containers.

Magnesium Gluconate

$[CH_2OH(CHOH)_4COO]_2Mg$

$C_{12}H_{22}MgO_{14}$ Formula wt, anhydrous 414.60
$C_{12}H_{22}MgO_{14} \cdot 2H_2O$ Formula wt, dihydrate 450.63

INS: 580 CAS: anhydrous [3632-91-5]
CAS: dihydrate [59625-89-7]

DESCRIPTION

Magnesium Gluconate occurs as a white to off white powder or granulate. It is anhydrous, the dihydrate, or a mixture of both. It is very soluble in water and is sparingly soluble in alcohol. It is insoluble in ether.

Function Nutrient.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Magnesium*, Appendix IIIA.

B. Dissolve a quantity of sample in water, heating in a water bath at 60° if necessary, to obtain a *Test Solution* containing 10 mg/mL. Similarly, prepare a *Standard Solution* of USP Potassium Gluconate Reference Standard in water, diluting to 10 mg/mL. Apply separate 5-μL portions of the *Test Solution* and the *Standard Solution* on a suitable thin-layer chromatographic plate (see *Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent system consisting of a mixture of alcohol, water, ammonium hydroxide, and ethyl acetate (50:30:10:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry it at 110° for 20 min. Allow to cool, and spray with a spray reagent prepared as follows: Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 *N* sulfuric acid to volume, and mix. After spraying, heat the plate at 110° for about 10 min. The principal spot obtained from the *Test Solution* corresponds in color, size, and R_f value to that obtained from the *Standard Solution*.
Assay Not less than 98.0% and not more than 102.0% of $C_{12}H_{22}MgO_{14}$, calculated on the anhydrous basis.
Chloride Not more than 0.05%.
Lead Not more than 2 mg/kg.
Reducing Substances Not more than 1.0%.
Sulfate Not more than 0.05%.
Water Between 3.0% and 12.0%.

TESTS

Assay Dissolve about 800 mg of sample, accurately weighed, in 20 mL of water, add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS,

and titrate with 0.05 *M* disodium EDTA to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 20.73 mg of $C_{12}H_{22}MgO_{14}$.

Chloride Determine as directed in the *Chloride Limit Test*, under *Chloride and Sulfate Limit Tests*, Appendix IIIB, dissolving 1 g of sample in 100 mL of water. Any turbidity produced by a 10-mL portion of this solution does not exceed that shown in a control containing 50 µg of chloride (Cl) ion.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Reducing Substances Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, dissolve it in 10 mL of water, add 25 mL of alkaline cupric citrate TS, and cover the flask with a small beaker. Boil gently for exactly 5 min and cool rapidly to room temperature. Add 25 mL of a 1:10 acetic acid solution, 10.0 mL of 0.1 *N* iodine, 10 mL of 2.7 *N* hydrochloric acid, and 3 mL of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in milligrams, of reducing substances (as D-glucose) by the formula

$$27(V_1N_1 - V_2N_2),$$

in which 27 is an empirically determined equivalence factor for D-glucose; V_1 and N_1 are the volume and normality, respectively, of the iodine solution; and V_2 and N_2 are the volume and normality, respectively, of the sodium thiosulfate solution.

Sulfate Determine as directed for *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 200-mg sample does not exceed that shown in a control containing 100 µg of sulfate (SO_4) ion.

Water Determine as directed for *Method 1b* in the *Karl Fischer Titrimetric Method* under *Water Determination*, Appendix IIB. Allow 30 min for the sample to dissolve, perform a blank determination (see *General Provisions*), and make any necessary correction.

Packaging and Storage Store in well-closed containers.

Magnesium Hydroxide

$Mg(OH)_2$ Formula wt 58.32

INS: 528 CAS: [1309-42-8]

DESCRIPTION

Magnesium Hydroxide occurs as a white, bulky powder. It is soluble in dilute acids but practically insoluble in water and in alcohol.

Function pH control; drying agent; color-retention agent.

REQUIREMENTS

Identification A 1:20 aqueous solution in 2.7 *N* hydrochloric acid gives positive tests for *Magnesium*, Appendix IIIA.

Assay Not less than 95.0% and not more than 100.5% of $Mg(OH)_2$ after drying.

Alkalies (Free) and Soluble Salts Passes test.

Calcium Oxide Not more than 1%.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 2.0%.

Loss on Ignition Between 30.0% and 33.0%.

TESTS

Assay Transfer about 400 mg of sample, previously dried at 105° for 2 h and accurately weighed, into an Erlenmeyer flask. Add 25.0 mL of 1 *N* sulfuric acid, and after solution is complete, add methyl red TS, and titrate the excess acid with 1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed, deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the sample taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the $Mg(OH)_2$ in the sample taken. Each milliliter of 1 *N* sulfuric acid is equivalent to 29.16 mg of $Mg(OH)_2$ and to 28.04 mg of CaO.

Alkalies (Free) and Soluble Salts Boil 2 g of sample with 100 mL of water for 5 min in a covered beaker, then filter while hot. Titrate 50 mL of the cooled filtrate with 0.1 *N* sulfuric acid, using methyl red TS as the indicator. Not more than 2 mL of the acid is consumed. Evaporate 25 mL of the filtrate to dryness, and dry at 105° for 3 h. Not more than 10 mg of residue remains.

Calcium Oxide Dissolve about 500 mg of sample, accurately weighed, in a mixture of 3 mL of sulfuric acid and 22 mL of water. Add 50 mL of alcohol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve them. Filter through a suitable tared, porous-bottom porcelain crucible, previously washed with 2 *N* sulfuric acid, water, and alcohol. Wash the crystals on the porous disk several times with a mixture of 2 volumes of alcohol and 1 volume of 2 *N* sulfuric acid. Ignite the crucible and contents at 450° ± 25° to constant weight. The weight of calcium sulfate thus obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

> **Note**: Avoid exposing the crucible to sudden temperature changes.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Loss on Ignition Transfer about 500 mg of sample, accurately weighed, into a tared platinum crucible, and ignite, increasing the heat gradually, to constant weight at 800° ± 25°.

Packaging and Storage Store in tight containers.

Magnesium Oxide

MgO Formula wt 40.30

INS: 530 CAS: [1309-48-4]

DESCRIPTION

Magnesium Oxide occurs as a very bulky, white powder known as *Light Magnesium Oxide* or as a relatively dense, white powder known as *Heavy Magnesium Oxide*. Five grams of *Light Magnesium Oxide* occupies a volume of approximately 40 to 50 mL, while 5 g of *Heavy Magnesium Oxide* occupies a volume of approximately 10 to 20 mL. It is soluble in dilute acids, practically insoluble in water, and insoluble in alcohol.

Function pH control; neutralizer; anticaking agent; free-flowing agent; firming agent.

REQUIREMENTS

Labeling Indicate whether it is *Light Magnesium Oxide* or *Heavy Magnesium Oxide*.
Identification A solution of sample in 2.7 *N* hydrochloric acid gives positive tests for *Magnesium*, Appendix IIIA.
Assay Not less than 96.0% and not more than 100.5% of MgO after ignition.
Acid-Insoluble Substances Not more than 0.1%.
Alkalies (Free) and Soluble Salts Passes test.
Arsenic Not more than 3 mg/kg.
Calcium Oxide Not more than 1.5%.
Lead Not more than 4 mg/kg.
Loss on Ignition Not more than 10.0%.

TESTS

Assay Ignite about 500 mg of sample to constant weight at 800° ± 25° in a tared platinum crucible, accurately weigh the residue, dissolve it in 30.0 mL of 1 *N* sulfuric acid, boil gently to remove any carbon dioxide, cool, add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the sample taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the MgO in the sample taken. Each milliliter of 1 *N* sulfuric acid is equivalent to 20.15 mg of MgO and to 28.04 mg of CaO.
Acid-Insoluble Substances Mix 2.0 g of sample with 75 mL of water, add hydrochloric acid in small portions, with agitation, until no more of the sample dissolves, and boil for 5 min. If an insoluble residue remains, filter it through a suitable tared porous-bottom porcelain crucible, wash well with water until the last washing is free from chloride, ignite at 800° ± 25° for 45 min, cool, and weigh.

> **Note**: Avoid exposing the crucible to sudden temperature changes.

Alkalies (Free) and Soluble Salts Boil 2 g of sample with 100 mL of water for 5 min in a covered beaker, and filter while hot. Add methyl red TS, and titrate 50 mL of the cooled filtrate with 0.1 *N* sulfuric acid. Not more than 2 mL of the acid is consumed. Evaporate 25 mL of the filtrate to dryness, and dry at 105° for 1 h. Not more than 10 mg of residue remains.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of 2.7 *N* hydrochloric acid.
Calcium Oxide Dissolve about 400 mg of sample, accurately weighed, in a mixture of 3 mL of sulfuric acid and 22 mL of water. Add 50 mL of alcohol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve them. Filter through a suitable tared, porous-bottom porcelain crucible, previously washed with 2 *N* sulfuric acid, water, and alcohol. Wash the crystals on the porous disk several times with a mixture of 2 volumes of alcohol and 1 volume of 2 *N* sulfuric acid. Ignite the crucible and contents at 450° ± 25° to constant weight. The weight of calcium sulfate so obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

> **Note**: Avoid exposing the crucible to sudden temperature changes.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 20 mL of 2.7 *N* hydrochloric acid, and 4 µg of lead (Pb) ion in the control.
Loss on Ignition Transfer about 500 mg of sample, accurately weighed, into a tared, covered, platinum crucible. Ignite at 800° ± 25° for 15 min, cool, and weigh.

Packaging and Storage Store in tight containers.

Magnesium Phosphate, Dibasic, Mixed Hydrates

$MgHPO_4 \cdot xH_2O$

DESCRIPTION

Magnesium Phosphate, Dibasic, Mixed Hydrates, occurs as a white, crystalline powder that is a partially dehydrated form of a mixture of magnesium phosphate, dibasic—in the trihydrate, dihydrate, and anhydrous forms—and of magnesium pyrophosphate. It is slightly soluble in water and insoluble in alcohol, but is soluble in dilute acids.

Function Nutrient; leavening agent; pH control agent.

REQUIREMENTS

Labeling When used as a magnesium supplement, state the amount, in milligrams per gram, of magnesium.

Identification

A. Dissolve about 200 mg of sample in 10 mL of 1.7 *N* nitric acid, and add, dropwise, ammonium molybdate TS. A green-yellow precipitate of ammonium phosphomolybdate forms that is soluble in 6 *N* ammonium hydroxide.

B. Dissolve about 100 mg of sample in 0.5 mL of 1 *N* acetic acid and 20 mL of water. Add 1 mL of ferric chloride TS, let the solution stand for 5 min, and filter. The filtrate gives a positive test for *Magnesium*, Appendix IIIA.

Assay Not less than 96.0% of $Mg_2P_2O_7$, calculated after ignition.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 25 mg/kg.

Lead Not more than 2 mg/kg.

Loss on Ignition Between 15.0% and 28.9%.

TESTS

Proceed as directed in the monograph for *Magnesium Phosphate, Dibasic, Trihydrate*.

Packaging and Storage Store in well-closed containers.

Magnesium Phosphate, Dibasic, Trihydrate

Dimagnesium Phosphate

$MgHPO_4 \cdot 3H_2O$ Formula wt 174.33

INS: 343(ii) CAS: [7782-75-4]

DESCRIPTION

Magnesium Phosphate, Dibasic, Trihydrate, occurs as a white, dibasic, crystalline powder. It contains three molecules of water of hydration. It is slightly soluble in water and insoluble in alcohol, but is soluble in dilute acids.

Function Nutrient; leavening agent; pH control agent.

REQUIREMENTS

Labeling When used as a magnesium supplement, state the amount, in milligrams per gram, of magnesium.

Identification

A. Dissolve about 200 mg of sample in 10 mL of 1.7 *N* nitric acid, and add, dropwise, ammonium molybdate TS. A green-yellow precipitate of ammonium phosphomolybdate forms that is soluble in 6 *N* ammonium hydroxide.

B. Dissolve 100 mg of sample in 0.5 mL of 1 *N* acetic acid and 20 mL of water. Add 1 mL of ferric chloride TS, let the solution stand for 5 min, and filter. The filtrate gives a positive test for *Magnesium*, Appendix IIIA.

Assay Not less than 96.0% of $Mg_2P_2O_7$, calculated after ignition.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 25 mg/kg.

Lead Not more than 2 mg/kg.

Loss on Ignition Between 29.0% and 36.0%.

TESTS

Assay Dissolve about 500 mg of the residue obtained in the test for *Loss on Ignition* (below), accurately weighed, by heating in a mixture of 50 mL of water and 2 mL of hydrochloric acid. Cool, dilute to 100.0 mL with water, and mix. Transfer 50.0 mL of this solution into a 400-mL beaker, add 100 mL of water, and heat to 55° to 60°. Add 15 mL of 0.1 *M* disodium EDTA from a buret, add a magnetic stirring bar, and while stirring, adjust with 1 *N* sodium hydroxide to pH 10. Add 10 mL of ammonia–ammonium chloride buffer TS and 12 drops of eriochrome black TS, and continue the titration with 0.1 *M* disodium EDTA until the wine-red color changes to pure blue. Calculate the weight, in milligrams, of $Mg_2P_2O_7$ in the residue taken by the formula

$$2 \times 11.13 \times V,$$

in which V is the volume, in milliliters, of 0.1 *M* disodium EDTA required in the titration of the 50.0-mL aliquot.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 5 mL of 2.7 *N* hydrochloric acid.

Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB, except in the *Procedure* use 10 mL of 1 *N* hydrochloric acid to dissolve the sample. Alternatively, use the following procedure: Prepare a 200-mL distilling flask connected with a condenser and carrying a thermometer and a dropping funnel equipped with a stopcock. Transfer 5.0 g of sample into the distilling flask, dissolve the sample in 25 mL of 1:4 sulfuric acid, add six glass beads, and connect the apparatus for distillation, using a 600-mL beaker to collect the distillate. Add 40 mL of the dilute sulfuric acid to the flask through the dropping funnel, then fill the funnel with water, heat the solution to boiling, and continue heating until the temperature reaches 165°. Adjust the stopcock of the dropping funnel so that the temperature is maintained at 165° ± 5°, and continue the distillation until about 300 mL has been collected. Rinse the condenser and condenser arm with water, collecting the rinsings in the beaker. Add 1 *N* sodium hydroxide to the distillate to make it alkaline to litmus paper, and then add 5 mL in excess. Add 5 mL of 30% hydrogen peroxide and six glass beads to the beaker, boil until a volume of about 30 mL is reached, and cool. Transfer the condensed distillate, including the glass beads, into a 125-mL distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Add 30 mL of perchloric acid, and continue as directed in *Method I* under *Fluoride Limit Test*, Appendix IIIB, beginning with ''Connect a small dropping funnel or a steam generator to the capillary tube. . . .''

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Ignition Ignite about 1 g of sample, accurately weighed, preferably in a muffle furnace, at 800° ± 25° to constant weight. Save the residue for the *Assay* (above).

Packaging and Storage Store in well-closed containers.

Magnesium Phosphate, Tribasic

Trimagnesium Phosphate

$Mg_3(PO_4)_2 \cdot xH_2O$ Formula wt, anhydrous 262.86

INS: 334(iii) CAS: [7757-87-1]

DESCRIPTION

Magnesium Phosphate, Tribasic, occurs as a white, crystalline powder. It may contain four, five, or eight molecules of water of hydration. It is readily soluble in dilute mineral acids, but is almost insoluble in water.

Function Nutrient.

REQUIREMENTS

Identification

A. Dissolve about 200 mg of sample in 10 mL of 1.7 *N* nitric acid, and add, dropwise, ammonium molybdate TS. A green-yellow precipitate of ammonium phosphomolybdate forms that is soluble in 6 *N* ammonium hydroxide.

B. Dissolve 100 mg of sample in 0.7 mL of 1 *N* acetic acid and 20 mL of water. Add 1 mL of ferric chloride TS, let the solution stand for 5 min, and filter. The filtrate gives a positive test for *Magnesium*, Appendix IIIA.

Assay Not less than 98.0% and not more than 101.5% of $Mg_3(PO_4)_2$, calculated on the ignited basis.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 10 mg/kg.

Lead Not more than 2 mg/kg

Loss on Heating *Tetrahydrate*: Between 15.0% and 23.0%; *Pentahydrate*: Between 20.0% and 27.0%; *Octahydrate*: Between 30.0% and 37.0%.

TESTS

Assay Transfer approximately 1.2 g of sample, previously heated at 425° to constant weight, cooled, and accurately weighed, into a glass funnel positioned in the neck of a 200-mL flat-bottom boiling flask. Wash down the wall of the funnel with a small amount of water and exactly 4 mL of concentrated hydrochloric acid. With the funnel still in place, pour 100 mL of water into the flask. Remove the funnel, and place the flask on a hotplate. Bring the solution to a boil, and while gently and frequently swirling the flask, let the solution boil for 5 min.

Caution: Use proper precautions when handling concentrated acids. When the flask is not swirled sufficiently, the solution may erupt unexpectedly from the neck of the flask. Wear protective clothing and goggles to avoid possible serious injury. Use flask tongs to hold the neck of the flask while swirling the contents.

Immediately after the boiling time has elapsed, carefully cool the flask in an ice bath to room temperature. Transfer the solution into a 200-mL volumetric flask, dilute to volume with water, mix, and pipet a 50-mL portion of the resulting solution into a 250-mL beaker. Add 50 mL of water, insert a 1-in. stirring bar, and place the flask on a hotplate equipped with temperature and stirring controls. Insert pH and temperature probes, and heat with stirring until the temperature reaches 50°. Add 25.0 mL of 0.1 *M* disodium ethylenediaminetetraacetate (EDTA) solution from a buret. Adjust the pH to 10.0 using 1 *N* sodium hydroxide TS. Add 10 mL of ammonia–ammonium chloride buffer TS and 12 drops of eriochrome black TS. Adjust the heat to maintain the temperature at 55° to 60°. Continue the titration with disodium EDTA to a blue to blue-green endpoint. Each milliliter of 0.1 *M* disodium EDTA consumed by the aliquot taken is equivalent to 8.76 mg of $Mg_3(PO_4)_2$.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of 2.7 *N* hydrochloric acid.

Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB, except in the *Procedure*, use 10 mL of 1 *N* hydrochloric acid to dissolve the sample.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Heating Heat about 2 g of sample, accurately weighed, at about 425° to constant weight.

Packaging and Storage Store in well-closed containers.

Magnesium Silicate

Synthetic Magnesium Silicate

INS: 553(i) CAS: [1343-88-0]

DESCRIPTION

Magnesium Silicate occurs as a very fine, white powder free from grittiness. It is a synthetic, usually amorphous form of magnesium silicate in which the molar ratio of magnesium oxide (MgO) to silicon dioxide (SiO_2) is approximately 2:5. It is insoluble in water and in alcohol, but is readily decomposed by mineral acids. The pH of a 1:10 slurry is between 7.0 and 10.8.

Function Anticaking agent; filter aid.

REQUIREMENTS

Identification

A. Mix about 500 mg of sample with 10 mL of 2.7 *N* hydrochloric acid, filter, and neutralize the filtrate to litmus

paper with 6 *N* ammonium hydroxide. The neutralized filtrate gives positive tests for *Magnesium*, Appendix IIIA.

B. Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with a sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a weblike structure.

Assay Not less than 15.0% of MgO and not less than 67.0% of SiO_2, calculated on the ignited basis.

Fluoride Not more than 10 mg/kg.

Free Alkali (as NaOH) Not more than 1%.

Lead Not more than 5 mg/kg.

Loss on Drying and **Loss on Ignition** Not more than the percentage stated or within the range claimed by the vendor.

Soluble Salts Not more than 3.0%.

TESTS

Assay for Magnesium Oxide Transfer about 1.5 g of sample, accurately weighed, into a 250-mL conical flask. Add 50.0 mL of 1 *N* sulfuric acid, and digest on a steam bath for 1 h. Cool to room temperature, add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sulfuric acid is equivalent to 20.15 mg of MgO.

Assay for Silicon Dioxide Transfer about 700 mg of sample, accurately weighed, into a 150-mL beaker. Add 20 mL of 1 *N* sulfuric acid, and heat on a steam bath for 1 h and 30 min. Decant the supernatant liquid through an ashless filter paper, and wash the residue, by decantation, three times with hot water. Add 25 mL of water to the residue, and digest on a steam bath for 15 min. Finally, transfer the residue to the filter paper, and wash thoroughly with hot water. Transfer the filter paper and its contents to a platinum crucible. Heat to dryness, incinerate, then ignite strongly for 30 min, cool, and weigh. Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 min, cool, and weigh. The loss in weight represents the weight of SiO_2.

Fluoride

0.2 N *EDTA/0.2* N *TRIS Solution* Weigh 18.6 g of disodium ethylenediaminetetraacetate (EDTA) and 6.05 g of tris(hydroxymethyl)aminomethane (TRIS), and transfer into a 250-mL beaker. Add 200 mL of hot, deionized water, and stir until dissolved. Adjust the pH to 7.5 to 7.6 by adding 5 *N* sodium hydroxide. Cool the solution, and adjust the pH to 8.0 with 5 *N* sodium hydroxide. Transfer the solution to a 250-mL volumetric flask, and dilute to volume with deionized water. Mix well, and store in a plastic container.

Fluoride Stock Solution (1000 mg/kg F) Dissolve 2.210 g of sodium fluoride, accurately weighed, in 50 mL of deionized water. Transfer to a 1-L volumetric flask, and dilute to volume. Store this solution and all fluoride solutions in plastic containers.

100 mg/kg Fluoride Solution Pipet 10 mL of *Fluoride Stock Solution* into a 100-mL volumetric flask, and dilute to volume with deionized water.

On the day of use prepare the following:

10 mg/kg Fluoride Solution Pipet 10 mL of *100 mg/kg Fluoride Solution* into a 100-mL volumetric flask, and dilute to volume with deionized water.

1 mg/kg Fluoride Solution Pipet 1 mL of *100 mg/kg Fluoride Solution* into a 100-mL volumetric flask, and dilute to volume with deionized water.

Calibration Curve Pipet 20 mL of *10 mg/kg Fluoride Solution* and 20 mL of *1 mg/kg Fluoride Solution* into separate 100-mL plastic beakers. Add 10 mL of *0.2* N *EDTA/0.2* N *TRIS Solution* to each beaker. Measure the potential, in millivolts, of each solution with a suitable fluoride-selective, ion-indicating electrode and a calomel reference electrode connected to a pH meter capable of measuring potentials with a reproducibility of ± 0.2 mV (Orion model 96-09 combination fluoride electrode, or equivalent). Generate a standard curve by plotting the logarithms of the fluoride ion concentrations, in milligrams per kilogram, of the *Fluoride Solutions* versus the potential, in millivolts, or calibrate an Orion Expandable Ion Analyzer EA-940 (or an equivalent instrument) for direct concentration reading.

Procedure Transfer 5 g of sample, accurately weighed, into a 150-mL Teflon beaker. Add 40 mL of deionized water and 20 mL of 1 *N* hydrochloric acid. Heat to near boiling for 1 min while stirring continuously. Cool in an ice bath, transfer to a 100-mL volumetric flask, and dilute to volume with deionized water. The sample does not dissolve completely. Pipet a 20-mL aliquot into a 100-mL plastic beaker, add 10 mL of *0.2* N *EDTA/0.2* N *TRIS Solution*, and measure the solution potential as described under *Calibration Curve*. From the measured potential of the sample solution, calculate the concentration, in milligrams per kilogram, of fluoride ion from the *Calibration Curve*.

Free Alkali Add 2 drops of phenolphthalein TS to 20 mL of diluted filtrate prepared in the test for *Soluble Salts* (below), representing 1 g of magnesium silicate. If a pink color develops, not more than 2.5 mL of 0.1 *N* hydrochloric acid discharges it.

Lead

Sample Solution Transfer 10.0 g of sample into a 250-mL flask, and add 50 mL of 0.5 *N* hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 3, or equivalent, filter paper, into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the original flask. Wash the slurry and flask with three 10-mL portions of hot water, decanting each washing through the filter into the volumetric flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Procedure Determine as directed under *Lead Limit Test*, Appendix IIIB, using 10 mL of the *Sample Solution*, and 5 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h. Retain the sample for determination of *Loss on Ignition*.

Loss on Ignition Ignite the sample retained from the test for *Loss on Drying* at 900° to 1000° for 20 min, and calculate the percent weight loss.

Soluble Salts Boil 10 g of sample with 150 mL of water for 15 min. Cool to room temperature, and add water to restore the original volume. Allow the mixture to stand for 15 min, and filter until clear. Reserve 20 mL of the filtrate for the test for *Free Alkali* (above). Add 25 mL of water to 75 mL of the clear filtrate. Evaporate 50 mL of this solution, representing 2.5 g of magnesium silicate, in a tared platinum dish on a steam bath to dryness, and ignite gently to constant weight. The weight of the residue does not exceed 75 mg.

Packaging and Storage Store in well-closed containers.

Magnesium Stearate

CAS: [557-04-0]

DESCRIPTION

Magnesium Stearate occurs as a fine, white, bulky powder that is unctuous and free from grittiness. It is a compound of magnesium with a mixture of solid organic acids obtained from edible sources and consists chiefly of variable proportions of Magnesium Stearate and magnesium palmitate. It is insoluble in water, in alcohol, and in ether.

Function Anticaking agent; binder; emulsifier.

REQUIREMENTS

Identification

A. Heat 1 g of sample with a mixture of 25 mL of water and 5 mL of hydrochloric acid. Liberated fatty acids float as an oily layer on the surface of the liquid. The water layer gives positive tests for *Magnesium*, Appendix IIIA.

B. Mix 25 g of sample with 200 mL of hot water, then add 60 mL of 2 *N* sulfuric acid, and while stirring frequently, heat the mixture until the fatty acids separate cleanly as a transparent layer. Wash the fatty acids with boiling water until they are free from sulfate, collect them in a small beaker, and warm them on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, then melt the acids, filter into a dry beaker, and dry at 105° for 20 min. The solidification point of the fatty acids so obtained is not below 54° (see *Solidification Point*, Appendix IIB).

Assay Not less than 6.8% and not more than 8.3% of MgO.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 4.0%.

TESTS

Assay Boil about 1 g of sample, accurately weighed, with 50 mL of 0.1 *N* hydrochloric acid for about 30 min, or until the separated fatty acid layer is clear, adding water if necessary to maintain the original volume. Cool, filter, and wash the filter and the container thoroughly with water until the last washing is not acid to litmus. Neutralize the filtrate to litmus with 1 *N* sodium hydroxide. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 5 mL of ammonia–ammonium chloride buffer TS and 0.15 mL of eriochrome black TS, and continue the titration with 0.05 *M* disodium EDTA to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 2.015 mg of MgO.

Lead

Sample Solution Ignite, in a muffle furnace at 475° to 500° for 15 to 20 min, 500 mg of sample contained in a silica crucible. Cool, add 3 drops of nitric acid, evaporate over a low flame to dryness, and re-ignite at 475° to 500° for 30 min. Dissolve the residue in 1 mL of a mixture of 1:1 (v/v) nitric acid:water, and wash into a separator with several successive portions of water.

Procedure (**Note**: Refer to *Lead Limit Test*, Appendix IIIB, for the solutions and the control.) Add 3 mL of *Ammonium Citrate Solution* and 0.5 mL of *Hydroxylamine Hydrochloride Solution* to the *Sample Solution*, and make the combined solutions alkaline to phenol red TS with ammonium hydroxide. Add 10 mL of *Potassium Cyanide Solution*. Immediately extract the solution with successive 5-mL portions of *Dithizone Extraction Solution*, draining off each extract into another separator, until the last portion of dithizone solution retains its green color. Shake the combined extracts for 30 s with 20 mL of 1:100 nitric acid, and discard the chloroform layer. Add exactly 4 mL of *Ammonia–Cyanide Solution* and 2 drops of *Hydroxylamine Hydrochloride Solution* to the acid solution. Add 10 mL of *Standard Dithizone Solution*, and shake the mixture for 30 s. Filter the chloroform layer through an acid-washed filter paper into a Nessler tube, and compare the color with that of a standard prepared as follows: Add 0.25 mL of the *Standard Lead Solution* containing 10 µg/mL of lead (Pb) ion, 4 mL of *Ammonia–Cyanide Solution*, and 2 drops of *Hydroxylamine Hydrochloride Solution* to 20 mL of 1:100 nitric acid, and shake for 30 s with 10 mL of *Standard Dithizone Solution*. Filter through an acid-washed filter paper into a Nessler tube. The color of the *Sample Solution* does not exceed that in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° to constant weight, weighing at 2-h increments.

Packaging and Storage Store in well-closed containers.

Magnesium Sulfate

Epsom Salt

$MgSO_4 \cdot xH_2O$ Formula wt, monohydrate 138.38
Formula wt, heptahydrate 246.47

INS: 518 CAS: monohydrate [14168-73-1]
CAS: heptahydrate [10034-99-8]
CAS: dried [15244-36-7]

DESCRIPTION

Magnesium Sulfate occurs as a colorless crystal or a granular crystalline powder. It is produced with one or seven molecules of water of hydration or in a dried form containing the equivalent of about 2.3 waters of hydration. It is readily soluble in water, slowly soluble in glycerine, and sparingly soluble in alcohol.

Function Nutrient.

REQUIREMENTS

Labeling Indicate whether it is the monohydrate, the heptahydrate, or the dried form.
Identification A 1:20 aqueous solution gives positive tests for *Magnesium* and for *Sulfate*, Appendix IIIA.
Assay Not less than 99.5% of $MgSO_4$ after ignition.
Lead Not more than 4 mg/kg.
Loss on Ignition *Monohydrate*: Between 13.0% and 16.0%; *Heptahydrate*: Between 40.0% and 52.0%; *Dried*: Between 22.0% and 28.0%.
Selenium Not more than 0.003%.

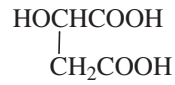
TESTS

Assay Accurately weigh about 500 mg of the residue obtained in the test for *Loss on Ignition* (below), dissolve it in a 1:50 mixture of hydrochloric acid:water, dilute with water to 100.0 mL, and mix. Transfer 50.0 mL of this solution into a 250-mL Erlenmeyer flask, add 10 mL of ammonia–ammonium chloride buffer TS and 12 drops of eriochrome black TS, and titrate with 0.1 *M* disodium EDTA until the wine red color changes to pure blue. Each milliliter of 0.1 *M* disodium EDTA is equivalent to 12.04 mg of $MgSO_4$.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Ignition Transfer about 1 g of sample, accurately weighed, and transfer it into a crucible, heat at 105° for 2 h, and then ignite in a muffle furnace at 450° ± 25° to constant weight.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.

Malic Acid

DL-Malic Acid; Hydroxysuccinic Acid; 2-Hydroxybutanedioic Acid



$C_4H_6O_5$ Formula wt 134.09

INS: 296 CAS: [617-48-1]

DESCRIPTION

Malic Acid occurs as a white or nearly white, crystalline powder or granules having a strongly acid taste. One gram dissolves in 0.8 mL of water and in 1.4 mL of alcohol. Its solutions are optically inactive. It melts at about 130°.

Function Acidifier; flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of a neat dispersion of the sample between two potassium bromide plates exhibits maxima only at the same wavelengths as those of a similar preparation of USP Malic Acid Reference Standard.
Assay Not less than 99.0% and not more than 100.5% of $C_4H_6O_5$.
Fumaric Acid Not more than 1.0%.
Lead Not more than 2 mg/kg.
Maleic Acid Not more than 0.05%.
Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between −0.10° and +0.10°.
Residue on Ignition Not more than 0.1%.
Water-Insoluble Matter Not more than 0.1%.

TESTS

Assay Dissolve about 2 g of sample, accurately weighed, in 40 mL of recently boiled and cooled water, and titrate with 1 *N* sodium hydroxide, using phenolphthalein TS as the indicator, to the first appearance of a faint pink color that persists for at least 30 s. Each milliliter of 1 *N* sodium hydroxide is equivalent to 67.04 mg of $C_4H_6O_5$.
Fumaric and Maleic Acids
Mobile Phase Prepare a filtered, degassed solution of 0.01 *N* sulfuric acid in water.

> **Note**: For all reference standards, do not dry before use, and keep the containers tightly closed and protected from light. Determine the water content of the USP Fumaric Acid Reference Standard titrimetrically before use, and make the necessary correction in preparing the *Standard Preparation*.

Standard Preparation Transfer about 5 mg of USP Fumaric Acid Reference Standard and about 2 mg of USP Maleic

Acid Reference Standard, both accurately weighed, into a 1000-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Test Preparation Transfer about 100 mg of sample, accurately weighed, into a 100-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Resolution Solution Transfer about 1 g of sample, about 10 mg of USP Fumaric Acid Reference Standard, and about 4 mg of USP Maleic Acid Reference Standard, all accurately weighed, into a 1000-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Procedure (See *Chromatography*, Appendix IIA.) Inject a portion of the *Resolution Solution* into a suitable high-performance liquid chromatograph, and obtain the chromatogram. Use a liquid chromatograph equipped with a 210-nm detector and a 30-cm × 6.5-mm (id) column packed with a strong cation exchange resin consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the hydrogen form (Polypore H from Brownlee Lab, or equivalent). Maintain the column at 37° ± 1°. Use a flow rate of about 0.6 mL/min. From the chromatogram so obtained, record the peak responses. The resolution of the maleic acid and sample peaks is not less than 2.5; the resolution of the fumaric acid and sample peaks is not less than 7.0; and the relative standard deviation of the *Sample Solution* peak for replicate injections is not more than 2.0%.

Separately inject about 20 µL each of the *Standard Preparation* and the *Test Preparation* into the chromatograph, record the chromatograms, and measure the peak responses. The relative retention times are approximately 0.6 for maleic acid, approximately 1.0 for Malic Acid, and approximately 1.5 for fumaric acid. Calculate the quantities, in milligrams, of maleic acid and fumaric acid, in the portion of the sample taken by the formula

$$100C \times (r_U/r_S),$$

in which C is the concentration, in milligrams per milliliter, of the corresponding *Reference Standard* in the *Standard Preparation*, and r_U and r_S are the responses of the corresponding peaks from the *Test Preparation* and the *Standard Preparation*, respectively.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, preparing the *Sample Solution* as follows: Dissolve 850 mg of sample in water, and dilute to 10 mL at 25°.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water-Insoluble Matter Dissolve 25 g of sample in 100 mL of water, and filter through a tared, sintered-glass filter crucible of suitable porosity. Wash the filter with hot water, dry at 100° to constant weight, cool, and weigh.

Packaging and Storage Store in well-closed containers.

Malt Syrup

Malt Extract

CAS: [8002-48-0]

DESCRIPTION

Malt is the product of barley (*Hordeum vulgare* L.) (Fam. Gramineae) germinated under controlled conditions. Malt Syrup and Malt Extract are interchangeable terms for a concentrate of the water extract of germinated barley grain with or without added food-grade preservatives. Malt Syrup is usually a yellow to brown, sweet, and viscous liquid containing varying amounts of amylolytic enzymes and plant constituents.

Function Color; enzyme; flavoring agent; humectant; nutritive sweetener; stabilizer; thickener; and texturizer.

REQUIREMENTS

Identification Add a few drops of a 1:10 aqueous solution to 5 mL of hot alkaline cupric tartrate TS. A red precipitate of cuprous oxide forms.

Assay Not less than 40.0% and not more than 65.0% of reducing sugar content, expressed as maltose.

Lead Not more than 0.5 mg/kg.

***N*-Nitrosodimethylamine** Not more than 0.005 mg/kg.

pH Between 4.5 and 5.5.

Protein Not more than 7.0%.

Sulfur Dioxide Not more than 10 mg/kg.

Total Solids Between 77.0% and 83.0%.

TESTS

Assay

Sample Solution Transfer about 5 g of sample, accurately weighed, into a 500-mL volumetric flask, dilute with water, and mix. (It is not necessary to remove any protein before analysis.)

Procedure Pipet 10.0 mL each of *Fehling Solutions A* and *B* (see *Alkaline Cupric Tartrate TS* under *Solutions and Indicators*) into a 250-mL flask. Add 20.0 mL (choose the size of the aliquot so that the sample titration will be about half that of the blank titration) of the *Sample Solution*, add water to make a total volume of 50 mL, and mix the contents of the flask by swirling gently. Add two small glass beads, and close the mouth of the flask with a small funnel or glass bulb. Heat the solution, preferably on a hot plate, at such a rate that the solution is brought to boiling within 3 min, and then continue boiling for exactly 2 min (total heating time, 5 min). Cool quickly to room temperature in an ice bath or with cold running water, and then rinse down the funnel or bulb and the walls of the flask with a few milliliters of water. Add 10 mL each of 30% potassium iodide solution and 28% sulfuric acid, and titrate rapidly with 0.1 *N* sodium thiosulfate until the iodine color almost disappears. Add 1 mL of starch TS, and titrate, dropwise, with continuous agitation, to the

disappearance of the blue color. Record the volume, in milliliters, of 0.1 *N* sodium thiosulfate required for the *Sample Solution* as *S*. Conduct two blank determinations (see *General Provisions*), substituting 20.0 mL (or the same volume as the aliquot of the *Sample Solution* taken) of water for the sample, and record the average volume, in milliliters, of the blanks as *B*. Obtain the titer difference (T_S), expressed as milliliters of 0.1 *N* sodium thiosulfate, for the sample by the equation

$$T_S = B - S.$$

Calculation By reference to the following table, determine the weight, in milligrams, of reducing sugars (as maltose) equivalent to the volume T_S, and record the value thus obtained as W_S. Calculate the total reducing sugars (as maltose), in milligrams, in the sample taken by the formula

$$25W_S.$$

(If the aliquot of the *Sample Solution* taken for assay differs from 20.0 mL, adjust the factor accordingly.)

Conversion of Titer Differences to Reducing Sugars Content

Titer Diff. (mL)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Reducing Sugars Content (as mg of Maltose)									
5.0	27.0	27.6	28.1	28.7	29.2	29.8	30.3	30.9	31.4	32.0
6.0	32.5	33.1	33.6	34.2	34.7	35.3	35.8	36.3	36.9	37.5
7.0	38.0	38.6	39.1	39.7	40.2	40.8	41.3	41.9	42.4	43.0
8.0	43.5	44.1	44.6	45.2	45.7	46.3	46.8	47.4	47.9	48.5
9.0	49.0	49.6	50.2	50.8	51.4	52.0	52.6	53.2	53.8	54.4
10.0	55.0	55.6	56.1	56.7	57.2	57.8	58.3	58.9	59.4	60.0
11.0	60.5	61.1	61.6	62.2	62.7	63.3	63.8	64.4	64.9	65.5
12.0	66.0	66.6	67.2	67.8	68.4	69.0	69.6	70.2	70.8	71.4
13.0	72.0	72.6	73.2	73.8	74.4	75.0	75.6	76.2	76.8	77.4
14.0	78.0	78.6	79.1	79.7	80.2	80.8	81.3	81.9	82.4	83.0
15.0	83.5	84.1	84.6	85.2	85.7	86.3	86.8	87.4	87.9	88.5
16.0	89.0	89.6	90.2	90.8	91.4	92.0	92.6	93.2	93.8	94.4
17.0	95.0	95.6	96.2	96.8	97.4	98.0	98.6	99.2	99.8	100.4
18.0	101.0	101.6	102.2	102.8	103.4	104.0	104.6	105.2	105.8	106.4
19.0	107.0	107.6	108.1	108.7	109.2	109.8	110.3	110.9	111.4	112.0
20.0	112.5	113.1	113.7	114.3	114.9	115.5	116.1	116.7	117.3	117.9

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

***N*-Nitrosodimethylamine** (Based on AOAC method 982.12.) (**Caution**: *N*-Nitrosamines are potent carcinogens. Take adequate precaution to avoid exposure. Carry out all steps in a well-ventilated fume hood, and wear protective gloves while handling nitrosamine standards. Because these compounds are highly photolabile, carry out all procedures under subdued light. Do not pipet solutions by mouth, and do not use the same pipet for other reagents. Destroy all nitrosamine solutions by boiling with hydrochloric acid, potassium iodide, and sulfamic acid before disposal.) (**Note**: Thoroughly clean all glassware before use. After normal cleaning and washing, wash with chromic acid. If contamination still exists, rinse all glassware with dichloromethane before use. Let the charred residue in the distillation flask soak with diluted alkali, and then wash in a normal manner.)

Apparatus Set up a distillation apparatus consisting of a 1000-mL distillation flask, a heating mantle, an adapter, and a 200-mm Graham condenser (Kontes, or equivalent) so that the connecting adapter slopes downward toward the vertical Graham condenser. Loosely wrap glass wool around the distillation flask and connecting adapter. Set up a 100-mL graduate under the condenser to collect the distillate. The temperature of the cooling water for the condenser should be ≤20°. Assemble a 250-mL Kuderna-Danish evaporative concentrator that has a 24/40 column connection and a 19/22 lower joint (Kontes, or equivalent) with a 4-mL Kuderna-Danish concentrator tube (Kontes, or equivalent) that has a 19/22 joint and 0.1-mL subdivisions from 0 to 2.0 mL at the bottom.

(See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a Thermal Energy Analyzer detector (Thermo Electron Corporation, or equivalent) and a 1.8-m × 3-mm (od) stainless steel column, or equivalent, packed with 20% Carbowax 20M, or equivalent, and 2% sodium hydroxide on 80- to 100-mesh, acid-washed Chromosorb P, or equivalent. Maintain the column at 170°. Set the injector port temperature to 220°. Use argon as the carrier gas, with a flow rate of 25 to 30 mL/min. Operate with a −110° to −130° slush bath. Adjust instrument parameters such as vacuum chamber pressure, oxygen flow, and calibration knob to obtain the proper sensitivity.

NDMA Standard Solutions Using an accurately weighed quantity of *N*-nitrosodimethylamine (NDMA) (Sigma), prepare a stock solution in dichloromethane having a concentration of 1 mg/mL. By serial dilution with dichloromethane, prepare a series of solutions containing 500, 200, 100, 40, 20, 10, and 5 ng/mL of NDMA. Store these solutions at −20°, and warm to room temperature before use. After 30 days, dispose of the *NDMA Standard Solutions*.

NDPA Standard Solution Prepare the initial solution as described above, but containing 250 μg of *N*-nitrosodi-*N*-propylamine (NDPA) per milliliter of anhydrous ethanol.

Sample Preparation Transfer 50 g of sample, accurately weighed, into a 1000-mL distillation flask, and add 1.0 mL of 10% sulfamic acid, 1.0 mL of *NDPA Standard Solution*, 1.0 mL of 1 *N* hydrochloric acid, and 15 mL of water. Mix the contents by gently swirling, and let the flask stand in the dark for 10 min. Add 10.0 mL of 3 *N* potassium hydroxide and two small boiling chips, mix, and connect the flask to the distillation apparatus.

Distillation During the initial 10 min of distillation, adjust the heating mantle so the mixture boils smoothly without too much frothing or bumping. Watch constantly for excessive foaming, and if necessary, turn off the heat for 1 to 2 min. After 10 min, increase the temperature, and continue distillation (watch for foaming) until approximately 55 mL of distillate is collected in a graduated cylinder. Do not boil the distilling flask to complete dryness; this may give erroneous results. Total distillation time should be ≤1 h. If any portion of the sample foams over during distillation, discontinue the distillation, and start over with a fresh sample.

Add 2.0 mL of 10 *N* potassium hydroxide to the distillate, and transfer the mixture into a 250-mL separatory funnel. Use the same graduated cylinder for all subsequent measuring of

dichloromethane. Rinse the condenser with 50 mL of dichloromethane, and collect the rinsing directly into the separatory funnel containing the distillate and potassium hydroxide. Extract the distillate with dichloromethane by shaking the funnel vigorously for 2 min. Drain off the lower, dichloromethane layer into a second separatory funnel. Extract the aqueous layer with two additional 50-mL portions of dichloromethane, and combine all dichloromethane extracts in the second separatory funnel. Discard the aqueous layer.

Place 40 g of anhydrous sodium sulfate into a coarse, sintered-glass Büchner funnel, wash with about 20 mL of dichloromethane, and discard the washing. Dry the combined dichloromethane extract by passing it through the sodium sulfate bed in the Büchner funnel, and collect the extract directly in the Kuderna-Danish evaporative concentrator. Wash the sodium sulfate bed with an additional 20 mL of dichloromethane, and collect the washing in the Kuderna-Danish evaporative concentrator.

Add a 1- to 2-mm boiling chip to the contents of the Kuderna-Danish evaporative concentrator, attach a three-section Snyder column with three chambers and a 24/40 joint (Kontes, or equivalent), and concentrate the extract by heating the flask in a 50° to 60° water bath. Initially maintain the outside water level close to the level of dichloromethane inside the flask, and continue heating until the amount of concentrated extract is about 4 mL (about 40 min). (If excessive boiling occurs during concentration, control it either by raising the flask slightly out of the water bath or by decreasing the bath temperature.) Finally, raise the flask above the water, and let condensed dichloromethane in the Snyder column drain into the flask. Add about 1 mL of dichloromethane to the top of the Snyder column, and let it drain into the flask. Disconnect the concentrator tube from the flask.

Add another boiling chip to the contents and attach a micro Snyder column with three chambers and a 19/22 joint (Kontes, or equivalent) to the concentrator tube. Concentrate the extract to about 0.8 mL by heating the concentrator tube in a 50° to 60° water bath. Lift out or immerse the tube in water to control the boiling rate, but do not lift the tube completely out of the water bath because this will stop the action of the boiling chip. Avoid overheating and excessive accumulation of dichloromethane in the column chambers. Stop concentration when the dichloromethane level reaches 0.8 mL; do not concentrate to less than 0.8 mL. Carry out this final concentrating step slowly, taking at least 30 min. Raise the tube, and with the bottom still touching the water, let the liquid drain, and note the volume to see if it is around 0.8 mL. If it is greater than 0.8 mL, continue the concentration as above. Finally, rinse the micro Snyder column with a few drops of dichloromethane, let the rinsing drain to the tube, disconnect the column, and dilute the extract to 1.0 or 1.1 mL, but not greater than 1.1 mL. (Do not use a nitrogen stream for concentrating the extract at any stage.)

Stopper the tube, mix in a vortex mixer, and store at 4° in the dark until analysis. Let the extract warm to room temperature, and note its volume before analyzing it.

To ensure the absence of contamination, carry the reagent blank taken through all of the steps mentioned above, except use 50 mL of 4% alcohol in water instead of 50 g of sample.

Procedure (See *Chromatography*, Appendix IIA.) Set attenuation (usually 4) of the chromatograph's thermal energy analyzer detector so that an injection of 30 pg of NDMA gives a definite peak with acceptable background. Using this attenuation, analyze 5- to 6-μL aliquots, in duplicate, of *NDMA Standard Solutions* of 5, 10, 20, and 40 ng/mL. (Note the volume injected.)

Next, choose a higher attenuation setting that gives an on-scale peak for 6 μL of *NDMA Standard Solution* at 500 μg/mL. Using this setting, analyze 6-μL aliquots, in duplicate, of *NDMA Standard Solutions* of 500, 200, 100, and 40 ng/mL.

Accurately measure the peak heights (±0.1 cm), and determine the average peak heights of two injections at each concentration. If the aliquots injected were not exactly 6 μL, make appropriate corrections, and convert all peak heights equivalent to 5-μL injections. Draw two standard curves, one for each attenuation setting, of peak heights versus picogram injection.

As above, inject a 6-μL aliquot of sample extract, in duplicate, using the lowest attenuation setting sensitive to 30 pg of NDMA. Measure, and determine the average peak height. Compare the sample response with the standard curve that produces the closest peak height at the same attenuation. Choose the *NDMA Standard Solution* that gives the closest peak height, inject 6-μL aliquots, in duplicate, and determine the average peak height.

For samples giving off-scale peaks at an attenuation of 32, dilute the extracts to 5.0 mL with dichloromethane contained in a volumetric flask, and reanalyze. For accurate results, analyze the sample extract and corresponding standard under the same attenuation setting, and all within 60 min.

If the extract gives a negative result for NDMA, or if the peak is too small to measure, inject 10-μL aliquots, in duplicate, using a 25-μL syringe. Similarly, inject duplicate 10-μL aliquots of the 5 ng/mL *NDMA Standard Solution* for quantitation. To achieve a 0.1 to 1 g/kg detection limit, analyze 10-μL aliquots of *Sample Preparation* under an attenuation setting that gives a detectable peak corresponding to 30 pg of NDMA.

> **Note**: If using a 25-μL syringe, which usually has a thick needle, watch for septum damage, and check for leaks. Use a new septum daily.

Calculate the concentration of uncorrected NDMA in the sample, in micrograms per kilogram, using the following formula:

$$(H_1PV_2)/(H_2GV_1),$$

in which H_1 is the average NDMA peak height, in centimeters, of the sample; P is the weight, in picograms, of NDMA producing the H_2 peak height; V_2 is the final volume, in milliliters, of *Sample Preparation*; H_2 is the average peak height, in centimeters, of the corresponding *NDMA Standard Solution*; G is the weight, in grams, of the sample taken for analysis; and V_1 is the volume, in microliters, of *Sample Preparation* injected.

Correction for Percent Recovery of NDPA Accurately measure the peak height of the NDPA peak on each sample chromatogram, and calculate the average peak height of two injections. Make appropriate corrections if the final volume of sample is not exactly 1.0 mL or the injection volume is not exactly 6.0 µL. Then inject, in duplicate, within 60 min, 6-µL of the *NDPA Standard Solution* under the same attenuation setting. Calculate the average peak height, and correct the value if exactly 6.0 µL is not injected. Calculate the percent recovery of NDPA for each sample. If recovery of NDPA is less than 80%, repeat the analysis from the beginning. Finally, correct the results as follows:

$$\text{Corrected NDMA, in mg/kg, in the sample} = (\text{uncorrected } \mu\text{g/kg/\% recovery of NDPA}) \times 0.1.$$

pH Determine as directed under *pH Determination*, Appendix IIB, using a 1:10 aqueous solution.

Protein Determine as directed under *Nitrogen Determination*, Appendix IIIC, using a 0.25-g sample. Percent nitrogen, N × 6.25, gives the percent of protein in the sample.

Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X, using a 100-g sample.

Total Solids Determine as directed under *Water Determination*, Appendix IIB, using an accurately weighed portion of sample. Calculate the percent of *Total Solids* by the formula

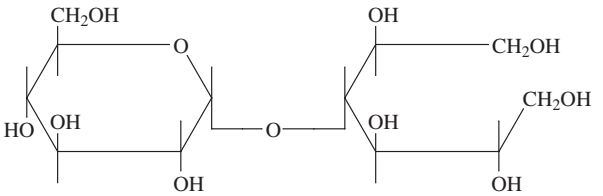
$$100[(W_U - W_W)/W_U],$$

in which W_U is the weight, in milligrams, of the sample, and W_W is the weight, in milligrams, of water determined.

Packaging and Storage Store in tight containers.

Maltitol

D-Maltitol; Hydrogenated Maltose; α-D-Glucopyranosyl-1,4-D-glucitol



$C_{12}H_{24}O_{11}$ Formula wt 344.31

INS: 965 CAS: [585-88-6]

DESCRIPTION

Maltitol occurs as a white, crystalline powder containing small amounts of sorbitol and related polyhydric alcohols. It is very soluble in water and slightly soluble in ethanol.

Function Sweetener; humectant; stabilizer.

REQUIREMENTS

Identification

Developing Solvent Prepare a mixture of *n*-propyl alcohol, ethyl acetate, and water (70:20:10).

Standard Solution Dissolve USP Maltitol Reference Standard in water to obtain a solution with a concentration of 2.5 mg/mL.

Test Solution Dilute a sample with water to obtain a solution containing, on the anhydrous basis, about 2.5 mg of sample per milliliter.

Procedure Separately apply 2 µL each of the *Standard Solution* and the *Test Solution* to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the plate in a developing chamber containing the *Developing Solvent* until the solvent front has moved about 17 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a 1:500 solution of sodium metaperiodate, air-dry for 15 min, and spray with a 1:50 solution of 4,4′-tetramethyldiaminodiphenylmethane in a 4:1 mixture of acetone and glacial acetic acid. The principal spot obtained from the *Test Solution* corresponds in R_f value and color to that obtained from the *Standard Solution*.

Assay Not less than 92.0% and not more than 100.5% of D-maltitol as $C_{12}H_{24}O_{11}$, calculated on the dried basis.

Lead Not more than 1 mg/kg.

Nickel Not more than 1 mg/kg.

Other Hydrogenated Saccharides Not more than 7.0%.

Reducing Sugars (as glucose) Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

Water Not more than 1.5%.

TESTS

Assay

Mobile Phase Use degassed water.

Standard Preparation Dissolve an accurately weighed quantity of USP Maltitol Reference Standard in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 10.0 mg/mL.

Assay Preparation Transfer about 0.7 g of sample, accurately weighed, into a 50-mL volumetric flask, dilute to volume with water, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with a refractive index detector maintained at a constant temperature and a 9-mm × 30-cm column packed with a strong cation-exchange resin, about 9 µm in diameter, or equivalent, consisting of sulfonated cross-linked styrene–(divinylbenzene copolymer in the calcium form (Aminex HPX-87c, or equivalent). Maintain the column temperature at 85° ± 0.5° and the flow rate of the *Mobile Phase* at about 0.5 mL/min. Chromatograph the *Standard Preparation*, and record the peak responses as directed under *Procedure*. Replicate injections show a relative standard deviation not greater than 2.0%.

Procedure Separately inject suitable portions (about 20 µL) of the *Assay Preparation* and the *Standard Preparation*

into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The elution pattern includes the higher-molecular-weight hydrogenated polysaccharides, followed by three individual peaks representing maltotriitol, Maltitol, and sorbitol. The principal peak is Maltitol, which elutes at about twice the retention time of the void volume, and the retention time for sorbitol is about 1.7 relative to Maltitol. Calculate the quantity, in milligrams, of D-Maltitol in the portion of the sample taken by the formula

$$50C(r_U/r_S),$$

in which C is the concentration, in milligrams per milliliter, of USP Maltitol Reference Standard in the *Standard Preparation*, and r_U and r_S are the peak responses of Maltitol obtained from the *Assay Preparation* and the *Standard Preparation*, respectively.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Nickel Determine as directed under *Nickel Limit Test*, Appendix IIIB, using a 20.0-g sample.

Other Hydrogenated Saccharides

Standard Preparation and *Assay Preparation* Proceed as directed under *Assay*.

Procedure Separately inject suitable portions (about 20 µL) of the *Assay Preparation* and the *Standard Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The elution pattern includes the higher-molecular-weight hydrogenated polysaccharides, followed by three individual peaks representing maltotriitol, Maltitol, and sorbitol. The principal peak is Maltitol, which elutes at about twice the retention time of the void volume, and the retention time for sorbitol is about 1.7 relative to Maltitol.

Calculation Calculate the quantity, in milligrams, of Maltitol and other hydrogenated saccharides in the *Assay Preparation* by the following formula:

$$50C(r_U/r_S),$$

in which C is the concentration, in milligrams per milliliter, of USP Maltitol Reference Standard in the *Standard Preparation*, and r_U and r_S are the peak responses of the hydrogenated saccharide in the *Assay Preparation* and the peak response for Maltitol in the *Standard Preparation*, respectively. Add the percentages of higher-molecular-weight hydrogenated polysaccharides, maltotriol, and sorbitol to obtain the total.

Reducing Sugars (as glucose) Dissolve 21 g of sample in 35 mL of water in a 400-mL beaker and mix. Add 25 mL of cupric sulfate TS and 25 mL of alkaline tartrate TS. Cover the beaker with a watch glass, heat the mixture at such a rate that it comes to a boil in approximately 4 min, and boil for exactly 2 min. Filter the precipitated cuprous oxide through a tared, sintered-glass filter crucible previously washed with hot water, ethanol, and ether and dried at 100° for 30 min. Thoroughly wash the collected cuprous oxide on the filter with hot water, then with 10 mL of ethanol, and finally with 10 mL of ether, and dry at 100° for 30 min. The weight of the cuprous oxide does not exceed 30 mg.

Residue on Ignition Determine as directed in *Method I* (for solids) under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Maltitol Syrup

Hydrogenated Glucose Syrup

INS: 965

DESCRIPTION

Maltitol Syrup occurs as a clear, colorless, syrupy liquid. It is a water solution of a hydrogenated, partially hydrolyzed starch containing maltitol, sorbitol, and hydrogenated oligo- and polysaccharides. It is miscible with water and with glycerin, and slightly miscible with alcohol.

Function Humectant; texturizing agent; stabilizer; sweetener.

REQUIREMENTS

Identification

A. Dissolve 1.4 g of sample in 75 mL of water. Transfer 3 mL of this solution into a 15-cm test tube, add 3 mL of a freshly prepared 1:10 catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for about 30 s. A deep pink or wine red color appears.

B. The retention time of the major peak in the chromatogram of the *Assay Preparation* corresponds to that in the chromatogram of the *Standard Preparation* as obtained from the *Assay*.

Assay Not less than 50.0%, by weight, of D-maltitol ($C_{12}H_{24}O_{11}$), calculated on the anhydrous basis, and not more than 8.0% of D-sorbitol ($C_6H_{14}O_6$), calculated on the anhydrous basis.

Lead Not more than 1 mg/kg, calculated on the anhydrous basis.

Nickel Not more than 1 mg/kg, calculated on the anhydrous basis.

pH of a 14% (w/w) Solution Between 5.0 and 7.5.

Reducing Sugars Not more than 0.3%, calculated on the anhydrous basis.

Residue on Ignition Not more than 0.1%, calculated on the anhydrous basis.

Water Not more than 31.5%.

TESTS

Assay

Mobile Phase Use degassed water.

Standard Preparation Dissolve accurately weighed quantities of USP Maltitol Reference Standard and USP Sorbitol Reference Standard in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 10.0 mg/g and 1.6 mg/g, respectively.

Assay Preparation Dissolve 0.4 g of sample, accurately weighed, in water, and dilute to about 20 g with water. Accurately record the final solution weight, and mix thoroughly.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a liquid chromatograph equipped with a refractive index detector that is maintained at a constant temperature of about 35° and a 10-cm × 7.8-mm column containing packing L34 (Bio-Rad Laboratories), or equivalent. Maintain the column temperature at about 60°, controlled within ±2°, and the flow rate of the *Mobile Phase* at about 0.5 mL/min. Chromatograph 10 µL of the *Standard Preparation*, and record the peak responses as directed under *Procedure*. Replicate injections show a relative standard deviation not greater than 2.0%, and the tailing factor for maltitol and sorbitol is not more than 1.2.

Procedure Separately inject suitable portions (about 10 µL) of the *Assay Preparation* and the *Standard Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The elution pattern includes the higher-molecular-weight hydrogenated polysaccharides, followed by three individual peaks representing maltotriitol, maltitol, and sorbitol. The relative retention times are about 0.38 for maltotriitol, 0.48 for maltitol, and 1.0 for sorbitol. Separately calculate the percentages, on the anhydrous basis, of maltitol and sorbitol in the portion of sample taken by the formula

$$[(C_S/C_U) \times (r_U/r_S) \times 10{,}000]/(100 - w),$$

in which C_S is the concentration, in milligrams per gram, of the appropriate USP Reference Standard in the *Standard Preparation;* C_U is the concentration, in milligrams per gram, of sample in the *Assay Preparation*; r_U and r_S are the peak responses of the corresponding analyte obtained from the *Assay Preparation* and the *Standard Preparation*, respectively; and w is the percent water as determined by the corresponding test.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Nickel Determine as directed under *Nickel Limit Test*, Appendix IIIB.

pH of a 14% (w/w) Solution Determine as directed under *pH Determination*, Appendix IIB, using a 14% (w/w) solution of sample in carbon dioxide-free water.

Reducing Sugars Add 3 mL of water, 20.0 mL of alkaline cupric citrate TS and a few glass beads to an amount of sample equivalent to 3.3 g on the anhydrous basis. Heat so that boiling begins after 4 min, and maintain the boiling for 3 min. Cool rapidly, and add 100 mL of diluted acetic acid TS and 20.0 mL of 0.05 *N* iodine VS, prepared by diluting 0.1 *N* iodine VS 1:1 with water. With continuous shaking, add 25 mL of a mixture of 6 mL of hydrochloric acid and 94 mL of water. When the precipitate has dissolved, titrate the excess iodine with 0.05 *N* sodium thiosulfate VS, prepared by diluting 0.1 *N* sodium thiosulfate VS 1:1 with water. Use 2 mL of starch TS, added toward the end of the titration, as an indicator. Not less than 12.8 mL of 0.05 *N* sodium thiosulfate VS is required (equivalent to not more than 0.3% reducing sugars, on the anhydrous basis, as glucose).

Residue on Ignition Determine as directed in *Method II* (for liquids) under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Maltodextrin

CAS: [9050-36-6]

DESCRIPTION

Maltodextrin occurs as a white, slightly hygroscopic powder, as granules of similar description, or as a clear to hazy solution in water. It is a purified, concentrated, nutritive mixture of saccharide polymers obtained by the partial hydrolysis of edible starch. Powders or granules are freely soluble or readily dispersible in water.

Function Anticaking and free-flowing agent; bulking agent; stabilizer and thickener; surface-finishing agent.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.

Identification Add a few drops of a 1:10 aqueous solution to 5 mL of hot alkaline cupric tartrate TS. A red precipitate of cuprous oxide forms.

Assay Less than 20.0% reducing sugar content (dextrose equivalent) expressed as D-glucose.

Lead Not more than 0.5 mg/kg.

Protein (Total) Not more than 0.5%, except not more than 1.0% in Maltodextrins produced from high-amylose starches.

Residue on Ignition Not more than 0.5%.

Sulfur Dioxide Not more than 0.0025%.

Total Solids *Powders and Granules*: Not less than 90.0%; *Liquids*: Not less than 50.0%.

TESTS

Assay Determine as directed under *Reducing Sugars Assay*, Appendix X.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Protein (Total) Determine as directed under *Nitrogen Determination*, Appendix IIIC. The protein content is N × 6.25.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X.
Total Solids

Powders and Granules Prepare an approximate 60% solution by dissolving 60 g of sample, accurately weighed, in water to a final total weight of 100 g. Heat the sample slightly, if necessary, to form a solution. Determine the refractive index of this solution at 20° or 45°, and use the tables for *Maltodextrin*, Appendix X, to obtain the percent *Total Solids* for the prepared solution. Calculate the *Total Solids* of the sample taken by the formula

$$(TS \times W_T/W_S),$$

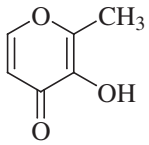
in which *TS* is the percent *Total Solids* for the prepared solution, W_T is the total weight, in grams, of the prepared solution, and W_S is the weight, in grams, of the sample taken.

Liquids Determine the refractive index of a sample at 20° or 45°, and use the tables for *Maltodextrin*, Appendix X, to obtain the percent *Total Solids*.

Packaging and Storage Keep dry, and store at ambient temperatures.

Maltol

3-Hydroxy-2-methyl-4-pyrone



$C_6H_6O_3$ Formula wt 126.11
INS: 636 CAS: [118-71-8]

DESCRIPTION

Maltol occurs as a white, crystalline powder with a characteristic caramel–butterscotch odor, and is suggestive of a fruity–strawberry aroma in dilute solution. One gram dissolves in about 82 mL of water, in 21 mL of alcohol, in 80 mL of glycerin, and in 28 mL of propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification A 1:100,000 solution in 0.1 *N* hydrochloric acid exhibits an absorbance maximum at 274 ± 2 nm.
Assay Not less than 99.0% of $C_6H_6O_3$, calculated on the anhydrous basis.
Melting Range Between 160° and 164°.
Residue on Ignition Not more than 0.2%.
Water Not more than 0.5%.

TESTS

Assay

Standard Solution Dissolve about 50 mg of USP Maltol Reference Standard, accurately weighed, in sufficient 0.1 *N* hydrochloric acid to make 250.0 mL, and mix. Transfer 5.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with 0.1 *N* hydrochloric acid, and mix.

Sample Solution Dissolve about 50 mg of sample, accurately weighed, in sufficient 0.1 *N* hydrochloric acid to make 250.0 mL, and mix. Transfer 5.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with 0.1 *N* hydrochloric acid, and mix.

Procedure Using 0.1 *N* hydrochloric acid as the blank, determine the absorbance of each solution in a 1-cm quartz cell at the wavelength of maximum absorption at about 274 nm, using a suitable spectrophotometer. Calculate the quantity, in milligrams, of $C_6H_6O_3$ in the sample taken by the formula

$$5C(A_U/A_S),$$

in which *C* is the concentration, in micrograms per milliliter, of USP Maltol Reference Standard in the *Standard Solution*; A_U is the absorbance of the *Sample Solution*; and A_S is the absorbance of the *Standard Solution*.
Melting Range Determine as directed in *Procedure for Class Ia* under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Mandarin Oil, Coldpressed

Mandarin Oil, Expressed

CAS: [8008-31-9]

FEMA: 2657

View IR

DESCRIPTION

Mandarin Oil, Coldpressed, occurs as a clear, dark orange to red-yellow or brown-orange liquid with a pleasant, orange odor. It often shows a blue fluorescence in diffused light. Oils produced from unripe fruit often show a green color. It is the oil obtained by expression of the peels of the ripe fruit of the mandarin orange, *Citrus reticulata* Blanco var. *Mandarin* (Fam. Rutaceae). It is soluble in most fixed oils and in mineral

oil, slightly soluble in propylene glycol, but insoluble in glycerin. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 0.4% and not more than 1.8% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{20}O$).
Angular Rotation Between +63° and +78°.
Refractive Index Between 1.473 and 1.477 at 20°.
Residue on Evaporation Between 2.0% and 5.0%.
Specific Gravity Between 0.846 and 0.852.

TESTS

Assay Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 10 g of sample, accurately weighed, and 156.26 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand for 30 min at room temperature before titrating.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Residue on Evaporation Determine as directed under *Residue on Evaporation*, Appendix VI, using about 5 g of sample, accurately weighed, and heating for 5 h.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Manganese Chloride

$MnCl_2$ Formula wt, anhydrous 125.84
$MnCl_2 \cdot 4H_2O$ Formula wt, tetrahydrate 197.91

CAS: anhydrous [7773-01-5]
CAS: tetrahydrate [13446-34-9]

DESCRIPTION

Manganese Chloride occurs as large, irregular, pink, translucent crystals. It is freely soluble in water at room temperature and very soluble in hot water.

Function Nutrient.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Manganese* and for *Chloride*, Appendix IIIA.
Assay Not less than 98.0% and not more than 102.0% of $MnCl_2 \cdot 4H_2O$.
Insoluble Matter Not more than 0.005%.
Iron Not more than 5 mg/kg.
Lead Not more than 4 mg/kg.
pH of a 5% Solution Between 4.0 and 6.0.
Substances Not Precipitated by Sulfide Not more than 0.2%, after ignition.
Sulfate Not more than 0.005%.

TESTS

Assay Transfer about 4 g of sample, accurately weighed, into a 250-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 25.0 mL of this solution into a 400-mL beaker, and add 10 mL of a 1:10 solution of hydroxylamine hydrochloride, 25 mL of 0.05 *M* disodium EDTA measured from a buret, 25 mL of ammonia–ammonium chloride buffer TS, and 5 drops of eriochrome black TS. Heat the solution to between 55° and 65°, and titrate from the buret to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 9.896 mg of $MnCl_2 \cdot 4H_2O$.
Insoluble Matter Dissolve about 20 g of sample, accurately weighed, in 200 mL of water, and allow to stand on a steam bath for 1 h. Filter through a tared, sintered-glass crucible, wash thoroughly with hot water, dry at 105° for 1 h, cool, and weigh.
Iron Dissolve 2.0 g of sample in 20 mL of water, add 1 mL of hydrochloric acid, and dilute to 50 mL with water. Add about 40 mg of ammonium persulfate crystals and 3 mL of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 mL of *Iron Standard Solution* (10 μg Fe) (see *Solutions and Indicators*, Appendix X) in an equal volume of a solution containing the quantities of the reagents used in the test.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample, accurately weighed, in 10 mL of water, and 4 μg of lead ion in the control.
pH of a 5% Solution Determine as directed under *pH Determination*, Appendix IIB.
Substances Not Precipitated by Sulfide Dissolve 2.0 g of sample in about 90 mL of water, add 4 mL of ammonium hydroxide, heat to 80°, and pass hydrogen sulfide through the solution to completely precipitate the manganese. Dilute to 100 mL, mix, and allow the precipitate to settle. Decant the supernatant liquid through a filter, and evaporate 50 mL of the filtrate to dryness in a tared dish. Add 0.5 mL of sulfuric acid, ignite to constant weight, cool, and weigh.
Sulfate Dissolve 10.0 g of sample in 100 mL of water, add 1 mL of 2.7 *N* hydrochloric acid, mix, and filter. Heat to boiling, then add 10 mL of barium chloride TS, and allow to stand overnight. Filter out any precipitate in a tared crucible, wash, ignite gently, cool, and weigh. The weight of the ignited

precipitate should not be more than 1.2 mg greater than the weight obtained in a complete blank test.

Packaging and Storage Store in well-closed containers.

Manganese Citrate

$Mn_3(C_6H_5O_7)_2 \cdot 10H_2O$ Formula wt, decahydrate 723.17
$Mn_3(C_6H_5O_7)_2$ Formula wt, anhydrous 543.02

CAS: [10024-66-5]

DESCRIPTION

Manganese Citrate occurs as a light pink or pink-white, fine, granular solid. It is very slightly soluble in water.

Function Nutrient.

REQUIREMENTS

Identification

A. A 1:20 solution in 20 mL of 1 *N* hydrochloric acid gives a positive test for *Manganese*, Appendix IIIA.

B. A sample gives a positive test for *Citrates*, Appendix IIIA.

Assay Not less than 96.5% and not more than 104.8% of $Mn_3(C_6H_5O_7)_2$, calculated on the dried basis.

Arsenic Not more than 3 mg/kg.

Lead Not more than 2 mg/kg.

Loss on Drying Between 23.0% and 26.0%.

Sulfates Not more than 0.02%.

TESTS

Assay Transfer about 350 mg of sample, accurately weighed, into a 250-mL beaker. Add 100 mL of water and 1 mL of hydrochloric acid. While stirring constantly, heat to approximately 75° to 80° on a hot plate. Add 25 mL of 0.05 *M* disodium EDTA, and if necessary, adjust the pH to 10.0 ± 0.2 with 1 *N* NaOH. Add 10 mL of ammonia–ammonium chloride buffer TS and approximately 8 drops of eriochrome black T indicator solution. Continue titrating with 0.05 *M* disodium EDTA until a true blue endpoint is reached and holds for at least 3 min. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 9.05 mg of $Mn_3(C_6H_5O_7)_2$.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.

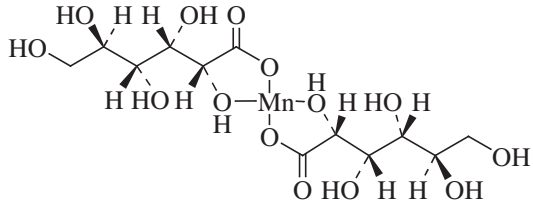
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 135° in vacuum for 16 h.

Sulfates Dissolve 10.0 g of sample in 200 mL of water and sufficient hydrochloric acid to make the solution acid to methyl red. Heat to boiling, add 10 mL of barium chloride TS, and allow to digest on a steam bath for 2 h. Filter the precipitate through a 1-μm tared filtering crucible, wash, ignite gently, cool, and weigh. The weight of the ignited precipitate multiplied by 0.4113 is not more than 0.02% of the original sample weight.

Packaging and Storage Store in well-closed containers.

Manganese Gluconate



$C_{12}H_{22}MnO_{14} \cdot 2H_2O$ Formula wt, dihydrate 481.27
$C_{12}H_{22}MnO_{14}$ Formula wt, anhydrous 445.24

CAS: [6485-39-8]

DESCRIPTION

Manganese Gluconate occurs as a slightly pink powder. The anhydrous form is obtained by spray-drying, and the dihydrate is obtained by crystallization. It is very soluble in hot water and is very slightly soluble in alcohol.

Function Nutrient.

REQUIREMENTS

Labeling Indicate whether it has been obtained through spray drying or from crystallization.

Identification

A. A 1:20 aqueous solution gives positive tests for *Manganese*, Appendix IIIA.

B. *Spray Reagent* Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute to volume with 2 *N* sulfuric acid, and mix.

Solvent System Mix 50 mL of alcohol, 30 mL of water, and 10 mL each of ammonium hydroxide and ethyl acetate.

Test Solution Dissolve a quantity of sample in water to obtain a solution containing 10 mg/mL, heating in a water bath at 60° if necessary.

Standard Solution Similarly, prepare a solution containing 10 mg/mL of USP Potassium Gluconate Reference Standard in water.

Procedure Apply separate 5-µL portions of the *Test Solution* and the *Standard Solution* on a suitable thin-layer chromatographic plate (see *Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow them to dry. Develop the chromatogram in the *Solvent System* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry it at 110° for 20 min. Allow the plate to cool, and spray it with *Spray Reagent*. Heat the plate at 110° for about 10 min. The principal spot obtained from the *Test Solution* corresponds in color, size, and R_f value to that obtained from the *Standard Solution*.

Assay Not less than 98.0% and not more than 102.0% of $C_{12}H_{22}MnO_{14}$, calculated on the anhydrous basis.

Arsenic Not more than 3 mg/kg.

Lead Not more than 2 mg/kg.

Reducing Substances Not more than 1.0%.

Water *Anhydrous*: Between 3.0% and 9.0%; *Dihydrate*: Between 6.0% and 9.0%.

TESTS

Assay Dissolve about 700 mg of sample, accurately weighed, in 50 mL of water, add 1 g of ascorbic acid, 10 mL of ammonia–ammonium chloride buffer TS, and 5 drops of eriochrome black TS, and titrate with 0.05 *M* disodium EDTA to a deep blue color. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 22.26 mg of $C_{12}H_{22}MnO_{14}$.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of hot water.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Reducing Substances Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, dissolve in 10 mL of water, add 25 mL of alkaline cupric citrate TS, and cover the flask with a small beaker. Boil gently for exactly 5 min, and cool rapidly to room temperature. Add 25 mL of 1:10 acetic acid solution, 10.0 mL of 0.1 *N* iodine, 10 mL of 2.7 *N* hydrochloric acid, and 3 mL of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in milligrams, of reducing substances (as D-glucose) by the formula

$$(V_1N_1 - V_2N_2)27,$$

in which V_1 and N_1 are the volume and normality, respectively, of the iodine solution, V_2 and N_2 are the volume and normality, respectively, of the sodium thiosulfate solution, and 27 is an empirically determined equivalence factor for D-glucose.

Water Determine as directed under *Water Determination*, Appendix IIB, except stir the mixture containing the test preparation, maintained at 50°, for 30 min before titrating with the reagent.

Packaging and Storage Store in well-closed containers.

Manganese Glycerophosphate

$C_3H_7MnO_6P{\cdot}xH_2O$ Formula wt, anhydrous 225.00

CAS: [1320-46-3]

DESCRIPTION

Manganese Glycerophosphate occurs as a white or pink-white powder. One gram dissolves in about 5 mL of 1:4 citric acid solution. It is slightly soluble in water, and is insoluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification A 1:20 solution in 2.7 *N* hydrochloric acid gives positive tests for *Manganese*, Appendix IIIA.

Assay Not less than 98.0% and not more than 100.5% of $C_3H_7MnO_6P$ after drying.

Lead Not more than 4 mg/kg.

Loss on Drying Not more than 12.0%.

TESTS

Assay Dissolve about 1 g of sample, previously dried at 110° to constant weight and accurately weighed, in 1.5 mL of nitric acid and 5 mL of warm water. Dilute to 125 mL with water, add 2.0 g of dibasic ammonium phosphate and a few drops of methyl red TS, and heat to boiling. While the solution is boiling, slowly add ammonium hydroxide, dropwise and with constant stirring, until the solution is alkaline, and then add 2.0 mL in excess. Let the solution stand for 2 h at room temperature. Filter through a tared, porous-bottom porcelain filter crucible, and wash the precipitate with 1:100 ammonia. Dry at 105°, and ignite to constant weight at 800° ± 25°. Each gram of manganese pyrophosphate so obtained is equivalent to 1.585 g of $C_3H_7MnO_6P$.

> **Note**: Avoid exposing the crucible to sudden temperature changes.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 25 mL of the following solution: Mix 1 g of sample with 3 mL of 1:2 nitric acid and 10 mL of water, and boil until brown fumes evolve. Add 10 mL of water, boil for 2 min, cool, and dilute to 100 mL with water. Use 100 mL of *Ammonium Citrate Solution*, 1 mL of *Potassium Cyanide Solution*, 0.5 mL of *Hydroxylamine Hydrochloride Solution*, and 4 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 110° to constant weight.

Packaging and Storage Store in well-closed containers.

Manganese Hypophosphite

$Mn(H_2PO_2)_2 \cdot xH_2O$ Formula wt, anhydrous 184.92

CAS: [10043-84-2]

DESCRIPTION

Manganese Hypophosphite occurs as a pink, granular or crystalline powder that is stable in air. One gram dissolves in about 6.5 mL of water at 25° or in about 6 mL of boiling water. It is soluble in alcohol.

> **Caution**: Mix Manganese Hypophosphite with nitrates, chlorates, or other oxidizing agents very carefully because an explosion may occur if it is triturated or heated.

Function Nutrient.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Manganese* and for *Hypophosphite*, Appendix IIIA.
Assay Not less than 97.0% and not more than 100.5% of $Mn(H_2PO_2)_2$ after drying.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 9.0%.

TESTS

Assay Transfer about 120 mg of sample, previously dried at 105° for 1 h and accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with water. Transfer 50.0 mL of this solution into a 250-mL glass-stoppered iodine flask, add 50.0 mL of 0.1 *N* bromine and 20 mL of 2 *N* sulfuric acid, and stopper the flask. Place a few milliliters of a saturated solution of potassium iodide in the lip around the stopper, shake the flask well, and allow it to stand for 3 h. Place the flask in an ice bath for 5 min, then carefully remove the stopper, and allow the potassium iodide solution to be drawn into the flask. Add 2 g of potassium iodide dissolved in 10 mL of recently boiled water, shake the flask, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each milliliter of 0.1 *N* bromine is equivalent to 2.311 mg of $Mn(H_2PO_2)_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as follows: Dissolve 625 mg of sample in 10 mL of water, add 2 mL of 1:2 nitric acid, and boil until brown fumes evolve. Add 10 mL of water, boil for 2 min, then cool and dilute with water to about 25 mL. Use 25 mL of *Ammonium Citrate Solution*, 1 mL of *Potassium Cyanide Solution*, 0.5 mL of *Hydroxylamine Hydrochloride Solution*, and 2.5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 1 h.

Packaging and Storage Store in well-closed containers.

Manganese Sulfate

$MnSO_4 \cdot H_2O$ Formula wt 169.02

CAS: [7785-87-7]

DESCRIPTION

Manganese Sulfate occurs as a pale-pink, granular powder. It is freely soluble in water, but is insoluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Manganese* and for *Sulfate*, Appendix IIIA.
Assay Not less than 98.0% and not more than 102.0% of $MnSO_4 \cdot H_2O$.
Arsenic Not more than 3 mg/kg.
Lead Not more than 4 mg/kg.
Loss on Heating Between 10.0% and 13.0%.
Selenium Not more than 0.003%.

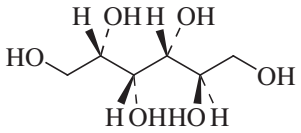
TESTS

Assay Transfer about 4 g of sample, accurately weighed, into a 250-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer a 25.0-mL portion of this solution into a 400-mL beaker, and add 10 mL of 1:10 hydroxylamine hydrochloride solution, 25 mL of 0.05 *M* disodium EDTA measured from a buret, 25 mL of ammonia–ammonium chloride buffer TS, and 5 drops of eriochrome black TS. Heat the solution to between 55° and 65°, and titrate from the buret to a blue endpoint. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 8.450 mg of $MnSO_4 \cdot H_2O$.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Heating Heat about 1 g of sample, accurately weighed, in a crucible tared in a stoppered weighing bottle, to constant weight at 400° to 500°. Cool in a desiccator, transfer to the stoppered weighing bottle, and weigh.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.

Mannitol

D-Mannitol; Mannite; 1,2,3,4,5,6-Hexanehexol



$C_6H_{14}O_6$ Formula wt 182.17

INS: 421 CAS: [69-65-8]

DESCRIPTION

Mannitol occurs as a white, crystalline powder or as free-flowing granules consisting of D-mannitol and a small quantity of sorbitol. It is soluble in water and in pyridine, very slightly soluble in alcohol, and practically insoluble in chloroform and ether.

Function Nutritive sweetener; texturizing agent.

REQUIREMENTS

Identification

A. Dissolve 1 g of sample in 75 mL of water. Transfer 3 mL of this solution into a 15-cm test tube, add 3 mL of a freshly prepared 1:10 catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for about 30 s. A deep pink or wine red color appears.

B. The retention time of the major peak in the chromatogram of the *Assay Preparation* corresponds to that in the chromatogram of the *Standard Preparation* as obtained from the *Assay*.

Assay Not less than 96.0% and not more than 101.5% of $C_6H_{14}O_6$, calculated on the dried basis.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 0.3%.

Nickel Not more than 1 mg/kg.

pH of a 10% (w/w) Solution Between 4.0 and 7.5.

Reducing Sugars Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

TESTS

Assay

Mobile Phase Use degassed water.

Resolution Solution Dissolve Sorbitol and USP Mannitol Reference Standard in water to obtain a solution having concentrations of about 4.8 mg/g of each.

Standard Preparation Dissolve an accurately weighed quantity of USP Mannitol Reference Standard in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 4.8 mg/g.

Assay Preparation Accurately weigh about 0.10 g of sample, dissolve in and dilute to about 20 g with water. Accurately record the final solution weight, and mix thoroughly.

Chromatographic System (see *Chromatography*, Appendix IIA) Use a liquid chromatograph equipped with a refractive index detector that is maintained at a constant temperature of about 35° and a 10-cm × 7.8-mm column containing packing L34 (Bio-Rad Laboratories), or equivalent. Maintain the column at a constant temperature of about 50°, controlled within ±2° of the selected temperature, and set the flow rate to about 0.7 mL/min. Chromatograph 10 µL of the *Standard Preparation*, and record the peak responses as directed under *Procedure*. The relative standard deviation for replicate injections is not more than 2.0%. In a similar manner, chromatograph 10 µL of the *Resolution Solution*. The resolution, *R*, between the sorbitol and Mannitol peaks is not less than 2.0.

Procedure Separately inject equal volumes (about 10 µL) of the *Assay Preparation* and the *Standard Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for Mannitol and 1.0 for sorbitol. Calculate the percentage, on the dried basis, of $C_6H_{14}O_6$ in the sample taken by the formula

$$[(C_S/C_U) \times (r_U/r_S) \times 10{,}000]/(100 - w),$$

in which C_S is the concentration, in milligrams per gram, of the USP Mannitol Reference Standard in the *Standard Preparation*; C_U is the concentration, in milligrams per gram, of sample in the *Assay Preparation*; r_U and r_S are the peak responses obtained from the *Assay Preparation* and the *Standard Preparation*, respectively; and w is the percent loss on drying obtained for Mannitol.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Nickel Determine as directed under *Nickel Limit Test*, Appendix IIIB.

pH of a 10% (w/w) Solution Determine as directed under *pH Determination*, Appendix IIB, using a 10% (w/w) solution of sample in carbon dioxide-free water.

Reducing Sugars Dissolve 3.3 g of sample in 25 mL of water with the aid of gentle heat. Cool, and add 20.0 mL of alkaline cupric citrate TS and a few glass beads. Heat so that boiling begins after 4 min, and maintain the boiling for 3 min. Cool rapidly, and add 100 mL of diluted acetic acid TS and 20.0 mL of 0.05 *N* iodine VS, prepared by diluting 0.1 *N* iodine VS 1:1 with water. With continuous shaking, add 25 mL of a mixture of 6 mL of hydrochloric acid and 94 mL of water. When the precipitate has dissolved, titrate the excess iodine with 0.05 *N* sodium thiosulfate VS, prepared by diluting 0.1 *N* sodium thiosulfate VS 1:1 with water. Use 2 mL of starch TS, added toward the end of the titration, as an indicator. Not less than 12.8 mL of 0.05 *N* sodium thiosulfate VS is required (equivalent to not more than 0.3% of reducing sugars, as glucose).

Residue on Ignition Determine as directed in *Method I* (for solids) under *Residue on Ignition*, Appendix IIC, igniting a 1.5-g sample.

Packaging and Storage Store in well-closed containers.

Marjoram Oil, Spanish Type

CAS: [8015-01-8]

View IR

DESCRIPTION

Marjoram Oil, Spanish Type, occurs as a slightly yellow liquid with a camphoraceous note. It is a volatile oil obtained by steam distillation from the flowering plant *Thymus mastichina* L. (Fam. Labiatae). It is soluble in most fixed oils, but it is insoluble in glycerin, in propylene glycol, and in mineral oil.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 49.0% and not more than 65.0% of cineole.
Acid Value Not more than 2.0.
Angular Rotation Between −5° and +10°.
Refractive Index Between 1.463 and 1.468 at 20°.
Saponification Value Between 5 and 20.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.904 and 0.920.

TESTS

Assay Determine as directed under *Solidification Point*, Appendix IIB, drying a sample over anhydrous sodium sulfate. Transfer 3 g of the dried oil, accurately weighed, into a test tube, and add 2.1 g of melted *o*-cresol. The *o*-cresol must be pure and dry and have a solidification point not below 30°. Insert the thermometer, stir, and warm the tube gently until the mixture is completely melted. Continue as directed in the method. Repeat the procedure until two successive readings agree within 0.10°. Compute the percentage of cineole from the table found under *Percentage of Cineole*, Appendix VI.
Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 10 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 80% alcohol and remains in solution on further addition of the alcohol to a total volume of 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Marjoram Oil, Sweet

View IR

DESCRIPTION

Marjoram Oil, Sweet, occurs as a yellow or green-yellow oil with a spicy or cardamom note. It is the volatile oil obtained by steam distillation of the dried herb of the marjoram shrub *Marjoram hortensis* L. (Fam. Labiatae). It is soluble in most fixed oils and in mineral oil (with turbidity). It is only partly soluble in propylene glycol, and it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 2.5.
Angular Rotation Between +14° and +24°.
Refractive Index Between 1.470 and 1.475 at 20°.
Saponification Value Between 23 and 40.
Saponification Value after Acetylation Between 68 and 86.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.890 and 0.906.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Saponification Value after Acetylation Determine as directed under *Total Alcohols*, Appendix VI, using about 2.5 g of acetylated oil, accurately weighed. Calculate the saponification value by the formula

$$28.05 \times A/B,$$

in which A is the number of milliliters of 0.5 N alcoholic potassium hydroxide consumed in the titration, and B is the weight, in grams, of the acetylated oil.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 80% alcohol.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers.

Masticatory Substances, Natural

Coagulated or Concentrated Latices of Vegetable Origin

DESCRIPTION

Masticatory Substances, Natural, of vegetable origin occur as a coagulated material that varies in color from white to brown, depending on its moisture content and heat treatment during purification. They consist of the gums from the trees of Sapotaceae, Apocynaceae, Moraceae, and Euphorbiaceae as listed below. The gums are purified by extensive treatment either alone or in combination with other gums or food-grade materials. They are heat treated and then clarified by centrifugation or by any appropriate means of filtration.

Family and Products	*Genus and Species*
Sapotaceae	
Chicle	*Manilkara zapotilla* Gilly and *Manilkara chicle* Gilly
Chiquibul	*Manilkara zapotilla* Gilly
Crown gum	*Manilkara zapotilla* Gilly and *Manilkara chicle* Gilly
Gutta hang kang	*Palaquium leiocarpum* Boerl. and *Palaquium oblongifolium* Burck
Gutta Katiau	*Palaquium ganua moteleyana* Clarke (also known as *Sideroxylon glabrescens*)
Massaranduba balata (and the solvent-free resin extract of Massaranduba balata)	*Manilkara huberi* (Ducke) Chevalier
Massaranduba chocolate	*Manilkara solimoesensis* Gilly
Nispero	*Manilkara zapotilla* Gilly and *Manilkara chicle* Gilly
Rosidinha (rosadinha)	*Micropholis* (also known as *Sideroxylon*) spp.
Venezuelan chicle	*Manilkara williamsii* Standley and related spp.
Apocynaceae	
Jelutong	*Dyera costulata* Hook. F. and *Dyera lowii* Hook. F.
Leche caspi (sorva)	*Couma macrocarpa* Barb. Rodr.
Pendare	*Couma macrocarpa* Barb. Rodr. and *Couma utilis* (Mart.) Muell. Arg.
Perillo	*Couma macrocarpa* Barb. Rodr. and *Couma utilis* (Mart.) Muell. Arg.
Moraceae	
Leche de vaca	*Brosimum utile* (H.B.K.) Pittier and *Poulsenia* ssp.; also *Lacmellea standleyi* (Woodson), *Monachino* (Apocynaceae)
Niger Gutta	*Ficus platyphylla* Del.
Tunu (tuno)	*Castilla fallax* Cook
Euphorbiaceae	
Chilte	*Cnidoscolus* (also known as *Jatropha*) *elasticus* Lundell and *Cnidoscolus tepiquensis* (Cost. and Gall.) McVaugh
Natural rubber (latex solids)	*Hevea brasiliensis*

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Arsenic Not more than 3 mg/kg.
Lead Not more than 3 mg/kg.

The following specifications, where applicable, should conform to the representations of the vendor: *Cleanliness*, *Color*, *Texture*, *Odor*, and *Loss on Drying*.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed under *Sample Solution for Arsenic Limit Test*, Appendix IV.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV.

Packaging and Storage Store in well-closed containers.

Menhaden Oil, Hydrogenated

HMO, FHMO, PHMO

CAS: [8016-14-6]

DESCRIPTION

Menhaden Oil, Hydrogenated, occurs as an opaque, white solid or semisolid. It belongs to a family of products obtained from menhaden fish, *Brevoortia* sp. (Fam. Clupeidae). It is available as a partially hydrogenated and as a fully hydrogenated product. It is rich in long-chain fatty acids.

Function Coating agent; crystal stabilizer. Used as a blend with other fats and oils.

REQUIREMENTS

Labeling Label to indicate whether it is Fully Hydrogenated Menhaden Oil or Partially Hydrogenated Menhaden Oil.

Identification Menhaden Oil can be differentiated from animal fats and vegetable oils by the distinctive significant amount of long-chain C20 and C22 fatty acids. Partially Hydrogenated Menhaden Oil exhibits the following typical composition profile of fatty acids, determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<12	12:0	14:0	16:0	16:1
Weight % Range:	Tr*	Tr	8–12	20–30	5–15
Fatty Acid:	18:0	18:1	18:2	18:3	20:0
Weight % Range:	5–18	Tr–13	Tr–4	Tr	Tr–15
Fatty Acid:	20:1	20:p*	22:0	22:1	22:p
Weight % Range:	Tr–10	Tr–10	8–12	Tr–6	Tr–10

Fully Hydrogenated Menhaden Oil exhibits the following typical composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<12	12:0	14:0	16:0	16:1
Weight % Range:	Tr	Tr	8–12	30–35	Tr–2
Fatty Acid:	18:0	18:1	18:2	20:0	20:1
Weight % Range:	18–21	Tr–2	Tr–1	14–18	Tr–2
Fatty Acid:	20:p	22:0	22:1	22:p	
Weight % Range:	Tr	10–12	Tr–3	Tr	

Arsenic Not more than 0.1 mg/kg.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Number *Partially Hydrogenated*: Between 11 and 119; *Fully Hydrogenated*: Not more than 10.
Lead Not more than 0.1 mg/kg.
Mercury Not more than 0.5 mg/kg.
Nickel Not more than 0.5 mg/kg.
Peroxide Value Not more than 5 meq/kg.
Saponification Value Between 180 and 200.
Unsaponifiable Matter Not more than 1.5%.

TESTS

Arsenic (**Note**: The atomic absorption spectrophotometric graphite furnace methods for arsenic, lead, mercury, and nickel in this monograph were developed at the National Marine Fisheries Service (NMFS) Southeast Fisheries Science Center, Charleston Laboratory, for the determination of these trace element contaminants in materials derived from fish oil. This method is intended for the quantitation of arsenic, lead, mercury, and nickel in marine oils at levels as low as 0.10 μg/g for arsenic and for lead and as low as 0.50 μg/g for mercury and for nickel.)

Apparatus For sample digestion, use a microwave oven (CEM Model MDS-2100, or equivalent) equipped with advanced composite vessels with 100-mL Teflon liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes.

For sample analysis, use a suitable graphite furnace atomic absorption spectrophotometer (GFAAS) equipped with an autosampler, pyrolytically coated graphite tubes, solid pyrolytic graphite platforms, and an adequate means of background correction. This method was developed using a Perkin-Elmer Model 5100, HGA-600 furnace, and an AS-60 autosampler with Zeeman effect background correction. An electrodeless discharge lamp serves as the source, argon as the purge gas, and air as the alternate gas. Set up the instrument according to manufacturers' specifications, with consideration of current good GFAAS practices. The instrument parameters are as follows—wavelength: 193.7 nm; lamp current: 300 (EDL) modulated; pyrolysis: 1000°; atomization: 2400°; slit: 0.7; characteristic mass: 15 pg.

Caution: Wear a full face shield and protective clothing and gloves at all times when working with acid baths.

Acid wash all glass, Teflon, and plastic vessels by soaking them in a nitric acid bath containing a 4:1 solution of water:nitric acid. After acid soaking, rinse acid-washed items in deionized water, dry them, and store them in clean, covered cabinets.

Calibration Standards A suitable stock standard [accuracy certified against National Institute of Standards and Technology (NIST) spectrometric standard solutions] may be purchased and used to prepare a *Working Standard* with a concentration of 100 μg/L. Prepare five calibration standards of 2.0, 5.0, 10.0, 25.0, and 50.0 μg/L by quantitative dilution of the *Working Standard* with 2% nitric acid.

Stock Solutions [Because of the difficulty in preparing matrix modifier stock solutions with the required purity, purchasing modifier stock solutions and using them to prepare working modifier solutions is recommended. A palladium (0.3%) and magnesium nitrate (0.2%) solution may be purchased from High Purity Standards, or equivalent.]

1% Palladium Stock Solution Transfer 1 g of ultrapure palladium metal, accurately weighed, into a Teflon beaker. Add 20 mL of water and 10 mL of nitric acid, and warm the solution on a hot plate to dissolve the palladium. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute to volume with deionized water.

1% Magnesium Nitrate Stock Solution Transfer 1 g of ultrapure magnesium nitrate, accurately weighed, into a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm the solution on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute to volume with deionized water.

Modifier Working Solution Transfer 3 mL of *1% Palladium Stock Solution* and 2 mL of *1% Magnesium Nitrate Stock Solution* into a 10-mL volumetric flask, and dilute to volume with 2% nitric acid. A volume of 5 μL provides 0.015 mg of palladium and 0.01 mg of magnesium nitrate.

Sample Preparation (**Caution**: Wear proper eye protection and protective clothing and gloves during sample preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.) Transfer approximately 500 mg of sample, accurately weighed to the nearest 0.1 mg, into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap,

*Tr indicates trace, and p indicates dienes and higher unsaturation.

and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature (a cool water bath may be used to speed the cooling process). Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of 30% hydrogen peroxide to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests into 25-mL volumetric flasks, and dilute to volume with deionized water.

Procedure The graphite furnace program is as follows: (1) dry at 115° using a 1-s ramp, a 65-s hold, and a 300-mL/min argon flow; (2) char the sample at 1000° using a 1-s ramp, a 20-s hold, and a 300-mL/min air flow; (3) cool down and purge the air from the furnace for 10 s using a 20° set temperature and a 300-mL/min argon flow; (4) atomize at 2400° using a 0-s ramp and a 5-s hold with the argon flow stopped; (5) clean out at 2600° with a 1-s ramp and a 5-s hold. Use the auto sampler to inject 20-µL aliquots of blanks, calibration standards, and sample solutions and 5 µL of *Modifier Working Solution*. Inject each solution in duplicate, and average the results. Use peak area measurement for all quantitations. After ensuring that the furnace is clean by running a 5% nitric acid blank, check the instrument's sensitivity by running a 20-µL aliquot of the 25-µg *Calibration Standard*. Compare the results obtained with the expected results for the equipment used, and take the necessary steps to correct any problems. Calculate the characteristic mass. Record and track the integrated absorbance and characteristic mass for reference and quality assurance.

Standard Curve Inject each *Calibration Standard* in duplicate. Use the algorithms provided in the instrument software to establish calibration curves. Recheck calibration periodically, and recalibrate if the recheck differs from the original calibration by more than 10%.

Sample Analysis Inject the *Sample Preparation* in duplicate, and record the integrated absorbance. If the instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample's response into the working range, and note the dilution factor (*DF*). All sample analyses should be blank corrected using a sample preparation blank.

Calculation If a computer-based instrument is used, the data output is reported as micrograms per liter. Calculate the concentration of arsenic, in micrograms per gram (equivalent to milligrams per kilogram), in the original sample taken by the formula

$$(C \times DF \times V)/W,$$

in which C is the concentration, in micrograms per liter, of arsenic in the sample aliquot injected; DF is the dilution factor; V is the volume, in liters, of the final *Sample Preparation*; and W is the weight, in grams, of the sample taken.

Quality Assurance To monitor recovery and ensure analytical accuracy, analyze blanks, spiked blanks, and a spiked oil with each digestion set.

Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (e) in the formula

Free fatty acids as oleic acid, e = 28.2.

Iodine Number Determine as directed under *Iodine Determination*, Appendix VII.

Lead

Apparatus For sample digestion, use the microwave apparatus as described for *Apparatus* in the *Arsenic* test (above). For sample analysis, see *Apparatus* for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Calibration Standards A suitable *Stock Standard* (accuracy certified against NIST spectrometric standard solutions) may be purchased and used to prepare a *Working Standard* with a concentration of 100 µg/L. Prepare five calibration standards of 2.0, 5.0, 10.0, 25.0, and 50.0 µg/L by quantitative dilution of the *Working Standard* with 2% nitric acid.

Stock Solutions [Because of the difficulty in preparing matrix modifier stock solutions with the required purity, purchasing modifier stock solutions and using them to prepare working solutions is recommended. An ammonium dihydrogen phosphate (4%) and magnesium nitrate (0.2%) solution may be purchased from High Purity Standards, or equivalent.]

10% Ammonium Dihydrogen Phosphate Stock Solution Transfer 10 g of ultrapure ammonium dihydrogen phosphate, accurately weighed, into a 100-mL volumetric flask. Add 40 mL of water and 1 mL of nitric acid to dissolve the phosphate. Dilute to 100 mL with deionized water.

1% Magnesium Nitrate Stock Solution Transfer 1 g of ultrapure magnesium nitrate, accurately weighed, into a Teflon beaker. Add 40 mL of water and 1 mL of nitric acid, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute to volume with deionized water.

Modifier Working Solution Transfer 4 mL of *10% Ammonium Dihydrogen Phosphate Stock Solution* and 2 mL of *1% Magnesium Nitrate Stock Solution* into a 10-mL volumetric flask, and dilute to volume with 2% nitric acid. A volume of 5 µL provides 0.2 mg of phosphate plus 0.01 mg of magnesium nitrate.

Sample Preparation Prepare as directed for *Sample Preparation* in the *Arsenic* test (above).

Procedure The graphite furnace program is as follows: (1) dry at 120° using a 1-s ramp, a 55-s hold, and a 300-mL/min argon flow; (2) char the sample at 850° using a 1-s ramp, a 30-s hold, and a 300-mL/min air flow; (3) cool down and purge the air from the furnace for 10 s using a 20° set temperature and a 300-mL/min argon flow; (4) atomize at 2100° using a 0-s ramp and a 5-s hold with the argon flow stopped; (5) clean out at 2600° with a 1-s ramp and a 5-s hold. Use the auto sampler to inject 20-µL aliquots of blanks, *Calibration Standards*, *Sample Solutions*, and 5 µL of *Modifier Working Solution*. Inject each solution in duplicate, and average the results. Use peak-area measurement for all quantitation. After ensuring that the furnace is clean by running a 5% nitric acid blank, check instrument sensitivity by running an aliquot of the 25-µg calibration standard. Compare the results obtained

with the expected results for the equipment used, and take the necessary steps to correct any problems. Calculate the characteristic mass, and record and track the integrated absorbance and characteristic mass for reference and quality assurance.

Standard Curve Inject each calibration standard in duplicate. Use the algorithms provided in the instrument software to establish calibration curves. Recheck the calibration periodically and recalibrate if recheck differs from the original calibration by more than 10%.

Sample Analysis Inject the *Sample Preparation* in duplicate, and record the integrated absorbance. If the instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample response into the working range, and note the dilution factor. All sample analyses should be blank corrected using a sample preparation blank.

Calculation If a computer-based instrument is used, the data output is reported as micrograms per liter. Calculate the concentration, in micrograms per gram (equivalent to milligrams per kilogram), of lead in the original sample by the following formula:

$$(C \times DF \times V)/W,$$

in which C is the concentration of lead, in micrograms per liter, in the sample aliquot injected; DF is the dilution factor; V is the final volume, in liters, of the *Sample Preparation*; and W is the weight, in grams, of the sample taken.

Quality Assurance To monitor recovery and ensure analytical accuracy, analyze blanks, spiked blanks, and a spiked oil with each digestion set.

Mercury

Apparatus For sample digestion, use the microwave apparatus as described for *Apparatus* in the *Arsenic* test (above).

For sample analysis, use a suitable atomic absorption spectrophotometer equipped with an atomic vapor assembly. This method was developed using a Perkin-Elmer Model 5100 and IL 440 Thermo Jarrell Ash atomic vapor assembly. An electrodeless discharge lamp serves as the source, with an inert gas such as argon or nitrogen as the purge gas. Set up the instrument according to manufacturer specifications. Instrument parameters are as follows—wavelength: 253.6 nm; slit: 0.7; reagent setting: 5; gas flow: 5 to 6 L/min; reaction time: 0.5 min.

Caution: Wear a full face shield and protective clothing and gloves at all times when working with acid baths.

Acid wash all glass, Teflon, and plastic vessels by soaking them in a nitric acid bath containing a 4:1 solution of water:nitric acid. After acid soaking, rinse acid-washed items in deionized water, dry, and store them in clean, covered cabinets.

Calibration Standards A suitable stock standard (accuracy certified against NIST spectrometric standard solutions) may be purchased and used to prepare a *Working Standard* containing 200 ng/g (ppb) of mercury. Prepare five calibration standards containing 20, 60, 100, 200, and 400 ng of mercury by quantitative dilution of the *Working Standard* with 1 *N* hydrochloric acid.

Reducing Reagent 5% stannous chloride in 25% hydrochloric acid (trace-metal grade) prepared daily.

Sample Preparation Prepare as directed for *Sample Preparation* in the *Arsenic* test (above).

Procedure Optimize the instrument settings for the spectrophotometer as described in the instrument manual. The instrument parameters for cold vapor generation are as follows—wavelength: 253.6 nm; slit: 0.70 nm; reagent setting: 5; gas flow: 5 to 6 L/min; reaction time: 0.5 min. Use a peak height integration method with a 40-s integration time and a 20-s read delay in an unheated absorption cell. Zero the instrument as follows: Place a Fleaker containing 50 mL of 1 *N* hydrochloric acid in the sample well of the hydride generator. Press ''start'' on the vapor generator and ''read'' on the atomic absorption spectrophotometer. The instrument will automatically flush the sample container with nitrogen, dispense the designated amount of reagent, stir the sample for a designated reaction time, and purge the head volume again with nitrogen, sweeping any vapor into the quartz cell for determination of absorption. The atomic absorption spectrophotometer will automatically zero on this sample when ''autozero'' is selected from the calibration menu.

Standard Curve Generate a standard curve of concentration versus absorption by analyzing the five *Calibration Standards* prepared as described for daily standards under *Standard Solutions*. Analyze each standard in duplicate, generate the calibration curve, and store, using procedures specific for the instrumentation.

Sample Analyses Transfer an appropriate aliquot of digest (usually 2 mL) in a Fleaker containing 50 mL of 1 *N* hydrochloric acid. Analyze samples in duplicate using the procedure specified in the instrument manual. Using the calibration algorithm provided in the instrument software, calculate and report the mercury concentration in nanograms of mercury in the aliquot analyzed.

Calculation Calculate the level of mercury as micrograms per gram (equivalent to milligrams per kilogram), in the original sample by the formula

$$(A \times DF)/(W \times 1000),$$

in which A is the amount of mercury, in nanograms, in the aliquot analyzed; DF is the dilution factor (final volume of digest/volume taken for analysis); and W is the weight, in grams, of the sample taken.

Quality Assurance To monitor recovery and ensure analytical accuracy, analyze blanks, spiked blanks, and a spiked oil with each digestion set.

Nickel

Apparatus For sample digestion, use the microwave apparatus as described for *Apparatus* in the *Arsenic* test (above).

For sample analysis, use a suitable graphite furnace atomic absorption spectrophotometer equipped with an autosampler, pyrolytically coated graphite tubes, solid pyrolytic graphite platforms, and an adequate means of background correction. This method was developed using a Perkin-Elmer Model 5100, HGA-600, furnace and an AS-60 autosampler with Zeeman effect background correction. A single-element, hollow-cathode lamp serves as the source, argon as the purge gas, and air as the alternate gas. Set up the instruments according to manufacturer specifications, with consideration of current good GFAAS practices. Instrument parameters are as fol-

lows—wavelength: 232.0 nm; slit: 0.2; lamp current: 25 ma; pyrolysis: 1400°; characteristic mass: 13 pg; and atomization: 2500°.

Caution: Wear a full face shield and protective clothing and gloves at all times when working with acid baths.

Acid wash all glass, Teflon, and plastic vessels by soaking them in a nitric acid bath containing a 4:1 solution of water:nitric acid. After acid soaking, rinse acid-washed items in deionized water, dry, and store them in clean, covered cabinets.

Calibration Standards A suitable stock standard (accuracy certified against NIST spectrometric standard solutions) may be purchased and used to prepare a *Working Standard* with a concentration of 100 μg/L. Prepare five calibration standards of 2.0, 5.0, 10.0, 25.0, and 50.0 μg/L by quantitative dilution of the *Working Standard* with 2% nitric acid.

Sample Preparation Prepare as directed for *Sample Preparation* in the *Arsenic* test (above).

Procedure The graphite furnace program is as follows: (1) dry at 120° using a 1-s ramp, a 50-s hold, and a 300-mL/min argon flow; (2) char the sample at 1400° using a 1-s ramp, a 20-s hold, and a 300-mL/min air flow; (3) cool down, and purge the air from the furnace for 15 s using a 20° set temperature and a 300-mL/min argon flow; (4) atomize at 2500° using a 0-s ramp and a 5-s hold with the argon flow stopped; (5) clean out at 2600° with a 1-s ramp and a 5-s hold. Use the auto sampler to inject 20-μL aliquots of blanks, calibration standards, and sample solutions. Inject each solution in duplicate, and average the results. Use the peak area measurement for all quantitations. After ensuring that the furnace is clean by running a 5% nitric acid blank, check instrument sensitivity by running a 20-μL aliquot of a 25-μg calibration standard. Compare the results obtained with the expected results for the equipment used, and take the necessary steps to correct any problems. Calculate the characteristic mass. Record and track the integrated absorbance and characteristic mass for reference and quality assurance.

Standard Curve Inject each calibration standard in duplicate. Use the algorithm provided in the instrument software to establish calibration curves. Recheck the calibration periodically, and recalibrate if the recheck differs from the original calibration by more than 10%.

Sample Analysis Inject the *Sample Preparation* in duplicate, and record the integrated absorbance. If the instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample response into the working range, and note the dilution factor. All sample analyses should be blank corrected using the *Sample Preparation* blank.

Calculation If a computer-based instrument is used, the data output is reported as micrograms per liter. Calculate the concentration of nickel, in micrograms per gram (equivalent to milligrams per kilogram), in the original sample taken by the formula

$$C \times DF \times V/W,$$

in which C is the concentration, in micrograms per liter, of nickel in the sample aliquot injected; DF is the dilution factor; V is the final volume, in liters, of the *Sample Preparation*; and W is the weight, in grams, of the sample taken.

Quality Assurance To monitor recovery and ensure analytical accuracy, analyze blanks, spiked blanks, and a spiked oil with each digestion set.

Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.

Packaging and Storage Store in well-closed containers.

Menhaden Oil, Refined

CAS: [8002-50-4]

DESCRIPTION

Menhaden Oil, Refined, is prepared from fish of the genus *Brevoortia,* commonly known as menhaden, by cooking, pressing, and refining. Winterization may separate the oil and produce a solid fraction.

Function A source of long-chain (greater than C18) ω-3 polyunsaturated fatty acids. It is used as a blend with other fats and oils.

REQUIREMENTS

Identification Menhaden Oil can be differentiated from animal fats and vegetable oils by the distinctive significant amount of the long-chain C20 and C22 fatty acids. Refined Menhaden Oil exhibits the following typical composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	≤12	12:0	14:0	16:0	16:1
Weight % Range:	Tr*	Tr	7–11	12–31	7–13
Fatty Acid:	16:p*	18:0	18:1	18:p	20:0
Weight % Range:	4–5	2–5	9–11	6–9	Tr
Fatty Acid:	20:1	20:4	20:5	22:0	22:1
Weight % Range:	1–2	1.5–2.5	11–14	Tr	Tr
Fatty Acid:	22:5	22:6			
Weight % Range:	1–3	7–11			

Arsenic Not more than 0.1 mg/kg.

Free Fatty Acids (as oleic acid) Not more than 0.1%.

Iodine Number Not less than 120.

Lead Not more than 0.1 mg/kg.

Mercury Not more than 0.5 mg/kg.

Peroxide Value Not more than 5 meq/kg.

Saponification Value Between 180 and 200.

Unsaponifiable Matter Not more than 1.5%.

*Tr indicates trace, and p indicates dienes and higher unsaturation.

TESTS

Arsenic, Free Fatty Acids, *Iodine Number*, *Lead*, *Mercury*, *Peroxide Value*, *Saponification Value*, and *Unsaponifiable Matter* Proceed as directed in the monograph for *Menhaden Oil, Hydrogenated*.

Packaging and Storage Store in well-closed containers.

Mentha Arvensis Oil, Partially Dementholized

Cornmint Oil, Partially Dementholized

CAS: [68919-18-0]

View IR

DESCRIPTION

Mentha Arvensis Oil, Partially Dementholized, occurs as a colorless to yellow liquid with a characteristic minty odor. It is the portion of oil remaining after the partial removal of menthol, by freezing operations only, from the oil of *Mentha arvensis* var. *piperascens* Holmes (forma piperascens Malinvaud) (Fam. Lamiaceae). It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 40.0% and not more than 60.0% of total alcohols, calculated as menthol ($C_{10}H_{20}O$).
Angular Rotation Between −30° and −10°.
Refractive Index Between 1.458 and 1.465 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.888 and 0.908.
Total Esters Between 5.0% and 20.0%, calculated as menthyl acetate ($C_{12}H_{22}O_2$).
Total Ketones Between 30.0% and 50.0%, calculated as menthone ($C_{10}H_{18}O$).

TESTS

Assay Determine as directed under *Total Alcohols*, Appendix VI, using about 1.5 g of the acetylated oil, accurately weighed, for the saponification. Calculate the percentage of alcohol, as menthol, in the sample by the formula

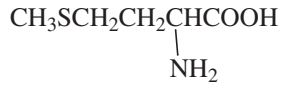
$$A \times 7.813(1 - 0.0021E)/(B - 0.021A),$$

in which A is the number of milliliters of 0.5 N alcoholic potassium hydroxide consumed in the saponification; E is the percentage of esters, as menthyl acetate, determined as directed under *Total Esters* (below); and B is the weight, in grams, of the acetylated oil taken.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2.5 to 4 mL of 80% alcohol and may become hazy upon further dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Total Esters Determine as directed in *Ester Determination* under *Esters*, Appendix VI, using about 10 g of sample, accurately weighed, and 99.15 as the equivalence factor (e) in the calculation.
Total Ketones Determine as directed for ketones in the *Hydroxylamine Method* under *Aldehydes and Ketones*, Appendix VI, using about 1 g of sample, accurately weighed, and 77.12 as the equivalence factor (e) in the calculation.

Packaging and Storage Store in a cool place protected from light in full, tight containers.

DL-Methionine

DL-2-Amino-4-(methylthio)butyric Acid



$C_5H_{11}NO_2S$ Formula wt 149.21

CAS: [59-51-8]

View IR

DESCRIPTION

DL-Methionine occurs as white crystalline platelets or a powder. One gram dissolves in about 30 mL of water. It is soluble in dilute acids and in solutions of alkali hydroxides. It is very slightly soluble in alcohol, and practically insoluble in ether. It is optically inactive. The pH of a 1:100 aqueous solution is between 5.6 and 6.1.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.5% and not more than 101.5% of $C_5H_{11}NO_2S$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.5%.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 140 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically.

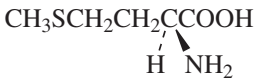
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

L-Methionine

L-2-Amino-4-(methylthio)butyric Acid



$C_5H_{11}NO_2S$ Formula wt 149.21

CAS: [63-68-3]

View IR

DESCRIPTION

L-Methionine occurs as colorless or white, lustrous plates, or as a white, crystalline powder. It is soluble in water, in alkali solutions, and in dilute mineral acids; slightly soluble in alcohol; and practically insoluble in ether.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.5% and not more than 101.5% of $C_5H_{11}NO_2S$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.5%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +21.0° and +25.0°, calculated on the dried basis; or $[\alpha]_D^{25^\circ}$: Between +21.1° and +25.1°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 140 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 2 g of sample in sufficient 6 *N* hydrochloric acid to make 100 mL.

Packaging and Storage Store in well-closed, light-resistant containers.

Methyl Alcohol

Methanol

CH_3OH Formula wt 32.04

CAS: [67-56-1]

DESCRIPTION

Methyl Alcohol occurs as a clear, colorless, flammable liquid. It is miscible with water, with ethyl alcohol, and with ether. Its refractive index at 20° is about 1.329.

Function Extraction solvent.

REQUIREMENTS

Assay Not less than 99.85% of CH_3OH, by weight.
Acetone and Aldehydes Not more than 0.003%.

Acidity (as formic acid) Not more than 0.0015%.
Alkalinity (as NH_3) Not more than 3 mg/kg.
Distillation Range Within a range of 1°, including 64.6° ± 0.1°.
Lead Not more than 1 mg/kg.
Nonvolatile Residue Not more than 10 mg/kg.
Readily Carbonizable Substances Passes test.
Solubility in Water Passes test.
Substances Reducing Permanganate Passes test.
Water Not more than 0.1%.

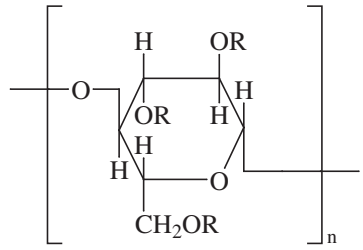
TESTS

Assay Determine the specific gravity of a sample by any reliable method (see *General Provisions*). The result is not greater than 0.7893 at 25°/25° (equivalent to 0.7928 at 20°/20°).
Acetone and Aldehydes Add 3.75 mL of water and 5.0 mL of alkaline mercuric-potassium iodide TS to 1.25 mL (about 1 g) of sample. Any turbidity does not exceed that produced in a standard containing 30 µg of acetone.
Acidity (as formic acid) Add 0.5 mL of phenolphthalein TS to a mixture of 10 mL of alcohol and 25 mL of water, and titrate with 0.02 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Add 19 mL (about 15 g) of sample, mix, and titrate with 0.02 *N* sodium hydroxide until the pink color is restored. Not more than 0.25 mL is required.
Alkalinity (as NH_3) Add 1 drop of methyl red TS to 25 mL of water, add 0.02 *N* sulfuric acid until a red color just appears, then add 29 mL (about 22.5 g) of sample, and mix. Not more than 0.2 mL of 0.02 *N* sulfuric acid is required to restore the red color.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Nonvolatile Residue Evaporate 125 mL (about 100 g) of sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.
Readily Carbonizable Substances Determine as directed under *Readily Carbonizable Substances*, Appendix IIB. A mixture of 25 mL of 95% sulfuric acid (cooled to 10°) and 25 mL of sample has no more color than 3.5 mL of platinum–cobalt CS, diluted to 50 mL with water (equivalent to not more than 35 APHA color units).
Solubility in Water Mix 15 mL of sample with 45 mL of water. After 1 h, the solution is as clear as an equal volume of water.
Substances Reducing Permanganate Transfer 20 mL of sample, previously cooled to 15°, to a glass-stoppered cylinder, add 0.1 mL of 0.1 *N* potassium permanganate, mix, and allow to stand for 5 min. The pink color is not entirely discharged.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers remote from heat, sparks, and open flames.

Methylcellulose

Modified Cellulose, MC



in which R = H or CH_3

INS: 461 CAS: [9004-67-5]

DESCRIPTION

Methylcellulose occurs as a white, fibrous powder or granules. It is the methyl ether of cellulose. It is soluble in water and in a limited number of organic solvent systems. Aqueous solutions of Methylcellulose are surface active, form films upon drying, and undergo a reversible transformation from sol to gel upon heating and cooling, respectively.

Function Thickener; stabilizer; emulsifier; bodying agent; bulking agent; binder; film former.

REQUIREMENTS

Identification

A. Add 1 g of sample to 100 mL of water. It swells and disperses to form a clear to opalescent, mucilaginous solution, depending upon the intrinsic viscosity, which is stable in the presence of most electrolytes and alcohol in concentrations up to 40%.

B. Heat a few milliliters of the solution prepared for *Identification Test A*. The solution becomes cloudy, and a flaky precipitate forms that redissolves as the solution cools.

C. Pour a few milliliters of the solution prepared for *Identification Test A* onto a glass plate, and allow the water to evaporate. A thin, self-sustaining film results.

Assay Not less than 27.5% or more than 31.5% of methoxyl groups (—OCH_3), calculated on the dried basis.
Lead Not more than 3 mg/kg.
Loss on Drying Not more than 5.0%.
Residue on Ignition Not more than 1.5%.
Viscosity The apparent viscosity of an aqueous solution containing 2 g of sample in each 100 mL is not less than 80% and not more than 120% of that stated on the label for

viscosity types of 100 centipoises or less, and not less than 75% and not more than 140% of that stated on the label for viscosity types higher than 100 centipoises.

TESTS

Assay (**Caution**: Perform all steps involving hydriodic acid carefully, in a well-ventilated hood. Use goggles, acid-resistant gloves, and other appropriate safety equipment. Be extremely careful when handling the hot vials because they are under pressure. In the event of hydriodic acid exposure, wash with copious amounts of water, and seek medical attention at once.)

Internal Standard Solution Transfer about 2.5 g of toluene, accurately weighed, into a 100-mL volumetric flask containing 10 mL of *o*-xylene, dilute with *o*-xylene to volume, and mix.

Standard Preparation Transfer about 135 mg of adipic acid into a suitable serum vial, add 4.0 mL of hydriodic acid followed by 4.0 mL of the *Internal Standard Solution*, and close the vial securely with a septum stopper. Accurately weigh the vial and its contents, add 90 µL of methyl iodide with a syringe through the septum, again weigh, and calculate the weight of methyl iodide added. Shake well, and allow the layers to separate.

Assay Preparation Transfer about 65 mg of sample, accurately weighed, into a 5-mL vial equipped with a pressure-tight septum closure, add an amount of adipic acid equal to the weight of the sample, and pipet 2 mL of the *Internal Standard Solution* into the vial. Cautiously pipet 2 mL of hydriodic acid into the mixture, immediately secure the closure, and accurately weigh. Shake the vial for 30 s, heat at 150° for 20 min, remove from the heat, shake again, using extreme caution, and heat at 150° for 40 min. Allow the vial to cool for about 45 min, and then weigh. If the weight loss is greater than 10 mg, discard the mixture and prepare another *Assay Preparation*.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph equipped with a thermal conductivity detector and a 1.8-m × 4-mm (id) glass column, or equivalent, packed with 10% methylsilicone oil (UCW 982, or equivalent) on 100- to 120-mesh flux-calcined chromatographic siliceous earth (Chromosorb WHP, or equivalent). Maintain the column at 100°, and the injection port and detector at 200°. Use helium as the carrier gas, with a flow rate of 20 mL/min.

Calibration Inject about 2 µL of the upper layer of the *Standard Preparation* into the chromatograph, and record the chromatogram. The retention times for methyl iodide, toluene, and *o*-xylene are approximately 3, 7, and 13 min, respectively. Calculate the relative response factor, *F*, of equal weights of toluene and methyl iodide by the formula

$$Q/A,$$

in which *Q* is the quantity ratio of methyl iodide to toluene in the *Standard Preparation*, and *A* is the peak area ratio of the methyl iodide to toluene obtained from the *Standard Preparation*.

Procedure Inject about 2 µL of the upper layer of the *Assay Preparation* into the chromatograph, and record the chromatogram. Calculate the percentage of methoxyl groups (—OCH_3) in the sample by the formula

$$2 \times (31/142) \times F \times a \times (W/w),$$

in which 31/142 is the ratio of the formula weights of methoxyl to methyl iodide; *a* is the ratio of the area of the methyl iodide peak to that of the toluene peak obtained from the *Assay Preparation*; *W* is the weight, in grams, of the toluene in the *Internal Standard Solution*; and *w* is the weight, in grams, of sample taken.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a sample solution prepared from a 2-g sample as directed for organic compounds, and 6 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 3-g sample at 105° for 2 h.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Viscosity Accurately weigh a sample, equivalent to 2 g of solids on the dried basis, transfer it to a wide-mouth, 250-mL centrifuge bottle, and add 98 g of water previously heated to between 80° and 90°. Stir with a mechanical stirrer for 10 min, then place the bottle in an ice bath until solution is complete, adjust the weight of the solution to 100 g, if necessary, and centrifuge it to expel any entrapped air. Adjust the temperature of the solution to 20° ± 0.1°, and determine as directed under *Viscosity of Methylcellulose*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Methylene Chloride

Dichloromethane; Methylene Dichloride

CH_2Cl_2 Formula wt 84.93

CAS: [75-09-2]

DESCRIPTION

Methylene Chloride occurs as a clear, colorless, nonflammable liquid. It is soluble in about 50 parts of water, and is miscible with alcohol, with acetone, with chloroform, and with ether. Its refractive index at 20° is about 1.424.

Function Extraction solvent.

REQUIREMENTS

Assay Not less than 99.0% of CH_2Cl_2.
Acidity (as HCl) Not more than 10 mg/kg.
Distillation Range Between 39.0° and 41.0°.
Free Halogens Passes test.
Lead Not more than 1 mg/kg.

Nonvolatile Residue Not more than 0.015%.
Specific Gravity Between 1.318 and 1.323.
Water Not more than 0.02%.

TESTS

Assay

Standard Solution Prepare a standard solution containing appropriate concentrations of methyl chloride, chloroform, methylene chloride, vinyl chloride, ethyl chloride, vinylidene chloride, 2-methyl-2-butene, *trans*-1,2-dichloroethylene, cyclohexane, and propylene oxide in high-purity DCM by adding each reagent to DCM in a glass bottle fitted with a silicone rubber septum. Inject the listed analytes into the bottle by accurately weighing a syringe containing the analyte, injecting the analyte through the septum, and reweighing the syringe to determine the amount of analyte added. Add sufficient amounts of each analyte to obtain these approximate concentrations: methyl chloride: 0.014% (w/w); vinyl chloride: 0.007; ethyl chloride: 0.0084; propylene oxide: 2.4; vinylidene chloride: 0.0098; *trans*-1,2-dichloroethylene: 0.017; chloroform: 0.012; cyclohexane: 0.047; 2-methyl-2-butene: 0.009. Dilute the standard stepwise with high-purity methyl chloride to create a series of standards in the range of approximately 10 to 300 mg/kg, except for propylene oxide, which should be in the range of 0.06 to 2.4 (w/w%). Assay the methyl chloride used to prepare the *Standard Solution* without the analytes to determine the possible presence of the analytes, and make any necessary corrections.

Procedure (See *Chromatography*, Appendix IIA.) Use a gas chromatograph capable of split and splitless capillary column injection and equipped with a flame ionization detector and a 25-m × 0.53-mm (id) fused-silica capillary column coated with a 2.0-μm film of 5% phenyl/95% methylsilicone liquid phase, or equivalent, and a 30-m × 0.32-mm (id) fused-silica capillary column, or equivalent, coated with 1.8-μm film of (6% cyanopropylphenyl) methylpolysiloxane liquid phase, or equivalent, connected in series, with the first column that was described placed behind the second. Set the injector temperature to 150°, the detector to 250°, and the oven to 40° isothermal. Use helium as the carrier gas at a flow rate of 4.4 mL/min. Set the split flow at a rate of 98 mL/min.

Using split injection mode, inject 1 to 5 μL of the standards into the chromatograph. Determine the peak areas by electronic integration. Plot peak area against concentration for each analyte corrected for the blank to construct a standard curve. Determine the concentration of additives and byproducts by comparison to the standard curve. The sum of the concentrations of the impurities and stabilizers is less than 1.0%. The order of elution and approximate retention times, in minutes, are as follows: methyl chloride: 2.8; vinyl chloride: 3.0; ethyl chloride: 3.5; propylene oxide: 4.1; 2-methyl-2-butene: 4.5; vinylidene chloride: 4.6; dichloromethane: 5.3; *trans*-1,2-dichloroethylene: 5.9; chloroform: 8.7; cyclohexane: 10.5; and carbon tetrachloride: 12.0.

Acidity (as HCl) Transfer 100 mL of sample into a separator, add 100 mL of neutralized water, and shake vigorously for 2 min. Allow the layers to separate, transfer the aqueous phase into an Erlenmeyer flask, add 4 drops of bromothymol blue TS, and titrate with 0.01 *N* sodium hydroxide: Not more than 3.6 mL is required.

Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.

Free Halogens Transfer 10 mL of sample to a separator, add 25 mL of water, and shake vigorously for 1 min. Allow the layers to separate, and then remove and discard the lower sample layer. Add 1 mL of potassium iodide TS and a few drops of starch TS to the aqueous phase, and allow it to stand for 5 min. A blue color does not appear.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Test* under *Lead Limit Test*, Appendix IIIB.

Nonvolatile Residue In a fume hood, evaporate 38 mL (about 50 g) of the sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Methyl Ester of Rosin, Partially Hydrogenated

View IR

DESCRIPTION

Methyl Ester of Rosin, Partially Hydrogenated, occurs as a light amber-colored liquid resin. It is soluble in acetone but insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a neat dispersion of the sample on a potassium bromide plate exhibits maxima only at the same wavelengths at those of a typical spectrum, as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Acid Number Between 4 and 8.

Lead Not more than 1 mg/kg.

Refractive Index Between 1.517 and 1.520 at 20°.

Viscosity Between 23 and 76 poises.

TESTS

Acid Number Determine as directed in the general method, Appendix IX.

Lead Determine as directed under *Chewing Gum Base*, Appendix IV. This solution meets the requirements of the *Lead*

Limit Test, Appendix IIIB, using 5 μg of lead (Pb) ion in the control.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Viscosity Determine as directed under *Viscosity*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Methyl Ethyl Cellulose

Modified Cellulose

INS: 465 CAS: [9004-69-7]

DESCRIPTION

Methyl Ethyl Cellulose occurs as a white or pale cream, fibrous solid or powder. It is the methyl ether of ethyl cellulose in which both the methyl and the ethyl groups are attached to the anhydroglucose units by ether linkages. It disperses in cold water to form aqueous sols that undergo a reversible transformation to gel upon heating and cooling, respectively.

Function Emulsifier; stabilizer; foaming agent.

REQUIREMENTS

Identification

A. Add 1 g of sample to 100 mL of water. It disperses to form an opalescent, fibrous sol.

B. Heat a few milliliters of the sol prepared for *Identification Test A* to about 60°. The sol becomes cloudy, and a gelatinous precipitate forms that redissolves upon cooling.

C. The remaining sol from *Identification Test A*, when whipped as for egg white in a kitchen-type mixer, produces a stable air/liquid foam.

Assay Not less than 14.5% and not more than 19.0% of ethoxyl groups (—OC_2H_5) and not less than 3.5% and not more than 6.5% of methoxyl groups (—OCH_3).

Lead Not more than 3 mg/kg.

Loss on Drying *Fibrous Form*: Not more than 15.0%; *Powdered Form*: Not more than 10.0%.

Residue on Ignition Not more than 0.6%.

Viscosity The apparent viscosity of a sol containing the equivalent of 2.5 g of dry sample in each 100 g of solution is not less than 80% and not more than 120% of that stated on the label or otherwise represented by the vendor. The usual range of viscosity types is between 20 and 60 centipoises.

TESTS

Assay for Ethoxyl Groups Determine as directed under *Hydroxypropoxyl Determination*, Appendix IIIC. Each milliliter of 0.02 *N* sodium hydroxide is equivalent to 0.9 mg of ethoxyl groups (—OC_2H_5).

Assay for Methoxyl Groups Determine as directed under *Methoxyl Determination*, Appendix IIIC, using about 50 mg of sample, previously dried at 105°, placed in a tared gelatin capsule, and accurately weighed, but calculate the total alkoxyl content as ethoxyl groups (—OC_2H_5). Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 0.7510 mg of ethoxyl groups (—OC_2H_5). Calculate the methoxyl groups (—OCH_3) by the formula

$$(A - B) \times 31/45,$$

in which A is the total alkoxyl content, calculated as —OC_2H_5; B is the —OC_2H_5 determined in the *Assay for Ethoxyl Groups*; 31 is the molecular weight of —OCH_3; and 45 is the molecular weight of —OC_2H_5.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared from 2 g of sample as directed for organic compounds, and 6 μg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 3-g sample at 105° for 4 h.

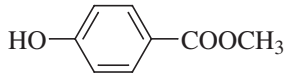
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Viscosity Transfer an accurately weighed sample, equivalent to 5.0 g on the dried basis, into a 250-mL beaker. Adjust the rotor of a variable-speed stirrer about 1 in. above the sample, add 195 mL of recently boiled and cooled water, and stir at a speed that will avoid undue aeration. Continue stirring for about 1.5 h, then either set aside for 3 h or overnight, or centrifuge to expel any entrapped air. Adjust the temperature to 20° ± 0.1°, and determine as directed under *Viscosity of Methylcellulose*, Appendix IIB, using a *Viscometer for High Viscosity*.

Packaging and Storage Store in well-closed containers.

Methylparaben

Methyl *p*-Hydroxybenzoate



$C_8H_8O_3$ Formula wt 152.15

INS: 218 CAS: [99-76-3]

DESCRIPTION

Methylparaben occurs as small, colorless crystals or as a white, crystalline powder. One gram dissolves in about 400 mL of water at 25°, in about 50 mL of water at 80°, in about 2.5 mL of alcohol, in about 7 mL of ether, and in about 4 mL of propylene glycol. It is slightly soluble in glycerin and in fixed oils.

Function Preservative; antimicrobial agent.

REQUIREMENTS

Identification Dissolve 500 mg of sample in 10 mL of 1 *N* sodium hydroxide, boil for 30 min, allow the solution to evaporate to a volume of about 5 mL, and cool. Acidify the solution with 2 *N* sulfuric acid, collect the crystals on a filter, wash several times with small portions of water, and dry in a desiccator over silica gel. The *p*-hydroxybenzoic acid so obtained melts between 212° and 217° (see *Melting Range or Temperature*, Appendix IIB).
Assay Not less than 99.0% and not more than 100.5% of $C_8H_8O_3$, calculated on the dried basis.
Acidity Passes test.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.5%.
Melting Range Between 125° and 128°.
Residue on Ignition Not more than 0.05%.

TESTS

Assay Transfer about 2 g of sample, accurately weighed, into a flask; add 40.0 mL of 1 *N* sodium hydroxide; and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h, cool, and titrate the excess sodium hydroxide with 1 *N* sulfuric acid to pH 6.5. Perform a blank determination (see *General Provisions*) with the same quantities of the same reagents in the same manner, and make any necessary correction. Each milliliter of 1 *N* sodium hydroxide is equivalent to 152.2 mg of $C_8H_8O_3$, calculated on the dried basis.
Acidity Mix 750 mg of sample with 15 mL of water, heat at 80° for 1 min, cool, and filter. The filtrate is acid or neutral to litmus. Add 0.2 mL of 0.1 *N* sodium hydroxide and 2 drops of methyl red TS to 10 mL of the filtrate. The solution is yellow, without even a light cast of pink.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying over silica gel for 5 h.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 4-g sample.

Packaging and Storage Store in well-closed containers.

Mineral Oil, White

Liquid Petrolatum; Liquid Paraffin

CAS: [8042-47-5]

DESCRIPTION

Mineral Oil, White, occurs as a colorless, transparent, oily liquid, free or nearly free from fluorescence. It is a mixture of refined liquid hydrocarbons, essentially paraffinic and naphthenic in nature, obtained from petroleum by solvent extraction and/or crystallization with subsequent purification by acid treatment and/or hydrogen treatment. It has an initial boiling point above 200°. Its average molecular weight is not less than 300. Its minimum carbon number at 5% distillation is 17. It is insoluble in water and in alcohol, is soluble in volatile oils, and is miscible with most fixed oils, but not with castor oil. It may contain any antioxidant permitted in food by the U.S. Food and Drug Administration, in an amount not greater than that required to produce its intended effect.

Function Defoaming agent; lubricant; release agent; protective coating; glazing agent; sealing agent.

REQUIREMENTS

Lead Not more than 1 mg/kg.
Readily Carbonizable Substances Passes test.
Specific Gravity Not less than that stated, or within the range claimed by the vendor.
Ultraviolet Absorbance (polynuclear hydrocarbons) Passes test.
Viscosity Not less than 3 centistokes at 100°.

TESTS

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Readily Carbonizable Substances

Chromic Acid Cleaning Mixture Dissolve 200 g of sodium dichromate in about 100 mL of water to which 1500 mL of sulfuric acid has been added, slowly with stirring.

Procedure Place 5 mL of sample in a glass-stoppered test tube that previously has been rinsed with *Chromic Acid Cleaning Mixture*, then rinsed with water, and dried. Add 5 mL of 94.5% to 94.9% sulfuric acid; while simultaneously starting a stopwatch, place the tube in a boiling water bath. After the test tube has been in the bath for 30 s, use a 3-s time span to remove it, and while holding the stopper in place, give three vigorous vertical shakes over an amplitude of about 5 in., then return it to the bath. Repeat every 30 s until exactly 10 min has passed, then remove the test tube. The sample remains unchanged in color, and the acid does not become darker than a standard color produced by mixing 3 mL of ferric chloride CS, 1.5 mL of cobaltous chloride CS, and 0.5 mL of cupric sulfate CS in a similar test tube, and overlaying this mixture with 5 mL of mineral oil.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Ultraviolet Absorbance (polynuclear hydrocarbons) Make all measurements in 1-cm cells using an ultraviolet spectrophotometer set in the wavelength range of 260 to 350 nm, under the same instrumental conditions. The standard reference absorbance is the absorbance, at 275 nm, of a standard reference solution of naphthalene (National Institute for Standards and Technology Standard Material No. 577, or a solution of equivalent purity) containing a concentration of 7.0 mg/

1000 mL in purified isooctane, measured against isooctane of the same spectral purity in 1-cm cells. (The absorbance will be approximately 0.30.)

Hexane Use a pure grade of hexane (predominantly *n*-hexane and methylcyclopentane) having an ultraviolet absorbance not exceeding 0.10 down to 220 nm and not exceeding 0.02 down to 260 nm. The purity should be such that the ''solvent control,'' as defined under *Procedure*, has an absorbance curve, compared to water, showing no extraneous impurity peaks and no absorbance exceeding that of dimethyl sulfoxide, compared to water, at any wavelength in the range 260 to 350 nm, inclusive. If necessary to obtain the prescribed purities, the hexane may be passed through activated silica gel.

Dimethyl Sulfoxide Use a pure grade of dimethyl sulfoxide (99.9%, melting point: 18°) that has a clear, water-white appearance; has an absorbance curve, compared with water, not exceeding 1.0 at 264 nm; and shows no extraneous impurity peaks in the wavelength range up to 350 nm. Store in glass-stoppered bottles.

Apparatus Use 125-mL glass-stoppered separators equipped with tetrafluoroethylene polymer stopcocks or other suitable stopcocks that will not contaminate the solvents.

Procedure Transfer 25 mL of sample and 25 mL of hexane to a separator, and mix. Add 5.0 mL of dimethyl sulfoxide, shake the mixture vigorously for at least 1 min, and allow it to stand until the lower layer is clear. Completely transfer the lower layer to a second separator, add 2 mL of hexane, and shake the mixture vigorously. Allow it to stand until the lower layer is clear, and then draw off the lower layer, designated as *mineral oil extract*. In a third separator, vigorously shake 5.0 mL of dimethyl sulfoxide with 25 mL of hexane for at least 1 min, allow it to stand until the lower layer is clear, and draw off this layer, designated as *solvent control*. Determine the absorbance of the *mineral oil extract* in a 1-cm cell in the range 260 to 350 nm, inclusive, compared to the *solvent control*. The absorbance of the *mineral oil extract* does not exceed that of the *solvent control* at any wavelength in the specified range by more than one-third of the standard reference absorbance.

> **Note**: Make suitable corrections of the absorbance when testing samples containing added antioxidants.

Viscosity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in tight containers.

Monoammonium L-Glutamate

Monoammonium Glutamate Monohydrate; Ammonium Glutamate

$$NH_4OOCCH_2CH_2CH(NH_2)COOH{\cdot}H_2O$$

$C_5H_{12}N_2O_4{\cdot}H_2O$ Formula wt 182.18

INS: 624 CAS: monohydrate [7558-63-6]

View IR

DESCRIPTION

Monoammonium L-Glutamate occurs as a white, free-flowing, crystalline powder. It is freely soluble in water, but practically insoluble in common organic solvents. The pH of a 1:20 aqueous solution is between 6.0 and 7.0.

Function Flavor enhancer; salt substitute.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_5H_{12}N_2O_4{\cdot}H_2O$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.5%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Not less than +25.4° and not more than +26.40, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 100 mL of glacial acetic acid. A few drops of water may be added first to speed dissolution. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1*N* perchloric acid is equivalent to 9.109 mg of $C_5H_{12}N_2O_4{\cdot}H_2O$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 50° for 4 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of sample in sufficient 2 *N* hydrochloric acid to make 100 mL.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight containers.

Monoammonium Glycyrrhizinate

Ammonium Glycyrrhizinate, Pentahydrate; Ammonium Glycyrrhizinate

$$C_{42}H_{61}O_{16}NH_4 \cdot 5H_2O$$

$C_{42}H_{65}NO_{16} \cdot 5H_2O$ Formula wt, anhydrous 839.98

INS: 958

DESCRIPTION

Monoammonium Glycyrrhizinate occurs as a white powder with an intensely sweet taste. It is obtained by extraction from ammoniated glycyrrhizin. It is soluble in ammonia water and is insoluble in glacial acetic acid.

Function Flavoring agent.

REQUIREMENTS

Identification A sample gives positive tests for *Ammonium*, Appendix IIIA.
Assay Not less than 85.0% and not more than 102.0% of $C_{42}H_{65}NO_{16}$, calculated on the dried basis.
Ash (Total) Not more than 0.5%.
Loss on Drying Not more than 6.0%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +45° and +53°, on the as-is basis.

TESTS

Assay (Based on AOAC method 982.19.)

Apparatus (See *Chromatography*, Appendix IIA.) Fit a high-performance liquid chromatograph operated at room temperature, with a 10-μm particle size, 30-cm × 4-mm (id), C18 reverse-phase column (μBondapak C18, Waters Corp., or equivalent). Maintain the *Mobile Phase* at a pressure and flow rate (typically 2.0 mL/min) capable of giving the required elution time (see *System Suitability* in *High-Performance Liquid Chromatography*). Use an ultraviolet detector that monitors absorption at 254 nm (0.2 to 0.1 AUFS range).

Mobile Phase Add 380 mL of acetonitrile and 10 mL of acetic acid to 610 mL of glass-distilled water that has been filtered through a 0.45-μm filter (Millipore, or equivalent). Mix, and de-gas thoroughly.

Standard Solution Transfer about 10 mg of Monoammonium Glycyrrhizinate Standard for analytical use (available from Sigma), accurately weighed, into 20 mL of a 1:1 solution of acetonitrile:water. Filter the solution through a 0.45-μm Millipore filter, or equivalent. Prepare fresh daily.

> **Note**: Correct the weight of Monoammonium Glycyrrhizinate Standard taken for the percent loss on drying shown on its label.

Assay Solution Dissolve about 10 mg of sample, accurately weighed, in 20 mL of a 1:1 solution of acetonitrile:water. Filter the solution through a 0.45-μm Millipore filter, or equivalent.

System Suitability Inject duplicate 10-μL portions of the *Standard Solution* into the chromatograph. The retention time of the Monoammonium Glycyrrhizinate is approximately 6 min. Adjust the operating conditions if necessary. The mean standard deviation for replicate injections is not more than 2.0%.

Procedure Separately inject, in duplicate, 10-μL volumes of the *Standard Solution* and the *Assay Solution* into the chromatograph, and determine the mean peak area for each solution. Calculate the percent Monoammonium Glycyrrhizinate, equivalent to $C_{42}H_{65}NO_{16}$, in the sample taken by the formula

$$100 \times (20C_S/W_U) \times (A_U/A_S),$$

in which C_S is the concentration, in milligrams per milliliter, of the *Standard Solution*; W_U is the weight, in milligrams, of sample taken; and A_U and A_S are the peak areas of the *Assay Solution* and the *Standard Solution*, respectively.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 1-g sample at 78° for 4 h at 1-mm Hg.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 1.5 g of undried sample in sufficient 40% ethanol to make 100 mL.

Packaging and Storage Store in a cool, dry place in a tight container.

Mono- and Diglycerides

DESCRIPTION

Mono- and Diglycerides occur as a substance that varies in consistency from yellow liquids through white- to pale yellow-colored plastics to hard, ivory-colored solids. They consist of mixtures of glycerol mono- and diesters, with minor amounts of triesters, and of edible fats or oils or edible fat-forming fatty acids. They are insoluble in water, but are soluble in alcohol, in ethyl acetate, and in chloroform and other chlorinated hydrocarbons.

Function Emulsifier; stabilizer.

REQUIREMENTS

Acid Value Not more than 6.
Arsenic Not more than 3 mg/kg.
Free Glycerin Not more than 7.0%.
Lead Not more than 2 mg/kg.
Residue on Ignition Not more than 0.5%.

The following specifications should conform to the representations of the vendor: *Hydroxyl Value*, *Iodine Value*, *1-Monoglyceride Content*, *Saponification Value*, and *Total Monoglycerides*.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Free Glycerin Determine as directed under *Free Glycerin or Propylene Glycol*, Appendix VII.
Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
1-Monoglyceride Content Determine as directed under *1-Monoglycrides*, Appendix VII.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 4 g of sample, accurately weighed.
Total Monoglycerides Determine as directed under *Total Monoglycerides*, Appendix VII.

Packaging and Storage Store in well-closed containers.

Monoglyceride Citrate

CAS: [36291-32-4]

DESCRIPTION

Monoglyceride Citrate occurs as a viscous, amber liquid. It is a mixture of glyceryl monooleate and its citric acid monoester, manufactured by the reaction of glyceryl monooleate with citric acid under controlled conditions. It is dispersible in most common fat solvents and in alcohol, and it is insoluble in water.

Function Solubilizer for antioxidants.

REQUIREMENTS

Acid Value Between 70 and 100.
Lead Not more than 2 mg/kg.
Residue on Ignition Not more than 0.3%.
Saponification Value Between 260 and 265.
Total Citric Acid Between 14.0% and 17.0%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII.
Total Citric Acid

Standard Solution Transfer about 35 mg of sodium citrate dihydrate, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Calculate the concentration (C), in micrograms per milliliter, of citric acid in the final solution by the formula

$$1000 \times 0.6533W/100,$$

in which 0.6533 is a factor converting sodium citrate dihydrate to citric acid and W is the weight, in milligrams, of the sodium citrate dihydrate taken.

Sample Solution Transfer about 150 mg of sample, accurately weighed, into a saponification flask, add 50 mL of 4% alcoholic potassium hydroxide solution, and reflux for 1 h. Acidify the reaction mixture with hydrochloric acid to a pH of 2.8 to 3.2, transfer into a 400-mL beaker, and evaporate to dryness on a steam bath. Quantitatively transfer the contents of the beaker into a separator, using no more than 50 mL of water, and then extract with three 50-mL portions of petroleum ether (b.p. 30° to 60°), discarding the extracts. Transfer the water layer to a 100-mL volumetric flask, dilute to volume with water, and mix.

Procedure Pipet 2.0 mL each of the *Standard Solution* and of the *Sample Solution* into separate, 40-mL graduated centrifuge tubes, and add 2 mL of 1:2 sulfuric acid and 11 mL of water to each tube. Boil for 3 min, cool, and add 5 mL of bromine TS to each tube. Dilute to 20 mL, allow to stand for 10 min, and centrifuge. Transfer 4.0 mL of each solution into separate 19- × 110-mm test tubes, add 1 mL of water, 0.5 mL of 1:2 sulfuric acid, and 0.3 mL of 1 *M* potassium bromide, and shake. Add 0.3 mL of 1.5 *N* potassium permanganate, shake, and allow to stand for 2 min. Add 1 mL of a saturated solution of ferrous sulfate, shake, allow to stand for 2 min, and then dilute to 10 mL with water. Add 10.0 mL of *n*-hexane (previously washed with sulfuric acid, followed by a water wash, and then dried over anhydrous sodium sulfate), shake vigorously for 2 min, and then centrifuge at a low speed for 1 min. Transfer 5.0 mL of the hexane extract into a 20- × 145-mm tube containing 10.0 mL of sodium sulfide solution (4 g of $Na_2S{\cdot}9H_2O$ in each 100 mL of water), and briefly shake vigorously (3 oscillations only). Centrifuge the mixture at low speed for 1 min. Immediately

determine the absorbance of each aqueous layer in a 1-cm cell at 450 nm with a suitable spectrophotometer, using a reagent blank in the reference cell. Calculate the quantity, in milligrams, of citric acid in the sample taken by the formula

$$0.1C \times A_U/A_S,$$

in which C is as defined under *Standard Solution*, A_U is the absorbance of the final solution from the *Sample Solution*, and A_S is the absorbance of the final solution from the *Standard Solution*.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Monopotassium L-Glutamate

Monopotassium Glutamate Monohydrate; Potassium Glutamate; MPG

$$KOOCCH_2CH_2CH(NH_2)COOH \cdot H_2O$$

$C_5H_8KNO_4 \cdot H_2O$ Formula wt 203.24

INS: 622 CAS: anhydrous [19473-49-5]

View IR

DESCRIPTION

Monopotassium L-Glutamate occurs as a white, free-flowing, crystalline powder. It is hygroscopic, is freely soluble in water, and is slightly soluble in alcohol. The pH of a 1:50 aqueous solution is between 6.7 and 7.3.

Function Flavor enhancer; salt substitute.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_5H_8KNO_4 \cdot H_2O$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.2%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Not less than +22.5° and not more than +24.0°, calculated on the dried basis.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 100 mL of glacial acetic acid. A few drops of water may be added first to speed dissolution. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

Caution: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 10.16 mg of $C_5H_8KNO_4 \cdot H_2O$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 80° in vacuum for 5 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of sample in sufficient 2 *N* hydrochloric acid to make 100 mL.

Packaging and Storage Store in tight containers.

Monosodium L-Glutamate

Monosodium Glutamate Monohydrate; Monosodium Glutamate; Sodium Glutamate; MSG

$$NaOOCCH_2CH_2CH(NH_2)COOH \cdot H_2O$$

$C_5H_8NNaO_4 \cdot H_2O$ Formula wt 187.13

INS: 621 CAS: monohydrate [6106-04-3]

View IR

DESCRIPTION

Monosodium L-Glutamate occurs as white, free-flowing crystals or crystalline powder. It is freely soluble in water, and is sparingly soluble in alcohol. The pH of a 1:20 aqueous solution is between 6.7 and 7.2.

Function Flavor enhancer.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_5H_8NNaO_4 \cdot H_2O$, calculated on the dried basis.
Chloride Not more than 0.2%.
Clarity and Color of Solution Passes test.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.5%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +24.5° and +25.5°, calculated on the dried basis; or $[\alpha]_{546.1\ nm}^{25^\circ}$: Between +29.4° and +30.4°, calculated on the dried basis.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 100 mL of glacial acetic acid. A few drops of

water may be added first to speed dissolution. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

Caution: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 9.356 mg of $C_5H_8NNaO_4 \cdot H_2O$.

Chloride Determine as directed in *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 10-mg sample does not exceed that shown in a control containing 20 µg of chloride (Cl) ion.

Clarity and Color of Solution A 1:10 aqueous solution is colorless and has no more turbidity than a standard mixture prepared as follows: Dilute 0.2 mL of *Standard Chloride Solution* (see *Chloride and Sulfate Limit Tests*, Appendix IIIB) with water to 20 mL; add 1 mL of 1:3 nitric acid, 0.2 mL of a 1:50 dextrin solution, and 1 mL of a 1:50 silver nitrate solution; mix; and allow to stand for 15 min.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.

Alternatively, determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

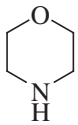
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 100° for 5 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of sample in sufficient 2 *N* hydrochloric acid to make 100 mL.

Packaging and Storage Store in tight containers.

Morpholine

Tetrahydro-2H-1,4-oxazine; Diethylene Oximide; Diethylene Imidoxide



C_4H_9NO Formula wt 87.12

CAS: [110-91-8]

View IR

DESCRIPTION

Morpholine occurs as a clear, colorless, mobile, hygroscopic liquid. It is miscible with water with the evolution of some heat. It is also miscible with acetone, with ether, with castor oil, with methanol, with alcohol, and with many oils such as linseed and pine.

Function Boiler water additive; component of coatings for fruits and vegetables.

REQUIREMENTS

Identification The infrared absorption spectrum of a neat dispersion of the sample between two sodium chloride plates exhibits maxima only at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 99.%.

Distillation Range Between 126.0° and 130.0°.

Lead Not more than 1 mg/kg.

Refractive Index Between 1.454 and 1.455 at 20°.

Specific Gravity Between 0.997 and 1.000 at 25°.

TESTS

Assay

Mixed Indicator Solution Prepare separate 0.1% solutions of bromocresol green in methanol and the sodium salt of methyl red in water. Mix 5 parts by volume of the bromocresol green solution with one part of the methyl red solution.

Procedure Transfer 50 mL of water into a 250-mL flask. Add 0.4 mL of *Mixed Indicator Solution*, and neutralize, adding 0.1 *N* hydrochloric acid dropwise just to the disappearance of the green color. Transfer 1.4 to 1.6 g of sample, accurately weighed, into the flask, and swirl to effect complete solution. Titrate with standard 0.5 *N* hydrochloric acid to the disappearance of the green color. Each milliliter of 0.5 *N* hydrochloric acid is equivalent to 43.56 mg of C_4H_9NO.

Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB.

Specific Gravity Determine at 25° by any reliable method (see *General Provisions*).

Packaging and Storage Store in tight containers.

Mustard Oil

DESCRIPTION

Mustard Oil occurs as a clear, pale yellow liquid with a sharp, pungent taste. It is the volatile oil obtained by the steam and water distillation of the comminuted presscakes of the seeds from *Brassica nigra* (Linnaeus) W.D.J. Koch or *Brassica*

juncea (Linnaeus) Czernjajev (Fam. Cruciferae). The essential oil forms upon maceration of the comminuted seeds in warm water, which releases sinigrin, a β-glucopyranoside, that is subsequently enzymatically hydrolyzed to allyl isothiocyanate.

Caution: Mustard Oil is a lacrimator.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the respective spectrum for allyl isothiocyanate in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 93.0%, as C_3H_5NCS (allyl isothiocyanate).
Refractive Index Between 1.524 and 1.534 at 20°.
Specific Gravity Between 1.008 and 1.019.

TESTS

Assay Determine as directed in method *M-1a—General Method, Polar Column*, under *Assay by Gas Chromatography*, Section 3.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using 20° as the determination temperature.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool, dry place protected from light in tight containers.

Myristic Acid

Tetradecanoic Acid

$$CH_3(CH_2)_{12}COOH$$

$C_{14}H_{28}O_2$ Formula wt 228.37

CAS: [544-63-8]

DESCRIPTION

Myristic Acid occurs as a hard, white or faintly yellow, somewhat glossy, crystalline solid or as a white or yellow-white powder. It is obtained from coconut oil and other fats. Myristic Acid is practically insoluble in water, but it is soluble in alcohol, in chloroform, and in ether.

Function Component in the manufacture of other food-grade additives; defoaming agent.

REQUIREMENTS

Acid Value Between 242 and 249.
Iodine Value Not more than 1.0.
Lead Not more than 2 mg/kg.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 242 and 251.
Solidification Point Between 48° and 55.5°.
Unsaponifiable Matter Not more than 1%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 3 g of sample, accurately weighed.
Solidification Point Determine as directed under *Solidification Point*, Appendix IIB.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Myrrh Oil

CAS: [9000-45-7]

FEMA: 2766

View IR

DESCRIPTION

Myrrh Oil occurs as a light brown or green liquid having the characteristic odor of the gum. It is the volatile oil obtained by steam distillation from myrrh gum obtained from several species of *Commiphora* (Fam. Burseraceae). It is soluble in most fixed oils, but is only slightly soluble in mineral oil. It is insoluble in glycerin and in propylene glycol. It becomes darker in color and more viscous under the influence of air and light.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those

of a typical spectrum as shown in the section on *Infrared Spectra* using the same test conditions as specified therein.
Acid Value Between 2 and 13.
Angular Rotation Between −60° and −98°.
Refractive Index Between 1.519 and 1.528 at 20°.
Saponification Value Between 9 and 35.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.985 and 1.014.

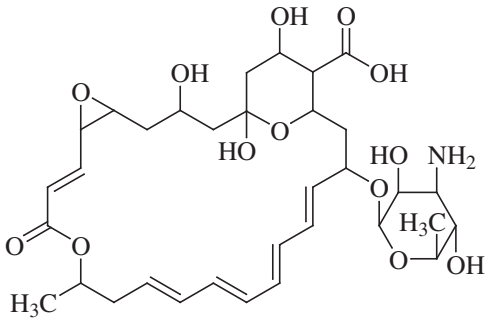
TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter dissolves in 10 mL of 90% alcohol, occasionally with opalescence or turbidity.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Natamycin

Pimaricin



$C_{33}H_{47}NO_{13}$ Formula wt 665.73

INS: 235 CAS: [7681-93-8]

DESCRIPTION

Natamycin occurs as an off white to cream colored powder that may contain up to 3 moles of water. It melts with decomposition at about 280°. It is practically insoluble in water, slightly soluble in methanol, and soluble in glacial acetic acid and in dimethylformamide.

Function Antimycotic.

REQUIREMENTS

Identification Transfer 50 mg of sample, accurately weighed, into a 200-mL volumetric flask, add 5.0 mL of water, and moisten the sample. Add 100 mL of a 1:1000 solution of glacial acetic acid in methanol, and shake by mechanical means in the dark until dissolved. Dilute to volume with the acetic acid–methanol solution, and mix. Transfer 2.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with the acetic acid–methanol solution, and mix. The ultraviolet absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as those of a similar solution of USP Natamycin Reference Standard, concomitantly measured.
Assay Not less than 97.0% and not more than 102.0% of $C_{33}H_{47}NO_{13}$, calculated on the anhydrous basis.
Lead Not more than 2 mg/kg.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +276° and +280°.
pH Between 5.0 and 7.5.
Water Between 6.0% and 9.0%.

TESTS

Assay (**Note**: Throughout this *Assay*, protect all solutions containing Natamycin from direct light.)

Mobile Phase Dissolve 3.0 g of ammonium acetate and 1.0 g of ammonium chloride in 760 mL of water, and mix. Add 5.0 mL of tetrahydrofuran and 240 mL of acetonitrile, mix, and filter through a 0.5-μm or finer porosity filter. If necessary, make adjustments to meet the system suitability requirements.

Standard Preparation Transfer about 20 mg of USP Natamycin Reference Standard, accurately weighed, into a 100-mL volumetric flask. Add 5.0 mL of tetrahydrofuran, and sonicate for 10 min. Add 60 mL of methanol, and swirl to dissolve. Add 25 mL of water, and mix. Allow to cool to room temperature. Dilute to volume with water, mix, and filter through a membrane filter of 5-μm or finer porosity.

Resolution Solution Dissolve 20 mg of sample in 99:1 (v/v) methanol:0.1 *N* hydrochloric acid mixture, and allow to stand for 2 h.

Note: Use this solution within 1 h.

Assay Preparation Transfer about 20 mg of sample, accurately weighed, into a 100-mL volumetric flask. Proceed as directed under *Standard Preparation*, beginning with ''add 5.0 mL of tetrahydrofuran. . . .''

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with an ultraviolet detector measuring at 303 nm and a 25-cm × 4.6-mm (id) column packed with octadecylsilanized silica (Supelcosil LC 18, or equiva-

lent). The flow rate is about 3 mL/min. Chromatograph the *Standard Preparation*, and record the peak responses. The column efficiency is not less than 3000 theoretical plates, the tailing factor is between 0.8 and 1.3, and the relative standard deviation for three replicate injections is not more than 1.0%. Chromatograph the *Resolution Solution.* The resolution between the sample and its methyl ester is not less than 2.5. The relative retention times are about 0.7 for Natamycin and 1.0 for its methyl ester.

Procedure Separately inject about 20 µL each of the *Standard Preparation* and the *Assay Preparation* into the chromatograph, and record the peak areas of the major peaks. Calculate the percentage of Natamycin in the portion of sample taken by the formula

$$0.1(W_S P_S/W_U)(r_U/r_S),$$

in which W_S is the weight, in milligrams, of USP Natamycin Reference Standard taken to prepare the *Standard Preparation*; P_S is the stated content, in micrograms per milligram, of USP Natamycin Reference Standard; W_U is the weight, in milligrams, of sample taken to prepare the *Assay Preparation*; and r_U and r_S are the peak area responses obtained with the *Assay Preparation* and the *Standard Preparation*, respectively.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 100 mg of sample in each 10 mL of glacial acetic acid.

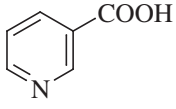
pH Determine as directed under *pH Determination*, Appendix IIB, using an aqueous suspension of sample containing 10 mg/mL.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight, light-resistant containers in a cool place.

Niacin

Nicotinic Acid; 3-Pyridinecarboxylic Acid



$C_6H_5NO_2$ Formula wt 123.11

CAS: [59-67-6]

DESCRIPTION

Niacin occurs as white or light yellow crystals or as a crystalline powder. One gram of sample dissolves in 60 mL of water. It is freely soluble in boiling water and in boiling alcohol and also in solutions of alkali hydroxides and carbonates. It is almost insoluble in ether.

Function Nutrient.

REQUIREMENTS

Identification

A. The infrared absorption spectrum of a mineral oil dispersion of the sample, previously dried at 105° for 1 h, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Niacin Reference Standard.

B. Dissolve about 50 mg of sample in 20 mL of water, neutralize to litmus paper with 0.1 *N* sodium hydroxide, and add 3 mL of cupric sulfate TS. A blue precipitate gradually forms.

C. Determine the absorbance of a solution containing 20 µg of sample in each milliliter of water, in a 1-cm cell, at 237 nm and 262 nm, using water as the blank. The ratio A_{237}/A_{262} is between 0.35 and 0.39.

Assay Not less than 99.5% and not more than 101.0% of $C_6H_5NO_2$, calculated on the dried basis.

Loss on Drying Not more than 1.0%.

Melting Range Between 234° and 238°.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 300 mg of sample, accurately weighed, in about 50 mL of water, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*). Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 12.31 mg of $C_6H_5NO_2$.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying the sample at 105° for 1 h.

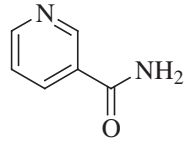
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.

Residue on Ignition Determine as directed under *Residue on Ignition*, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

Niacinamide

Nicotinamide



$C_6H_6N_2O$ Formula wt 122.13

CAS: [98-92-0]

DESCRIPTION

Niacinamide occurs as a white, crystalline powder. One gram dissolves in about 1 mL of water, in about 1.5 mL of alcohol,

and in about 10 mL of glycerin. Its solutions are neutral to litmus.

Function Nutrient.

REQUIREMENTS

Identification

A. Transfer about 20 mg of sample into a 1000-mL volumetric flask, dissolve in and dilute to volume with water, mix, and determine the absorbance of the solution in a 1-cm cell at 245 nm and at 262 nm with a suitable spectrophotometer, using water as the blank. The ratio A_{245}/A_{262} is 0.65 ± 0.02.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Niacinamide Reference Standard.

Assay Not less than 98.5% and not more than 101.0% of $C_6H_6N_2O$ after drying.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 0.5%.

Melting Range Between 128° and 131°.

Readily Carbonizable Substances Passes test.

Residue on Ignition Not more than 0.1%.

TESTS

Assay (**Note**: Use either this *Assay* test or the *Assay* test in the monograph for *Niacinamide Ascorbate*.)

Mobile Phase Prepare a filtered and degassed solution containing 70% 0.005 *M* sodium 1-heptanesulfonate and 30% methanol.

Standard Preparation (**Note**: Use low-actinic glassware, and prepare fresh solutions daily.) Transfer about 50 mg of USP Niacinamide Reference Standard, accurately weighed, into a 100-mL volumetric flask, dissolve in about 3 mL of water, dilute with *Mobile Phase* to volume, and mix. Pipet 4.0 mL of the resulting solution into a 50-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Assay Preparation (**Note**: Use low-actinic glassware, and prepare fresh solutions daily.) Prepare as directed under *Standard Preparation*, using sample instead of the Reference Standard.

Resolution Solution Prepare a solution containing equal volumes of the *Standard Preparation* and of a niacin solution similarly prepared and having the same concentration.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with a 254-nm detector and a 30-cm × 3.9-mm column packed with octadecylsilanized silica (μBondapak C18, or equivalent). Adjust the flow rate to about 2 mL/min.

Procedure Chromatograph the *Resolution Solution*. The resolution, *R*, between the niacin and Niacinamide peaks is not less than 3.0. Chromatograph replicate injections of the *Standard Preparation*, record the chromatograms, and measure the responses for the major peaks. The relative standard deviation is not more than 2.0%. Separately inject equal volumes (about 20 μL) of the *Standard Preparation* and the *Assay Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in milligrams, of $C_6H_6N_2O$ in the portion of sample taken by the formula

$$1250C(r_U/r_S),$$

in which *C* is the concentration, in milligrams per milliliter, of USP Niacinamide RS in the *Standard Preparation*, and r_U and r_S are the peak responses for the *Assay Preparation* and the *Standard Preparation*, respectively.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample over silica gel for 4 h.

Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.

Readily Carbonizable Substances Determine as directed under *Readily Carbonizable Substances*, Appendix IIB, but dissolve 200 mg of sample in 5 mL of 95% sulfuric acid. The solution has no more color than *Matching Fluid A*.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight containers.

Niacinamide Ascorbate

Nicotinamide Ascorbate

CAS: [1987-71-9]

DESCRIPTION

Niacinamide Ascorbate occurs as a yellow colored powder that may gradually darken upon exposure to air. It is a complex of ascorbic acid ($C_6H_8O_6$) and niacinamide ($C_6H_6N_2O$). One gram is soluble in 3.5 mL of water and in about 20 mL of alcohol. It is very slightly soluble in chloroform and in ether and is sparingly soluble in glycerin.

Function Nutrient.

REQUIREMENTS

Identification

A. A 1:50 aqueous solution slowly reduces alkaline cupric tartrate TS at 25°, but more readily upon heating.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits maxima only at the same wavelengths as those of a similar preparation of USP Ascorbic Acid Reference Standard and of a similar preparation of USP Niacinamide Reference Standard.

Assay Not less than 73.5% of ascorbic acid ($C_6H_8O_6$) and not less than 24.5% of niacinamide ($C_6H_6N_2O$), calculated

on the anhydrous basis. The total of ascorbic acid and niacinamide is not less than 99.0%.

Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.5%.
Melting Range Between 141° and 145°.
Residue on Ignition Not more than 0.1%.

TESTS

Assay for Ascorbic Acid Dissolve about 400 mg of sample, accurately weighed, in a mixture of 100 mL of water, recently boiled and cooled, and 25 mL of 2 *N* sulfuric acid. Titrate the solution immediately with 0.1 *N* iodine, adding starch TS near the endpoint. Each milliliter of 0.1 *N* iodine is equivalent to 8.806 mg of $C_6H_8O_6$.

Assay for Niacinamide Dissolve about 300 mg of sample, accurately weighed, in 20 mL of glacial acetic acid, warming slightly if necessary to effect solution. Add 100 mL of benzene and 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 12.21 mg of $C_6H_6N_2O$.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample to constant weight at 75°.

Melting Range Determine as directed in *Procedure for Class Ia* under *Melting Range or Temperature*, Appendix IIB.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in tight, light-resistant containers.

Nickel

Nickel Catalysts

Ni — Atomic wt 58.69

CAS: [7440-02-0]

DESCRIPTION

Nickel metal occurs as a lustrous, white, hard, ferromagnetic, metallic solid. Nickel is commonly used as a catalyst for hydrogenation reactions for food chemicals. Depending on the use, Nickel catalysts fall into two general categories: *Sponge Nickel Catalyst* and *Supported Nickel Catalyst*.

Sponge Nickel Catalyst is typically used in the manufacture of amines and polyols. It is prepared by chemically treating a Nickel–aluminum amalgam with sodium hydroxide to remove the majority of aluminum, thus leaving a highly porous (skeletal) Nickel solid. The resulting *Sponge Nickel Catalyst* is extremely pyrophoric in air and must be stored under an inert liquid.

Supported Nickel Catalyst is typically used in the manufacture of edible oils. It is prepared from a Nickel salt deposited onto an inert carrier consisting of various types of acceptable silicas, aluminas, or combinations thereof. The Nickel salt–carrier complex is not catalytically active and is converted to *Supported Nickel Catalyst* in a stream of hydrogen at elevated temperatures. After activation, *Supported Nickel Catalyst* also is pyrophoric and must be protected from air, typically by suspending it in a food-grade stearine. It usually is supplied as droplets or flakes.

Function Catalyst for hydrogenation reactions.

REQUIREMENTS

Identification

Sponge Nickel Catalyst Dissolve approximately 100 mg of sample in about 2 mL of hydrochloric acid, and dilute to about 20 mL with water. Place 5 mL of this solution into a test tube, add a few drops of bromine water, and make it slightly alkaline with ammonium hydroxide. Add 2 to 3 mL of a 1% solution of dimethylglyoxime in alcohol. An intense red color or precipitate forms.

Supported Nickel Catalyst Ash as described under *Assay* (below). Transfer a 5-mL aliquot of the ashed sample solution into a test tube, and complete the identification as described above.

Assay

Sponge Nickel Catalyst Not less than 83.0% of Ni, calculated on the dried basis.

> **Caution**: Sponge Nickel is pyrophoric when dried. Handle with extreme care.

Supported Nickel Catalyst Not less than 20.0% and not more than 27.0% of Ni, calculated on the as-is basis.

TESTS

Assay **(Caution:** Because of the danger of combustion, wear goggles and other appropriate protective gear.)

Sponge Nickel Catalyst

Sample Solution Place about 5 g of wet sample into a 20-mL beaker, add 10 mL of ethanol, and decant the supernatant fluid. Repeat the sequence of adding ethanol and decanting the fluid five times. Weigh a clean, 30-mL round-bottom flask, and record that weight as W_F. Transfer the catalyst sample into the tared flask, and dry for 5 h under vacuum, warming on a 60° water bath. Using nitrogen, return the flask to atmospheric pressure, and cool it to room temperature. Weigh the flask with the dried catalyst sample, and record that weight as W_C. Calculate the weight, in grams, of the dried catalyst, W_S, by the formula $W_C - W_F$.

Place 30 mL of water into a 500-mL beaker, and add the dried catalyst. Wash the flask with 50 mL of 1:1 hydrochloric

acid, and add the wash to the beaker. Heat the beaker gently to dissolve the catalyst, then cool to room temperature. Filter the solution through Whatman number 5, or equivalent, filter paper into a 250-mL volumetric flask. Fill the flask to volume with water to prepare the *Sample Solution*.

Procedure Place 5 mL of the *Sample Solution* into a 200-mL beaker. Add 2 g of tartaric acid and 100 mL of water, heat to about 80°, and add 30 mL of 1% dimethylglyoxime in ethanol. Add ammonium hydroxide until the solution is slightly basic, and place the mixture on a steam bath for 20 min. Filter the precipitated material into a tared, fritted-glass, medium-porosity filter crucible, and wash with hot water until the filtrate is free of chloride. Dry the precipitate at 120° for 2 h, and then dry to constant weight, and weigh.

Calculate the percent Nickel by the following formula:

$$50(W_P \times 20.32)/W_S,$$

in which W_P is the weight, in grams, of the precipitate; 20.32 is the percent Nickel in the precipitate; and W_S is the weight, in grams, of the dried sample taken.

Supported Nickel Catalyst

Sample Solution Fill a 100-mL porcelain crucible half-full of ashless filter paper pulp. Place 2 g of the finished catalyst, in droplet or flake form and accurately weighed, on top of the paper pulp. Transfer the crucible to a muffle furnace set at room temperature, and slowly raise the temperature to 650° so that the stearine melts into the paper, and the organic mass burns and chars slowly. Continue heating at 650° for 2 h or until the carbon is burned off. Cool, add 20 mL of hydrochloric acid, quantitatively transfer the solution or suspension into a 400-mL beaker, and carefully evaporate to dryness on a steam bath. Cool, add 20 mL of hydrochloric acid, warm to aid dissolution (catalysts containing silica will not dissolve completely), transfer into a 500-mL volumetric flask, dilute to volume with water, and mix. Allow any solids to settle, pipet a clear, 50-mL aliquot into a 400-mL beaker, and dilute to 250 mL with water. (If there is suspended matter in the volumetric flask, filter a portion through a dry, medium-speed filter paper into a dry receiver, and pipet from the receiver.)

Procedure Determine as directed under *Procedure for Sponge Nickel Catalyst*, beginning with ''Add 2 g of tartaric acid. . . .''

Calculate the percent Nickel by the following formula:

$$10(W_P \times 20.32)/W_S,$$

in which W_P is the weight, in grams, of the precipitate; 20.32 is the percent Nickel in the precipitate; and W_S is the weight, in grams, of the sample taken.

Packaging and Storage

Sponge Nickel Catalyst Store under liquids such as water, alcohol, or methylcyclohexane in a cool, dry place.

Supported Nickel Catalyst Store in tight containers in a cool, dry place.

Nisin Preparation

contains 34 amino acids and has an approximate empirical formula of $C_{143}H_{230}O_{37}N_{42}S_7$ Formula wt ~3348

INS: 234 CAS: [1414-45-5]

DESCRIPTION

Nisin Preparation occurs as a white, free-flowing powder. It is a mixture of closely related polypeptides produced by strains of the *Lactococcus lactis* subsp. *lactis* Lancefield Group N in a sterilized milk-culture medium. Nisin in the fermentation broth can be recovered by various methods, such as injecting sterile, compressed air (froth concentration); acidification; salting out; and spray-drying.

The product comprises Nisin and sodium chloride that is adjusted to an activity level of not less than 900 IU/mg by the addition of sodium chloride and nonfat milk solids.

Function Antimicrobial agent.

REQUIREMENTS

Assay Not less than 900 IU of Nisin per milligram of Nisin Preparation.

Differentiation of Nisin from Other Antimicrobial Substances Passes tests.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 3.0%.

Microbial Limits:

Aerobic Plate Count Not more than 10 CFU per gram.

E. coli Negative in 25 g.

Salmonella Negative in 25 g.

Sodium Chloride Content Not less than 50.0%.

TESTS

Assay

Assay Medium Dissolve 10 g of bacteriological peptone, 3 g of beef extract, 3 g of sodium chloride, 1.5 g of autolyzed yeast, 1 g of brown sugar, and 15 g of agar in distilled water to a final volume of 1000 mL. Sterilize in an autoclave at 121° for 15 min. The medium can be stored in a covered container at room temperature until use. At the time of use, melt the medium, and cool to approximately 50°. Add 2% of a 1:1 mixture of Tween 20 (polyoxyethylene sorbitan monolaurate) and distilled water, previously held for 20 to 30 min at 48°.

Assay Organism Maintain *Micrococcus luteus* (ATCC 10240,[1] NCIMB 8166[2]) by subculturing on agar slants of the *Assay Medium* and incubating at 30° for 48 h. Prepared slants

[1]ATCC is the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110.

[2]NCIMB Ltd. is located at 23 St. Machar Drive, Aberdeen, Scotland AB24 3RY.

may be stored for a maximum of 14 days at 4° until required. When required for use, the growth on the slant cultures is suspended in 7 mL of sterilized normal saline solution.

Nisin Standard Solutions Suspend 100 mg of Nisin International Reference Preparation[3] (1000 IU of Nisin per milligram), accurately weighed, in 80 mL of 0.02 *N* hydrochloric acid. Set aside at room temperature for 2 h. Dilute the suspension to a final volume of 100.0 mL with 0.02 *N* hydrochloric acid. This standard stock solution contains 1000 IU of Nisin per milliliter. From this solution, pipet 0.5, 1.0, 2.5, 5.0, and 10.0 mL into separate 1000-mL volumetric flasks. Dilute each flask to volume with 0.02 *N* hydrochloric acid to obtain *Nisin Standard Solutions* with concentrations of 0.5, 1.0, 2.5, 5.0 and 10.0 IU/mL. Store the standard stock solution for up to 7 days at 4°, or prepare a fresh solution each day.

Preparation of the Standard Curve Using normal saline solution, dilute the suspension of the *Assay Organism* to 1:10, and mixing thoroughly, add 2 mL of this dilution to each 100 mL of melted *Assay Medium* held at 48°. Pour the inoculated medium to a depth of 3 to 4 mm (approximately 15 mL) into 5 sterile, flat-bottomed Petri dishes, and allow to solidify. Invert the plates, and store at 4° for l h. Bore four 8-mm holes on 30-mm centers in each plate of the agar medium with the aid of a sterile, hollow-steel borer, 8 to 9 mm in diameter, and discard the agar discs. Transfer, in quadruplicate, 0.20-mL volumes in the concentration range of 0.5 to 10.0 IU/mL of the *Nisin Standard Solutions* into the holes, one concentration to a plate. Cover the plates, and incubate them overnight at 30°. Measure the zones of inhibition to the nearest 0.1 mm by means of calipers or other appropriate devices. Plot the log of the Nisin concentration in the critical range against the zone diameters, and draw the best straight line through the plotted points.

Procedure Suspend 100 mg of sample in 80 mL of 0.02 *N* hydrochloric acid in a 100-mL volumetric flask, and set aside at room temperature for 2 h. Dilute the solution to volume by adding 0.02 *N* hydrochloric acid. Dilute to a 1:200 solution with 0.02 *N* hydrochloric acid. Proceed as described above for the *Standard Curve*, transferring in quadruplicate a measured volume of this solution into the holes of four agar discs. After incubation, measure the zones of inhibition. From the *Standard Curve*, determine the Nisin concentrations, and average the results.

Differentiation of Nisin from Other Antimicrobial Substances

Stability to Acid Suspend a 100-mg sample in 0.02 *N* hydrochloric acid as described in the preparation of the *Nisin Standard Solutions* under the *Assay*. Boil this solution for 5 min. Using the *Assay* method described above, determine the Nisin concentration. No significant loss of activity is noted following this heat treatment. The calculated Nisin concentration of the boiled sample is 100% ± 5% of the *Assay* value. Adjust the pH of the Nisin solution to 11.0 by adding 5 *N* sodium hydroxide. Heat the solution at 65° for 30 min, and then cool. Adjust the pH to 2.0 by adding hydrochloric acid dropwise. Again determine the Nisin concentration using the *Assay* method described above. Complete loss of the antimicrobial activity of Nisin is observed following this treatment.

Tolerance of Lactococcus lactis *to High Concentrations of Nisin* Prepare cultures of *Lactococcus lactis* (ATCC 11454, NCIMB 8586) in sterile skim milk by incubating for 18 h at 30°. Prepare one or more flasks containing 100 mL of litmus milk, and sterilize at 121° for 15 min. Suspend 0.1 g of sample in the sterilized litmus milk, and allow to stand at room temperature for 2 h. Add 0.1 mL of the *L. lactis* culture, and incubate at 30° for 24 h. *L. lactis* will grow in this concentration of sample (about 1000 IU/mL); however, it will not grow in similar concentrations of other antimicrobial substances. This test will not differentiate Nisin from subtilin.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 2-g sample at 105° for 2 h, and continuing to dry to a constant weight.

Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

Aerobic Plate Count

E. coli

Salmonella

Sodium Chloride Content Accurately weigh about 20 mg of sample, and dissolve it in 50 mL of water contained in a glass-stoppered flask. Add, while agitating, 3 mL of nitric acid, 5 mL of nitrobenzene, 50.0 mL of 0.1 *N* silver nitrate, and 2 mL of ferric ammonium sulfate TS. Shake well, and titrate the excess silver nitrate with 0.2 *N* ammonium thiocyanate. The titration endpoint is indicated by the appearance of a red color. Perform a blank determination (see *General Provisions*). Calculate the content of sodium chloride in the sample by the formula

$$(2 \times 5.844)(A - B),$$

in which 2 and 5.844 are equivalence factors; *A* is the volume, in milliliters, of 0.2 *N* ammonium thiocyanate consumed by the blank; and *B* is the volume, in milliliters, of 0.2 *N* ammonium thiocyanate consumed by the sample preparation.

Packaging and Storage Store in well-closed containers at temperatures not exceeding 22°.

[3]The Nisin International Reference Preparation is available from the WHO International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries & Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England.

Nitrogen

N_2 Formula wt 28.01

INS: 941 CAS: [7727-37-9]

DESCRIPTION

Nitrogen occurs as a colorless gas that is not combustible and does not support combustion. It may be condensed to a colorless liquid boiling at −195.8° or to a white solid melting at −209.8°. One liter of the gas weighs about 1.25 g at 0° and at 760 mm Hg. One volume of the gas dissolves in about 62 volumes of water and in about 8 volumes of alcohol at 20° and at 760 mm Hg.

Function *Gas*: air and oxygen displacer; propellant and aerating agent; packaging gas; *Liquid*: direct-contact freezing agent.

REQUIREMENTS

Note: Reduce the sample gas cylinder pressure with a regulator. Measure the sample gas with a gas volume meter downstream from the detector tubes to minimize contamination of or change to the gas samples. The detector tubes called for in certain tests are described under *Solutions and Indicators*.

Identification Insert a burning wood splinter into a test tube filled with sample gas. The flame is extinguished.

Note: Use caution.

Assay Not less than 99.0% of N_2, by volume.
Carbon Dioxide Not more than 0.03%, by volume.
Carbon Monoxide Not more than 10 ppm, by volume.
Oxygen Not more than 1.0%, by volume.
Water Passes test.

TESTS

Assay (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a thermal conductivity detector and a 3-m × 4-mm (id) column packed with a molecular sieve, or equivalent, prepared from a synthetic alkali–metal aluminosilicate capable of absorbing molecules with diameters of up to 0.5 nm, which permits complete separation of oxygen from nitrogen. Use industrial-grade helium (99.99%) as the carrier gas. Introduce a gas sample into the gas-sampling valve. Select the operating conditions of the gas chromatograph so that the standard peak signal resulting from the following procedure corresponds to not less than 70% of the full-scale reading. The peak response produced by the sample gas exhibits a retention time corresponding to that produced by an oxygen–helium certified standard (a mixture of 1.0% oxygen in industrial-grade helium is available from most suppliers) and is equivalent to not more than 1.0% of oxygen, by volume, when compared with the peak response of the oxygen–helium certified standard, indicating not less than 99.0% of N_2, by volume.

Carbon Dioxide Pass 1050 ± 50 mL of sample gas through a carbon dioxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 0.03%, by volume.
Carbon Monoxide Pass 1050 ± 50 mL of sample gas through a carbon monoxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 10 ppm, by volume.
Oxygen Determine as directed under *Assay* (above).
Water Pass 24,000 mL of sample gas through a suitable water-absorption tube not less than 100 mm long, which previously has been flushed with about 500 mL of sample gas and weighed. Regulate the flow so that about 60 min will be required for passage of the gas. The gain in weight of the absorption tube does not exceed 1.0 mg.

Packaging and Storage Store in tight cylinders.

Nitrogen Enriched Air

N_2 Formula wt 28.01

DESCRIPTION

Nitrogen Enriched Air occurs as a colorless gas. It is produced from air *in situ* by physical separation methods. It contains not less than 90% and not more than 99% nitrogen, by volume. The remaining components are noble gases and, primarily, oxygen.

Function Air and oxygen displacer.

REQUIREMENTS

Note: Reduce the sample gas cylinder pressure with a regulator. Measure the sample gas with a gas volume meter downstream from the detector tubes to minimize contamination of or change to the gas samples. The detector tubes called for in certain tests are described under *Solutions and Indicators*.

Labeling Where the gas is piped from cylinders or directly from the collecting tank to the point of use, label each outlet ''Nitrogen Enriched Air.''
Identification Insert a burning wood splinter into a test tube filled with sample gas. The flame is extinguished.

Note: Use caution.

Assay Not less than 90.0% and not more than 99.0% of N_2, by volume.
Carbon Dioxide Not more than 0.03%, by volume.
Carbon Monoxide Not more than 10 ppm, by volume.
Nitric Oxide and Nitrogen Dioxide Not more than 2.5 ppm, by volume.
Oxygen Not less than 1.0% and more than 10.0%, by volume.

Sulfur Dioxide Not more than 5 ppm, by volume.
Water Passes test.

TESTS

Assay (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a thermal conductivity detector and a 3-m × 4-mm (id) column packed with a molecular sieve, or equivalent, prepared from a synthetic alkali–metal aluminosilicate capable of absorbing molecules with diameters of up to 0.5 nm, which permits complete separation of oxygen from nitrogen. Use industrial-grade helium (99.99%) as the carrier gas. Introduce a gas sample into the gas-sampling valve. Select the operating conditions of the gas chromatograph so that the peak signal of a 5.0% oxygen–helium certified standard (a mixture of 5.0% oxygen, by volume, in industrial-grade helium is available from most suppliers) corresponds to approximately 45% of the full-scale reading. The peak response produced by the sample gas exhibits a retention time corresponding to that produced by the 5.0% oxygen–helium standard and, when compared with the peak response of that standard, is equivalent to not less than 1.0% and not more than 10.0% of oxygen, indicating not less than 90.0% and not more than 99.0% of N_2, by volume.
Carbon Dioxide Pass 1050 ± 50 mL of sample gas through a carbon dioxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 0.03%, by volume.
Carbon Monoxide Pass 1050 ± 50 mL of sample gas through a carbon monoxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 10 ppm, by volume.
Nitric Oxide and Nitrogen Dioxide Pass 550 ± 50 mL of sample gas through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 2.5 ppm, by volume.
Oxygen Determine as directed under *Assay* (above).
Sulfur Dioxide Pass 1050 ± 50 mL of sample gas through a sulfur dioxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 5 ppm, by volume.
Water Pass 24,000 mL of sample gas through a suitable water-absorption tube, not less than 100 mm long, that previously has been flushed with about 500 mL of sample gas and weighed. Regulate the flow of sample gas so that about 60 min will be required for it to pass through the tube. The weight gain of the absorption tube does not exceed 1.0 mg.

Packaging and Storage Store in metal cylinders or in a low-pressure collecting tank.

Nitrous Oxide

Nitrogen Oxide

N_2O Formula wt 44.01

INS: 942 CAS: [10024-97-2]

DESCRIPTION

Nitrous Oxide occurs as a colorless gas. One liter at 0° and 760 mm Hg weighs about 1.97 g. One volume dissolves in about 1.4 volumes of water at 20° and 760 mm Hg. It is freely soluble in alcohol and soluble in ether and in oils.

Function Propellant; aerating agent; packaging gas.

REQUIREMENTS

> **Note**: The following tests are designed to reflect the quality of Nitrous Oxide in both its vapor and its liquid phases, which are present in previously unopened cylinders. Reduce the sample gas cylinder pressure with a regulator. Withdraw the samples for the tests with the least possible release of sample gas consistent with proper purging of the sample apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination of or change to the samples. The detector tubes called for in certain tests are described at the end of *Solutions and Indicators*.

Perform the tests in the sequence in which they are listed below.

Identification

A. With the cylinder temperatures the same and maintained between 15° and 25°, concomitantly read the pressure of the cylinder of sample gas and of a cylinder of 99.9% Nitrous Oxide certified standard (available from most suppliers).

> **Note**: Do not use the Nitrous Oxide certified standard if its cylinder has been depleted to less than half of its full capacity.

The pressure of the sample gas cylinder is within 50 psi of that of the Nitrous Oxide certified standard cylinder.

B. Pass 100 ± 5 mL of sample gas released from the vapor phase of the contents of the sample gas cylinder through a carbon dioxide detector tube at the rate specified for the tube. No color change occurs (distinction from carbon dioxide).
Carbon Monoxide Not more than 10 ppm, by volume.
Nitric Oxide Not more than 1 ppm, by volume.
Nitrogen Dioxide Not more than 1 ppm, by volume.
Halogens (as Cl) Not more than 1 ppm, by volume.
Carbon Dioxide Not more than 0.03%, by volume.
Ammonia Not more than 0.0025%, by volume.
Water Not more than 150 mg/m^3.
Odor Passes test.
Air Not more than 1.0%, by volume.
Assay Not less than 99.0% of N_2O, by volume.

TESTS

Carbon Monoxide Pass 1050 ± 50 mL of sample gas, released from the vapor phase of the contents of the sample gas cylinder, through a carbon monoxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 10 ppm, by volume.

Nitric Oxide Pass 550 ± 50 mL of sample gas, released from the vapor phase of the contents of the sample gas cylinder, through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 1 ppm, by volume.

Nitrogen Dioxide Arrange a sample gas cylinder so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it and to prevent frost from reaching the inlet of the detector tube. Release a flow of liquid into the tubing sufficient to provide 550 mL of the vaporized sample plus any excess necessary to ensure adequate flushing of air from the system. Pass 550 ± 50 mL of this gas through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 1 ppm, by volume.

Halogens Pass 1050 ± 50 mL of sample gas, released from the vapor phase of the contents of the sample gas cylinder, through a chlorine detector tube at the rate specified for the tube. The indicator change corresponds to not more than 1 ppm, by volume.

Carbon Dioxide Pass 1050 ± 50 mL of sample gas, released from the vapor phase of the contents of the sample gas cylinder, through a carbon dioxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 0.03%, by volume.

Ammonia Pass 1050 ± 50 mL of the sample gas, released from the vapor phase of the contents of the sample gas cylinder, through an ammonia detector tube at the rate specified for the tube. The indicator change corresponds to not more than 0.0025%, by volume.

Water Flush the sample gas regulator with 5 or more liters of the sample gas. Pass 50 ± 5 L, released from the vapor phase of the contents of the sample gas cylinder, through a water vapor detector tube connected to the regulator with a minimal length of metal or polyethylene tubing. Measure the gas passing through the detector tube with a gas flowmeter set at a flow rate of 2 L/min. The corrected indicator change corresponds to not more than 150 mg/m^3.

Odor Carefully open the sample gas cylinder valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose. No appreciable odor is discernible.

Air Determine as directed under *Assay*, below.

Assay (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a thermal-conductivity detector and a 6-m × 4-mm (id) column, or equivalent, packed with porous polymer beads, or equivalent, that permit complete separation of nitrogen and oxygen from Nitrous Oxide, although the nitrogen and oxygen may not be separated from each other. Use industrial-grade helium (99.99%) as the carrier gas, and control the column temperature. Introduce sample gas taken from the liquid phase, as directed under *Nitrogen Dioxide* (above), into the gas chromatograph by means of a gas-sampling valve. Select the operating conditions of the gas chromatograph so that the peak response resulting from the following procedure corresponds to not less than 70% of the full-scale reading. The sample's peak response exhibits a retention time corresponding to that produced by an air–helium certified standard (a mixture of 1.0% air in industrial-grade helium is available from most suppliers) and is equivalent to not more than 1.0% of air, by volume, when compared with the peak response of the air–helium certified standard, indicating not less than 99.0% of N_2O, by volume.

Packaging and Storage Preserve in cylinders.

Nutmeg Oil

Myristica Oil

CAS: [8008-45-5]

View IR

DESCRIPTION

Nutmeg Oil occurs as a colorless or pale yellow liquid with the characteristic odor and taste of nutmeg. It is the volatile oil obtained by steam distillation from the dried kernels of the ripe seed of *Myristica fragrans* Houttuyn (Fam. Myristicaceae). Two types of oil, the *East Indian* and the *West Indian*, are commercially available. It is soluble in alcohol.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is the East Indian or West Indian type.

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Angular Rotation *East Indian Type*: Between +8° and +30°; *West Indian Type*: Between +25° and +45°.

Refractive Index *East Indian Type*: Between 1.474 and 1.488; *West Indian Type*: Between 1.469 and 1.476 at 20°.

Residue on Evaporation *East Indian Type*: Not more than 60 mg per 3 mL; *West Indian Type*: Not more than 50 mg per 3 mL.

Solubility in Alcohol Passes test.

Specific Gravity *East Indian Type*: Between 0.880 and 0.910; *West Indian Type*: Between 0.854 and 0.880.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Residue on Evaporation Determine as directed under *Residue on Evaporation*, Appendix VI, using 3 mL of sample. Heat on a steam bath for 5 h, and then heat at 105° for 1 h.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. *East Indian Type*: One milliliter dissolves in 3 mL of 90% alcohol; *West Indian Type*: One milliliter dissolves in 4 mL of 90% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Octanoic Acid

Caprylic Acid

$$CH_3(CH_2)_6COOH$$

$C_8H_{16}O_2$ Formula wt 144.21

CAS: [124-07-2]

FEMA: 2799

DESCRIPTION

Octanoic Acid occurs as a colorless, oily liquid. It is slightly soluble in water and soluble in most organic solvents. Its specific gravity is about 0.910.

Function Component in the manufacture of other food-grade additives; defoaming agent; flavoring agent.

REQUIREMENTS

Acid Value Between 366 and 396.
Lead Not more than 0.1 mg/kg.
Iodine Value Not more than 2.0.
Residue on Ignition Not more than 0.1%.
Titer (Solidification Point) Between 8° and 15°.
Unsaponifiable Matter Not more than 0.2%.
Water Not more than 0.4%.

TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 10-g sample.
Titer (Solidification Point) Determine as directed under *Solidification Point*, Appendix IIB.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Oleic Acid

(*Z*)-9-Octadecenoic Acid

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$$

$C_{18}H_{34}O_2$ Formula wt 282.47

CAS: [112-80-1]

DESCRIPTION

Oleic Acid occurs as a colorless to pale yellow, oily liquid when freshly prepared, but upon exposure to air it gradually absorbs oxygen and darkens. It is an unsaturated acid obtained from fats. When strongly heated in air, it decomposes and produces acrid vapors. Its specific gravity is about 0.895. It is practically insoluble in water, but is miscible with alcohol, with ether, and with fixed and volatile oils.

Function Component in the manufacture of other food-grade additives; defoaming agent; lubricant; binder.

REQUIREMENTS

Acid Value Between 196 and 204.
Iodine Value Between 83 and 103.
Lead Not more than 0.1 mg/kg.
Residue on Ignition Not more than 0.01%.
Saponification Value Between 196 and 206.
Titer (Solidification Point) Not above 10°.
Unsaponifiable Matter Not more than 2.0%.
Water Not more than 0.4%.

TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 10-g sample.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 3 g of sample, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, Appendix IIB.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Olestra

DESCRIPTION

Olestra occurs as a solid, soft gel, or liquid at room temperature depending on the fatty acids used in manufacture. It is a mixture of the octa-, hepta-, and hexa-esters of sucrose prepared by the reaction of sucrose with edible C12 to C20 and higher fatty acid methyl esters. It is insoluble in water and soluble in common lipid solvents.

Function Calorie-free substitute for fats and oils.

> **Note**: Use of Olestra in foods requires the addition of specific amounts of vitamins A, D, E, and K to these foods.

REQUIREMENTS

Identification

A. A sample exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	C12	C14	C16–18
Weight % (Range):	≤1	≤1	≥78
Fatty Acid:	C20 and longer	Unsaturated	
Weight % (Range):	≤20		25–83

Determine the composition profile of fatty acids as directed under *Fatty Acid Composition*, Appendix VII, with the following modifications: (1) In the *Sample Preparation*, use about 55 mg of sample per 10 mL, and (2) in the *Procedure*, use a suitable capillary gas chromatograph (see *Chromatography*, Appendix IIA), equipped with a flame ionization detector, a 60-m × 0.25-mm (id) column, or equivalent, coated with a 0.20-μm layer of 2-cyanopropylpolysiloxane (Supelco SP-2340, or equivalent), a capillary injection port (split mode, operated at a split ratio of 1:100), and an integrator. Set the initial column temperature at 150°, heat at a rate of 1.3°/min to 225°, and hold at 225° for 10 min. Set the injection port temperature to 210° and the detector to 230°. Set the carrier gas flow rate at 25 cm/s.

B. A sample exhibits the following typical distribution of sucrose esters constrained by a minimum octa-ester content, a maximum lower ester content, and the hepta-ester as the remainder.

Ester Distribution:	Octa-	Hexa-	Penta-
Weight % (Range):	≥70	≤1	≤0.5

Mobile Phases (*A*) Hexane and (B) methyl-*tert*-butyl ether (use HPLC grade filtered through 0.2-μm filter).

Standard Preparation Transfer about 250 mg of an Olestra sample with known amounts of various esters (from Sigma Chemical, Nu-Chek-Prep, or equivalent), accurately weighed, into a 25-mL volumetric flask. Dissolve and dilute to volume with *Mobile Phase A*. Filter the solution through a 0.5-μm filter.

Sample Preparation Prepare a test sample as directed under *Standard Preparation*.

Procedure Use a high-performance liquid chromatograph, or equivalent, suitable for programmed gradient mobile phase delivery with an 80-mm × 4-mm (id), 5-μm silica Zorbax Reliance, or equivalent, normal phase column maintained in a column oven at 37°. Maintain the *Mobile Phase* at a flow rate of 2 mL/min. Use a programmed gradient increasing from an initial concentration of 4.8% *B* in *A* to 100% *B* in four steps (16% at 5 min, 25% at 8 min, 50% at 10 min, and 100% at 12 min) to achieve suitable separation of sucrose esters. Use an evaporative light-scattering detector (Applied Chromatography Systems 750/14, or equivalent) and an electronic integrator.

Chromatograph 20 μL of the *Standard Preparation*. Ensure that the results are within two standard deviations of the known values before proceeding.

Similarly chromatograph 20 μL of the *Sample Preparation.* Identify the peaks for the octa-, hepta-, hexa-, and penta-esters in the chromatogram by comparison with the *Standard Preparation* chromatogram. Calculate the percentage of each ester, *i*, by the formula

$$\%\ \text{ester}_i = \frac{OA_i}{\sum_{i=1}^{n} OA_i} \times 100$$

in which OA_i is the peak area of each ester in the chromatogram of the *Sample Preparation* that has a retention time corresponding to that of the same esters in the chromatogram of the *Standard Preparation*.

Assay Not less than 97% of the combined octa-, hepta-, and hexa-esters of sucrose.
Free Fatty Acids Not more than 0.5%.
Lead Not more than 0.1 mg/kg.
Methanol Not more than 300 mg/kg.
Peroxide Value Not more than 10 meq/kg.
Residue on Ignition Not more than 0.5%.
Thixotropy (Stiffness) Not less than 50 kPa/s.
Water Not more than 0.1%.

TESTS

Assay

Mobile Phase Tetrahydrofuran (use HPLC grade filtered through a 0.2-μm filter).

Standard Preparation Transfer about 750 mg of an Olestra sample with a known amount of Olestra monomer (from Sigma Chemical, Nu-Chek-Prep, or equivalent), accurately weighed, into a 25-mL volumetric flask. Dissolve and dilute to volume with *Mobile Phase*. Filter the solution through a 0.5-μm filter.

System Suitability Test

Sample Preparation Prepare as directed under *Standard Preparation*.

Procedure (See *Chromatography*, Appendix IIA.) Use a liquid chromatograph suitable for size-exclusion chromatography and equipped with a refractive index detector and a 60-cm × 7.5-mm (id) column packed with 5-μm, 500A porosity PL-Gel, or equivalent, both operated at 40°. Operate the chromatograph at 500 to 1500 psi at a flow rate of 1.0 mL/min.

Chromatograph 20 μL of the *Standard Preparation*. Ensure that the results are within two standard deviations of the known values before proceeding.

Similarly chromatograph 20 μL of the *Sample Preparation.* Identify the peak for the Olestra monomer in the *Standard Preparation* chromatogram. Calculate the percentage of Olestra monomer, *m,* in the *Sample Preparation* by the formula

$$100(r_M/r_T),$$

in which r_M is the peak area of the Olestra monomer and r_T is the sum of all peaks eluting in about 17.5 min.

Free Fatty Acids Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Methanol

Alkaline Alumina Mix 500 g of 60- to 200-mesh neutral alumina (Brockman Activity 1) with 166.5 g of a 40% potassium hydroxide solution contained in a 1-qt wide-mouth polypropylene jar by shaking vigorously until the alumina becomes uniform. Allow the mixture to equilibrate for 16 h before using.

Internal Standard Solution Transfer 100 mg of butyl stearate (from Sigma Chemical, or equivalent), accurately weighed, into a 100-mL volumetric flask, dilute to volume with hexane, and mix.

Standard Addition Solution Transfer 400 mg of methyl oleate (from Nu-Chek-Prep, or equivalent), accurately weighed, into a 10-mL volumetric flask, dilute to volume with hexane, and mix.

Sample Preparation Prepare four 50-mL serum bottles. Pipet 2.0 mL of *Internal Standard Solution* into each bottle. Pipet 5.0, 10.0, or 20.0 μL of the *Standard Addition Solution*, equivalent to about 21.6, 43.2, and 86.3 μg of methanol, into three of the bottles, and evaporate the solvent at room temperature under nitrogen. Transfer 300 mg of sample, accurately weighed, into each of the four serum bottles. Heat the mixture for about 5 min at 90°, and immediately add 5 g of the *Alkaline Alumina*. Mix, cap each serum bottle with a Teflon-backed rubber septum, and hold at 70.0° for 3 h.

Procedure (See *Chromatography*, Appendix IIA.) Use a gas chromatograph suitable for headspace analysis equipped with a flame ionization detector and a 1.8-m × 2-mm (id) column, or equivalent, packed with 100- to 120-mesh styrene–divinyl copolymer (Chromosorb 101, or equivalent). Maintain the column at 100° for 2 min then heat at 10°/min to 195°. Set the injection port and detector temperatures to 225°. Separately inject the *Internal Standard Solution* and each of the four *Sample Preparations* and obtain the chromatogram. Using a 5-mL gas-tight syringe, withdraw 3.0 mL of the headspace from each serum bottle, and inject into the gas chromatograph. Measure the peak areas for methanol and butanol eluting at about 3 and 10 min, respectively.

Prepare a plot of the ratio of peak area responses of methanol to those of the *Internal Standard Solution* versus the amount, in micrograms, of methanol in each of the four *Sample Preparations*. Calculate the quantity, in milligrams per kilogram, of methanol in the sample taken by the formula

$$3.3A,$$

in which *A* is the number of micrograms of methanol determined from the standard addition plot.

Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.

Thixotropy (Stiffness)

Sample Preparation Prepare samples in quadruplicate. Transfer about 8.0 g of sample, accurately weighed, into a 57-mm aluminum pan. Heat the sample to above 113° until completely liquid, then temper it by cooling to 29° with agitation. The controlled cooling rate should follow the profile shown within 8° at each time noted:

Time in min:	0	30	60	90	120	150	180
Temperature:	113	70	49	40	35	33	29

Hold the sample at 21° for 7 days.

Procedure Use a suitable cone and plate rheometer [Contraves Rheomat 115A (cone CP-6), Physica Rheolab MC 100 (cone MK23), or equivalent] maintained at 37.8° and capable of measuring the non-Newtonian flow curve hysteresis for ascending and descending shear rates programmed from 0 to 800 s^{-1}. Hold the rheometer at 0 s^{-1} for 120 s, raise it to 800 s^{-1} in 7.5 min, hold for 1 s, then decrease to 0 s^{-1} in 7.5 min to measure the thixotropic area. Check the accuracy of the rheometer with viscosity standards (Cannon ASTM Certified Viscosity Standards, S-2000 and N-350, or equivalent). The measured viscosity must be within 0.20% of the stated viscosity at 37.8°, or the rheometer's cone factor must be recalculated.

Place a sufficient amount of the *Sample Preparation* on the rheometer plate to fill the gap between the plate and cone. Measure the thixotropic area. The relative standard deviation

of the mean area for the replicate samples must be within 15% or the *Sample Preparation* must be repeated.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 30 mL of chloroform followed by 10 mL of methanol to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Olibanum Oil

Oil of Frankincense

CAS: [8016-36-2]

View IR

DESCRIPTION

Olibanum Oil occurs as a pale yellow liquid with a balsamic odor with a faint lemon note. It is the volatile oil distilled from a gum obtained from the tree *Boswellia carterii* Birdw. and other *Boswellia* species (Fam. Burseraceae). It is soluble in most fixed oils and, with a slight haze, in mineral oil. It is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 4.0.
Angular Rotation Between −15° and +35°.
Ester Value Between 4 and 40.
Refractive Index Between 1.465 and 1.482 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.862 and 0.889.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value Determine as directed in *Ester Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 6 mL of 90% alcohol, occasionally with opalescence.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Onion Oil

CAS: [8002-72-0]

View IR

DESCRIPTION

Onion Oil occurs as a clear, amber yellow to amber orange liquid with a strong, pungent odor and taste characteristic of onion. It is the volatile oil obtained by steam distillation of the bulbs of *Allium cepa* L. (Fam. Liliaceae). It is soluble in most fixed oils, in mineral oil, and in alcohol. It is insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Refractive Index Between 1.549 and 1.570 at 20°.
Specific Gravity Between 1.050 and 1.135.

TESTS

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Orange Oil, Bitter, Coldpressed

View IR

DESCRIPTION

Orange Oil, Bitter, Coldpressed, occurs as a pale yellow or yellow-brown liquid with the characteristic aromatic odor of the Seville orange and an aromatic, somewhat bitter taste. It is the volatile oil obtained by expression, without the use of heat, from the fresh peel of the fruit of *Citrus aurantium* L. (Fam. Rutaceae). It is miscible with absolute alcohol and with an equal volume of glacial acetic acid. It is soluble in fixed

oils and in mineral oil. It is slightly soluble in propylene glycol, but it is relatively insoluble in glycerin. It is affected by light, and its alcohol solutions are neutral to litmus. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Aldehydes Not less than 0.5% and not more than 1.0% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{20}O$).
Angular Rotation Between +88° and +98°.
Refractive Index Between 1.472 and 1.476 at 20°.
Residue on Evaporation Between 2.0% and 5.0%.
Specific Gravity Between 0.845 and 0.851.

TESTS

Aldehydes Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 10 g of sample, accurately weighed, and 78.14 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand for 30 min at room temperature before titrating.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Residue on Evaporation Determine as directed under *Residue on Evaporation*, Appendix VI, using a 5-g sample, and heat for 4.5 h.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Orange Oil, Coldpressed

Sweet Orange Oil

CAS: [8028-48-6]

View IR

DESCRIPTION

Orange Oil, Coldpressed, occurs as an intensely yellow, orange, or deep orange liquid with the characteristic odor and taste of the outer part of fresh, sweet orange peel. It is the volatile oil obtained by expression, without the use of heat, from the fresh peel of the ripe fruit of *Citrus sinensis* L. Osbeck (Fam. Rutaceae). It is miscible with dehydrated alcohol and with carbon disulfide. It is soluble in glacial acetic acid. It may contain a suitable antioxidant.

Note: Do not use Coldpressed Orange Oil that has a terebinthine odor.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is the California or Florida type.
Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 1.2% and not more than 2.5% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{20}O$).
Angular Rotation Between +94° and +99°.
Refractive Index Between 1.472 and 1.474 at 20°.
Specific Gravity Between 0.842 and 0.846.
Ultraviolet Absorbance *California Type*: Not less than 0.130; *Florida Type*: Not less than 0.240.

TESTS

Assay Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 10 g of sample, accurately weighed. Allow the mixture to stand for 15 min, with occasional shaking, before titrating, and use 78.14 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Ultraviolet Absorbance Determine as directed under *Ultraviolet Absorbance of Citrus Oils*, Appendix VI, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 330 ± 3 nm.

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Orange Oil, Distilled

View IR

DESCRIPTION

Orange Oil, Distilled, occurs as a colorless to pale yellow liquid with a mild citrus floral odor. It is the volatile oil obtained by distillation from the fresh peel or juice of the

fruit of *Citrus sinensis* L. Osbeck (Fam. Rutaceae), with or without the previous separation of the juice, pulp, or peel. It is soluble in most fixed oils, in mineral oil, and in alcohol (with haze). It is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Aldehydes Between 1.0% and 2.5% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{20}O$).
Angular Rotation Between +94° and +99°.
Refractive Index Between 1.471 and 1.474 at 20°.
Specific Gravity Between 0.840 and 0.844.
Ultraviolet Absorbance Not more than 0.01.

TESTS

Aldehydes Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 5 mL of sample, accurately weighed, and 78.14 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand at room temperature for 1 h before titrating.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Ultraviolet Absorbance Determine as directed under *Ultraviolet Absorbance of Citrus Oils*, Appendix VI, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 330 ± 3 nm.

Packaging and Storage Store in a cool place protected from light in full, tight containers.

Origanum Oil, Spanish Type

CAS: [8007-11-2]

View IR

DESCRIPTION

Origanum Oil, Spanish Type, occurs as a yellow-red to a dark, brown-red liquid with a pungent, spicy odor suggestive of thyme oil. It is the volatile oil obtained by steam distillation from the flowering herb *Thymus capitatus* Hoffm. et Link and various species of *Origanum* (Fam. Labiatae). It is soluble in most fixed oils and in propylene glycol. It is soluble, with turbidity, in mineral oil, but it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 60.0% and not more than 75.0%, by volume, of phenols.
Angular Rotation Between −2° and +3°.
Refractive Index Between 1.506 and 1.512 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.935 and 0.960.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI, using a suitable quantity of sample shaken with about 2% of powdered tartaric acid and filtered.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube. Occasionally the oil is too dark to read in a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample is soluble in 2 mL of 70% alcohol. The solution may become cloudy on dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Orris Root Oil

View IR

DESCRIPTION

Orris Root Oil occurs as a light yellow to brown-yellow mass at room temperature and melts to form a yellow to yellow-brown liquid. It is the volatile oil obtained by steam distillation from the peeled, dried, and aged rhizomes of *Iris pallida* Lam. (Fam. Iridaceae). It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 9.0% and not more than 20.0% of ketones, calculated as irone ($C_{14}H_{22}O$).
Acid Value Between 175 and 235.
Ester Value Between 4 and 35.
Melting Range Between 38° and 50°.

TESTS

Assay Determine as directed under *Aldehydes*, Appendix VI, using about 1 g of sample, accurately weighed, and 103.2 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand for 1 h at room temperature before titrating.
Acid Value Determine as directed under *Acid Value*, Appendix VI, using about 1 g of sample, accurately weighed.
Ester Value Determine as directed in *Ester Value* under *Esters*, Appendix VI, using about 1 g of sample, accurately weighed.
Melting Range Determine as directed in *Procedure for Class II* under *Melting Range or Temperature*, Appendix IIB.

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Ox Bile Extract

Sodium Choleate; Purified Oxgall

$C_{24}H_{39}NaO_5$ Formula wt 430.56 (as sodium choleate)

DESCRIPTION

Ox Bile Extract occurs as a yellow-green powder. It contains ox bile acids, chiefly glycocholic and taurocholic, as sodium salts, equivalent to not less than 45.0% cholic acid, $C_{24}H_{40}O_5$. It is the purified portion of the bile of an ox, obtained by evaporating the alcohol extract of concentrated bile. It is soluble in water and in alcohol.

Function Surfactant.

REQUIREMENTS

Identification
A. A sample gives a positive test for *Sodium*, Appendix IIIA.
B. A 1:10 aqueous solution in alcohol, when mixed with 0.5 mL of iodine TS, turns blue.
Assay Not less than 45.0% of cholic acid ($C_{24}H_{40}O_5$).
Ash (Total) Not more than 10.0%.
Loss on Drying Not more than 6.0%.
Microbial Limits:
Aerobic Plate Count Not more than 20,000 CFU per gram.
E. coli Not more than 3 CFU per gram.
Salmonella Negative in 25 g.
Yeasts and Molds Not more than 10 CFU per gram.
pH Between 6.3 and 7.0.

TESTS

Assay
Standard Solution Using an accurately weighed quantity of USP Cholic Acid Reference Standard, prepare a solution in 60% acetic acid with a known concentration of about 0.5 mg/mL. When stored in a refrigerator, this solution may be used for several months.
Assay Preparation Dissolve about 50 mg of sample, accurately weighed, in 100 mL of 60% acetic acid, and mix. Filter the solution, discarding the first 10 mL of the filtrate.
Procedure Transfer 1.0 mL each of the *Standard Solution* and the *Assay Preparation* into separate containers. Add 1.0 mL of a freshly prepared 1:100 furfural solution to each container. Cool the containers in an ice bath for 5 min. Add 13 mL of a dilute sulfuric acid solution, prepared by cautiously mixing 50 mL of sulfuric acid with 65 mL of water. Thoroughly mix the contents in each container, and place them for 10 min in a water bath maintained at 70°. Immediately place the containers in an ice bath for 2 min. Determine the absorbance of each solution in a 1-cm cell at the wavelength of maximum absorbance at about 650 nm. Calculate the quantity, in milligrams, of cholic acid in the sample by the formula

$$100C(A_U/A_S),$$

in which C is the concentration, in milligrams per milliliter, of USP Cholic Acid Reference Standard in the *Standard Solution*; and A_U and A_S are the maximum absorbances at identical wavelengths of the *Assay Preparation* and the *Standard Solution*, respectively.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 16 h.
Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):
Aerobic Plate Count
E. coli
Salmonella
Yeasts and Molds
pH Determine as directed under *pH Determination*, Appendix IIB, using a 1:20 aqueous solution.

Packaging and Storage Store in tight containers.

Oxystearin

INS: 387 CAS: [8028-45-3]

DESCRIPTION

Oxystearin occurs as a tan to light brown, fatty or waxlike substance. It is a mixture of the glycerides of partially oxidized stearic and other fatty acids. It is soluble in ether, in hexane, and in chloroform.

Function Crystallization inhibitor in salad and cooking oils; sequestrant; defoaming agent.

REQUIREMENTS

Acid Value Not more than 15.
Hydroxyl Value Between 30 and 45.
Iodine Value Not more than 15.
Lead Not more than 2 mg/kg.
Refractive Index (butyro) Between 59 and 61 at 48° (equivalent to 1.465 to 1.467 on the Abbé scale).
Saponification Value Between 225 and 240.
Unsaponifiable Matter Not more than 0.8%.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII, using about 5 g of sample, accurately weighed.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Refractive Index (butyro) Determine as directed under *Refractive Index*, Appendix IIB, using a melted sample filtered through filter paper. Determine the refractive index at 48° with an Abbé or butyro refractometer.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 3 g of sample, accurately weighed.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.

Packaging and Storage Store in well-closed containers.

Ozone

Triatomic Oxygen

O_3 Formula wt 48.00

CAS: [10028-15-6]

DESCRIPTION

Ozone occurs as an unstable, colorless gas. It is produced *in situ* from oxygen either by ultraviolet irradiation of air or by passing a high-voltage discharge through air. It is a potent oxidizing agent that decomposes at ambient temperature to molecular oxygen.

Function Antimicrobial in the treatment, processing, and storage or display of fish, meat, and poultry and in preparing, packing, or holding raw agricultural commodities. Disinfectant for water to be used for direct consumption or to make ice.

REQUIREMENTS

Identification

Reagent Solution Disperse 124.5 mg of alizarin violet 3R in 500 mL of water contained in a 1-L volumetric flask. Mechanically stir overnight. Add 20 mg of sodium hexametaphosphate, 48.5 g of ammonium chloride, and 6.2 mL of ammonium hydroxide (equivalent to 1.6 g of NH_3). Dilute to volume with water, and stir overnight. A 10-fold aqueous dilution of this solution in a 1-cm cell has an absorbance of 0.155 at 548 nm. The pH of dilutions with sample waters is between 8.1 and 8.5.

Procedure Introduce 20 mL of the *Reagent Solution* into each of two 200-mL volumetric flasks. Fill one flask with Ozone-free water to serve as the blank. Fill the other with the sample water by directly introducing it, with the aid of a long-stemmed funnel or pipet, below the surface of the *Reagent Solution* to prevent Ozone loss by degassing. Immediately measure the absorbance of both solutions at 548 nm, using 1- to 5-cm cells. The presence of Ozone is indicated if the sample solution has a lower absorbance than the blank.
Assay 0.01 to 0.5 mg of O_3 per liter.

TESTS

Assay

Indigo Stock Solution Dissolve 0.770 g of potassium indigotrisulfonate in 500 mL of water and 1 mL of phosphoric acid in a 1-L volumetric flask, dilute to volume with water, and mix. A 1:100 dilution of this reagent has an absorbance of 0.20 ± 0.010 cm^{-1} at 600 nm.

Indigo Reagent I Just before use, transfer 20 mL of *Indigo Stock Solution*, 10 g of monobasic sodium phosphate, and 7 mL of phosphoric acid into a 1-L volumetric flask, dilute to volume with water, and mix.

Indigo Reagent II Proceed as directed for *Indigo Reagent I*, using 100 mL of *Indigo Stock Solution* instead of 20 mL.

Malonic Acid Reagent Dissolve 5 g of malonic acid in water, and dilute to 100 mL.

Procedure (for a concentration range of 0.01 to 0.1 mg of Ozone per liter) Add 10.0 mL of *Indigo Reagent I* to each of two 100-mL flasks. Fill one flask with Ozone-free water to serve as the blank. Fill the other with the sample water by directly introducing it, with the aid of a long-stemmed funnel or pipet, below the surface of the dye solution to prevent Ozone loss by degassing. Without delay, mix, and measure the absorbance of each solution at 600 nm, preferably in 10-cm cells. (For a concentration range of 0.05 to 0.5 mg of Ozone per liter, use *Indigo Reagent II*, and proceed as above.)

Control of Interferences In the presence of chlorine, add 1 mL of *Malonic Acid Reagent* to both flasks before adding the samples. Proceed as above, but measure absorbance immediately.

Calculate the concentration of Ozone in the sample water, in milligrams per liter, by the formula

$$100D/(f \times b \times V),$$

in which D is the difference in absorbance between the sample solution and blank solution; b is the path length, in centimeters; V is the volume of sample, in milliliters (normally 90 mL); and f is 0.42.

Palmarosa Oil

Geranium Oil, East Indian Type; Geranium Oil, Turkish Type

CAS: [8014-19-5]

FEMA: 2831

View IR

DESCRIPTION

Palmarosa Oil occurs as a light yellow to yellow oil that is often hazy and brown with a rosy, floral, geranium odor. It is the volatile oil obtained by steam distillation from the partially dried grass *Cymbopogon martini* Stapf. var. *motia* (Fam. Gramineae). It is soluble in most fixed oils and in propylene glycol. It is soluble, usually with opalescence or turbidity, in mineral oil. It is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay for Alcohols Not less than 88.0% of total alcohols.
Assay for Esters Not less than 4.0% and not more than 18.0% of esters, calculated as geranyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −2° and +3°.
Refractive Index Between 1.470 and 1.476 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.879 and 0.892.

TESTS

Assay for Alcohols Determine as directed under *Total Alcohols*, Appendix VI, using about 1 g of the acetylated oil, accurately weighed, for the saponification, and 77.13 as the equivalence factor (*e*) in the calculation.
Assay for Esters Determine as directed for *Ester Determination* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed, and 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Palmitic Acid

Hexadecanoic Acid

$C_{16}H_{32}O_2$ Formula wt 256.43

CAS: [57-10-3]

DESCRIPTION

Palmitic Acid occurs as a hard, white or faintly yellow, somewhat glossy crystalline solid, or as a white or pale yellow powder. It is a mixture of solid organic acids obtained from fats consisting chiefly of Palmitic Acid ($C_{16}H_{32}O_2$) with varying amounts of stearic acid ($C_{16}H_{36}O_2$). Palmitic Acid is practically insoluble in water. It is soluble in alcohol, in ether, and in chloroform.

Function Component in the manufacture of other food-grade additives; defoaming agent.

REQUIREMENTS

Acid Value Between 204 and 220.
Iodine Value Not more than 2.0.
Lead Not more than 0.1 mg/kg.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 205 and 221.
Titer (Solidification Point) Between 53.3° and 62°.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 3 g of sample, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, Appendix IIB.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Palm Kernel Oil (Unhydrogenated)

CAS: [8023-79-8]

DESCRIPTION

Palm Kernel Oil (Unhydrogenated) is a fat obtained from the kernel of the fruit of the oil palm *Elaeis guineensis* Jacq. (Fam. Arecaceae) by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. Like coconut oil, it has a more abrupt melting range than other fats and oils.

Function Coating agent; texturizer.

REQUIREMENTS

Identification Palm Kernel Oil (Unhydrogenated) exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	6:0	8:0	10:0	12:0	14:0	16:0	16:1
Weight % (Range):	0–1.5	3–5	2.5–6	40–52	14–18	7–10	0–1
Fatty Acid:	18:0	18:1	18:2	20:0			
Weight % (Range):	1–3	11–19	0.5–4	tr. –1			

Color (AOCS-Wesson) Not more than 20 yellow/2.0 red.
Free Fatty Acids *As Oleic Acid*: Not more than 0.1%; *As Lauric Acid*: Not more than 0.07%.
Iodine Value Between 13 and 23.
Lead Not more than 0.1 mg/kg.
Melting Range Between 27° and 29°.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Color (AOCS-Wesson) Proceed as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factors (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.
Free fatty acids as lauric acid, $e = 20.0$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Melting Range Determine as directed under *Melting Range*, Appendix VII.
Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Palm Oil (Unhydrogenated)

CAS: [8002-75-3]

DESCRIPTION

Palm Oil (Unhydrogenated) is a deep orange-red fat obtained from the pulp of the fruit of the oil palm *Elaeis guineensis* Jacq. (Fam. Aracaceae) usually by boiling, centrifugation, and mechanical expression. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a semisolid at 21° to 27°.

Function Coating agent; emulsifying agent; texturizer.

REQUIREMENTS

Identification Palm Oil (Unhydrogenated) exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	14:0	16:0	18:0	18:1	18:2
Weight % (Range):	0.5–5.9	32–47	2–8	34–44	7–12

Color (AOCS-Wesson) Not more than 35 yellow/5.0 red.
Free Fatty Acids *As Oleic Acid*: Not more than 0.1%; *As Palmitic Acid*: Not more than 0.09%.
Iodine Value Between 50 and 55.

Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.
Stability (Active Oxygen Method) Not less than 50 h.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factors (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.
Free fatty acids as palmitic acid, $e = 25.6$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.
Stability (Active Oxygen Method) Determine as directed under *Stability*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

DL-Panthenol

DL-Pantothenyl Alcohol; Racemic Pantothenyl Alcohol

$$HOCH_2C(CH_3)_2CH(OH)CONH(CH_2)_2CH_2OH$$

$C_9H_{19}NO_4$ Formula wt 205.25

CAS: [16485-10-2]

DESCRIPTION

DL-Panthenol occurs as a white to creamy white, crystalline powder. It is a racemic mixture of the dextrorotatory (active) and levorotatory (inactive) isomers of panthenol, the alcohol analogue of pantothenic acid. It is freely soluble in water, in alcohol, and in propylene glycol. It is soluble in chloroform and in ether, and is slightly soluble in glycerin. Its solutions are neutral or alkaline to litmus.

Note: The physiological activity of DL-Panthenol is one-half that of dexpanthenol (D-Panthenol).

Function Nutrient.

REQUIREMENTS

Identification

A. Add 5 mL of 1 *N* sodium hydroxide and 1 drop of cupric sulfate TS to 1 mL of a 10% aqueous solution of sample, and shake vigorously. A deep blue color appears.

B. Add 1 mL of 1 *N* hydrochloric acid to 1 mL of a 1% aqueous solution of sample, and heat on a steam bath for about 30 min. Cool, add 100 mg of hydroxylamine hydrochloride, mix, and add 5 mL of 1 *N* sodium hydroxide. Allow to stand for 5 min, then adjust the pH to within a range of 2.5 to 3.0 with 1 *N* hydrochloric acid, and add 1 drop of ferric chloride TS. A purple-red color appears.

C. The infrared absorption spectrum of a film of the sample exhibits maxima only at the same wavelengths as those of a similar preparation of USP Dexpanthenol Reference Standard.
Assay Not less than 99.0% and not more than 102.0% of $C_9H_{19}NO_4$ (DL-Panthenol), calculated on the dried basis.
Aminopropanol Not more than 0.1%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.5%.
Melting Range Between 64.5° and 68.5°.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 400 mg of sample, accurately weighed, into a 300-mL reflux flask fitted with a standard-taper glass joint. Add 50.0 mL of 0.1 *N* perchloric acid in glacial acetic acid, and reflux for 5 h.

Caution: Handle perchloric acid in an appropriate fume hood.

Cool, covering the condenser with foil to prevent contamination by moisture, and rinse the condenser with glacial acetic acid. Add 5 drops of crystal violet TS, and titrate with 0.1 *N* potassium acid phthalate in glacial acetic acid to a blue-green endpoint. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 20.53 mg of $C_9H_{19}NO_4$.
Aminopropanol Transfer about 10 g of sample, accurately weighed, into a 50-mL flask, and dissolve in 25 mL of water. Add bromothymol blue TS, and titrate with 0.01 *N* sulfuric acid from a microburet to a yellow endpoint. Each milliliter of 0.01 *N* sulfuric acid is equivalent to 0.75 mg (750 μg) of aminopropanol.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 56° for 4 h in vacuum over phosphorus pentoxide.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight containers.

Paraffin, Synthetic

Fischer-Tropsch Paraffin

CAS: [8002-74-2]

View IR

DESCRIPTION

Paraffin, Synthetic, occurs as a white wax that is very hard at room temperature. It is synthesized by the Fischer-Tropsch process from carbon monoxide and hydrogen, which are catalytically converted to a mixture of paraffin hydrocarbons; the lower-molecular-weight fractions are removed by distillation, and the residue is hydrogenated and further treated by percolation through activated charcoal. It is soluble in hot hydrocarbon solvents.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of the melted sample on a potassium bromide plate exhibits maxima only at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Absorptivity Less than 0.01 at 290 nm in decahydronaphthalene at 88°.
Congealing Point Between 200° and 210°F (93.3° and 98.9° C).
Lead Not more than 3 mg/kg.
Oil Content Not more than 0.50%.

TESTS

Absorptivity

Sample Solution Transfer about 100 mg of sample, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with decahydronaphthalene previously heated to 88°, and mix.

Procedure Use an accurately calibrated spectrophotometer capable of measuring absorbance with a repeatability of ±0.1% or better from an average of 0.4 absorbance level at 290 nm, having a spectral bandwidth of 2 nm or less, capable of making wavelength measurements repeatable within ±0.2 nm, and having cell holders with temperature control. Determine the absorbance of the *Sample Solution* in a 10-cm cell at 290 nm, maintaining the temperature of the sample cell and the reference cell at 88°. Use decahydronaphthalene at 88° in a matched cell as the blank (see *General Provisions*). Cell lengths should be known to within ±0.5% or better of the nominal pathlength. Calculate the absorptivity of the *Sample Solution* by the formula

$$A/bc,$$

in which A is the absorbance of the *Sample Solution*, corrected for the solvent blank; b is the exact pathlength, in centimeters, of the sample cell; and c is the exact concentration, in grams per liter, of the *Sample Solution*.

Congealing Point (The temperature at which the molten sample, when allowed to cool under the prescribed conditions, ceases to flow.)

Procedure Place a representative sample in a casserole or other suitable dish, and heat slowly in a water bath to a temperature approximately 15°F above the expected congealing point. Use an ASTM Congealing Point Thermometer with a temperature range of 68° to 213°F and that conforms to the requirements for an ASTM 54 F thermometer (see *Thermometers*, Appendix I). Using a cork, as needed, fit the thermometer into a jacket consisting of a 1-oz glass vial that has a 25-mm diameter and is 55 mm long, and adjust the thermometer so that the bottom of the bulb is 10 to 15 mm from the bottom of the vial. Heat the thermometer jacket assembly to approximately the same temperature as the prepared sample. When both the sample and the assembly have reached the required temperature, remove the assembly from the bath, then immediately remove the thermometer from its jacket, and immerse the thermometer bulb into the molten sample until the bulb is completely covered, taking care not to cover any part of the thermometer stem with the sample. As rapidly as possible, remove the thermometer and any adhering sample from the sample dish and place the thermometer in the jacket, holding both the thermometer and its jacket in a horizontal position during this operation. Rotate the thermometer horizontally at the rate of approximately one revolution every 2 s, pausing momentarily at the completion of each revolution to inspect the drop of sample on the thermometer bulb. When the drop rotates with the bulb, record the thermometer reading as the congealing point, reported to the nearest 0.5°F. Repeat the determination. If the variation is greater than 1°F, make a third determination, and record the average of the three determinations as the congealing point.
Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, Appendix IV. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 10 µg of lead (Pb) ion in the control.
Oil Content Determine as directed under *Oil Content of Synthetic Paraffin*, Appendix IIC.

Packaging and Storage Store in well-closed containers.

Parsley Herb Oil

CAS: [8000-68-8]

View IR

DESCRIPTION

Parsley Herb Oil occurs as a yellow to light brown liquid with the odor of parsley herb. It is the oil obtained by steam distillation of the aboveground parts of the plant *Petroselinum crispum* (Fam. Umbelliferae), including the immature seed. It is soluble in most fixed oils, in mineral oil, and in alcohol

(with opalescence). It is slightly soluble in propylene glycol, but it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 2.0.
Angular Rotation Between +1° and −9°.
Refractive Index Between 1.503 and 1.530 at 20°.
Specific Gravity Between 0.908 and 0.940.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers.

Parsley Seed Oil

CAS: [8000-68-8]

View IR

DESCRIPTION

Parsley Seed Oil occurs as a yellow to light brown liquid with a green, herbal odor. It is the oil obtained by steam distillation of the ripe seed of *Petroselinum crispum* (Fam. Umbelliferae). It is soluble in most fixed oils and in mineral oil. It is slightly soluble in propylene glycol, but it is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 4.0.
Angular Rotation Between −4° and −10°.
Refractive Index Between 1.513 and 1.522 at 20°.
Saponification Value Between 2 and 10.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.040 and 1.080.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VII.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 6 mL of 80% alcohol, occasionally with slight haziness.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full containers that are made from steel or aluminum and that are suitably lined.

Partially Hydrolyzed Proteins

Enzyme-Hydrolyzed (Source) Protein; Partially Hydrolyzed (Source) Protein; (Source) Peptone; Enzyme-Modified (Source) Protein; Partial Enzymatic Digest of (Source) Protein; Partial Acid Digest of (Source) Protein

INS: 429

DESCRIPTION

Partially Hydrolyzed Proteins occur as liquid, paste, powder, or granules. They are composed of peptides and polypeptides resulting from the partial or incomplete hydrolysis of peptide bonds present in edible proteinaceous materials catalyzed by heat, food-grade proteolytic enzymes, and/or suitable food-grade acids. Their degree of hydrolysis typically ranges from 3% to 85% on the basis of peptide bond cleavage. During processing, the proteinaceous raw material may be treated with safe and suitable alkaline materials. The edible proteinaceous materials used as raw materials are derived from casein and other milk products such as whey protein; from animal tissue, including gelatin, defatted animal tissue, and egg albumen; from yeast; and from soy protein products, wheat protein products, or other suitable and safe plant sources.

> **Note**: Depending on the protein source and the degree of hydrolysis, Partially Hydrolyzed Proteins may present an allergenic risk to sensitized individuals.

Function Binder; dough conditioner; emulsifier and emulsifier salt; flavoring agent; flavor enhancer; nutrient; fermentation aid; surface-active agent; texturizer.

REQUIREMENTS

Note: Calculate all analyses on the dried basis. Evaporate liquid and paste samples to dryness in a suitable tared container on a steam bath, then, as for the powdered and granular forms, dry to constant weight at 105° (see *General Provisions*).

Labeling Indicate the source of protein, including type.
Assay (Total Nitrogen; TN) Not less than 7.0%.
α-Amino Nitrogen (AN) Not less than 90.0% and not more than 110.0% of the amount claimed on the label.
α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Not less than 2.0% and not more than 62.0%, when calculated on an ammonia nitrogen-free basis.
Ammonia Nitrogen (NH_3-N) Not more than 1.5%.
Ash (Total) Not more than 40.0%.
Glutamic Acid Not more than 20.0% as $C_5H_9NO_4$ and not more than 35.0% of the total amino acids.
Lead Not more than 2 mg/kg.

TESTS

Assay (Total Nitrogen; TN) Determine as directed under *Nitrogen Determination*, Appendix IIIC.
α-Amino Nitrogen (AN) Transfer 7 to 25 g of sample, accurately weighed, into a 500-mL volumetric flask with the aid of several 50-mL portions of warm, ammonia-free water, dilute to volume with water, and mix. Neutralize 20.0 mL of the solution with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide, using phenolphthalein TS as the indicator, and add 10 mL of freshly prepared phenolphthalein–formol solution (50 mL of 40% formaldehyde containing 1 mL of 0.05% phenolphthalein in 50% alcohol neutralized exactly to pH 7 with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide). Titrate with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide to a distinct red color, add a small, but accurately measured, volume of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide in excess, and back titrate to neutrality with 0.2 *N* hydrochloric acid. Conduct a blank titration (see *General Provisions*), using the same reagents, with 20 mL of water in place of the test solution. Each milliliter of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide is equivalent to 2.8 mg of α-amino nitrogen.
α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Calculate by dividing the percent α-amino nitrogen (*AN*) by the percent total nitrogen (*TN*) as corrected for ammonia nitrogen (NH_3-*N*) according to the formula

$$100[(AN - NH_3\text{-}N)/(TN - NH_3\text{-}N)].$$

Ammonia Nitrogen (NH_3-N) (**Caution**: Provide adequate ventilation.) (**Note**: Use nitrogen-free reagents, where available, or reagents very low in nitrogen content.) Transfer between 700 mg and 2.2 g of sample into a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass. If desired, wrap the sample, if solid or semisolid, in nitrogen-free filter paper to facilitate the transfer.

Add about 200 mL of water, and mix. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets, or a 2:5 sodium hydroxide solution, down the inside of the flask so that it forms a layer under the solution, using a sufficient amount (usually about 25 g of solid sodium hydroxide) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser that has a delivery tube extending well beneath the surface of a measured excess of 0.5 *N* hydrochloric or sulfuric acid contained in a 500-mL flask. Add 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and then heat until all of the ammonia has distilled, collecting at least 150 mL of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*), substituting 2 g of sucrose for the sample, and make any necessary correction. Each milliliter of 0.5 *N* acid consumed is equivalent to 7.003 mg of ammonia nitrogen.

Note: If it is known that the substance to be determined has a low nitrogen content, 0.1 *N* acid and alkali may be used, in which case each milliliter of 0.1 *N* acid consumed is equivalent to 1.401 mg of nitrogen.

Calculate the percent of ammonia nitrogen by the formula

$$100(NH_3\text{-}N/S),$$

in which NH_3-*N* is the weight, in milligrams, of ammonia nitrogen, and *S* is the weight, in milligrams, of the sample.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC, using a 1-g sample.
Glutamic Acid

Apparatus Use an ion-exchange amino acid analyzer equipped with sulfonated polystyrene columns, in which the effluent from the sample is mixed with ninhydrin reagent and the absorbance of the resultant color is measured continuously and automatically at 570 and 440 nm by a recording photometer.

Standard Solution Weigh 1250 ± 2 mg of reagent-grade glutamic acid, and place it into a 500-mL volumetric flask. Fill the flask half-full with water, and add 5 mL of hydrochloric acid to help dissolve the amino acid, dilute to volume with water, and mix. Dilute 1 mL of this solution with 4 mL of 0.2 *N* sodium citrate, pH 2.2, buffer. This *Standard Solution* contains 0.5 mg of glutamic acid per milliliter (C_S).

Sample Solution Accurately weigh 5 mg of sample, and dilute it to exactly 5 mL with 0.2 *N* sodium citrate, pH 2.2, buffer. Remove any insoluble material by centrifugation or filtration.

Procedure Using 2-mL aliquots of both the *Standard Solution* and the *Sample Solution*, proceed according to the apparatus manufacturer's instructions. From the chromatograms thus obtained, match the retention times produced by the *Standard Solution* with those produced by the *Sample Solution*, and identify the peak produced by glutamic acid. Record the area of the glutamic acid peak from the *Sample Solution* as A_U, and that from the *Standard Solution* as A_S.

Calculations Calculate the concentration, C_A, in milligrams per milliliter, of glutamic acid in the *Sample Solution* by the formula

$$A_U \times C_S/A_S,$$

in which C_S is the concentration, in milligrams per milliliter, of the glutamic acid in the *Standard Solution*.

Calculate the percentage of glutamic acid, on the basis of total protein, by the formula

$$(100 \times C_A)/(6.25 \times N_T),$$

in which N_T is the total nitrogen, in percent, determined in the *Assay*, and 6.25 is the conversion factor for protein and amino acids.

Calculate the percentage of glutamic acid in the sample by the formula

$$100 \times C_A/S_W,$$

in which S_W is the weight, in milligrams, of the sample taken.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Packaging and Storage Store in tight containers.

Peanut Oil (Unhydrogenated)

CAS: [8002-03-7]

DESCRIPTION

Peanut Oil (Unhydrogenated) is a pale-yellow oil obtained from the kernel of the peanut plant *Arachis hypogaea* L. (Fam. Fabaceae) by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21° to 27°, but solidifies to a gel-like consistency at 2° to 4°. It is free from visible foreign matter at 21° to 27°, but sometimes clouds at temperatures above 21°.

Function Coating agent; texturizer.

REQUIREMENTS

Identification Peanut Oil (Unhydrogenated) exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<14	14:0	16:0	16:1	18:0
Weight % (Range):	<0.1	<0.2	6–15	<1.0	1.3–6.5
Fatty Acid:	18:1	18:2	18:3	20:0	20:1
Weight % (Range):	36–72	13–45	<2.0	<1.0–2.5	0.5–2.1
Fatty Acid:	22:0	22:1	24:0		
Weight % (Range):	1.5–4.8	<0.1	1.0–2.5		

Color (AOCS-Wesson) Not more than 5.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 84 and 100.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 2.0%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (e) in the formula given in the procedure:

Free fatty acids as oleic acid, e = 28.2.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Linolenic Acid Determine as directed under *Fatty Acid Composition*, Appendix VII.
Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Pectins

INS: 440 CAS: [9000-69-5]

DESCRIPTION

Pectins occur as a white, yellow, light gray, or light brown powder. They consist mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium, and ammonium salts. They are obtained by extraction in an aqueous medium of appropriate edible plant material, usually citrus fruits or apples. No organic precipitants shall be used other than methanol, ethanol, and isopropanol. In some types of Pectins, a portion of the methyl esters may have been converted to primary amides by treatment with ammonia under alkaline conditions. Pectins dissolve in water, forming an opalescent, colloidal dispersion. They are practically insoluble in ethanol. The commercial product is normally diluted with sugars for standardization purposes. In addition to sugars, Pectins may be mixed with suitable food-grade salts required for pH control and desirable setting characteristics.

Note: The following *Requirements* and *Tests* apply to the Pectins as supplied, whether standardized or not, except for specifications covering amide substitution and the weight percent of total galacturonic acid in the Pectin component, in which cases the test procedures include provisions for removing the sugars and soluble salts before analysis of the Pectin component.

Function Gelling agent; thickener; stabilizer.

REQUIREMENTS

Labeling Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.

Identification

Sample Preparation Add 0.05 g of sample into a 100-mL volumetric flask, and moisten with pure 2-propanol. Add 50 mL of water, and mix with a magnetic stirrer. Using 0.5 *M* sodium hydroxide, adjust the pH to 12, stop the stirrer, and leave the solution undisturbed and at room temperature for 15 min. Reduce the pH to 7.0 with 0.5 *M* hydrochloric acid. Dilute to 100.0 mL with water.

Tris Buffer Solution Dissolve 6055 g of Tris(Hydroxylmethyl)aminomethane and 0.147 g of calcium chloride dihydrate in 1 L of water. Adjust the pH to 7.0 with 1 *M* hydrochloric acid.

Enzyme Solution Mix pectate lyase with *Tris Buffer Solution* to achieve a 1:100 solution.

Procedure Set out three quartz cuvettes suitable for use with a UV spectrophotometer. For each of the following solutions, shake the contents of each cuvette well and, using the spectrophotometer, measure the absorbance at 235 nm at 0 and 10 min.

Mix 0.5 mL of *Tris Buffer Solution*, 1.0 mL of *Sample Preparation*, and 1.0 mL of water in a quartz cuvette to prepare the *Sample Blank*. Mix 0.5 mL of *Tris Buffer Solution*, 1.5 mL of water, and 0.5 mL of *Enzyme Solution* in a second quartz cuvette to prepare the *Enzyme Blank*. Mix 0.5 mL of *Tris Buffer Solution*, 1.0 mL of *Sample Preparation*, 0.5 mL of water, and 0.5 mL of *Enzyme Solution* in a third quartz cuvette to prepare the *Sample Solution*. Shake the solutions, and measure at 235 nm immediately (time 0) and 10 min after adding the *Enzyme Solution*.

Calculation Calculate the corrected absorbance at 0 and 10 min (A_0 and A_{10}, respectively) using the absorbance values measured for each solution and time and the following equations:

$$A_0 = \text{Sample} - (\text{enzyme blank} + \text{sample blank}),$$

$$A_{10} = \text{Sample} - (\text{enzyme blank} + \text{sample blank}).$$

Calculate the amount of unsaturated product (U) produced as follows:

$$U = (A_{10} - A_0)/(4600 \times I),$$

in which I is the thickness, in centimeters, of the sample.

Report the value of U as $U \times 10^5$. For Pectins, the value of U is greater than 0.5. Other gums show essentially no change.

Ash (acid-insoluble) Not more than 1.0%.

Degree of Amide Substitution in the Pectin Component Not more than 25% of total carboxylic groups.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 12.0%.

Methanol, **Ethanol**, and **Isopropanol** Not more than 1.0% total.

Sodium Methyl Sulfate Not more than 0.1%.

Sulfur Dioxide Not more than 0.005%.

Total Galacturonic Acid in the Pectin Component Not less than 65.0%, calculated on the ash-free, dried basis.

Total Insoluble Substances Not more than 3.0%.

TESTS

Ash (acid-insoluble) Determine as directed under *Ash (Acid Insoluble)*, Appendix IIC.

Degree of Amide Substitution and Total Galacturonic Acid in the Pectin Component

Clark's Solution Mix 100 g of magnesium sulfate heptahydrate with 0.3 mL of sulfuric acid and sufficient water to make 180 mL of solution.

Apparatus Use a distillation apparatus consisting of a steam generator connected to a round-bottom flask attached to a condenser. Both the steam generator and the round-bottom flask are equipped with heating mantles.

Procedure Weigh 5 g of sample to the nearest 0.1 mg, and transfer it to a suitable beaker. Add a mixture of 5 mL of 2.7 *N* hydrochloric acid and 100 mL of 60% ethanol, and stir for 10 min. Transfer the solution to a fritted-glass filter tube (30- to 60-mL capacity), and wash the filtrate with six 15-mL portions of the same hydrochloric acid–60% ethanol mixture, followed by 60% ethanol, until the filtrate is free of chlorides. Finally, wash with 20 mL of ethanol, dry for 2.5 h in an oven at 105°, cool, and weigh. Transfer exactly one-tenth of the total net weight of the now ash-free, dried sample (representing 0.5 g of the original, unwashed sample) to a 250-mL conical flask, and moisten the sample with 2 mL of ethanol. Add 100 mL of recently boiled and cooled water, stopper, and swirl occasionally until a complete solution is formed. Add 5 drops of phenolphthalein TS, titrate with 0.1 *N* sodium hydroxide, and record the results as the initial titer (V_1).

Add exactly 20 mL of 0.5 *N* sodium hydroxide to the flask, stopper, shake vigorously, and let it stand for 15 min. Add exactly 20 mL of 0.5 *N* hydrochloric acid, and shake until the pink color disappears. Titrate with 0.1 *N* sodium hydroxide to a faint pink color that persists after vigorous shaking; record this value as the saponification titer (V_2).

Quantitatively transfer the contents of the conical flask into a 500-mL distillation flask fitted with a Kjeldahl trap and a water-cooled condenser, the delivery tube of which extends well beneath the surface of a mixture of 150 mL of carbon dioxide-free water and 20.0 mL of 0.1 *N* hydrochloric acid in a receiving flask. Add 20 mL of a 1:10 sodium hydroxide solution to the distillation flask, seal the connections, and then begin heating carefully to avoid excessive foaming. Continue heating until 80 to 120 mL of distillate has been collected. Add a few drops of methyl red TS to the receiving flask,

titrate the excess acid with 0.1 *N* sodium hydroxide, and record the volume required, in milliliters, as *S*. Perform a blank determination (see *General Provisions*) on 20.0 mL of 0.1 *N* hydrochloric acid, and record the volume of 0.1 *N* sodium hydroxide required, in milliliters, as *B*. Record the amide titer ($B - S$) as V_3.

Transfer exactly one-tenth of the total net weight of the dried sample (representing 0.5 g of the original, unwashed sample) to a 50-mL beaker, and wet with about 2 mL of ethanol. Dissolve the sample in 25 mL of 0.125 *M* sodium hydroxide. Agitate the solution for 1 h at room temperature. Quantitatively transfer the saponified sample solution to a 50-mL volumetric flask, and dilute to volume with water. Transfer 25.0 mL of the diluted sample solution to the round-bottom flask of the distillation apparatus, and add 20 mL of Clark's solution. Start the distillation by heating the round-bottom flask. Collect the first 15 mL of distillate separately in a measuring cylinder. Then start the steam supply, and continue distillation until 150 mL of distillate has been collected in a 200-mL beaker. Quantitatively combine the distillates, titrate with 0.05 *M* sodium hydroxide to pH 8.5, and record the volume of titrant required, in milliliters, as *S*.

Perform a blank determination (see *General Provisions*) using 20 mL of water. Record the required volume of 0.05 *M* sodium hydroxide, in milliliters, as *B*. Record acetate ester titer ($S - B$) as V_4.

Calculation Calculate the degree of amidation (as the percent of total carboxyl groups) by the formula

$$100[V_3/(V_1 + V_2 + V_3 - V_4)].$$

Calculate the milligrams of galacturonic acid by the formula

$$19.41(V_1 + V_2 + V_3 - V_4).$$

The milligrams of galacturonic acid obtained in this way is the content of one-tenth of the weight of the washed and dried sample. To calculate the percent galacturonic acid on a moisture- and ash-free basis, multiply the number of milligrams obtained by $1000/x$, in which x is the weight, in milligrams, of the washed and dried sample. If the sample is known to be of the nonamidated type, only V_1 and V_2 need to be determined, and V_3 may be regarded as zero in the formula for calculating milligrams of galacturonic acid.

Lead (**Note**: Use deionized water throughout this procedure.)

Diluted Standard Lead Solution (2 μg Pb per milliliter) Immediately before use, pipet 0.10 mL of a certified commercially available 1000 ppm (1000 μg/mL) lead stock solution into a 50-mL volumetric flask containing 30 mL of water, 4 mL of 20% (v/v) hydrochloric acid, and 4 mL of 0.1 *M* EDTA. Dilute to volume with water, and mix.

Control Lead Solution (0.4 μg Pb per milliliter) Pipet 5.0 mL of the *Diluted Standard Lead Solution* into a 25-mL volumetric flask containing 10 mL of water, 2 mL of 20% (v/v) hydrochloric acid, and 2 mL of 0.1 *M* EDTA. Dilute to volume with water, and mix.

Standard Lead Blank Solution Add 30 mL of water, 4 mL of 20% (v/v) hydrochloric acid, and 4 mL of 0.1 *M* EDTA into a 50-mL volumetric flask. Dilute to volume with water, and mix.

Sample Preparation Transfer 2.0 g of sample into a clean, 100-mL glass beaker, add 25 mL of 70% (v/v) nitric acid, cover with a watch glass, and heat at low to moderate heat on a hot plate in a fume hood for 2 h. Remove the watch glass, and continue to heat until the sample is dry with no visible fumes. Add 0.5 mL of 70% (v/v) nitric acid, and heat to dryness. Cool to room temperature, and add 2 mL of 20% (v/v) hydrochloric acid and 2 mL of 0.1 *M* EDTA. Quantitatively transfer the solution to a 25-mL volumetric flask, dilute to volume with water, and mix.

Procedure Set up an inductively coupled plasma emission spectrometer according to manufacturer's instructions, using the lead emission line at 220.35 nm. Calibrate the instrument using the *Standard Lead Blank Solution* and the *Diluted Standard Lead Solution*. Then analyze the *Sample Preparation* and the *Control Lead Solution*. The sample passes the test if the lead concentration found in the *Sample Preparation* is equal to or less than that in the *Control Lead Solution*.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Methanol, **Ethanol**, and **Isopropanol**

Internal Standard Solution Dissolve 50 mg of *n*-propanol in 1 L of water.

Sample Solution Dissolve 100 mg of sample in 10 mL of water, and as necessary, use sodium chloride as a dispersing agent.

Standard Alcohol Solution Using a micropipet, transfer 50 mg each of methanol (corresponding to 63.21 μL), ethanol (corresponding to 63.35 μL), and isopropanol (corresponding to 63.65 μL) into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Sodium Nitrite Solution Dissolve 250 g of sodium nitrite in 1000 mL of water.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph equipped with a flame-ionization detector and a 90-cm × 4-mm (id) glass column, or equivalent, with the first 15 cm packed with Chromopack (or equivalent) and the remainder packed with 120- to 150-mesh Porapak R (or equivalent). Maintain the column at 150°, isothermal. Set the injection port temperature to 250°. Use nitrogen as the carrier gas with a flow rate of 80 mL/min.

Procedure Weigh 200 mg of urea, and place it in a 25-mL amber-glass vial (Reacti-Flasks, or equivalent). Purge the urea with nitrogen for 5 min, add 1 mL of saturated oxalic acid solution, close the vial with a rubber stopper, and swirl. Add 1 mL of *Sample Solution* and 1 mL of *Internal Standard Solution*, and simultaneously start a stopwatch ($t = 0$). Swirl the vial, and recap it with an open screw cap fitted with a silicone rubber septum. Swirl the vial until $t = 30$ s. At $t = 45$ s, inject 0.5 mL of *Sodium Nitrite Solution* through the septum. Swirl until $t = 70$ s, and at $t = 150$ s, use a pressure lock syringe (Precision Sampling Corporation, or equivalent) to withdraw 1.0 mL of the headspace through the septum. Inject the 1.0 mL into the injection port of the gas chromatograph. Repeat this procedure, but use 1 mL of the *Standard Alcohol Solution* instead of the *Sample Solution*.

Calculation Quantify the total methanol, ethanol, and isopropanol present in 1 mL of the *Sample Solution* taken by the following equation:

$$T = [V_{MS}\,(R_{MU}/R_{MS}) \times 0.791] + [V_{ES}\,(R_{EU}/R_{ES}) \times 0.7893] + [V_{IS}\,(R_{IU}/R_{IS}) \times 0.7855],$$

in which T is the total amount, in milligrams, of methanol, ethanol, and isopropanol in 1 mL of the *Sample Solution*; the subscripts M, E, and I refer to methanol, ethanol, and isopropanol, respectively; V_S is the volume, in milliliters, of the corresponding alcohol in the *Standard Alcohol Solution*; R_U is the ratio of the peak area of the corresponding alcohol in the *Sample Solution* to that of *n*-propanol in the *Internal Standard Solution*; R_S is the ratio of the peak area of the corresponding alcohol in the *Standard Alcohol Solution* to that of *n*-propanol in *Internal Standard Solution*; and 0.791, 0.7893, and 0.7855 are the densities, in grams per milliliter, for methanol, ethanol, and isopropanol, respectively. Calculate the percent methanol, ethanol, and isopropanol present in the sample by the following formula:

$$(1000T)/W,$$

in which W is the weight, in milligrams, of the sample.

Sodium Methyl Sulfate

Mobile Phase Prepare a 0.04 *M* potassium hydrogen phthalate solution by transferring 16.4 g of potassium hydrogen phthalate into a 2-L volumetric flask, diluting to volume with water, and mixing. Filter the solution through a 0.45-µm pore-size filter (Millipore, or equivalent).

Standard Preparation Transfer 10.0 mg of anhydrous sodium methyl sulfate into a 100-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Assay Preparation Suspend about 1 g of sample, accurately weighed, in 10.0 mL of 50% (v/v) ethanol solution. Stir for 30 min using a Teflon-coated stirring bar. Allow the suspension to precipitate, and filter. Evaporate a 1.0-mL aliquot to dryness using reduced pressure (10 mm Hg), and heat at 60°. Redissolve the residue in 1.0 mL of the *Mobile Phase*.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with a refractive index detector and a 25-cm × 4.6-mm (id) column packed with Nucleosil 10SB (or equivalent) and maintained at 40°. Set the flow rate at 1 mL/min.

System Suitability Make three replicate injections of the *Standard Preparation*. They should show a relative standard deviation of not more than 4.0% for the response factor of the sodium methyl sulfate peak obtained using the formula

$$(A_S/C_S),$$

in which A_S is the peak area response of the *Standard Preparation*, and C_S is the concentration, in milligrams per milliliter, of sodium methyl sulfate in the *Standard Preparation*.

Procedure Determine the peak area in the chromatograms for the *Standard Preparation* and *Assay Preparation* by separately injecting 20 µL of each into the chromatograph. Calculate the quantity in percent of sodium methyl sulfate in the sample by the formula

$$(C_S A_U)/(A_S W),$$

in which C_S is the concentration, in milligrams per milliliter, of sodium methyl sulfate in the *Standard Preparation*; A_U and A_S are the peak area responses obtained from the *Assay Preparation* and *Standard Preparation*, respectively; and W is the weight, in grams, of the sample taken.

Sulfur Dioxide Determine as directed under *Sulfur Dioxide Determination*, Appendix X, except use the following method under *Sample Introduction and Distillation*: Transfer about 20 g of sample, accurately weighed, into the round-bottom flask, *C*, and add 20 mL of ethanol to moisten the sample. Add 400 mL of water, swirling vigorously to disperse the sample. Reassemble the apparatus, making sure that the tapered joints are clean and greased with stopcock grease, and proceed as directed under *Sample Introduction and Distillation*, beginning with, ''the nitrogen flow through the *3% Hydrogen Peroxide Solution*. . . ,'' in the first paragraph.

Total Insoluble Substances (**Note**: Use deionized water free from dust on insoluble particles throughout this procedure.) Dry 70-mm glass fiber filter paper GF/B (Whatman 1821 070, or equivalent) for 1 h in an oven equipped with a fan and set to 105°. Transfer the paper to a desiccator containing silica gel, and allow it to cool. Weigh the filter paper, and record the weight as M_1.

Transfer an accurately weighed 1-g sample into a 250-m beaker, recording the sample weight as S. Add 5 mL of isopropanol to the beaker and, while stirring, add 100 mL of a previously mixed and filtered solution of 0.03 *M* sodium hydroxide containing 0.1% (w/w) tetrasodium ethylene diamine tetraacetic acid (EDTA). Stir the mixture for 30 min at room temperature, then heat to boiling.

Caution: Some Pectins foam when heated.

Filter the hot solution through the previously dried filter paper under a vacuum. Rinse the beaker and filter five times with 100 mL of warm (approximately 50°) water. Dry the filter paper in the oven at 105° for 1 h. Transfer it to the desiccator, and leave it to cool. Weigh the paper and record the weight (M_2).

Calculate the percentage of insoluble material using the formula

$$[(M_2 - M_1)/S] \times 100.$$

Packaging and Storage Store in well-closed containers.

Pennyroyal Oil

CAS: [8013-99-8]

FEMA: 2839

View IR

DESCRIPTION

Pennyroyal Oil occurs as a light yellow to yellow, aromatic liquid with a minty odor. It is the volatile oil obtained by

steam distillation from the fresh or partly dried plant *Mentha pulegium* L. (Fam. Labiatae). It is soluble in most fixed oils and in propylene glycol. It is soluble, with slight cloudiness, in mineral oil, but it is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 88.0% and not more than 96.0%, by volume, of ketones.
Angular Rotation Between +18° and +25°.
Refractive Index Between 1.483 and 1.488 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.928 and 0.940.

TESTS

Assay Determine as directed in the *Neutral Sulfite Method* under *Aldehydes and Ketones*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Pentaerythritol Ester of Partially Hydrogenated Wood Rosin

View IR

DESCRIPTION

Pentaerythritol Ester of Partially Hydrogenated Wood Rosin occurs as a hard, amber-colored resin (color K or paler as determined by ASTM Designation D 509). It is soluble in acetone, but insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Number Between 7 and 18.
Lead Not more than 1 mg/kg.
Ring-and-Ball Softening Point 94° or higher.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV.
Ring-and-Ball Softening Point Determine as directed in *Ring-and-Ball Softening Point* under *Softening Point*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Pentaerythritol Ester of Wood Rosin

View IR

DESCRIPTION

Pentaerythritol Ester of Wood Rosin occurs as a hard, pale amber colored resin (color M or paler as determined by ASTM Designation D 509). It is soluble in acetone, but is insoluble in water and in alcohol.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Number Between 6 and 16.
Lead Not more than 1 mg/kg.
Ring-and-Ball Softening Point 100° or higher.

TESTS

Acid Number Determine as directed under *Acid Number*, Appendix IX.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV.
Ring-and-Ball Softening Point Determine as directed in *Ring-and-Ball Softening Point* under *Softening Point*, Appendix IX.

Packaging and Storage Store in well-closed containers.

Peppermint Oil

CAS: [8006-90-4]

FEMA: 2848

View IR

DESCRIPTION

Peppermint Oil occurs as a colorless or pale yellow liquid with a strong, penetrating odor of peppermint and a pungent taste that is followed by a sensation of coldness when air is drawn into the mouth. It is the essential oil obtained by steam distillation from the fresh overground parts of the flowering plant of *Mentha piperita* L. (Fam. Labiatae); it may be rectified by distillation, but is neither partially nor wholly dementholized. It is soluble in alcohol and in most vegetable oils, but it is insoluble in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is natural or rectified.

Identification

A. The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

B. Mix 3 drops of sample with 5 mL of a 1:300 solution of nitric acid:glacial acetic acid in a dry test tube, and place the tube in a beaker of boiling water. A blue color appears within 5 min, which, on continued heating, deepens and shows a copper-colored fluorescence, and then fades, leaving a golden yellow solution.

Assay for Total Esters Not less than 5.0% of esters, calculated as menthyl acetate ($C_{12}H_{22}O_2$).

Assay for Total Menthol Not less than 50.0% of menthol ($C_{10}H_{20}O$).

Angular Rotation Between −18° and −32°.

Dimethyl Sulfide *Rectified Oil*: Passes test; *Natural Oil*: Fails test.

Refractive Index Between 1.459 and 1.465 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.896 and 0.908.

TESTS

Assay for Total Esters Determine as directed in *Ester Determination* under *Esters*, Appendix VI, using about 10 g of sample, accurately weighed, and 99.16 as the equivalence factor (*e*) in the calculation.

Assay for Total Menthol Determine as directed under *Total Alcohols*, Appendix VI, using 2.5 g of acetylated sample. Calculate the percentage of total menthol by the formula

$$7.814A(1 - 0.0021E)/(B - 0.021A),$$

in which *A* is the difference between the number of milliliters of 0.5 *N* hydrochloric acid required in the titration and the number of milliliters of 0.5 *N* hydrochloric acid required in the residual blank titration; *E* is the percentage of total esters determined and calculated as menthyl acetate ($C_{12}H_{22}O_2$); and *B* is the weight, in grams, of the acetylated sample.

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Dimethyl Sulfide Using 25 mL of sample, distill 1 mL, and carefully superimpose the distillate on 5 mL of mercuric chloride TS in a test tube. A white film does not form at the zone of contact within 1 min if the sample is rectified.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 70% alcohol.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers.

Perlite

Expanded Perlite

DESCRIPTION

In its natural state, Perlite occurs as a dense, gray to brown, glassy volcanic rock consisting essentially of fused sodium potassium aluminum silicate plus 3% to 5% water. When fractured and heated at high temperature (900° to 1100°) under proper conditions, it pops like popcorn, caused by the presence of occluded water, expanding to 20 or more times its original volume. The expanded material is crushed to yield a white, nonhygroscopic powder having a bulk density of 32 to 400 kg/m^3 (2 to 25 lb/ft^3) and a particle size ranging from less than one to several hundred micrometers. It is in this latter expanded and powdered state that Perlite is used as a filter aid in food processing. Acceptable food-grade free-flowing agents such as sodium carbonate and sodium silicate may be added. The powder is slightly soluble in water and sparingly soluble in dilute acids and alkalies.

Function Filter aid in food processing.

REQUIREMENTS

Identification

A. Mix about 1 g of sample with 25 mL of 2.7 *N* hydrochloric acid contained in a beaker, cover with a watch glass, heat on a steam bath for 15 min, and cool. Filter, and neutralize the filtrate to litmus paper with 6 *N* ammonium hydroxide. The neutralized filtrate gives positive tests for *Aluminum*, for *Potassium*, and for *Sodium*, Appendix IIIA.

B. Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a burner. Place the hot, transparent bead in contact with a sample, and fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a weblike structure.
Arsenic Not more than 10 mg/kg.
Lead Not more than 10 mg/kg.
Loss on Drying (powdered form) Not more than 3.0%.
Loss on Ignition (glassy form) Not more than 7.0%.
pH (filtrate from a 10% suspension) Between 5 and 11.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using 3.0 mL of *Sample Solution*, prepared as follows: Transfer 10.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat at 70° for 15 min. Cool, and decant through Whatman No. 3, or equivalent, filter paper into a 100-mL volumetric flask. Wash the slurry with three 10-mL portions of hot water and the filter paper with 15 mL of hot water, dilute the solution to volume with water, and mix. Retain a 10.0-mL portion of this solution for the *Lead* test (below).
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 10.0-mL portion of the solution prepared in the *Arsenic* test (above), and using 10 µg of lead (Pb) ion in the control.
Loss on Drying (powdered form) Determine as directed under *Loss on Drying*, Appendix IIC, drying the powdered sample at 105° for 2 h.
Loss on Ignition (glassy form) Ignite 250 mg of a crushed sample of the glassy form to constant weight at 1000°.
pH (filtrate from a 10% solution) Determine as directed under *pH Determination*, Appendix IIB, using the filtrate from the following procedure: Boil 10 g of sample with 100 mL of water for 30 min, dilute to 100 mL with water, and filter through a fine-pore, sintered-glass funnel.

Packaging and Storage Store in well-closed containers.

Petitgrain Oil, Paraguay Type

CAS: [8014-17-3]

View IR

DESCRIPTION

Petitgrain Oil, Paraguay Type, occurs as a yellow to brown-yellow liquid with a somewhat harsh, bittersweet, floral odor. It is the volatile oil obtained by steam distillation from the leaves and small twigs of the bitter orange tree, *Citrus aurantium* L. subspecies *amara* (Fam. Rutaceae). It is soluble in most fixed oils and is soluble, with opalescence or turbidity, in mineral oil and in propylene glycol. It is relatively insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 45.0% and not more than 60.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −4° and +1°.
Refractive Index Between 1.455 and 1.462 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.878 and 0.889.

TESTS

Assay Determine as directed in *Ester Determination* under *Esters*, Appendix VI, using about 2 g of sample, accurately weighed, and 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 4 mL of 70% alcohol. The solution usually develops opalescence or turbidity upon further dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Petrolatum

White Petrolatum; Yellow Petrolatum; Petroleum Jelly

INS: 905b

CAS: [8009-03-8]
CAS: [92045-77-7]
CAS: [100684-33-1]

DESCRIPTION

Petrolatum occurs as an unctuous mass varying in color from white to yellow or light amber. It is transparent in thin layers, and has not more than a slight fluorescence, even after being melted. It is a purified mixture of semisolid saturated hydrocarbons, mainly paraffinic in nature, obtained from petroleum.

In general, it has a viscosity of not less than 3 centistokes at 100°. At 5% initial distillation, 95% of material has a carbon number equal to or greater than 18. It has a minimum average molecular weight of 350. It is insoluble in water, and is almost insoluble in cold or hot alcohol and in cold absolute alcohol. It is partially soluble in ether, in solvent hexane, and in most fixed and volatile oils, and is freely soluble in chloroform and in turpentine oil. It may contain any antioxidant permitted by the U.S. Food and Drug Administration in an amount not greater than that required to produce its intended effect.

Function Defoaming agent; lubricant; protective coating; release agent.

REQUIREMENTS

Acidity or Alkalinity Passes test.
Color Passes test.
Consistency Passes test.
Fixed Oils, Fats, and Rosin Passes test.
Lead Not more than 1 mg/kg.
Melting Range Between 38° and 60°.
Organic Acids Passes test.
Residue on Ignition Passes test.
Specific Gravity Between 0.815 and 0.880 at 60°.
Ultraviolet Absorption (polynuclear hydrocarbons) Passes test.

TESTS

Acidity or Alkalinity Transfer 35 g of sample into a 250-mL separator, add 100 mL of boiling water, and shake vigorously for 5 min. After the sample and water have separated, draw off the water into a casserole, wash the sample in the separator with two 50-mL portions of boiling water, and add the washings to the casserole. Add 1 drop of phenolphthalein TS to the accumulated 200 mL of water, and boil. The solution does not develop a pink color. If the addition of phenolphthalein produces no pink color, add 0.1 mL of methyl orange TS. No red or pink color appears.

Color Melt about 10 g of sample on a steam bath, and pour about 5 mL of the liquid into a 150- × 50-mm, clear-glass, bacteriological test tube, keeping the sample liquid. The sample is not darker than a solution made by mixing 3.8 mL of ferric chloride CS and 1.2 mL of cobaltous chloride CS in a similar tube. Make the comparison in reflected light against a white background, holding the sample tube directly against the background at an angle such that there is no fluorescence.

Consistency

Apparatus Use a penetrometer fitted with a polished, cone-shaped, metal plunger weighing 150 g, and having a detachable steel tip of the following dimensions: The tip of the cone has a 30° angle, the point being truncated to a 0.38- ± 0.03-mm diameter; the base of the tip has a 8.38- ± 0.05-mm diameter; and the tip is 15 ± 0.25 mm long. The remaining portion of the cone has a 90° angle, is 28.2 mm long, and has a 65.1-mm maximum diameter at the base. The containers for the test are flat-bottomed metal or glass cylinders that have a 100-mm diameter and a height of not less than 65 mm.

Procedure Melt a quantity of sample at 82° ± 2.5°, and pour the liquid into one or more of the containers, filling each to within 6 mm of the rim. Cool at 25° ± 2.5° for at least 16 h, protecting from drafts. Two hours before the test, place the containers in a water bath at 25° ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25° ± 0.5° by placing it in the water bath as well.

Without disturbing the surface of the sample, place a container on the penetrometer table, and lower the plunger until the tip of the cone just touches the top surface of the sample at a spot 25 to 38 mm from the edge of the container. Adjust the zero setting, and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration, to the nearest 0.1 mm, from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. If the penetration exceeds 20 mm, use a separate container of the sample for each trial. Calculate the average of the three or more readings, and conduct further trials to a total of ten if the individual results differ from the average by more than ±3%. The final average of the trials is not less than 10.0 mm and not more than 30.0 mm, indicating a consistency value between 100 and 300.

Fixed Oils, Fats, and Rosin Use 10 g of sodium hydroxide and 50 mL of water to digest 10 g of sample at 100° for 30 min. Separate the water layer, and add an excess of 2 *N* sulfuric acid to it. No oily or solid matter separates.

Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV, using 5 μg of lead (Pb) ion in the control.

Melting Range Determine as directed in *Procedure for Class III*, under *Melting Range or Temperature*, Appendix IIB.

Organic Acids Weigh 20 g of sample, add 50 mL of alcohol, previously neutralized to phenolphthalein TS with sodium hydroxide, and 50 mL of water. Agitate thoroughly, and heat to boiling. Add 1 mL of phenolphthalein TS, and while vigorously agitating the solution, titrate rapidly to the appearance of a sharp-pink endpoint, noting the change in the alcohol–water layer. Not more than 0.4 mL of 0.1 *N* sodium hydroxide is required.

Residue on Ignition Heat 4 g of sample in an open porcelain or platinum dish over a Bunsen burner. The sample volatilizes without emitting any acrid odor and, on ignition, yields not more than 0.05% of residue.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Ultraviolet Absorption (polynuclear hydrocarbons) A sample meets the ultraviolet absorbance specifications required by the U.S. Food and Drug Administration for Petrolatum.

Packaging and Storage Store in tight containers.

Petroleum Wax

Refined Paraffin Wax; Refined Microcrystalline Wax

INS: 905c

CAS: [8002-74-2]
CAS: Hydrotreated Paraffin Wax [64742-51-4]
CAS: Hydrotreated Microcrystalline Wax [64742-60-5]
CAS: Microcrystalline Wax [63231-60-7]

View IR

DESCRIPTION

Petroleum Wax occurs as a translucent wax that ranges in color from amber to almost white. It is a refined mixture of solid hydrocarbons, paraffinic in nature, obtained from petroleum. It may be prepared as ''refined paraffin wax'' or as ''refined microcrystalline wax.'' The refined paraffin wax is usually obtained from a lower-molecular-weight fraction of petroleum and has lower viscosities when molten than the refined microcrystalline wax. The refined microcrystalline wax is usually higher in molecular weight, in flash point, and in melting point than the refined paraffin wax. These waxes are graded and sold according to color and to melting point, which ranges from about 48° to 102°. They exhibit low solubility in organic solvents, but they are most soluble in aromatic hydrocarbons and least soluble in ketones, in esters, and in alcohols.

Function Masticatory substance in chewing gum base; protective coating; defoaming agent; microcapsules for spices and flavoring agents.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits maxima only at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Lead Not more than 1 mg/kg.
Ultraviolet Absorbance (polynuclear hydrocarbons) *280 to 289 nm*: Not more than 0.15; *290 to 299 nm*: Not more than 0.12; *300 to 359 nm*: Not more than 0.08; *360 to 400 nm*: Not more than 0.02.

The following additional specifications, where applicable, should conform to the representations of the vendor: *Color*, *Melting Point*, and *Odor*.

TESTS

Color Determine by any suitable method, such as ASTM D 1500.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV, using 5 μg of lead (Pb) ion in the control.
Melting Point Determine by any suitable method, such as ASTM D 127.
Odor Determine by any suitable method, such as ASTM D 1833.
Ultraviolet Absorbance (polynuclear hydrocarbons) Determine as directed in the U.S. Food and Drug Administration regulation for Petroleum Wax (**21** *CFR* 172.886).

Packaging and Storage Store in well-closed containers that are properly vented for liquid materials.

Petroleum Wax, Synthetic

Synthetic Wax (Ethylene Polymer or Ethylene Copolymer with Alpha-Olefins)

View IR

DESCRIPTION

Petroleum Wax, Synthetic, occurs as an off white to white wax. It is a refined mixture of solid hydrocarbons, paraffinic in nature, prepared by the catalytic polymerization of ethylene or copolymer of ethylene with linear (C3–C12) alpha-olefins. Synthetic Petroleum Wax ranges in melting point from about 77° to 116° (170° to 240°F). It is most soluble in aromatic hydrocarbons and least soluble in ketones, in esters, and in alcohols.

Function Masticatory substance in chewing gum base; protective coating; defoaming agent.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits maxima only at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Lead Not more than 1 mg/kg.
Molecular Weight (average) Between 500 and 1200.
Ultraviolet Absorbance (polynuclear hydrocarbons) *280 to 289 nm*: Not more than 0.15; *290 to 299 nm*: Not more than 0.12; *300 to 359 nm*: Not more than 0.08; *360 to 400 nm*: Not more than 0.02.

The following additional specifications, where applicable, should conform to the representations of the vendor: *Color*, *Melting Point*, and *Odor*.

TESTS

Color Determine by any suitable method, such as ASTM D 1500.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV, using 5 μg of lead (Pb) ion in the control.
Melting Point Determine by any suitable method, such as ASTM D 127.

Molecular Weight (average)

Apparatus Use a suitable vapor pressure osmometer, such as the Hewlett-Packard Model 302A, or equivalent, equipped with dual thermistor beads.

Calibration Standards Dissolve accurately weighed amounts of benzil ($C_6H_5COCOC_6H_5$) in *o*-dichlorobenzene to produce solutions containing approximately 3, 7, 10, and 15 mg of benzil, respectively, per gram of solution, and heat to 100° on a steam bath.

Sample Preparations Dissolve accurately weighed amounts of sample in *o*-dichlorobenzene to produce solutions containing approximately 10, 20, 35, and 50 mg of sample, respectively, per gram of solution, and heat to 100° on a steam bath. (Other suitable concentrations that give ΔR readings between 5 and 25 may be used in the *Procedure* below.)

Procedure Following the manufacturer's instructions, balance the osmometer to zero with *o*-dichlorobenzene on both thermistor beads, and establish the calibration constant, K_S, at 100°, using the four *Calibration Standards*. When the temperature within the osmometer has re-equilibrated to 100°, place an aliquot of the most concentrated *Sample Preparation* on the sample thermistor bead. After 4.0 min, balance the instrument to zero with the potentiometer, and record the ΔR value. Repeat this procedure with the same *Sample Preparation* two or three times, and average the ΔR values for that concentration. In a similar manner, obtain the average ΔR values for each of the other three concentrations of the *Sample Preparation*. Plot the four average ΔR values for the *Sample Preparations* as a function of ΔR/concentration, and extrapolate the line to zero to obtain the constant, K_U, for the sample. Divide K_S by K_U to obtain the molecular weight of the sample tested.

Odor Determine by any suitable method, such as ASTM D 1833.

Ultraviolet Absorbance (polynuclear hydrocarbons) Determine as directed in the U.S. Food and Drug Administration regulation for Petroleum Wax (**21** *CFR* 172.886).

Packaging and Storage Store in well-closed containers.

DL-Phenylalanine

DL-α-Amino-β-phenylpropionic Acid

$C_6H_5CH_2CH(NH_2)COOH$

$C_9H_{11}NO_2$ Formula wt 165.19

CAS: [150-30-1]

View IR

DESCRIPTION

DL-Phenylalanine occurs as white crystalline platelets. It is soluble in water, in dilute mineral acids, and in solutions of alkali hydroxides. It is very slightly soluble in alcohol. It is optically inactive.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_9H_{11}NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.2%.
Residue on Ignition Not more than 0.3%.

TESTS

Assay Transfer about 500 mg of sample, previously dried at 105° for 3 h and accurately weighed, into a 250-mL flask. Dissolve the sample in 75 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint.

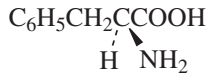
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 16.52 mg of $C_9H_{11}NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

L-Phenylalanine

L-α-Amino-β-phenylpropionic Acid



$C_9H_{11}NO_2$ Formula wt 165.19

CAS: [63-91-2]

View IR

DESCRIPTION

L-Phenylalanine occurs as colorless or white, platelike crystals or as a white crystalline powder. One gram is soluble in about 35 mL of water. It is slightly soluble in alcohol, in dilute mineral acids, and in alkali hydroxide solutions. It melts with decomposition at about 283°. The pH of a 1:100 aqueous solution is between 5.4 and 6.0.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_9H_{11}NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.2%.
Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between −33.2° and −35.2°, calculated on the dried basis; or $[\alpha]_D^{25^\circ}$: Between −32.7° and −34.7°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 300 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 16.52 mg of $C_9H_{11}NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying* Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 2 g of previously dried sample in sufficient water to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Phosphoric Acid

Orthophosphoric Acid

H_3PO_4 Formula wt 98.00

INS: 338 CAS: [7664-38-2]

DESCRIPTION

Phosphoric Acid occurs as a colorless, aqueous solution, usually available in concentrations ranging from 75.0% to 85.0%. It is miscible with water and with alcohol.

Function Acidifier; sequestrant.

REQUIREMENTS

Labeling Indicate the percent, or the percent range, of Phosphoric Acid (H_3PO_4).
Identification A 1:10 aqueous solution gives positive tests for *Phosphate*, Appendix IIIA.
Assay Not less than the minimum or within the range of percentage claimed by the vendor.
Arsenic Not more than 3 mg/kg.
Cadmium Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Lead Not more than 3 mg/kg.

TESTS

Assay Transfer about 1.5 g of sample, accurately weighed, into a tared glass-stoppered flask, and dilute to 120 mL with water. Add 0.5 mL of thymolphthalein TS, mix, and titrate with 1 *N* sodium hydroxide to the first appearance of a blue color. Each milliliter of 1 *N* sodium hydroxide is equivalent to 49.00 mg of H_3PO_4.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.
Cadmium Determine as directed under *Cadmium Limit Test*, Appendix IIIB. Alternatively, use the *Inductively Coupled Plasma Emission Method* below:

Apparatus Use a suitable Inductively Coupled Plasma Emission Spectrophotometer set to 226.502 nm for cadmium and to 371.029 for yttrium (internal standard) with an axial view mode. (This method was developed using a Perkin-Elmer Model 3300 DV equipped with a sapphire injector, low-flow GemCone nebulizer, cyclonic spray chamber, and yttrium internal standard.) Use acid-rinsed plastic volumetric flasks and other labware.

Standard Solutions and Reagents Use commercially available certified stock standard solutions of 10-, 100-, or 1000-μg/mL cadmium in 2% to 5% nitric acid. Use higher purity nitric acid for standards and samples. Where possible, match the sample matrix by adding a material of known high purity to the standards. Prepare an *Internal Standard* solution of 10-μg/mL yttrium in 2% nitric acid, using a certified stock solution. Prepare *Working Calibration Standards* (monthly) of 0.250-, 0.050-, and 0-μg/mL cadmium containing 5% nitric acid; 0.100-μg/mL yttrium; and 5% high-purity sample matrix matching reagent (if available).

Sample Preparation Dissolve 2.5 g of sample in water, and add 2.5 mL of nitric acid and 500 μL of 10-μg/mL yttrium. Dilute to 50 mL.

Procedure Set up the instrumental method to measure the area of the *0-μg/mL Working Calibration Standard* (blank) peaks and then the net intensities of the *0.050-* and *0.250-μg/mL Working Calibration Standards* with the yttrium *Internal Standard*. The calibration curve should be linear. Examine the spectra of the cadmium and yttrium, and make any necessary adjustments to the exact peak locations and baselines to ensure proper integration of the areas under the respective peaks. Analyze the sample and calculate the concentration, in micrograms per milliliter, of the cadmium in the *Sample Solution*.

Calculate the quantity, in milligrams per kilogram, of cadmium in the sample by multiplying this value by 20.

Procedure Notes Some sample types may naturally contain significant levels of yttrium. In these cases, choose a suitable alternative internal standard, or run the test without an internal standard. Use of the internal standard is not required, but it is helpful when there are variations in the viscosity among sample types. Samples may be prepared in higher or lower concentrations as needed. Standard concentrations may be adjusted as needed. Alternative procedures should be validated before use.

Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 1-g sample and 0.2 mL of the 50-mg/kg *Fluoride Limit Solution*.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Packaging and Storage Store in tight containers.

Pimenta Leaf Oil

Pimento Leaf Oil

CAS: [8016-45-3]

FEMA: 2901

View IR

DESCRIPTION

Pimenta Leaf Oil occurs as a pale yellow to light brown-yellow liquid when freshly distilled, becoming darker with age. In contact with iron, it acquires a blue shade, turning to dark brown on extended contact. It has a spicy odor. It is the volatile oil obtained by steam distillation from the leaves of the evergreen shrub *Pimenta officinalis* Lindl. (Fam. Myrtaceae). It is soluble in propylene glycol, and it is soluble, with slight opalescence, in most fixed oils. It is relatively insoluble in glycerin and in mineral oil.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 80.0% and not more than 91.0%, by volume, of phenols.
Angular Rotation Between −2° and +0.5°.
Refractive Index Between 1.531 and 1.536 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.037 and 1.050.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI, using a suitable quantity of sample shaken with about 2% of powdered tartaric acid for about 2 min and filtered. Modify the test by heating the flask on a boiling water bath for 10 min and cooling, after shaking the mixture of sample and 1 *N* potassium hydroxide.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol; a slight opalescence may occur when additional solvent is added.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Pimenta Oil

Pimenta Berries Oil; Pimento Oil; Allspice Oil

View IR

DESCRIPTION

Pimenta Oil occurs as a colorless, yellow, or orange liquid that becomes darker with age. It has the characteristic odor and taste of allspice. It is the volatile oil distilled from the fruit of *Pimenta officinalis*, Lindley (Fam. Myrtaceae). It is soluble in alcohol, in propylene glycol, and in most vegetable oils.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 65.0%, by volume, of phenols.
Angular Rotation Between −4° and 0°.
Refractive Index Between 1.527 and 1.540 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.018 and 1.048.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI, modified by heating on a steam bath for 10 min after shaking

for 5 min. Then cool and let stand overnight, or until the liquids are clear.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 70% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Pine Needle Oil, Dwarf

Pine Needle Oil

CAS: [8000-26-8]

FEMA: 2904

View IR

DESCRIPTION

Pine Needle Oil, Dwarf, occurs as a colorless or yellow liquid with a pleasant, aromatic odor and a bitter, pungent taste. It is the volatile oil obtained by steam distillation of fresh leaves of *Pinus mugo* Turra var. *pumilio* (Haenke) Zenari (Fam. Pinaceae). It is soluble in most vegetable oils, but insoluble in alcohol and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 3.0% and not more than 10.0% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −5° and −15°.
Distillation Range Not more than 10% distills below 165°.
Refractive Index Between 1.475 and 1.480 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.853 and 0.871.

TESTS

Assay Determine as directed in *Ester Determination* under *Esters*, Appendix VI, using about 10 g of sample, accurately weighed, and 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 10 mL of 90% alcohol, often with turbidity.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Pine Needle Oil, Scotch Type

CAS: [8023-99-2]

View IR

DESCRIPTION

Pine Needle Oil, Scotch Type, occurs as a colorless or yellow liquid with an aromatic, turpentine odor. It is the volatile oil obtained by steam distillation from the needles of *Pinus sylvestris* L. (Fam. Pinaceae). It is soluble in most fixed oils; soluble, with faint opalescence, in mineral oil; and slightly soluble in propylene glycol. It is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 1.5% and not more than 5.0% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −4° and +10°.
Refractive Index Between 1.473 and 1.479 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.857 and 0.885.

TESTS

Assay Accurately weigh about 10 g of sample, and proceed as directed under *Ester Determination*, Appendix VI, using 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 6 mL of 90% alcohol, occasionally with slight opalescence.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Poloxamer 331

α-Hydro-*omega*-hydroxy-poly(oxyethylene)-poly(oxypropylene)(51-57 moles)poly(oxyethylene) Block Copolymer

Formula wt (avg.) 3800

DESCRIPTION

Poloxamer 331 occurs as a practically colorless liquid. It is a block copolymer condensate of ethylene oxide and propylene oxide with an average formula weight of 3800, a specific gravity of about 1.02, and a refractive index of about 1.452. It is very slightly soluble in water at 25°, but is freely soluble at 0°; it is freely soluble in alcohol, but is insoluble in propylene glycol and in ethylene glycol.

Function Solubilizing and stabilizing agent in flavor concentrates.

REQUIREMENTS

Cloud Point of a 1:10 Solution Between 9° and 12°.
Ethylene Oxide, Propylene Oxide, and 1,4-Dioxane Not more than 5 mg/kg of each.
Hydroxyl Value Between 27.2 and 32.1.
Lead Not more than 2 mg/kg.
Molecular Weight Between 3500 and 4125.
pH of a 2.5% Solution Between 6.0 and 7.4.

TESTS

Cloud Point of a 1:10 Solution Prepare a 10% aqueous solution at a temperature below the expected cloud point, and transfer about 100 mL of this solution into a 50- × 120-mm test tube. Immerse the tube in a water bath, previously cooled to at least 10° below the expected cloud point, so that the water level is a few millimeters above that of the test solution. Place a suitable thermometer (see *Thermometers*, Appendix I) in the test solution, and position it so that the immersion line will be at the surface of the liquid. Stir the solution slowly with a mechanical stirrer (about 200 rpm), and heat gradually so that the test solution is heated at a rate of about 1°/min. Do not allow the temperature of the water bath to rise more than 10° above that of the test solution at any time. Continue heating in this manner, and record the temperature (cloud point) at which the test solution becomes cloudy.

Ethylene Oxide, Propylene Oxide, and 1,4-Dioxane

Stripped Poloxamer Place between 100 and 300 g of sample into a suitable four-neck, round-bottom flask, equipped with a stirrer, a thermometer, a gas dispersion tube, a dry ice trap, a vacuum outlet, and a heating mantle. At room temperature, evacuate the flask carefully to a pressure of less than 1 mm Hg, applying the vacuum slowly while observing for excessive foaming caused by entrapped gases. After any foaming has subsided, sparge with nitrogen, allowing the pressure to rise to 10 mm Hg. Heat the flask to 130° while increasing the pressure to about 60 mm Hg. Continue stripping for 4 h, then cool to room temperature. Shut off the vacuum pump, and bring the flask back to atmospheric pressure while maintaining nitrogen sparging. Remove the sparging tube with the gas still flowing, then turn off the gas flow. Transfer the *Stripped Poloxamer* to a suitable, nitrogen-filled container.

Standard Preparations (**Caution**: Ethylene oxide, propylene oxide, and 1,4-dioxane are toxic and flammable. Prepare these solutions in a well-ventilated fume hood.) (**Note**: Ethylene oxide is a gas at room temperature. It is usually stored in a lecture-type gas cylinder or small metal pressure bomb. Chill the cylinder in a refrigerator before use.) Place about 50 g of *Stripped Poloxamer*, accurately weighed, into a vial that can be sealed, and add suitable quantities of propylene oxide and 1,4-dioxane. Determine the amounts added by weight difference after each addition. Transfer about 5 mL of liquid ethylene oxide into a 100-mL beaker chilled in wet ice. Using a gas-tight gas chromatographic syringe that has been chilled in a refrigerator, transfer a suitable amount of the liquid ethylene oxide into the mixture. Immediately seal the vial, and shake. Determine the amount added by weight difference. By appropriate dilution with *Stripped Poloxamer*, prepare four solutions, covering the range from 1 to 20 mg/kg for the three components added to the matrix (e.g., 5, 10, 15, and 20 mg/kg). Transfer 1 ± 0.01 g of each of these solutions to separate 22-mL pressure headspace vials, seal each with a silicone septum, star spring, and pressure-relief safety aluminum sealing cap, and crimp the cap closed with a cap-sealing tool.

Test Preparation Transfer 1 ± 0.01 g of sample to a 22-mL pressure headspace vial, and seal, cap, and crimp as directed for the *Standard Preparations*.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a balanced pressure automatic headspace sampler and a flame-ionization detector and containing a 50-m × 0.32-mm fused silica capillary column bonded with a 5-μm film of 5% phenyl–95% methylsiloxane, or equivalent. Program the column temperature to ramp from 70° to 250° at 10°/min, with the transfer line at 140° and the detector at 250°. Use helium as the carrier gas, flowing at a rate of about 0.8 mL/min. The resolution, *R*, of ethylene oxide and propylene oxide, when the *Standard Preparations* are run through the chromatograph as directed under *Calibration*, is not less than 2.0. On the three *Calibration* plots, no point digresses from its line by more than 10%.

Calibration Place the vials containing the *Standard Preparations* in the automated sampler, and start the sequence so that each vial is heated at a temperature of 110° for 30 min before a suitable portion of its headspace is injected into the chromatograph. Set the automatic sampler for a needle withdrawal time of 0.3 min, a pressurization time of 1 min, an injection time of 0.08 min, and a vial pressure of 22 psig with the vial vent off. Obtain the peak areas for ethylene oxide, propylene oxide, and 1,4-dioxane, which have relative retention times of about 1.0, 1.3, and 3.1, respectively. Plot the area versus milligram per kilogram on linear graph paper, and draw the best straight line through the points.

Procedure Place the vial containing the *Test Preparation* in the automatic sampler, and chromatograph its headspace as done for the *Standard Preparations*. Obtain the peak areas of each of the components, and read the concentrations directly from the *Calibration* plots.

Hydroxyl Value

Distilled Pyridine Distill pyridine over phthalic anhydride (about 60 g for each 1000 mL), discarding the first 25 mL and the last 50 mL of distillate from each 1000 mL distilled.

Phenolphthalein Indicator Prepare a 1% solution of phenolphthalein in undistilled pyridine.

Phthalation Reagent Dissolve 14.4 g of phthalic anhydride in sufficient *Distilled Pyridine* to make 100 mL, mix vigorously, and store in a brown bottle. Allow to stand at least 2 h before use. Determine the suitability of *Phthalation Reagent* as follows: Mix 10.0 mL of *Phthalation Reagent* with 25 mL of undistilled pyridine and 50 mL of water, allow to stand for 15 min, then add a few drops of *Phenolphthalein Indicator*, and titrate with 0.5 *N* sodium hydroxide. Multiply the volume, in milliliters, of the alkali solution required by its exact normality; if the result is not within the range 18.8 to 20.0, adjust the concentration of *Phthalation Reagent* accordingly.

Procedure Transfer about 15 g of sample, accurately weighed, into a 250-mL hydroxyl flask, and add 25.0 mL of *Phthalation Reagent*, using a pipet previously rinsed with *Phthalation Reagent* and touching the tip of the pipet against the protrusion of the flask approximately 15 s after the pipet has drained. In the same manner, transfer the same volume of *Phthalation Reagent* into a second flask to serve as the blank. Add a few glass beads to each flask, swirl to dissolve the sample, and reflux for 1 h. Cool the flasks to room temperature, and wash each condenser with two 10-mL portions of undistilled pyridine. Disconnect the condensers, add 10 mL of water to each flask, stopper, swirl, and allow to stand for 10 min. Add 50.0 mL of approximately 0.66 *N* sodium hydroxide to each flask, then add 0.5 mL of *Phenolphthalein Indicator*, and titrate with 0.5 *N* sodium hydroxide to the first pink color that persists for at least 15 s. Calculate the uncorrected hydroxyl value, *h*, by the formula

$$(B - S) \times (N \times 56.1/W),$$

in which *B* is the volume, in milliliters, of 0.5 *N* sodium hydroxide required for the blank; *S* is the volume, in milliliters, of 0.5 *N* sodium hydroxide required for the sample; *N* is the exact normality of the sodium hydroxide solution; and *W* is the weight, in grams, of the sample.

If the sample contains significant acidity or alkalinity, correct the results as follows: Dissolve approximately 15 g of sample, accurately weighed, in 40 mL of undistilled pyridine, and add 60 mL of water and 0.5 mL of *Phenolphthalein Indicator*. If the solution is colorless, titrate with 0.1 *N* sodium hydroxide to a light pink endpoint, recording the volume required, in milliliters, as *v*. If the solution is pink, titrate with 0.1 *N* hydrochloric acid to the disappearance of the pink color, recording the volume required, in milliliters, as *v′*. Calculate the acidity correction factor, *A*, by the formula

$$v \times N \times 56.1/w,$$

in which *N* is the exact normality of the sodium hydroxide solution, and *w* is the weight, in grams, of the sample. Calculate the alkalinity correction factor, *A′*, by the formula

$$v' \times N' \times 56.1/w,$$

in which *N′* is the exact normality of the hydrochloric acid solution.

Finally, calculate the corrected hydroxyl value, *H*, by whichever formula, below, is appropriate:

$$h + A, \text{ or } h - A'.$$

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Molecular Weight Take the corrected *Hydroxyl Value*, *H*, from the above test, and calculate the molecular weight by the formula

$$56{,}100 \times (2/H).$$

pH of a 2.5% Solution Determine as directed under *pH Determination*, Appendix IIB, using a 2.5% aqueous solution.

Packaging and Storage Store in tight containers.

Poloxamer 407

α-Hydro-*omega*-hydroxy-poly(oxyethylene)-poly(oxypropylene)(63-71 moles)poly(oxyethylene) Block Copolymer

Formula wt (avg.) 12,500

DESCRIPTION

Poloxamer 407 occurs as a white solid. It is a copolymer condensate of ethylene oxide and propylene oxide with a melting range of about 52° to 56°. It is freely soluble in alcohol and in water, but is insoluble in propylene glycol and in ethylene glycol.

Function Solubilizing and stabilizing agent in flavor concentrates.

REQUIREMENTS

Cloud Point of a 1:10 Solution Above 100°.
Ethylene Oxide, Propylene Oxide, and 1,4-Dioxane Not more than 5 mg/kg of each.
Hydroxyl Value Between 8.5 and 11.5.
Lead Not more than 2 mg/kg.
Molecular Weight Between 9760 and 13,200.
pH of a 2.5% Solution Between 6.0 and 7.4.

TESTS

Cloud Point of a 1:10 Solution Dissolve about 5 g of sample in 50 mL of water contained in a test tube, place the tube in a boiling water bath, and heat to 100°. The solution does not become cloudy.
Ethylene Oxide, Propylene Oxide, and 1,4-Dioxane Determine as directed in the monograph for *Poloxamer 331*.
Hydroxyl Value Determine as directed in the monograph for *Poloxamer 331*, except in the *Procedure*, use about 45 g of sample, accurately weighed, and add 25 mL of *Distilled Pyridine* to both the sample flask and the blank flask before refluxing.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Molecular Weight Determine the *Hydroxyl Value*, *H*, from the above test, and calculate the molecular weight by the formula

$$56{,}100 \times (2/H).$$

pH of a 2.5% Solution Determine as directed under *pH Determination*, Appendix IIB, using a 2.5% aqueous solution.

Packaging and Storage Store in tight containers.

Polydextrose

INS: 1200 CAS: [68424-04-4]

DESCRIPTION

Polydextrose occurs as an off white to light tan solid. It is a randomly bonded polymer prepared by the condensation of a melt that consists of approximately 90% D-glucose, 10% sorbitol, and 1% citric acid or 0.1% phosphoric acid on a weight basis. The 1,6-glycosidic linkage predominates in the polymer, but other possible bonds are present. The product contains small quantities of free glucose, sorbitol, and D-anhydroglucoses (levoglucosan), with traces of citric acid or phosphoric acid. It may be partially reduced by transition metal catalytic hydrogenation in an aqueous solution. It may be neutralized with any food-grade base and decolorized and deionized for further purification. It is very soluble in water.

Function Bulking agent; humectant; texturizer.

REQUIREMENTS

Identification

A. Add 4 drops of 5% aqueous phenol solution to 1 drop of a 1:10 aqueous solution, then rapidly add 15 drops of sulfuric acid. A deep yellow to orange color appears.

B. While vigorously swirling (vortex mixer), add 1.0 mL of acetone to 1.0 mL of a 1:10 aqueous solution. The solution remains clear. Retain this solution for *Identification Test C*.

C. While vigorously swirling, add 2.0 mL of acetone to the solution from *Identification Test B*. A heavy, milky turbidity develops immediately.

D. Add 4 mL of alkaline cupric citrate TS to 1 mL of a 1:50 aqueous solution. Boil vigorously for 2 to 4 min. Remove from heat, and allow the precipitate (if any) to settle. The supernatant liquid is blue or blue-green.

Assay Not less than 90.0% polymer, calculated on the anhydrous, ash-free basis.
5-Hydroxymethylfurfural and Related Compounds Not more than 0.1%, calculated on the anhydrous, ash-free basis.
Lead Not more than 0.5 mg/kg.
Molecular Weight Limit Passes test.
Monomers *D-Anhydroglucoses*: Not more than 4.0%, calculated on the anhydrous, ash-free basis; *Glucose* and *Sorbitol*: Not more than 6.0%, calculated on the anhydrous, ash-free basis.
Nickel Not more than 2 mg/kg for hydrogenated Polydextrose.
pH of a 10% Solution *Untreated Polydextrose*: Between 2.5 and 7.0; *Neutralized or Decolorized Polydextrose*: Between 5.0 and 6.0.
Residue on Ignition *Untreated Polydextrose*: Not more than 0.3%; *Neutralized or Decolorized Polydextrose*: Not more than 2.0%.
Water Not more than 4.0%.

TESTS

Assay

Glucose Standard Solutions Transfer 100 mg of α-D-glucose (Aldrich, or equivalent), accurately weighed, into a 500-mL volumetric flask, and dilute to volume with water. Dilute 5 aliquots of the solution with water to obtain the following concentrations of standard: 50, 40, 20, 10, and 5 μg/mL.

Phenol Solution Add 20 mL of water to 80 g of phenol.

Sample Solution Transfer approximately 250 mg of sample, accurately weighed, into a 250-mL volumetric flask, and dilute to volume with water.

Standard Curve On a daily basis, pipet 2.0 mL of each *Glucose Standard Solution* into separate, acetone-free, 15-mL screw-cap vials. Add 0.12 mL of the *Phenol Solution*, and mix gently. Uncap each vial and rapidly add 5.0 mL of sulfuric acid. Immediately recap each vial, and shake vigorously.

Caution: Wear rubber gloves and a safety shield while adding sulfuric acid.

Let the vials stand at room temperature for 45 min, then determine the absorbance of each sample at 490 nm in a suitable spectrophotometer, using a *Phenol Solution*–sulfuric acid reagent blank in the reference cell. Plot mean absorbance values versus concentrations, in micrograms per milliliter, obtained from triplicate samples.

Procedure Transfer a 10.0-mL aliquot of *Sample Solution* into a 250-mL volumetric flask and dilute to volume with water. Proceed as directed under *Standard Curve* (above). Calculate the percent polymer by the formula

$$1.05[100(A - Y)/(S \times C) - P_G - 1.11\ P_L],$$

in which 1.05 is an experimentally derived correction factor to account for the polymer (which also contains a small amount of sorbitol) not giving the exact amount of color given by an equivalent amount of glucose monomers; A is the sample absorbance; Y is the y intercept of the standard curve; S is the slope (approximately 0.02) of absorbance versus glucose concentration, in grams per milliliter, obtained from the *Standard Curve*; C is the concentration, in grams per milliliter (adjusted for ash and moisture), of the *Sample Solution*; P_G and P_L are the percentages of glucose and levoglucosan, determined respectively, under *Monomers* (below); and 1.11 is a conversion factor from levoglucosan, which gives an equivalent amount of color to an equivalent weight of glucose.

5-Hydroxymethylfurfural and Related Compounds Transfer approximately 1 g of sample, accurately weighed, into a 100-mL volumetric flask, and dilute to volume with water. Read the absorbance of this solution against a water blank at 283 nm in a 1-cm quartz cell in a spectrophotometer. Calculate the percent 5-hydroxymethylfurfural and related compounds by the formula

$$(0.749 \times A)/C,$$

in which 0.749 is a composite proportionality constant that includes the extinction coefficient and other molecular weight, unit, and volume conversions; A is the absorbance of the *Sample Solution*, and C is the concentration, in milligrams per milliliter, of *Sample Solution*, corrected for ash and moisture.

Lead

Apparatus Use a suitable spectrophotometer (Perkin-Elmer Model 6000, or equivalent), a graphite furnace containing a L'vov platform (Perkin-Elmer Model HGA-500, or equivalent), and an autosampler (Perkin-Elmer Model AS-40, or equivalent). Use a lead hollow-cathode lamp (lamp current of 10 mA), a slit width of 0.7 mm (set low), the wavelength set at 283.3 nm, and a deuterium arc lamp for background correction.

Note: For this test, use reagent-grade chemicals with as low a lead content as is practicable, as well as high-purity water and gases. Before use in this analysis, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse them thoroughly with high-purity water, preferably obtained from a mixed-bed, strong-acid, strong-base, ion-exchange cartridge capable of producing water with an electrical resistivity of 12 to 15 megohms.

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade Lead Nitrate (alternatively, use NIST Standard Reference Material containing 10 mg of lead per kilogram, or equivalent) in 100 mL of water containing 1 mL of nitric acid. Dilute to 1000.0 mL with water, and mix. Prepare and store this solution in glass containers that are free from lead salts. Each milliliter of this solution contains the equivalent of 100 µg of lead (Pb) ion.

Standard Lead Solution On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* to 100.0 mL with water, and mix. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 µg of lead (Pb) ion.

Standard Solutions Prepare a series of lead standard solutions serially diluted from the *Standard Lead Solution* with water. Pipet 0.2, 0.5, 1, and 2 mL, respectively, of *Standard Lead Solution* into separate 100-mL volumetric flasks, dilute to volume with water, and mix. The *Standard Solutions* contain, respectively, 0.02, 0.05, 0.1, and 0.2 µg of lead per milliliter.

Matrix Modifier Transfer 100.0 mg of ammonium phosphate, dibasic, into a 10-mL volumetric flask, dilute to volume with water, and mix.

Sample Solution Transfer about 1 g of sample, accurately weighed, into a 10-mL volumetric flask, add 5 mL of water, and mix. Dilute to volume with water, and mix.

Spiked Sample Solution Prepare a solution as directed under *Sample Solution*, but add 100 µL of the *Standard Lead Solution*, dilute to volume with water, and mix. This solution contains 0.1 µg of lead per milliliter.

Procedure With the use of an autosampler, atomize 10-µL aliquots of the four *Standard Solutions*, using the following sequence of conditions: (1) dry at 130° with a 20-s ramp period, a 40-s hold time, and a 300-mL/min argon flow rate; (2) char at 800° with a 20-s ramp period, a 40-s hold time, and a 300-mL/min argon flow rate; (3) atomize at 2400° for 6 s with a 50-mL/min argon flow rate; (4) clean at 2600° with a 1-s ramp period, a 5-s hold time, and a 300-mL/min argon flow rate; and (5) recharge at 20° with a 2-s ramp period, a 20-s hold time, and a 300-mL/min argon flow rate. Atomize 10 µL of the *Matrix Modifier* in combination with either 10 µL of the *Sample Solution* or 10 µL of the *Spiked Sample Solution* under identical conditions used for the *Standard Solutions*.

Plot a standard curve using the concentration, in micrograms per milliliter, of each *Standard Solution* versus its maximum absorbance value compensated for background correction, and draw the best straight line. From the standard curve, determine the concentrations C_S and C_A, in micrograms per milliliter, of the *Sample Solution* and the *Spiked Sample Solution*, respectively. Calculate the quantity, in milligrams per kilogram, of lead in the sample by the formula

$$10C_S/W,$$

in which W is the weight, in grams, of sample taken. Calculate the recovery by the formula

$$100[(C_A - C_S)/0.1],$$

in which 0.1 is the amount of lead, in micrograms per milliliter, added to the *Spiked Sample Solution*.

Molecular Weight Limit

Apparatus (See *Chromatography*, Appendix IIA.) Use a suitable high-performance liquid chromatograph equipped with a differential refractometer, either a loop injector or suitable autosampler, a column heating block or oven and a computing integrator, or a computer data handling system with molecular weight determination capabilities. Use a Waters Ultrahydrogel 250 A size exclusion column, or equivalent. Maintain the column at 45°; use the HPLC pump to supply eluent to it at 0.8 mL/min, reproducible to 0.5%. Set the differential refractometer at a sensitivity of 4×10^{-6} refractive index units full scale, and set the plotter of the integrator to 64 mV full scale. Maintain the detector cell at 35° ± 0.1°. Noise attributable to the detector and electronics should be less than 0.1% full scale.

Eluent Dissolve 35.0 g of sodium nitrate and 1.0 g of sodium azide in 100 mL of HPLC-grade water. Filter through a 0.45-μm filter into a 4-L flask. Dilute to volume with HPLC-grade water. Degas by applying an aspirator vacuum for 30 min. The resulting eluent is 0.1 *N* sodium nitrate containing 0.025% sodium azide.

Standard Solution Transfer 20 mg each of dextrose; stachyose; and 5800, 23,700, and 100,000 molecular weight (MW) pullulan standards into a 10-mL volumetric flask. Dissolve in and dilute to volume with *Eluent*. Filter through a 0.45-μm syringe filter into a suitable autosampler vial, and seal (all components of the *Standard Solution* are available from Polymer Laboratories, Inc., Technical Center, Amherst Fields Research Park, 160 Old Farm Road, Amherst, MA 01002).

Sample Preparation Transfer 50 mg of sample, accurately weighed, into a 10-mL volumetric flask. Dissolve in and dilute to volume with *Eluent*. Filter through a 0.45-μm syringe filter into a suitable autosampler vial.

Column Equilibration After installing a new column in the HPLC, pump *Eluent* through it overnight at 0.3 mL/min. Before calibration or analysis, increase the flow slowly to 0.8 mL/min over a 1-min period, then pump at 0.8 mL/min for at least 1 h before the first injection. Check the flow gravimetrically, and adjust it if necessary. Reduce the flow to 0.1 mL/min when the system is not in use.

Data System Setup Set the integrator or computerized data-handling system as its respective manual instructs for normal gel permeation chromatographic determinations. Set the integration time to 15 min.

Column Standardization After equilibrating the HPLC system at a flow rate of 0.8 mL/min for at least 1 h, inject 50 μL of the *Standard Solution* five times, allowing 15 min between injections. Record the retention times of the various components in the *Standard Solution*. Retention times for each component should agree within ±2 s. Insert the average retention time along with the molecular weight of each component into the calibration table of the molecular weight distribution software.

System Suitability Check the regression results for a cubic fit of the calibration points. They should have an R^2 value of 0.9999+. Dextrose and stachyose should be baseline resolved from one another and from the 5800 MW pullulan standard. Elevated valleys are usually observed between the 5800, the 23,700, and the 100,000 MW pullulan standards.

Procedure Inject 50 μL of the *Sample Preparation*, following the same conditions and procedure as described under *Column Standardization*. Using the Molecular Weight Distribution software of the data-reduction system, generate a molecular weight distribution curve of the sample. There is no measurable peak above a molecular weight of 22,000.

Monomers

Apparatus (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph equipped with a 250-cm × 2-mm (id) glass column, or equivalent, packed with 3% OV-1 stationary phase on 100- to 120-mesh Gas Chrom Q, or equivalent, and with a flame ionization detector. Maintain the column at 175°, the injection port at 210°, and the detector at 230°. Relative retention times (min): D-anhydroglucoses (levoglucosan), pyranose form (3.7), furanose form (not present in standard) (4.3); *n*-octadecane (5.1); α-D-glucose (8.7); D-sorbitol (11.3); β-D-glucose (13.3).

Standard Solution Transfer 50 mg of α-D-glucose (NIST), 40 mg of anhydrous D-sorbitol, and 35 mg of D-anhydroglucoses, all accurately weighed, into a 100-mL volumetric flask; dissolve in and dilute to volume with pyridine.

Octadecane Solution Transfer 50 mg of *n*-octadecane, accurately weighed, into a 100-mL volumetric flask; dissolve in and dilute to volume with pyridine.

Silylation of Standard Solution Transfer 1.0 mL of *Standard Solution* to a screw-cap vial, and add 1.0 mL of *Octadecane Solution* and 0.5 mL of *N*-trimethylsilylimidazole. Cap the vial, and immerse it in an ultrasonic bath at 70° for 60 min.

Sample Solution Transfer 20 mg of sample, accurately weighed, into a screw-cap vial, and add 1.0 mL of *Octadecane Solution*, 1 mL of pyridine, and 0.5 mL of *N*-trimethylsilylimidazole. Cap the vial, and immerse it in an ultrasonic bath at 70° for 60 min.

Procedure Before sample analysis, inject 3 μL of the *Silylated Standard Solution* into the gas chromatograph. Repeat twice, then inject duplicate 3-μL portions of the *Sample Solution*. Calculate the percentage of each monomer by the formula

$$(R \times W_S)/(R_S \times W),$$

in which R is the ratio of the area of the monomer peak to the area of the octadecane peak in the sample injection; W_S is the weight, in milligrams, of the respective monomer in the *Standard Solution*; R_S is the mean ratio of the area of the monomer peak to the area of the octadecane peak in the standard injections; and W is the weight, in milligrams, of sample, adjusted for ash and moisture.

Nickel Determine as directed in the *Nickel Limit Test*, Appendix IIIB.

pH of a 10% Solution Determine as directed under *pH Determination*, Appendix IIB.

Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC.

Water Determine as directed under *Water Determination*, Appendix IIB, using pyridine instead of methanol in the titration vessel.

Packaging and Storage Store in tight, light-proof containers.

Polydextrose Solution

DESCRIPTION

Polydextrose Solution occurs as a clear, straw-colored liquid. It is a 70% to 80% water solution of polydextrose.

Function Bulking agent; humectant; texturizer.

REQUIREMENTS

Identification

A. Add 4 drops of 5% aqueous phenol solution to 1 drop of a 1:10 aqueous solution, then rapidly add 15 drops of sulfuric acid. A deep yellow to orange color appears.

B. While vigorously swirling (vortex mixer), add 1.0 mL of acetone to 1.0 mL of a 1:10 aqueous solution. The solution remains clear.

C. While vigorously swirling, add 2.0 mL of acetone to the solution from *Identification Test B*. A heavy, milky turbidity develops immediately.

D. Add 4 mL of alkaline cupric citrate TS to 1 mL of a 1:50 aqueous solution. Boil vigorously for 2 to 4 min. Remove from heat, and allow the precipitate (if any) to settle. The supernatant liquid is blue or blue-green.

Assay Not less than 90.0% polymer, calculated on the anhydrous, ash-free basis.

5-Hydroxymethylfurfural Not more than 0.1%, calculated on the anhydrous, ash-free basis.

Lead Not more than 0.5 mg/kg.

Molecular Weight Limit Passes test.

Monomers *1,6-Anhydro-D-glucose*: Not more than 4.0%, calculated on the anhydrous, ash-free basis; *Glucose* and *Sorbitol*: Not more than 6.0%, calculated on the anhydrous, ash-free basis.

Nickel Not more than 2 mg/kg.

pH of a 10% Solution Between 3.5 and 6.5.

Residue on Ignition Not more than 2.0%.

Water Within the range of 27.5% to 32.5%.

TESTS

Assay Determine as directed in the monograph for *Polydextrose*, using approximately 360 mg of sample, accurately weighed.

5-Hydroxymethylfurfural Determine as directed in the monograph for *Polydextrose*, using 1.4 g of sample, accurately weighed.

Lead Determine as directed in the monograph for Polydextrose, using an amount of sample equivalent to 1 g of Polydextrose.

Molecular Weight Limit Determine as directed in the monograph for *Polydextrose*, using 720 mg of sample, accurately weighed.

Monomers

1,6-Anhydro-D-glucose; *Glucose*; and *Sorbitol* Transfer 30 mg of sample, accurately weighed, into a screw-cap vial, and add about 2 mL of pyridine. While flushing the vial with a stream of dry air or nitrogen, heat at 80° to 90° until the solution volume is reduced to 0.2 to 0.5 mL. Add a second portion of pyridine, and repeat the evaporation procedure. Continue as directed in the monograph for *Polydextrose*.

Nickel Determine as directed under *Nickel Limit Test*, Appendix IIIB.

pH of a 10% Solution Determine as directed under *pH Determination*, Appendix IIB, using a mixed solution comprising 1.4 g of sample diluted to 10 mL with water.

Residue on Ignition Determine as directed in *Method II* under *Residue on Ignition*, Appendix IIC.

Water Transfer 1 to 2 mL of sample into a dropper vial, and accurately weigh the dropper, vial, and sample combined. Add 50 mL of pyridine to a clean, dry reaction jar previously flushed with dry air for 1 min. Titrate the pyridine with Karl Fischer reagent (see *Reagent* in *Method 1a* under *Water Determination*, Appendix IIB) to the endpoint to consume any water present. Transfer one drop of sample (50 to 100 mg) from the weighed sample vial to the reaction jar. Accurately reweigh the dropper, vial, and remaining sample. Stir the pyridine–sample mixture for 5 to 10 min. Titrate with Karl Fischer reagent to the endpoint. For each determination, calculate the percentage of water (W) in the sample by the equation

$$W = (V \times F \times 100)/S,$$

in which V is the volume, in milliliters, of Karl Fischer reagent consumed in the second titration; F is the Karl Fischer reagent standardization factor, in milligrams per milliliter; and S is the sample weight, in milligrams, equal to the difference between the initial and final weighings of the dropper, vial, and sample combination. Calculate the water content of the sample as the average of two determinations.

Packaging and Storage Store in tight, light-resistant containers.

Polyethylene

$(C_2H_4)_x$

CAS: [9002-88-4]

View IR

DESCRIPTION

Polyethylene occurs as a white, translucent, partially crystalline and partially amorphous resin. It is produced by the direct

polymerization of ethylene at high temperatures and high pressure. Various grades and types, differing from one another in molecular weight, molecular weight distribution, degree of chain branching, and extent of crystallization, are available. It is insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a sample dissolved in hot toluene and evaporated on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Lead Not more than 3 mg/kg.
Molecular Weight Between 2000 and 21,000.
Volatiles Not more than 0.5%.

TESTS

Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV.
Molecular Weight Determine as directed under *Molecular Weight*, Appendix IV.
Volatiles (**Caution**: To reduce explosion hazard, pass carbon dioxide or nitrogen into the lower part of the drying oven at a rate of about 100 mL/min.) Determine as directed under *Loss on Drying*, Appendix IIC, drying a 4-g sample for 45 min at 105°.

Packaging and Storage Store in well-closed containers.

Polyethylene Glycols

PEG

$$HOCH_2-(CH_2-O-CH_2)_n-CH_2OH$$

in which n = average number of oxyethylene groups

INS: 1521 CAS: [25322-68-3]

DESCRIPTION

Polyethylene Glycols are addition polymers of ethylene oxide and water, ranging in molecular weight from about 200 to about 9500. Commercially available Polyethylene Glycols are usually designated by a number that roughly corresponds to the nominal molecular weight. Polyethylene Glycols having a nominal molecular weight of 600 or below occur as clear to slightly hazy, colorless or practically colorless, viscous, slightly hygroscopic liquids that are miscible with water. Polyethylene Glycols having a nominal molecular weight of 1000 or above are freely soluble in water and occur as creamy white, waxy solids or as flakes resembling paraffin. The Polyethylene Glycols are soluble in many organic solvents, including aliphatic ketones and alcohols, chloroform, glycol ethers, esters, and aromatic hydrocarbons; they are insoluble in ether and in most aliphatic hydrocarbons. As their molecular weight increases, water solubility, vapor pressure, hygroscopicity, and solubility in organic solvents decrease, while solidification point, specific gravity, flash point, and viscosity increase. They may contain a suitable antioxidant.

Function Dispersing, coating, binding, plasticizing agent; lubricant; flavoring adjuvant.

REQUIREMENTS

Labeling Indicate the nominal average molecular weight.
Completeness and Color of Solution Passes test.
Ethylene Glycol and Diethylene Glycol Not more than 0.25%, combined.
Ethylene Oxide and 1,4-Dioxane Not more than 10 mg/kg each.
Lead Not more than 1 mg/kg.
Nominal Molecular Weight *Polyethylene Glycols having nominal molecular weights below 1000*: Not less than 95.0% and not more than 105.0% of the labeled value; *Polyethylene Glycols having nominal molecular weights between 1000 and 7000*: Not less than 90.0% and not more than 110.0% of the labeled value; *Polyethylene Glycols having nominal molecular weights above 7000*: Not less than 87.5% and not more than 112.5% of the labeled value.
pH Between 4.5 and 7.5.
Residue on Ignition Not more than 0.2%.
Viscosity Passes test.

TESTS

Completeness and Color of Solution A solution of 5 g of sample in 50 mL of water is colorless. It is clear for liquid grades and not more than slightly hazy for solid grades.
Ethylene Glycol and Diethylene Glycol

POLYETHYLENE GLYCOLS HAVING NOMINAL MOLECULAR WEIGHTS BELOW 450

Apparatus (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector (Varian Aerograph 600D, or equivalent), containing a 1.5-m × 3-mm (id) stainless-steel column, or equivalent, packed with sorbitol 12%, by weight, on 60- to 80-mesh nonacid-washed diatomaceous earth (Chromosorb W, or equivalent).

Operating Conditions The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: *column temperature*, 165°; *inlet temperature*, 260°; *carrier gas*, nitrogen (or other suitable inert gas), flowing at a rate of 70 mL/min; *recorder*, 0.5 to +1.05 mV, full span, 1-s full-response time; *hydrogen and air flow to burner*, optimize to give maximum sensitivity.

Standard Solutions Prepare chromatographic standards by dissolving accurately weighed amounts of commercial ethylene glycol and diethylene glycol, previously purified by distillation if necessary, in water. Suitable concentrations range from 0.2 to 1 mg of each glycol per milliliter.

Sample Preparation Transfer about 4 g of sample, accurately weighed, into a 10-mL volumetric flask, dilute to volume with water, and mix.

Procedure Inject a 2-µL portion of each of the *Standard Solutions* into the chromatograph, and obtain the chromatogram for each solution. Under the stated conditions, the elution time is approximately 2.0 min for ethylene glycol and 6.5 min for diethylene glycol. Measure the peak heights, and record the values as follows: A = height, in millimeters, of the ethylene glycol peak; B = weight, in milligrams, of ethylene glycol per milliliter of *Standard Solution*; C = height, in millimeters, of the diethylene glycol peak; and D = weight, in milligrams, of diethylene glycol per milliliter of *Standard Solution*.

Similarly, inject a 2-µL portion of the *Sample Preparation* into the chromatograph, and obtain the chromatogram, recording the height of the ethylene glycol peak as E and that of the diethylene glycol peak as F. Calculate the percentage of ethylene glycol in the sample by the formula

$$(E \times B)/(A \times \text{sample weight, in grams});$$

calculate the percentage of diethylene glycol in the sample by the formula

$$(F \times D)/(C \times \text{sample weight, in grams}).$$

Not more than 0.25% of total ethylene and diethylene glycols is found.

POLYETHYLENE GLYCOLS HAVING NOMINAL MOLECULAR WEIGHTS OF 450 OR HIGHER

Sample Preparation Dissolve 50.0 g of sample in 75 mL of diphenyl ether in a 250-mL distillation flask. Warm the mixture, if necessary, just enough to melt the crystals. Slowly distill at a pressure of 1 to 2 mm Hg into a receiver graduated to 100 mL in 1-mL subdivisions, until 25 mL of distillate has been collected. Add 20.0 mL of water to the distillate, shake vigorously, and allow the layers to separate. Cool in an ice bath to solidify the diphenyl ether and facilitate its removal. Filter the water layer, wash the diphenyl ether with 5.0 mL of ice-cold water, pass the washings through the filter, and collect the filtrate and washings in a 25-mL volumetric flask. Warm to room temperature, dilute to volume with water, if necessary, and mix. Mix this solution with 25.0 mL of freshly distilled acetonitrile in a 125-mL glass-stoppered flask.

Standard Preparation Transfer 62.5 mg of diethylene glycol to a 25-mL volumetric flask, dilute to volume with a 1:1 mixture of freshly distilled acetonitrile and water, and mix.

Procedure Transfer 10.0 mL each of the *Sample Preparation* and of the *Standard Preparation* into separate 50-mL flasks, each containing 15 mL of ceric ammonium nitrate TS, and mix. Within 2 to 5 min, using a suitable spectrophotometer, determine the absorbance of each solution in a 1-cm cell at the wavelength of maximum absorbance occurring between 400 and 600 nm, using a blank consisting of 15 mL of ceric ammonium nitrate TS and 10 mL of a 1:1 mixture of acetonitrile and water. The absorbance of the solution from the *Sample Preparation* does not exceed that from the *Standard Preparation*.

Ethylene Oxide and 1,4-Dioxane

Stripped Polyethylene Glycol 400 Place 3000 g of Polyethylene Glycol 400 into a 5000-mL, 4-neck, round-bottom flask equipped with a stirrer, a thermometer, a gas dispersion tube, a dry ice trap, a vacuum outlet, and a heating mantle. At room temperature, evacuate the flask carefully to a pressure of less than 1 mm Hg, applying the vacuum slowly while observing for excessive foaming due to entrapped gases. After any foaming has subsided, sparge with nitrogen, allowing the pressure to rise to 10 mm Hg. Heat the flask to 60° while increasing the pressure to about 60 mm Hg. Continue stripping for 4 h, then cool to room temperature. Shut off the vacuum pump, and bring the flask pressure back to atmospheric while maintaining nitrogen sparging. Remove the sparging tube with the gas still flowing, then turn off the gas flow. Transfer the *Stripped Polyethylene Glycol 400* to a suitable nitrogen-filled container.

Standard Preparations (**Caution**: Ethylene oxide and 1,4-dioxane are toxic and flammable. Prepare these solutions in a well-ventilated fume hood.) Add a suitable quantity of 1,4-dioxane to a known weight of organic-free water in a vial that can be sealed. Determine the amount added by weight difference. Using the special handling described in the following, complete the preparation. Ethylene oxide is a gas at room temperature. It is usually stored in a lecture-type gas cylinder or small metal pressure bomb. Chill the cylinder in a refrigerator before use. Transfer about 5 mL of the liquid ethylene oxide to a 100-mL beaker chilled in wet ice. Using a gas-tight gas chromatographic syringe that has been chilled in a refrigerator, transfer a suitable amount of the liquid ethylene oxide into the mixture. Immediately seal the vial, and shake. Determine the amount added by weight difference. By appropriate dilution with *Stripped Polyethylene Glycol 400*, prepare four solutions, covering the range from 1 to 20 mg/kg for the two components added to the matrix (e.g., 5, 10, 15, and 20 mg/kg). Transfer 10 mL of each of these solutions to separate 22-mL pressured headspace vials, seal each with a silicone septum, star spring, and pressure-relief safety aluminum sealing cap, and crimp the cap closed with a cap-sealing tool. Shake for 2 min.

Sample Preparation Transfer 10 ± 0.01 g of sample to a 22-mL pressure headspace vial, and seal, cap, and crimp as directed for the *Standard Preparations*.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a balanced pressure automatic headspace sampler and a flame-ionization detector and that contains a 50-m × 0.32-mm fused silica capillary column bonded with a 5-µm film of 5% phenyl–95% methylsiloxane, or equivalent. Program the column temperature from 70° to 250° at 10°/min, with the transfer line at 140° and the detector at 250°. Use helium as the carrier gas, flowing at a rate of about 0.8 mL/min. On

the two *Calibration* plots, no point digresses from its line by more than 10%.

Calibration Place the vials containing the *Standard Preparations* in the automated sampler, and start the sequence so that each vial is heated at a temperature of 50° for 30 min before a suitable portion of its headspace is injected into the chromatograph. Set the automatic sampler for a needle withdrawal time of 0.3 min, a pressurization time of 1 min, an injection time of 0.08 min, and a vial pressure of 22 psig with the vial vent off. Obtain the peak areas for ethylene oxide and 1,4-dioxane, which have relative retention times of about 1.0 and 3.1, respectively. Plot the area versus milligram per kilogram on linear graph paper, and draw the best straight line through the points.

Procedure Place the vial containing the *Sample Preparation* in the automatic sampler, and chromatograph its headspace as done for the *Standard Preparations*. Obtain the peak areas of each of the components, and read the concentrations directly from the *Calibration* plots. Not more than 10 mg/kg of ethylene oxide or 1,4-dioxane is found.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Nominal Molecular Weight

Phthalic Anhydride Solution Place 49.0 g of phthalic anhydride in an amber bottle, and dissolve it in 300 mL of pyridine that has been freshly distilled over phthalic anhydride. Shake the bottle vigorously until solution is effected, and allow to stand overnight before using.

Sample Preparation for Liquid Polyethylene Glycols Carefully introduce 25.0 mL of the *Phthalic Anhydride Solution* into a clean, dry, heat-resistant pressure bottle. Add an accurately weighed amount of the sample equivalent to its expected average molecular weight divided by 160 to the bottle. (Thus, a sample of about 1.3 g would be taken for Polyethylene Glycol 200, or about 3.8 g for Polyethylene Glycol 600.) Stopper the bottle, and wrap it securely in a fabric bag.

Sample Preparation for Solid Polyethylene Glycols Carefully introduce 25.0 mL of the *Phthalic Anhydride Solution* into a clean, dry, heat-resistant pressure bottle. Add an accurately weighed amount of the sample, previously melted, equivalent to its expected molecular weight divided by 160 to the bottle; because of limited solubility, however, do not use more than 25 g of any sample. Add 25 mL of pyridine, freshly distilled over phthalic anhydride, swirl to effect solution, stopper the bottle, and wrap it securely in a fabric bag.

Procedure Immerse the sample bottle in a water bath, maintained between 96° and 100°, to the same depth as that of the mixture in the bottle. Heat in the water bath for 30 to 60 min, using 60 min for Polyethylene Glycols having molecular weights of 3000 or higher, then remove the bottle from the bath and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove the bottle from the fabric bag, add 5 drops of a 1:100 phenolphthalein:pyridine solution, and titrate with 0.5 *N* sodium hydroxide to the first pink color that persists for 15 s, recording the volume, in milliliters, of 0.5 *N* sodium hydroxide required as *S*. Perform a blank determination on 25.0 mL on the *Phthalic Anhydride Solution* plus any additional pyridine added to the sample bottle, and record the volume, in milliliters, of 0.5 *N* sodium hydroxide required as *B*. Calculate the nominal molecular weight of the sample by the formula

$$2000W/(B - S)N,$$

in which W is the weight of the sample, in grams; $(B - S)$ is the difference between the volume of 0.5 *N* sodium hydroxide consumed by the blank and by the sample, respectively; and N is the exact normality of the sodium hydroxide solution.

pH Determine potentiometrically in a solution prepared by dissolving 5 g of the sample in 100 mL of carbon dioxide-free water and adding 0.3 mL of saturated potassium chloride solution.

Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition,* igniting a 25-g sample.

Viscosity Determine as directed under *Viscosity of Dimethylpolysiloxane*, Appendix IIB, maintaining the constant-temperature bath at 100° ± 0.3° and using a capillary viscometer having a flow time of at least 200 s for the sample being tested. The viscosity is within the limits specified in the table below. (For Polyethylene Glycols not listed in the table, calculate the limits by interpolation.)

Nominal Average Mol Wt	Viscosity Range (centistokes)	Nominal Average Mol Wt	Viscosity Range (centistokes)
200	3.9–4.8	2400	49–65
300	5.4–6.4	2500	51–70
400	6.8–8.0	2600	54–74
500	8.3–9.6	2700	57–78
600	9.9–11.3	2800	60–83
700	11.5–13.0	2900	64–88
800	12.5–14.5	3000	67–93
900	15.0–17.0	3250	73–105
1000	16.0–19.0	3350	76–110
1100	18.0–22.0	3500	87–123
1200	20.0–24.5	3750	99–140
1300	22.0–27.5	4000	110–158
1400	24–30	4250	123–177
1450	25–32	4500	140–200
1500	26–33	4750	155–228
1600	28–36	5000	170–250
1700	31–39	5500	206–315
1800	33–42	6000	250–390
1900	35–45	6500	295–480
2000	38–49	7000	350–590
2100	40–53	7500	405–735
2200	43–56	8000	470–900
2300	46–60		

Packaging and Storage Store in tight containers.

Polyglycerol Esters of Fatty Acids

INS: 475

DESCRIPTION

Polyglycerol Esters of Fatty Acids are mixed partial esters formed by reacting polymerized glycerols with edible fats, oils, or fatty acids. Minor amounts of mono-, di-, and triglycerides; free glycerol and polyglycerols; free fatty acids; and sodium salts of fatty acids may be present. The polyglycerols vary in degree of polymerization, which is specified by a number (such as tri-, penta-, deca-, etc.) that is related to the average number of glycerol residues per polyglycerol molecule. A specified polyglycerol consists of a distribution of molecular species characteristic of its nominal degree of polymerization. By varying the proportions as well as the nature of the fats or fatty acids to be reacted with the polyglycerols, a large and diverse class of products may be obtained. They include light yellow to amber, oily to very viscous liquids; light tan to medium brown, plastic or soft solids; and light tan to brown, hard, waxy solids. The esters range from very hydrophilic to very lipophilic, but as a class tend to be dispersible in water and soluble in organic solvents and oils.

Function Emulsifier.

REQUIREMENTS

Lead Not more than 2 mg/kg.

The following specifications should conform to the representations of the vendor: *Acid Value*, *Hydroxyl Value*, *Iodine Value*, *Residue on Ignition*, *Saponification Value*, and *Sodium Salts of Fatty Acids*.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

Hydroxyl Value Transfer an accurately weighed sample, approximately equivalent in grams to 561 divided by the expected hydroxyl value, ±10%, into a 300-mL Erlenmeyer flask. Mix 9 volumes of pyridine with 1 volume of acetic anhydride, pipet 25.0 mL of this solution into the sample flask, and pipet 25.0 mL into a separate 300-mL Erlenmeyer flask to serve as the blank. Add boiling stones to each flask, and fit the flasks with air condensers, lubricating the joints only with a few drops of pyridine. Reflux the sample solution gently by heating on a hot plate, confining the vapors in the lower portion of the condenser, and continue refluxing for 45 min. Do not heat the blank. Cool the sample flask to room temperature, and rinse the condenser, the condenser tip, and the sides of the flask with 25 mL of pyridine. Add about 50 mL of 0.55 *N* sodium hydroxide to each flask, mix by swirling for about 45 s, then add 1 mL of phenolphthalein TS and 75 mL of isopropanol, and continue the titration with stirring to the first pink color that persists for at least 30 s. Calculate the hydroxyl value by the formula

$$AV + [(56.1)(B - S)(N/W)],$$

in which *AV* is the *Acid Value*, determined as directed above; $(B - S)$ is the difference between the volume, in milliliters, of 0.55 *N* sodium hydroxide required for the blank and for the sample, respectively; *N* is the exact normality of the sodium hydroxide solution; and *W* is the weight of the sample, in grams.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using an accurately weighed amount of sample approximately equivalent to 700 divided by the expected saponification value.

Sodium Salts of Fatty Acids Dissolve about 5 g of sample, accurately weighed, in 75 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to an emerald green endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction, recording the net volume of perchloric acid consumed as *V*. Calculate the number of milligrams of potassium hydroxide equivalent to the sodium salts per gram of sample by the formula

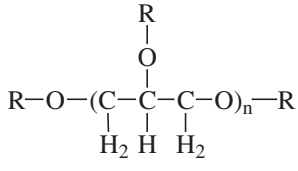
$$56.1 \times (VN/W),$$

in which *N* is the exact normality of the perchloric acid, and *W* is the weight of the sample, in grams.

Packaging and Storage Store in well-closed containers.

Polyglycerol Polyricinoleic Acid

Glycerol Esters of Condensed Castor Oil Fatty Acids; Polyglycerol Esters of Interesterified Ricinoleic Acid; Polyglycerol Polyricinoleate



in which n = 2-6 and R = H
or polyricinoleic acid ester

INS: 476

DESCRIPTION

Polyglycerol Polyricinoleic Acid occurs as a clear, light brown, viscous liquid. It is prepared by esterification of poly-

glycerol with condensed castor oil fatty acids. The castor oil fatty acids are mainly composed of 80% to 90% ricinoleic acid. It is soluble in ether, in hydrocarbons, and in halogenated hydrocarbons. It is insoluble in water and in alcohol.

Function Emulsifier.

REQUIREMENTS

Identification (**Caution**: Conduct these tests in a fume hood.)

A. *Fatty Acids* Reflux 1 g of sample with 15 mL of 0.5 *N* ethanolic potassium hydroxide for 1 h. Add 15 mL of water, acidify with dilute hydrochloric acid TS (about 6 mL). Oily drops or a white to yellow-white solid is produced that is soluble in 5 mL of hexane.

B. *Ricinoleic Acid* Remove the hexane layer obtained in *Identification Test A*, extract again with 5 mL of hexane, and remove the hexane layer. The fatty acids thus extracted have a hydroxyl value corresponding to that of castor oil fatty acids (about 150 to 170).

Assay Not less than 75% of di-, tri-, and tetraglycerols, and not more than 10% of polyglycerols equal to or higher than heptaglycerol.

Acid Value Not more than 6.

Hydroxyl Value Between 80 and 100.

Iodine Value Between 72 and 103.

Lead Not more than 1 mg/kg.

Refractive Index Between 1.463 and 1.467.

Saponification Value Between 170 and 210.

TESTS

Assay

Sample Preparation Reflux about 0.5 g of sample with 20 mL of ethanolic 1 *N* potassium hydroxide solution for 2 h. Reduce the volume of ethanol by evaporation at 45° to 50° in a stream of nitrogen. Add 10 mL of water, and acidify with concentrated hydrochloric acid. Extract the fatty acids from the aqueous phase with successive 20-mL volumes of hexane. Wash the hexane extracts with 20 mL of water, and combine the wash with the aqueous phase. With the aid of a pH meter, adjust the aqueous polyol solution to pH 7.0 with aqueous potassium hydroxide solution. Evaporate to 2 to 3 mL under reduced pressure, and extract three times with 30 mL of boiling ethanol. Filter off any residue, and evaporate the ethanol under reduced pressure to yield a viscous liquid mixture of polyols. Transfer 0.1 g of the mixture into a 10-mL capped vial containing 0.5 mL of warm pyridine previously dried over potassium hydroxide, and dissolve. Add 0.2 mL of hexamethyldisilazane, shake, add 2 mL of trimethylchlorosilate, and shake again. Place the vial on a warm plate at about 80° for 3 to 5 min. Check that white fumes are evolving, indicating an excess of reagent.

Procedure (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph equipped with a flame ionization detector (FID) and a 1.5-m × 4-mm (id) column packed with 3% OV-1 on 100- to 120-mesh diatomite CQ or 100- to 120-mesh Gas Chrom Q, or equivalent. Program the oven temperature to increase from 90° to 330° at 4° to 6°/min. Set the injection block temperature at 275° and the detector block temperature at 350°. Use nitrogen as the carrier gas with a flow rate of 86 mL/min.

Inject 2.0 µL of the *Sample Preparation* into the chromatograph. The resultant chromatogram displays the following sequence of peaks:

Identity	Description	Elution Sequence of Peaks (and Typical Attenuation Settings)
1	Solvent	Overloaded
2	Glycerol	Single peak (2×10^3)
3	Cyclic diglycerols	Single peak (2×10^3)
4	Diglycerols	Single peak (32×10^3)
5	Cyclic triglycerols	Single peak (2×10^3)
6	Triglycerols	Single peak (16×10^3)
7	Cyclic tetraglycerols	Single peak (2×10^3)
8	Tetraglycerols	Multiple peaks (8×10^3)
9	Pentaglycerols	Single peak (4×10^3)
10	Hexaglycerols	Single peak (2×10^3)
11	Heptaglycerols	Single peak (2×10^3)
12	Octaglycerols	Single peak (1×10^3)
13	Nonaglycerols	Barely discernible in the tail of peak 12

Calculation Measure each peak area, and correct for attenuation changes to obtain the corrected area (S_N) of each peak. Calculate the percentage of the total di-, tri-, and tetraglycerols using the following formula:

$$[(\sum S_3 \text{ to } S_8)/(\sum S_3 \text{ to } S_{13})]100.$$

Calculate the percentage of polyols equal to or greater than heptaglycerol using the following formula:

$$[(\sum S_{11} \text{ to } S_{13})/(\sum S_3 \text{ to } S_{13})]100.$$

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using a 4-g sample.

Packaging and Storage Store in well-closed containers.

Polyisobutylene

CAS: [9003-27-4]

View IR

DESCRIPTION

Polyisobutylene is a synthetic polymer. Low-molecular-weight grades are soft and gummy; high-molecular-weight

grades are tough and elastic. All grades are light in color, are soluble in diisobutylene and in benzene, but are insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification Prepare the sample by dissolving it in hot toluene and evaporating on a potassium bromide plate. The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Lead Not more than 3 mg/kg.
Molecular Weight Not less than 37,000.
Volatiles Not more than 0.3%.

TESTS

Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV, using 3 µg of lead (Pb) ion in the control.
Molecular Weight Determine as directed in *Polyisobutylene (Flory Method)* under *Molecular Weight*, Appendix IV.
Volatiles (**Caution**: To reduce explosion hazard, pass carbon dioxide or nitrogen into the lower part of the drying oven at a rate of about 100 mL/min.) Determine as directed under *Loss on Drying*, Appendix IIC, drying a 5-g sample for 2 h at 105°.

Packaging and Storage Store low-molecular-weight grades in boxes or drums with a release liner or coating; store high-molecular-weight grades wrapped in polyethylene film.

Polypropylene Glycol

CAS: [25322-69-4]

DESCRIPTION

Polypropylene Glycol occurs as a clear, colorless or practically colorless, viscous liquid. It is an addition polymer of propylene glycol and water represented by the formula $HO(C_3H_6O)_n$-C_3H_6OH, in which *n* represents the average number of oxypropylene groups. It is soluble in water and in such organic solvents as aliphatic ketones and alcohols, but it is insoluble in ether and in most aliphatic hydrocarbons.

Function Defoaming agent.

REQUIREMENTS

Lead Not more than 1 mg/kg.
Nominal Molecular Weight Not less than 90.0% and not more than 110.0% of the labeled value.
pH Between 6.0 and 9.0.
Propylene Oxide Not more than 0.02%.
Residue on Ignition Not more than 0.01%.
Viscosity Passes test.

TESTS

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Nominal Molecular Weight

Phthalic Anhydride Solution Place 49.0 g of phthalic anhydride into an amber bottle, and dissolve it in 300 mL of pyridine that has been freshly distilled over phthalic anhydride. Shake the bottle vigorously until solution is effected, and allow it to stand overnight before using.

Test Preparation Carefully introduce 25.0 mL of the *Phthalic Anhydride Solution* into a clean, dry, heat-resistant pressure bottle. Add an accurately weighed amount of sample, equivalent to its expected nominal molecular weight divided by 160, into the bottle, cap the bottle tightly, and encase it in a fabric bag.

Procedure Immerse the capped bottle from *Test Preparation* in a water bath, maintained between 96° and 100°, to the same depth as that of the mixture in the bottle. Heat the water bath for 30 min, then remove the bottle from the bath, and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove the bottle from the fabric bag, add 5 drops of a 1:100 solution of phenolphthalein:pyridine, and titrate with 0.5 *N* sodium hydroxide to the first pink color that persists for 15 s, recording the volume, in milliliters, of 0.5 *N* sodium hydroxide required as *S*. Perform a blank determination (see *General Provisions*) on 25.0 mL of the *Phthalic Anhydride Solution* plus any additional pyridine added to the sample bottle. Make any necessary correction, and record the volume, in milliliters, of 0.5 *N* sodium hydroxide required as *B*. Calculate the nominal molecular weight of the sample by the formula

$$2000W/N(B - S),$$

in which *W* is the weight, in grams, of the sample; *N* is the exact normality of the sodium hydroxide solution; and $(B - S)$ is the difference, in milliliters, between the volume of 0.5 *N* sodium hydroxide consumed by the blank and that consumed by the sample.
pH Using a pH meter, neutralize 100 mL of methanol with either 0.1 *N* hydrochloric acid or 0.1 *N* sodium hydroxide. Add 10 mL of sample, and dissolve. Record the pH of the resulting solution.
Propylene Oxide

Magnesium Chloride Solution Add 100 mL of 10 *N* hydrochloric acid to 950 g of magnesium chloride ($MgCl_2 \cdot 6H_2O$), dissolve in and dilute to 1000 mL with water, and mix. Carefully add 400 mL of anhydrous methanol to 100 mL of the stock solution, and allow the mixture to come to room temperature before using.

Indicator Dissolve 100 mg of bromocresol green in 100 mL of anhydrous methanol.

Procedure Place 150 mL of anhydrous methanol into each of two 500-mL glass-stoppered, conical flasks, the second of which is used as the reagent blank. Pressure-pipet 25.0 mL of the *Magnesium Chloride Solution* into each flask, allowing the same drainage time for each transfer, and mix thoroughly. Add about 50 g of sample, accurately weighed, to the first flask, and

dissolve it by swirling. Add about 1 mL of the *Indicator* to each flask, and titrate the contents of the sample flask with 0.1 *N* alcoholic potassium hydroxide to a brilliant blue endpoint, recording the volume required, in milliliters, as *S*. Titrate the reagent blank flask in the same manner, and record the volume required, in milliliters, as *B*.

To correct for the alkalinity in the sample, place 150 mL of anhydrous methanol into a 500-mL conical flask, add about 50 g of sample, accurately weighed, and swirl to effect solution. Add 1 mL of *Indicator*, and titrate with 0.1 *N* hydrochloric acid to a yellow endpoint, recording the volume required, in milliliters, as *C*. Calculate the percent of propylene oxide in the sample taken by the formula

$$5.81 \times \{[(B - S)N_1]/W_1\} - (CN_2/W_2),$$

in which N_1 is the exact normality of the potassium hydroxide; W_1 is the weight, in grams, of the sample taken for the reaction; N_2 is the exact normality of the hydrochloric acid; and W_2 is the weight, in grams, of the sample taken for the alkalinity correction.

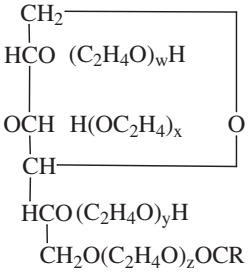
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 25-g sample.

Viscosity Determine as directed under *Viscosity of Dimethylpolysiloxane*, Appendix IIB, maintaining the constant-temperature bath at 37.8° ± 0.2° and using a capillary viscometer having a flow time of at least 200 s for the sample being tested. The viscosity of a sample having a nominal molecular weight of 1000 is between 85 and 97 centistokes, and that of a sample having a nominal molecular weight of 2000 is between 150 and 175 centistokes.

Packaging and Storage Store in tight containers.

Polysorbate 20

Polyoxyethylene (20) Sorbitan Monolaurate; Sorbitan Monododecanoate; Poly(oxy-1,2-ethanediyl) Derivative



in which w + x + y + z = approximately 20, and R is $CH_3(CH_2)_{10}COO$.

INS: 432 CAS: [9005-64-5]

DESCRIPTION

Polysorbate 20 occurs as a yellow- to amber-colored liquid. It is a mixture of laurate partial esters of sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and its mono- and dianhydrides. It is soluble in water, in alcohol, in ethyl acetate, in methanol, and in dioxane, but it is insoluble in mineral oil and in mineral spirits.

Function Emulsifier; stabilizer.

REQUIREMENTS

Identification Add 5 mL of 1 *N* sodium hydroxide to 5 mL of a 1:20 aqueous solution, boil for a few minutes, cool, and acidify with 2.7 *N* hydrochloric acid. The solution is strongly opalescent.

Assay for Oxyethylene Content Not less than 70.0% and not more than 74.0% of oxyethylene groups (—C_2H_4O—), equivalent to between 97.3% and 103.0% of Polysorbate 20, calculated on the anhydrous basis.

Acid Value Not more than 2.

1,4-Dioxane Not more than 10 mg/kg.

Hydroxyl Value Between 96 and 108.

Lauric Acid Between 15 and 17 g per 100 g of sample.

Lead Not more than 2 mg/kg.

Residue on Ignition Not more than 0.25%.

Saponification Value Between 40 and 50.

Water Not more than 3.0%.

TESTS

Assay for Oxyethylene Content Determine as directed under *Oxyethylene Determination*, Appendix VII, using a 65-mg sample, accurately weighed.

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

1,4-Dioxane

Stripped Polysorbate Prepare an appropriate quantity of 1,4-dioxane-free sample by stripping a sample of polysorbate at 10 mm Hg, with nitrogen sparge at 130° for 4 h or until, when tested as directed under *Procedure*, no 1,4-dioxane is detected.

1,4-Dioxane Standard Preparation By appropriate quantitative dilutions using *Stripped Polysorbate* and water, prepare a standard preparation containing 10 µg/mL of HPLC-grade (99.8%) 1,4-dioxane. Transfer 5.0 g, accurately weighed, of the *1,4-Dioxane Standard Preparation* into a 22-mL pressure headspace vial; seal with a silicone septum, star spring, and pressure-relief safety aluminum sealing cap; and crimp the cap closed with a cap sealing tool.

Test Preparation Transfer 5.0 g of sample, accurately weighed, into a 22-mL pressure headspace vial, and seal the cap and crimp as directed for the *1,4-Dioxane Standard Preparation*.

Chromatographic System (See *Chromatography*, Appendix IIA.) Chromatographic conditions may vary depending on the type of headspace unit used. Use a gas chromatograph equipped with a headspace sampler, flame ionization detector, backflush valve, 1-mL gas sample loop, and a 1-m × 3.2-mm (id) nickel precolumn and a 6-m × 3.2-mm (id) nickel analytical column, or equivalent, containing 60- to 80-mesh

TENAX TA support, or equivalent. Maintain the column at 190°. Set the detector and the injector at 250°. Use helium as the carrier gas, with a flow rate of about 30 mL/min. Program the backflush valve to initiate backflushing after 1,4-dioxane elutes into the analytical column.

Procedure Place the vial containing the *1,4-Dioxane Standard Preparation* in the automated sampler, and start the operating sequence so that each vial is heated at 90° for a minimum of 30 min. Using appropriate headspace sampler settings, inject the *1,4-Dioxane Standard Preparation*, and measure the peak area for 1,4-dioxane. Similarly, place the vial containing the *Test Preparation* in the automatic sampler, obtain a chromatogram as done for the *1,4-Dioxane Standard Preparation*, and measure the peak area for 1,4-dioxane. The sample passes the test if the peak area of the *Test Preparation* is not greater than that of the *1,4-Dioxane Standard Preparation*.

Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.

Lauric Acid Transfer about 25 g of sample, accurately weighed, into a 500-mL round-bottom boiling flask, add 250 mL of alcohol and 7.5 g of potassium hydroxide, and mix. Connect a suitable condenser to the flask, reflux the mixture for 1 to 2 h, then transfer to an 800-mL beaker, rinsing the flask with about 100 mL of water and adding it to the beaker. Heat on a steam bath to evaporate the alcohol, adding water occasionally to replace the alcohol, and evaporate until the odor of alcohol can no longer be detected. Use hot water to adjust the final volume to about 250 mL. Neutralize the soap solution with 1:2 sulfuric acid, add 10% in excess, and heat, while stirring, until the fatty acid layer separates. Transfer the fatty acids into a 500-mL separator, wash with three or four 20-mL portions of hot water, and combine the washings with the original aqueous layer from the saponification. Extract the combined aqueous layer with three 50-mL portions of petroleum ether, add the extracts to the fatty acid layer, evaporate to dryness in a tared dish, cool, and weigh. The lauric acid so obtained has an acid value between 250 and 275 when determined as directed in *Method I* under *Acid Value*, Appendix VII.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.

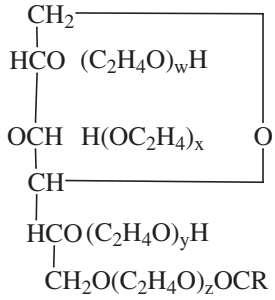
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 8 g of sample, accurately weighed.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Polysorbate 60

Polyoxyethylene (20) Sorbitan Monostearate; Sorbitan Monooctadecanoate; Poly(oxy-1,2-ethanediyl) Derivative



in which w + x + y + z = approximately 20, and R is $CH_3(CH_2)_{16}COO$.

INS: 435 CAS: [9005-67-8]

DESCRIPTION

Polysorbate 60 occurs as a yellow to orange colored, oily liquid or semigel. It is a mixture of stearate and palmitate partial esters of sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and its mono- and dianhydrides. It is soluble in water, in aniline, in ethyl acetate, and in toluene, but it is insoluble in mineral oil and in vegetable oils.

Function Emulsifier; stabilizer.

REQUIREMENTS

Identification

A. Add 5 mL of 1 *N* sodium hydroxide to 5 mL of a 1:20 aqueous solution, boil for a few minutes, cool, and acidify with 2.7 *N* hydrochloric acid. The solution is strongly opalescent.

B. A 60:40 (v/v) mixture of Polysorbate 60:water at 25° or cooler yields a gelatinous mass.

Assay for Oxyethylene Content Not less than 65.0% and not more than 69.5% of oxyethylene groups (—C_2H_4O—), equivalent to between 97.0% and 103.0% of Polysorbate 60, calculated on the anhydrous basis.

Acid Value Not more than 2.

1,4-Dioxane Not more than 10 mg/kg.

Hydroxyl Value Between 81 and 96.

Lead Not more than 2 mg/kg.

Residue on Ignition Not more than 0.25%.

Saponification Value Between 45 and 55.

Stearic and Palmitic Acids Between 21.5 and 26.0 g per 100 g of sample.

Water Not more than 3.0%.

TESTS

Assay for Oxyethylene Content Determine as directed under *Oxyethylene Determination*, Appendix VII, using a 65-mg sample, accurately weighed.
Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
1,4-Dioxane Determine as directed under *1,4-Dioxane* in the monograph for *Polysorbate 20*.
Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 8 g of sample, accurately weighed.
Stearic and Palmitic Acids Isolate the fatty acids as directed under *Lauric Acid* in the monograph for *Polysorbate 20*, and determine the weight of the acids. The product so obtained has an acid value between 200 and 212 when determined as directed in *Method I* under *Acid Value*, Appendix VII, and a solidification point not below 52° when determined as directed under *Solidification Point*, Appendix IIB.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Polysorbate 65

Polyoxyethylene (20) Sorbitan Tristearate

CAS: [9005-71-4]

DESCRIPTION

Polysorbate 65 occurs as a tan, waxy solid. It is a mixture of stearate and palmitate partial esters of sorbitol and its anhydrides condensed with approximately 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and its mono- and dianhydrides. It is soluble in mineral oil and in vegetable oils, in mineral spirits, in acetone, in ether, in dioxane, in alcohol, and in methanol, and it is dispersible in water.

Function Emulsifier; stabilizer.

REQUIREMENTS

Identification Add 5 mL of 1 *N* sodium hydroxide to 5 mL of a 1:20 aqueous solution, boil for a few minutes, cool, and acidify with 2.7 *N* hydrochloric acid. The solution is strongly opalescent.

Assay for Oxyethylene Content Not less than 46.0% and not more than 50.0% of oxyethylene groups (—C_2H_4O—), equivalent to between 96.0% and 104.0% of Polysorbate 65, calculated on the anhydrous basis.
Acid Value Not more than 2.
1,4-Dioxane Not more than 10 mg/kg.
Hydroxyl Value Between 44 and 60.
Lead Not more than 2 mg/kg.
Residue on Ignition Not more than 0.25%.
Saponification Value Between 88 and 98.
Stearic and Palmitic Acids Between 42 and 44 g per 100 g of sample.
Water Not more than 3.0%.

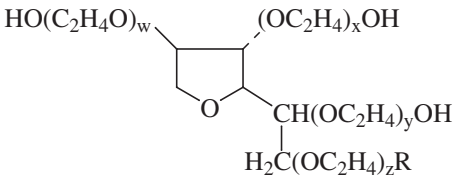
TESTS

Assay for Oxyethylene Content Determine as directed under *Oxyethylene Determination*, Appendix VII, using 90 mg of sample, accurately weighed.
Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
1,4-Dioxane Determine as directed under *1,4-Dioxane* in the monograph for *Polysorbate 20*.
Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 6 g of sample, accurately weighed.
Stearic and Palmitic Acids Isolate the fatty acids as directed under *Lauric Acid* in the monograph for *Polysorbate 20*, and determine the weight of the acids. The product so obtained has an *Acid Value* between 200 and 212 when determined as directed in *Method I* under *Acid Value*, Appendix VII, and a *Solidification Point* not below 52° when determined as directed under *Solidification Point*, Appendix IIB.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Polysorbate 80

Polyoxyethylene (20) Sorbitan Monooleate; Sorbitan Mono-9-octadecenoate; Poly(oxy-1,2-ethanediyl) Derivative



in which w + x + y + z = 20,
and R is $(C_{17}H_{33})COO$.

INS: 433 CAS: [9005-65-6]

DESCRIPTION

Polysorbate 80 occurs as a yellow to orange colored, oily liquid. It is a mixture of oleate partial esters of sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and its mono- and dianhydrides. It is very soluble in water, producing a nearly colorless solution, and it is soluble in alcohol, in fixed oils, in ethyl acetate, and in toluene. It is insoluble in mineral oil.

Function Emulsifier; stabilizer.

REQUIREMENTS

Identification

A. Add 5 mL of 1 *N* sodium hydroxide to 5 mL of a 1:20 aqueous solution, boil for a few minutes, cool, and acidify with 2.7 *N* hydrochloric acid. The solution is strongly opalescent.

B. Add bromine TS, dropwise, to a 1:20 aqueous solution. The bromine is decolorized.

C. A 60:40 (v/v) mixture of Polysorbate 80:water at 25° or below yields a gelatinous mass.

Assay for Oxyethylene Content Not less than 65.0% and not more than 69.5% of oxyethylene groups (—C_2H_4O—), equivalent to between 96.5% and 103.5% of Polysorbate 80, calculated on the anhydrous basis.
Acid Value Not more than 2.
1,4-Dioxane Not more than 10 mg/kg.
Hydroxyl Value Between 65 and 80.
Lead Not more than 2 mg/kg.
Oleic Acid Between 22 and 24 g per 100 g of sample.
Residue on Ignition Not more than 0.25%.
Saponification Value Between 45 and 55.
Water Not more than 3.0%.

TESTS

Assay for Oxyethylene Content Determine as directed under *Oxyethylene Determination*, Appendix VII, using a 65-mg sample, accurately weighed.
Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
1,4-Dioxane Determine as directed under *1,4-Dioxane* in the monograph for *Polysorbate 20*.
Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Oleic Acid Isolate the fatty acids as directed under *Lauric Acid* in the monograph for *Polysorbate 20*, and determine the weight of the acid. With the product so obtained, determine the acid value (between 193 and 206) as directed in *Method I* under *Acid Value*, Appendix VII. Similarly determine the iodine value (between 80 and 92) as directed under *Iodine Value*, Appendix VII.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 8 g of sample, accurately weighed.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Polyvinyl Acetate

CAS: [9003-20-7]

View IR

DESCRIPTION

Polyvinyl Acetate occurs as a clear, water white to pale yellow, solid resin. It is prepared by the polymerization of vinyl acetate. After completion of polymerization, the resin is freed of traces of residual catalyst (usually a peroxide), monomer, and/or solvent by vacuum drying, steam sparging, washing, or any combination of these treatments. The resin is soluble in acetone, but it is insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Free Acetic Acid Not more than 0.05%.
Lead Not more than 3 mg/kg.
Loss on Drying Not more than 1.0%.
Molecular Weight Not less than 2000.

TESTS

Free Acetic Acid Transfer 10.0 g of sample into a 250-mL glass-stoppered Erlenmeyer flask, dissolve in 75 mL of ethylene dichloride, add 60 mL of specially denatured ethanol formula 2B, and mix. Add phenolphthalein TS, and titrate with 0.02 *N* methanolic potassium hydroxide to a faint pink endpoint. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.02 *N* methanolic potassium hydroxide is equivalent to 1.201 mg of $C_2H_4O_2$.

Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV, using 10 μg of lead (Pb) ion in the control.

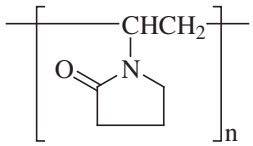
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 1.5-g sample at 100° for 2 h in vacuum.

Molecular Weight Determine as directed under *Molecular Weight*, Appendix IV.

Packaging and Storage Store in well-closed containers.

Polyvinylpolypyrrolidone

Crospovidone; PVPP; 1-Vinyl-2-pyrrolidone Crosslinked Insoluble Polymer



INS: 1202

DESCRIPTION

Polyvinylpolypyrrolidone occurs as a white to off white, hygroscopic, free-flowing powder. It is a crosslinked homopolymer of purified vinylpyrrolidone, produced catalytically. It is insoluble in water and in other common solvents.

Function Clarifying agent; stabilizer.

REQUIREMENTS

Identification Add 0.1 mL of iodine TS to a suspension of 1 g of sample in 10 mL of water, and shake the mixture for 30 s. The reagent is discolored (distinction from polyvinylpyrrolidone, which produces a red color). Add 1 mL of starch TS, and shake the mixture. No blue color appears.

Assay Not less than 11.0% and not more than 12.8% of nitrogen (N).

Lead Not more than 2 mg/kg.

pH of a 1:100 Suspension Between 5.0 and 11.0.

Residue on Ignition Not more than 0.4%.

Soluble Substances Not more than 0.5% in water, and not more than 1.0% in an acid–alcohol medium.

Unsaturation (as vinylpyrrolidone) Not more than 0.1%.

Water Not more than 6.0%.

TESTS

Assay Determine as directed in *Method II* under *Nitrogen Determination*, Appendix IIIC, using a 100-mg sample. In the wet-digestion step, repeat the addition of hydrogen peroxide (usually three to six times) until a clear, light green solution is obtained, then heat for an additional 4 h, and continue as directed, beginning with ''Cautiously add 2 mL of water. . . .''

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

pH of a 1:100 Suspension Determine as directed under *pH Determination*, Appendix IIB, using a 1-g sample suspended in 100 mL of water.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Soluble Substances

Solubility in Water Place 10 g of sample into a 200-mL flask containing 100 mL of water. Shake the flask, and allow the contents to rest for 24 h. Filter on a filter screen with a porosity of 2.5 μm, then on a filter screen with a porosity of 0.8 μm. The residue left by evaporating the filtrate over a water bath until dry must be less than 50 mg.

Solubility in an Acid–Alcohol Medium Place 1 g of sample into a flask containing 500 mL of the following mixture: 3 g of glacial acetic acid, 10 mL of ethanol, and sufficient water to make up the volume to 100 mL. Allow the contents of the flask to rest for 24 h. Filter on a filter screen with a porosity of 2.5 μm, then on a filter screen with a porosity of 0.8 μm. Concentrate the filtrate over a water bath. Finish evaporation over the water bath in a 70-mm diameter tared silica capsule. The dry residue remaining after evaporation must be less than 10 mg, taking into account any residue left by the evaporation of 500 mL of the acetic acid–ethanol mixture.

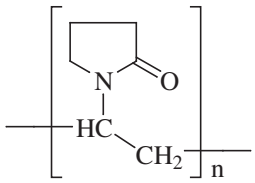
Unsaturation (as vinylpyrrolidone) Suspend 4 g of sample in 30 mL of water, stir for 15 min, and filter through a sintered-glass filter having a porosity between 9 and 15 μm, collecting the filtrate in a 250-mL flask. Wash the residue with 100 mL of water, add 500 mg of sodium acetate to the combined filtrates, and titrate with 0.1 *N* iodine until the color of iodine no longer fades. Add an additional 3.0 mL of 0.1 *N* iodine, allow to stand for 10 min, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination (see *General Provisions*), and make any necessary correction. Not more than 0.72 mL is consumed.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Polyvinylpyrrolidone

PVP; Povidone; Poly[1-(2-oxo-1-pyrrolidinyl)ethylene]



$(C_6H_9NO)_x$ Lower mol wt range product ~40,000
Higher mol wt range product ~360,000

INS: 1201 CAS: [9003-39-8]

DESCRIPTION

Polyvinylpyrrolidone occurs as a white to tan powder. It is a polymer of purified 1-vinyl-2-pyrrolidone produced catalytically. It is soluble in water, in alcohol, and in chloroform, and is insoluble in ether. The pH of a 1:20 aqueous solution is between 3 and 7.

Function Clarifying agent; separation/filtration aid; stabilizer; bodying agent; tableting aid; dispersant; coating on fresh fruit.

REQUIREMENTS

Labeling Indicate the K-value or the K-value range.

Identification

A. Add 20 mL of 1 *N* hydrochloric acid and 5 mL of potassium dichromate TS to 10 mL of a 1:50 aqueous solution. An orange-yellow precipitate forms.

B. Add 5 mL of a 1:50 aqueous solution to 75 mg of cobalt nitrate and 300 mg of ammonium thiocyanate dissolved in 2 mL of water, mix, and then make the resulting solution acid with 2.7 *N* hydrochloric acid. A pale blue precipitate forms.

C. Add a few drops of iodine TS to 5 mL of a 1:200 aqueous solution. A deep red color appears.

Assay Not less than 11.5% and not more than 12.8% as nitrogen (N), calculated on the anhydrous basis.

Aldehydes (as acetaldehyde) Not more than 0.05%.

Hydrazine Not more than 1 mg/kg.

K-Value Between 27 and 32 for the lower-molecular-weight-range product, and between 81 and 97 for the higher-molecular-weight-range product.

Lead Not more than 2 mg/kg.

Residue on Ignition Not more than 0.1%.

Unsaturation (as vinylpyrrolidone) Not more than 0.1%.

Water Not more than 5.0%.

TESTS

Assay Determine as directed in *Method II* under *Nitrogen Determination*, Appendix IIIC, using a 100-mg sample. In the wet-digestion step, omit the use of hydrogen peroxide, and use 5 g of a 33:1:1 mixture of potassium sulfate:cupric sulfate:titanium dioxide instead of the 10:1 potassium sulfate:cupric sulfate mixture. Heat until a clear, light green solution appears, heat for an additional 45 min, and continue as directed, beginning with ''Cautiously add 20 mL of water, cool, then. . . ,'' except use 70 mL of water instead of 20.

Aldehydes (as acetaldehyde)

Phosphate Buffer Transfer 50.0 g of potassium pyrophosphate into a 500-mL volumetric flask, and dissolve in 400 mL of water. Adjust, if necessary, to a pH of 9.0 with 1 *N* hydrochloric acid, dilute to volume with water, and mix.

Aldehyde Dehydrogenase Solution Transfer a quantity of lyophilized aldehyde dehydrogenase (Sigma A550, or equivalent) to 70 units into a glass vial, dissolve it in 10.0 mL of water, and mix.

Note: This solution is stable for 8 h at 4°.

NAD Solution Transfer 40 mg of nicotinamide adenine dinucleotide (B-NAD, Grade III-C, from Sigma Chemical Co.) into a glass vial, dissolve it in 10.0 mL of *Phosphate Buffer*, and mix.

Standard Solution Add about 2 mL of water to a glass weighing bottle, and accurately weigh. Add about 100 mg (about 0.13 mL) of freshly distilled acetaldehyde, and accurately weigh. Transfer this solution into a 100-mL volumetric flask. Rinse the weighing bottle with several small portions of water, transferring each rinsing into the 100-mL volumetric flask. Dilute the solution to volume with water, and mix. Store at 4° for about 20 h. Pipet 1 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix.

Test Preparation Transfer about 2 g of sample, accurately weighed, into a 100-mL volumetric flask, dissolve it in 50 mL of *Phosphate Buffer*, dilute to volume with *Phosphate Buffer*, and mix. Insert a stopper into the flask, heat at 60° for 1 h, and cool to room temperature.

Procedure Pipet 0.5 mL each of the *Standard Solution*, the *Test Preparation*, and water (the reagent blank) into separate 1-cm cells. Using water as the reference, determine the absorbance of the solutions at a wavelength of 340 nm. Add 2.5 mL of *Phosphate Buffer* and 0.2 mL of *NAD Solution* to each cell. Cover the cells to exclude oxygen. Mix by inversion, and allow them to stand for 2 to 3 min at 22° ± 2°. Determine the absorbances of the solutions as before. Calculate the percentage of aldehydes, expressed as acetaldehyde, in the sample taken by the formula

$$10(C/W)\{[(A_{U2} - A_{U1}) - (A_{B2} - A_{B1})]/[(A_{S2} - A_{S1}) - (A_{B2} - A_{B1})]\},$$

in which C is the concentration, in milligrams per milliliter, of acetaldehyde in the *Standard Solution*; W is the weight, in grams, of sample taken; A_{U1}, A_{S1}, and A_{B1} are the absorbances of the solutions obtained from the *Test Preparation*, the *Standard Solution*, and the water reagent blank, respectively, before the *Phosphate Buffer* and *NAD Solution* were added; and A_{U2}, A_{S2}, and A_{B2} are the absorbances of the solutions obtained from the *Test Preparation*, the *Standard Solution*, and the water reagent blank, respectively, after the *Phosphate Buffer* and *NAD Solution* were added. Not more than 0.05% of aldehydes is found.

Hydrazine

Salicylaldazine Standard Solution Dissolve 300 mg of hydrazine sulfate in 5 mL of water, add 1 mL of glacial acetic acid and 2 mL of a freshly prepared 20% (v/v) solution of salicylaldehyde in isopropyl alcohol, mix, and allow the solution to stand until a yellow precipitate forms. Extract the mixture with two 15-mL portions of methylene chloride. Combine the methylene chloride extracts, and dry over anhydrous sodium sulfate. Decant the methylene chloride solution, and evaporate it to dryness. Recrystallize the residue of salicylaldazine from a 60:40 mixture of warm toluene and methanol by cooling. Filter, and dry the crystals in a vacuum. The crystals have a melting range of 213° to 219°, but the range between the beginning and end of melting is not to exceed 10°. Prepare a salicylaldazine solution containing 9.38 μg/mL of toluene.

Procedure Transfer 2.5 g of sample into a 50-mL centrifuge tube, add 25 mL of water, and mix to dissolve. Add 500 μL of a 1:20 solution of salicylaldehyde:methanol, swirl, and heat in a water bath at 60° for 15 min. Allow the solution to cool, add 2.0 mL of toluene, insert a stopper in the tube, shake vigorously for 2 min, and centrifuge. On a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of dimethylsilanized chromatographic silica gel mixture, apply 10 μL of the clear upper toluene layer in the centrifuge tube and 10 μL of the *Salicylaldazine Standard Solution*. Allow the spots to dry, and develop the chromatogram in a solvent system of 2:1 methanol:water until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under UV light at a wavelength of 365 nm. Salicylaldazine appears as a fluorescent spot having an R_f value of about 0.3, and the fluorescence of any salicylaldazine spot from the test specimen is not more intense than that produced by the spot obtained from the *Salicylaldazine Standard Solution*.

K-Value The molecular weight of the sample is characterized by its viscosity in aqueous solution, relative to that of water, expressed as a K-value. Determine the relative viscosity as follows: Transfer an accurately weighed portion of the as-is sample, equivalent to approximately 1 g on the anhydrous basis, into a 100-mL volumetric flask, dissolve it in about 50 mL of water, dilute to volume with water, mix thoroughly, and allow it to stand for 1 h, then pipet 15 mL of filtrate into a clean, dry Ubbelholde-type viscometer, and place the viscometer in a water bath maintained at 25° ± 0.2°. After allowing the viscometer and the sample solution to warm in the water bath for 10 min, draw the solution by means of very gentle suction up through the capillary until the meniscus is above the upper etched mark. Release suction, and after the meniscus reaches the upper etched mark, begin timing the flow through the capillary. Record the exact time when the meniscus reaches the lower etched mark, and calculate the flow time to the nearest 0.01 s. Repeat this operation until at least three readings are obtained. The readings must agree within 0.1 s; if not, repeat the determination with additional 15-mL portions of the sample solution after recleaning the viscometer with sulfuric acid–dichromate cleaning solution or with a suitable laboratory cleaning compound that will remove oils, greases, waxes, and other impurities. Calculate the average flow time for the sample solution, and then obtain the flow time in a similar manner for 15 mL of water for the same viscosity pipet. Calculate the relative viscosity, z, of the sample by dividing the average flow time of the sample solution by that of the water sample, and then calculate the K-value by the formula

$$\left[\sqrt{300c \log z + (c + 1.5c \log z)^2} + 1.5c \log z - c\right] / (0.15c + 0.003c^2),$$

in which c is the weight, in grams, on the anhydrous basis, of the sample in each 100.0 g of solution, and z is as defined above.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting about 2 g of sample, accurately weighed.

Unsaturation (as vinylpyrrolidone) Dissolve about 10 g of sample, accurately weighed, in 80 mL of water in a 125-mL round-bottom flask, add 1.0 g of sodium acetate, mix, and begin titrating with 0.1 *N* iodine. When the iodine color no longer fades, add 3 additional milliliters of iodine, and allow the solution to stand for 5 to 10 min. Add starch TS, and titrate the excess iodine with 0.1 *N* sodium thiosulfate. Perform a blank determination (see *General Provisions*), using the same volume of 0.1 *N* iodine, accurately measured, as was used for the sample, and make any necessary correction. Each milliliter of 0.1 *N* iodine is equivalent to 5.556 mg of vinylpyrrolidone.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Pork Collagen

DESCRIPTION

Pork Collagen occurs as a light tan powder. It is a mixture of proteins containing 40% to 50% collagen, a scleroprotein occurring in animal tendons, ligaments, and connective tissue. It is derived from porcine fatty trimmings gathered during the production of fresh pork meat. During processing, the trimmings are ground, heated, and stabilized, followed by centrifugal separation to reduce the fat. The partially defatted tissue is then dried and further reduced in fat content, resulting

in a high-protein material that may be milled or ground into powder or granular form. It is dispersible in water, and forms thermally reversible gels.

Function Binder; purge reduction.

REQUIREMENTS

Identification Pork Collagen contains not less than 5.52% hydroxyproline, corresponding to 40.0% collagen, as determined under *Assay* (below).
Assay Not less than 40.0% collagen.
Ash (Total) Not more than 3.0%.
Fat Not more than 14.0%.
Lead Not more than 1 mg/kg.
Loss on Drying Not more than 5.0%.
Microbial Limits
Salmonella Negative in 25 g.
Protein Not less than 85.0% (including collagen).

TESTS

Assay (Based on AOAC Method 990.26)

Buffer Solution Transfer 30 g of citric acid monohydrate, 15 g of sodium hydroxide, and 90 g of sodium acetate trihydrate into a 1-L volumetric flask containing 500 mL of water. Add 290 mL of 1-propanol. Adjust the pH to 6.0 with acid or base, and dilute to volume with water.

Oxidant Solution Dissolve 1.41 g of chloramine-T, accurately weighed, in 100 mL of the *Buffer Solution*. The solution is stable for 1 week when stored in dark bottles at 4°.

Color Reagent Dissolve 10 g of 4-dimethylaminobenzaldehyde in 35 mL of 60% (w/w) perchloric acid. Prepare daily.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Hydroxyproline Standard Solution (600 μg/mL) Transfer 60 mg of *trans*-4-hydroxyproline (Sigma, or equivalent) accurately weighed, into a 100-mL volumetric flask. Dissolve and dilute to volume with water. The solution is stable for up to 2 months at 4°.

Standard Curve Pipet 5 mL of the *Hydroxyproline Standard Solution* into a 500-mL volumetric flask, and dilute to volume with water. Prepare this solution on the day of use. Pipet 10-, 20-, 30-, and 40-mL volumes of the solution into separate 100-mL volumetric flasks, and dilute each to volume with water. These solutions contain 0.6, 1.2, 1.8, and 2.4 μg/mL of hydroxyproline, respectively, and must be prepared on the day of use. Transfer 2.0 mL of each solution into four separate test tubes, and 2.0 mL of water into a fifth tube to use as a blank. Add 1.0 mL of *Oxidant Solution* to each tube, shake, and let stand for about 30 min. Add 2.0 mL of *Color Reagent* to each tube, mix thoroughly, cap the tubes with foil or screw caps, and place them in a water bath at 60° ± 0.5° for exactly 15 min. Cool the tubes in running water for 3 min, and dry the outside of the tubes. Measure the absorbance of each solution against the blank in 10-mm glass cells, using an appropriate spectrophotometer, at 558 nm. Draw a standard curve by plotting the absorbance values on the Y-axis and the hydroxyproline, in micrograms per 2 mL, on the X-axis.

Procedure Transfer 4.0 g of sample, accurately weighed, into an Erlenmeyer flask. Add 30 mL of 7 *N* sulfuric acid, cover with a watch glass, and place the flasks in a drying oven at 105° for 16 h.

> **Caution**: Use an oven resistant to corrosion by acids such as those used in analysis involving perchloric acid. Use caution in handling the hot hydrolysate.

Transfer the hot hydrolysate quantitatively into a 500-mL volumetric flask with the aid of water. Dilute to volume with water, and mix. Filter some of the solution into a 100-mL Erlenmeyer flask. The filtrate is stable for up to 2 weeks at 4°. Dilute the filtrate with water in a volumetric flask so that the concentration of hydroxyproline in the final dilution will be in the range of 0.5 to 2.4 μg/2 mL (dilution of 5 mL of filtrate to 100 mL is usually suitable). Record the volume of filtrate used as *V*. Determine as directed under *Standard Curve* (above), beginning with ''Transfer 2.0 mL of each solution. . . .''

Calculation Calculate the percent hydroxyproline content in the sample by the formula

$$(X \times 2.5)/(W \times V),$$

in which *X* is the hydroxyproline content, in micrograms per 2 mL, obtained from the *Standard Curve*; *W* is the weight, in grams, of the sample; and *V* is the volume, in milliliters, of filtrate used.

Calculate the percent collagen in the sample by multiplying the percent hydroxyproline by 7.25.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.

Fat Transfer 1 g of sample, accurately weighed, into a fat-extraction flask, add 10 mL of water, and shake until homogeneous (warm if necessary). Add approximately 1 mL of ammonium hydroxide, and heat in a water bath for 15 min at 60° to 70°, shaking occasionally. Add 10 mL of alcohol, and mix well. Add 25 mL of peroxide-free ether, stopper, and shake vigorously for 1 min, allow to cool if necessary, add 25 mL of petroleum ether, and shake vigorously. Allow the layers to separate and clarify or centrifuge to expedite the process. Decant the organic layer into a suitable flask or dish, and repeat the extraction twice with 15 mL each of peroxide-free ether and petroleum ether for each extraction. Evaporate the combined ether extractions on a steam bath, and dry the residue to a constant weight at 102°, or 70° to 75° at less than 50 mm Hg. Calculate the percent of fat in the sample taken by the formula

$$(R \times 100)/S,$$

in which *R* is the weight, in milligrams, of the residue and *S* is the weight, in milligrams, of the sample taken.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC.

Microbial Limits (**Note:** The current method for the following test may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

Salmonella

Protein Determine as directed under *Nitrogen Determination*, Appendix IIIC. The percent protein equals percent N × 6.25.

Packaging and Storage Store in tight containers.

Potassium Acid Tartrate

Potassium Bitartrate; Cream of Tartar

KOOCCH(OH)CH(OH)COOH

$C_4H_5KO_6$ Formula wt 188.18

INS: 336 CAS: [868-14-4]

DESCRIPTION

Potassium Acid Tartrate occurs as colorless or slightly opaque crystals, or as a white, crystalline powder. It is a salt of L(+)-tartaric acid. One gram dissolves in 165 mL of water at 25°, in 16 mL of boiling water, and in 8820 mL of alcohol. A saturated solution is acid to litmus.

Function Acidifier; buffer.

REQUIREMENTS

Identification

A. When sufficiently heated, a sample chars and emits flammable vapors having an odor resembling that of burning sugar. At a higher temperature and with free access to air, the heat consumes the carbon of the black residue, and a white, fused mass of potassium carbonate remains that imparts a red-purple color to a nonluminous flame.

B. Mix a saturated solution of sample with sodium cobaltinitrite TS. A yellow-orange precipitate forms.

C. Neutralize a saturated solution of sample with 1 *N* sodium hydroxide in a test tube, add silver nitrate TS, then just sufficient 6 *N* ammonium hydroxide to dissolve the white precipitate, and boil the solution. A mirror forms on the inner surface of the test tube from the silver deposited there.

Assay Not less than 99.0% and not more than 101.0% of $C_4H_5KO_6$ after drying.

Ammonia Passes test.

Insoluble Matter Passes test.

Lead Not more than 2 mg/kg.

TESTS

Assay Dissolve about 6 g of sample, previously dried at 105° for 3 h and accurately weighed, in 100 mL of boiling water, and titrate with 1 *N* sodium hydroxide, using phenolphthalein TS as the indicator. Each milliliter of 1 *N* sodium hydroxide is equivalent to 188.2 mg of $C_4H_5KO_6$.

Ammonia Heat 500 mg of sample with 5 mL of 1 *N* sodium hydroxide. No odor of ammonia is detected.

Insoluble Matter Agitate 500 mg of sample with 3 mL of 6 *N* ammonium hydroxide. No undissolved residue remains.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Packaging and Storage Store in tight containers.

Potassium Alginate

Algin

$(C_6H_7O_6K)_n$ Equiv wt, calculated, 214.22
Equiv wt, actual (avg.) 238.00

INS: 402 CAS: [9005-36-1]

DESCRIPTION

Potassium Alginate occurs as a white to yellow, fibrous or granular powder. It is the potassium salt of alginic acid (see the monograph for *Alginic Acid*). It dissolves in water to form a viscous, colloidal solution. It is insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater than 30% by weight. It is insoluble in chloroform, in ether, and in acids having a pH lower than about 3.

Function Stabilizer; thickener; gelling agent.

REQUIREMENTS

Identification

A. Add 1 mL of calcium chloride TS to 5 mL of a 1:100 aqueous solution. A voluminous, gelatinous precipitate forms.

B. Add 1 mL of 2 *N* sulfuric acid to 10 mL of a 1:100 aqueous solution. A heavy, gelatinous precipitate forms.

C. Place about 5 mg of sample into a test tube, add 5 mL of water, 1 mL of a freshly prepared 1:100 naphtholresorcinol:ethanol solution, and 5 mL of hydrochloric acid. Heat the mixture to boiling, boil gently for about 3 min, and then cool to about 15°. Transfer the contents of the test tube into a 30-mL separator with the aid of 5 mL of water, and extract with 15 mL of isopropyl ether. Perform a blank determination (see *General Provisions*), and make any necessary correction. The isopropyl ether extract from the sample exhibits a deeper purple hue than that from the blank.

Assay A sample yields not less than 16.5% and not more than 19.5% of carbon dioxide (CO_2), corresponding to between 89.2% and 105.5% of potassium alginate (equiv wt 238.00), calculated on the dried basis.

Arsenic Not more than 3 mg/kg.

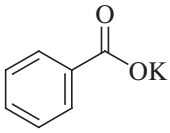
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 15.0%.

TESTS

Assay Determine as directed under *Alginates Assay*, Appendix IIIC. Each milliliter of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 28.75 mg of potassium alginate (equiv wt 238.00).
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Packaging and Storage Store in well-closed containers.

Potassium Benzoate



$C_7H_5KO_2$ Formula wt 160.22
INS: 212 CAS: [582-25-2]

DESCRIPTION

Potassium Benzoate occurs as white granules, crystalline powder, or flakes. One gram dissolves in 2 mL of water, in 75 mL of alcohol, and in 50 mL of 90% alcohol.

Function Preservative; antimicrobial agent.

REQUIREMENTS

Identification A 1:5 aqueous solution responds to the flame test for *Potassium* and gives positive tests for *Benzoate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of $C_7H_5KO_2$, calculated on the anhydrous basis.
Alkalinity (as KOH) Not more than 0.06%.
Lead Not more than 2 mg/kg.
Water Not more than 1.5%.

TESTS

Assay Transfer about 600 mg of sample, accurately weighed, into a 250-mL beaker, add 100 mL of glacial acetic acid, and stir until the sample is completely dissolved. Add crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid. Each milliliter of 0.1 *N* perchloric acid is equivalent to 16.02 mg of $C_7H_5KO_2$.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Alkalinity Dissolve 2 g of sample in 20 mL of hot water, and add 2 drops of phenolphthalein TS. If a pink color appears, not more than 0.2 mL of 0.1 *N* sulfuric acid is required to discharge it.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Potassium Bicarbonate

$KHCO_3$ Formula wt 100.12
INS: 501(ii) CAS: [298-14-6]

DESCRIPTION

Potassium Bicarbonate occurs as colorless, transparent, monoclinic prisms or as a white, granular powder. It is stable in air. Its solutions are neutral or alkaline to phenolphthalein TS. One gram dissolves in 2.8 mL of water. It is almost insoluble in alcohol.

Function pH control; leavening agent.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Potassium* and for *Bicarbonate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 101.5% of $KHCO_3$, calculated on the dried basis.
Carbonate Passes test.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.25%.

TESTS

Assay Dissolve about 4 g of sample, accurately weighed, in 100 mL of water. Add 2 drops of methyl red TS, and while constantly stirring, slowly titrate with 1 *N* hydrochloric acid until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the pink color no longer fades after boiling. Each milliliter of 1 *N* hydrochloric acid is equivalent to 100.1 mg of $KHCO_3$.
Carbonate Dissolve 1 g of sample, without agitation, in 20 mL of water at a temperature not above 5°, then add 2 mL

of 0.1 *N* hydrochloric acid and 2 drops of phenolphthalein TS. The solution does not immediately turn more than a faint pink color.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample over silica gel for 4 h.

Packaging and Storage Store in well-closed containers.

Potassium Bromate

$KBrO_3$ Formula wt 167.00

INS: 924a CAS: [7758-01-2]

DESCRIPTION

Potassium Bromate occurs as white crystals or as a granular powder. It is soluble in water and slightly soluble in alcohol. The pH of a 1:20 aqueous solution is between 5 and 9.

Function Maturing agent; oxidizing agent.

REQUIREMENTS

Identification
A. A 1:20 aqueous solution imparts a violet color to a nonluminous flame.
B. Add sulfurous acid, dropwise, to a 1:20 aqueous solution. A yellow color appears that disappears upon the addition of an excess of sulfurous acid.
Assay Not less than 99.0% and not more than 101.0% of $KBrO_3$ after drying.
Chloride Not more than 0.05%.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 0.1%.
Sulfate Not more than 0.01%.

TESTS

Assay Dissolve about 100 mg of sample, previously dried to constant weight over a suitable desiccant and accurately weighed, in 50 mL of water contained in a 250-mL glass-stoppered Erlenmeyer flask. Add 3 g of potassium iodide, followed by 3 mL of hydrochloric acid. Allow the mixture to stand for 5 min, add 100 mL of cold water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the endpoint is approached. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate consumed is equivalent to 2.783 mg of $KBrO_3$.
Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 100-mg sample is not greater than that shown in a control containing 50 μg of chloride (Cl) ion.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample over a suitable desiccant to constant weight.
Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 100-mg sample is not greater than that shown in a control containing 10 μg of sulfate (SO_4) ion.

Packaging and Storage Store in well-closed containers.

Potassium Carbonate

K_2CO_3 Formula wt, anhydrous 138.21
$K_2CO_3 \cdot 1½H_2O$ Formula wt, hydrated 165.23

INS: 501(i) CAS: [584-08-7]

DESCRIPTION

Potassium Carbonate is anhydrous or contains 1.5 molecules of water of crystallization. The anhydrous form occurs as a white, granular powder, and the hydrated form as small, white, translucent crystals or granules. It is very deliquescent, and its solutions are alkaline. One gram dissolves in 1 mL of water at 25° and in about 0.7 mL of boiling water. It is insoluble in alcohol.

Function pH control.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Potassium* and for *Carbonate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of K_2CO_3 after drying.
Insoluble Substances Passes test.
Lead Not more than 2 mg/kg.
Loss on Drying *Anhydrous*: Not more than 1%; *Hydrated*: Between 10.0% and 16.5%.

TESTS

Assay Transfer about 1 g, accurately weighed, of the dried sample obtained as directed under *Loss on Drying* (below) into a beaker, and dissolve it in 50 mL of water. Add 2 drops of methyl red TS, and while constantly stirring, slowly titrate with 1 *N* hydrochloric acid until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the faint pink color no longer fades after boiling. Each milliliter of 1 *N* hydrochloric acid is equivalent to 69.11 mg of K_2CO_3.
Insoluble Substances Dissolve a 1-g sample in 20 mL of water. No residue remains.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Alternatively, determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying about 3 g of sample, accurately weighed, at 180° for 4 h.

Packaging and Storage Store in tight containers.

Potassium Carbonate Solution

INS: 501(i)

DESCRIPTION

Potassium Carbonate Solution occurs as a clear or slightly turbid, colorless, alkaline solution that absorbs carbon dioxide when exposed to air, forming potassium bicarbonate. It is available as solutions with concentrations of about 50.0% (w/w).

Function pH control.

REQUIREMENTS

Identification A sample gives positive tests for *Potassium* and for *Carbonate*, Appendix IIIA.

Assay Not less than 97.0% and not more than 103.0%, by weight, of the labeled amount of K_2CO_3.

Lead Not more than 2 mg/kg, calculated on the basis of potassium carbonate (K_2CO_3) determined in the *Assay* (below).

TESTS

Assay Based on the labeled percentage of K_2CO_3, accurately weigh a volume of the sample solution equivalent to about 1 g of potassium carbonate, and add it to 50.0 mL of 1 *N* sulfuric acid. Add 2 drops of methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sulfuric acid is equivalent to 69.11 mg of K_2CO_3.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using the equivalent of 1 g of potassium carbonate (K_2CO_3), calculated on the basis of the *Assay* (above).

Packaging and Storage Store in tight containers.

Potassium Chloride

KCl — Formula wt 74.55

INS: 508 — CAS: [7447-40-7]

DESCRIPTION

Potassium Chloride occurs as colorless, elongated, prismatic, or cubical crystals, or as a white, granular powder. It is stable in air. Its solutions are neutral to litmus. It may contain up to 1.0% (total) of suitable food-grade anticaking, free-flowing, or conditioning agents such as calcium stearate or silicon dioxide, either singly or in combination. One gram dissolves in 2.8 mL of water at 25°, and in about 2 mL of boiling water. Potassium Chloride containing anticaking, free-flowing, or conditioning agents may produce cloudy solutions or dissolve incompletely. It is insoluble in alcohol.

Function Nutrient; gelling agent; salt substitute; yeast food.

REQUIREMENTS

Labeling Indicate the name and quantity of any added substance(s) if the material contains such substances.

Identification A 1:20 aqueous solution gives positive tests for *Potassium* and for *Chloride*, Appendix IIIA.

Assay Not less than 99.0% of KCl after drying; and not less than 98.0% of KCl after drying when a sample contains added substance(s).

Acidity or Alkalinity A sample containing no added substance(s) passes test.

Heavy Metals (as Pb) Not more than 5 mg/kg.

Iodide and/or Bromide Passes test.

Loss on Drying Not more than 1.0%.

Sodium Passes test.

TESTS

Assay Dissolve about 250 mg of sample, dried at 105° for 2 h and accurately weighed, in 150 mL of water. Add 1 mL of nitric acid, and immediately titrate with 0.1 *N* silver nitrate, determining the endpoint potentiometrically, using silver–calomel electrodes and a salt bridge containing 4% agar in a saturated potassium nitrate solution. Perform a blank determination, and make any necessary correction (see *General Provisions*). Each milliliter of 0.1 *N* silver nitrate is equivalent to 7.455 mg of KCl.

Acidity or Alkalinity Add 3 drops of phenolphthalein TS to a solution of 5 g of sample in 50 mL of recently boiled and cooled water. No pink color appears. Add 0.3 mL of 0.02 *N* sodium hydroxide. A pink color appears.

Heavy Metals (as Pb)

> The Committee on Food Chemicals Codex notes that this method may be used only until the First Supplement to this edition is released in 2004. At that time, the committee will set a lead limit as low as practicable for potassium chloride. Manufacturers are encouraged to develop and validate methods for use in industrial settings and that are sensitive enough to detect lead in the amounts typically present in potassium chloride, and to propose such methods to the committee in a timely manner.

This test is designed to limit the content of common metallic impurities colored by sulfide ion (Ag, As, Bi, Cd, Cu, Hg, Pb, Sb, Sn) by comparing the color with a standard containing lead (Pb) ion under the specified test conditions. It demon-

strates that the test substance is not grossly contaminated by such heavy metals, and within the precision of the test, that it does not exceed the *Heavy Metals* limit given as determined by concomitant visual comparison with a control solution. In the specified pH range, the optimum concentration of lead (Pb) ion for matching purposes by this method is 20 μg in 50 mL of solution.

The most common limitation of the *Heavy Metals Test* is that the color the sulfide ion produces in the *Sample Solution* depends on the metals present and may not match the color in the *Lead Solution* used for matching purposes. Lead sulfide is brown, as are Ag, Bi, Cu, Hg, and Sn sulfides. While it is possible that ions not mentioned here may also yield non-matching colors, among the nine common metallic impurities listed above, the sulfides with different colors are those of As and Cd, which are yellow, and that of Sb, which is orange. If a yellow or orange color is observed, the following action is indicated: Because this monograph does not include an arsenic requirement, As should be determined. Any As found should not exceed 3 mg/kg. If these criteria are met, Cd may be a contributor to the yellow color, so the Cd content should be determined. If an orange color is observed, the Sb content should be determined. These additional tests are in accord with the section on *Trace Impurities* in the *General Provisions* of this book, as follows: ''if other possible impurities may be present, additional tests may be required, and should be applied, as necessary, by the manufacturer, vendor, or user to demonstrate that the substance is suitable for its intended application.''

Determine the amount of heavy metals by *Method I* or *Method II* as the following criteria specify: Use *Method I* for samples that yield clear, colorless solutions before adding sulfide ion. Use *Method II* for samples that do not yield clear, colorless solutions under the test conditions specified for *Method I*. Use *Method III*, a wet digestion method, only in those cases where neither *Method I* nor *Method II* can be used.

Special Reagents

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL with water, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* to 100.0 mL with water. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 μg of lead (Pb) ion.

Procedure

(**Note**: In the following procedures, failure to accurately adjust the pH of the solution within the specified limits may result in a significant loss of test sensitivity.)

Method I

Sample Solution Dilute 4 g of sample in 25 mL of water.

Solution A Pipet 2.0 mL of *Standard Lead Solution* (20 μg of Pb) into a 50-mL color-comparison tube, and add water to make 25 mL. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute to 40 mL with water, and mix.

Solution B Place 25 mL of the *Sample Solution* into a 50-mL color-comparison tube that matches the one used for *Solution A*, adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute to 40 mL with water, and mix.

Solution C Place 25 mL of the *Sample Solution* into a third color-comparison tube that matches those used for *Solutions A* and *B*, and add 2.0 mL of *Standard Lead Solution*. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute to 40 mL with water, and mix.

Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*, and the intensity of the color of *Solution C* is equal to or greater than that of *Solution A*. If the color of *Solution C* is lighter than that of *Solution A*, the sample is interfering with the test procedure and *Method II* must be used.

Method II

Solution A Prepare as directed under *Method I*.

Solution B Place a quantity of sample, accurately weighed, into a suitable crucible, add sufficient sulfuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the sample is thoroughly carbonized, add 2 mL of nitric acid and 5 drops of sulfuric acid, cautiously heat until white fumes no longer evolve, then ignite, preferably in a muffle furnace, at 500° to 600° until all of the carbon is burned off. Cool, add 4 mL of 1:2 hydrochloric acid, cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 min. Add 6 *N* ammonia dropwise until the solution is just alkaline to litmus paper, dilute to 25 mL with water, and adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid. Filter if necessary, rinse the crucible and the filter with 10 mL of water, transfer the solution and rinsings into a 50-mL color-comparison tube, dilute to 40 mL with water, and mix.

Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*.

Method III

Solution A Transfer an 8:10 (v/v) mixture of sulfuric acid:nitric acid into a 100-mL Kjeldahl flask, clamp the flask at an angle of 45°, and then add, in small increments, an additional volume of nitric acid equal to that added in the preparation of *Solution B* (below). Heat the solution to dense, white fumes, cool, and cautiously add 10 mL of water. Add a volume of 30% hydrogen peroxide equal to that added in the preparation of *Solution B* (below) then boil gently to dense, white fumes, and cool. Cautiously add 5 mL of water, mix, and boil gently to dense, white fumes. Continue boiling until the volume is reduced to about 2 or 3 mL, then cool, and dilute cautiously with a few milliliters of water. Pipet 2.0 mL

of *Standard Lead Solution* into this solution, and mix. Transfer the solution into a 50-mL color-comparison tube, rinse the flask with water, add the rinsings to the tube until the volume is 25 mL, and mix. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper), initially with ammonium hydroxide and then with 6 *N* ammonia as the desired range is neared, dilute to 40 mL with water, and mix.

Solution B Transfer 4 mg of sample, accurately weighed, into a 100-mL Kjeldahl flask (or into a 300-mL flask if the reaction foams excessively), clamp the flask at an angle of 45°, and add a sufficient amount of an 8:10 (v/v) mixture of sulfuric acid:nitric acid to moisten the sample thoroughly. Warm gently until the reaction begins, allow the reaction to subside, and then add additional portions of the acid mixture, heating after each addition, until all of the 18 mL of acid mixture has been added. Increase the heat, and boil gently until the reaction mixture darkens. Remove the flask from the heat, add 2 mL of nitric acid, and heat to boiling again. Continue the intermittent heating and addition of 2-mL portions of nitric acid until no further darkening occurs, then heat strongly to dense, white fumes, and cool. Cautiously add 5 mL of water, mix, boil gently to dense, white fumes, and continue heating until the volume is reduced to about 2 or 3 mL. Cool, cautiously add 5 mL of water, and examine. If the solution is yellow, cautiously add 1 mL of 30% hydrogen peroxide, and again evaporate to dense, white fumes and to a volume of about 2 or 3 mL. Cool, dilute cautiously with a few milliliters of water, and mix. Transfer into a 50-mL color-comparison tube, rinse the flask with water, add the rinsings to the tube until the volume is 25 mL, and mix. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper), initially with ammonium hydroxide and then with 6 *N* ammonia as the desired range is neared, dilute to 40 mL with water, and mix.

Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*.

Iodide and/or Bromide Dissolve 2 g of sample in 6 mL of water, add 1 mL of chloroform, and then add, dropwise and with constant agitation, 5 mL of a mixture of equal parts of chlorine TS and water. The chloroform is free from even a transient violet or permanent orange color.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Sodium

Sample without Added Substances: A 1:20 aqueous solution, tested on a platinum wire, does not impart a pronounced yellow color to a nonluminous flame.

Sample with Added Substances:

Standard Dilutions Transfer 1.2711 g of reagent-grade sodium chloride, accurately weighed, into a 500-mL volumetric flask, and dissolve and dilute to volume with water to obtain a solution containing 1000 μg of sodium per milliliter. Prepare *Standard Dilutions* from this solution to cover the range 0 to 10 μg/mL of sodium at intervals of 2 μg/mL using 500-mL volumetric flasks and adding 0.5 g of reagent-grade potassium chloride to each volumetric flask before diluting to volume.

Sample Solution Transfer 2.50 g of sample, accurately weighed, into a 250-mL volumetric flask, dilute to volume with water, and mix. Pipet 25 mL of this solution into a 250-mL volumetric flask, dilute to volume with water, and mix.

Standard Curve Atomize portions of the *Standard Dilutions* as described under *Procedure* (below) until readings for the series are reproducible, adjusting the instrument so that the solution containing 10 μg/mL gives a full-scale reading. Prepare a standard curve by plotting the absorbance against the concentration.

Procedure Determine the absorbances of the *Standard Dilutions* and the *Sample Solution* at the sodium emission line of 589.6 nm with a flame atomic absorption spectrophotometer equipped with a sodium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Determine the concentration, *C*, in micrograms per milliliter, of sodium from the standard curve, and calculate the percent sodium in the sample taken by the formula

$$C/10.$$

Not more than 0.5% is present.

Packaging and Storage Store in well-closed containers.

Potassium Citrate

Tripotassium Citrate

$$KOOCCH_2C(OH)(COOK)CH_2COOK \cdot H_2O$$

$C_6H_5K_3O_7 \cdot H_2O$ Formula wt 324.41

INS: 332(ii) CAS: [6100-05-6]

DESCRIPTION

Potassium Citrate occurs as transparent crystals or as a white, granular powder. It is deliquescent when exposed to moist air. One gram dissolves in about 0.5 mL of water. It is almost insoluble in alcohol.

Function Buffer; sequestrant; stabilizer.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Potassium* and for *Citrate*, Appendix IIIA.

Assay Not less than 99.0% and not more than 100.5% of $C_6H_5K_3O_7$ after drying.

Alkalinity Passes test.

Lead Not more than 2 mg/kg.

Loss on Drying Between 3.0% and 6.0%.

TESTS

Assay Dissolve about 250 mg of sample, previously dried at 180° to constant weight and accurately weighed, in 40 mL of glacial acetic acid, warming slightly to effect solution. Cool the solution to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 10.213 mg of $C_6H_5K_3O_7$.

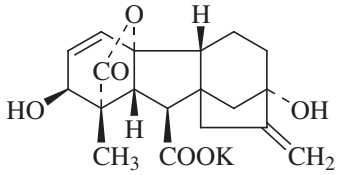
Alkalinity A 1:20 aqueous solution is alkaline to litmus, but after the addition of 0.2 mL of 0.1 *N* sulfuric acid to 10 mL of this solution, no pink color appears after the addition of 1 drop of phenolphthalein TS.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 180° to constant weight.

Packaging and Storage Store in tight containers.

Potassium Gibberellate



$C_{19}H_{21}KO_6$ Formula wt 384.47

CAS: [125-67-7]

DESCRIPTION

Potassium Gibberellate occurs as a white to slightly off white, crystalline powder. It is soluble in water, in alcohol, and in acetone. The pH of a 1:20 aqueous solution is about 6. It is deliquescent.

Function Enzyme activator.

REQUIREMENTS

Identification

A. Dissolve a few milligrams of sample in 2 mL of sulfuric acid. A red solution having a green fluorescence forms.

B. A 1:10 aqueous solution gives positive tests for *Potassium*, Appendix IIIA.

Assay Not less than 80.0% and not more than 87.0% of $C_{19}H_{21}KO_6$, equivalent to between 72.1% and 78.4% of $C_{19}H_{22}O_6$ (gibberellic acid).

Lead Not more than 5 mg/kg.

Loss on Drying Between 5.0% and 13.0%.

Optical (Specific) Rotation $[\alpha]_D^{20^\circ}$: Between +43.0° and +60.0°.

Residue on Ignition Between 19.0% and 23.0%.

TESTS

Assay

Standard Preparation Transfer an accurately weighed quantity of USP Gibberellic Acid Reference Standard, equivalent to about 25 mg of pure gibberellic acid (corrected for phase purity and volatiles content), into a 50-mL volumetric flask, dissolve in and dilute to volume with methanol, and mix. Transfer 10.0 mL of this solution into a second 50-mL volumetric flask, dilute to volume with methanol, and mix.

Assay Preparation Transfer about 65 mg of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in and dilute to volume with methanol, and mix. Transfer 10.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with methanol, and mix.

Procedure Transfer 5.0 mL of the *Assay Preparation* into a 25- × 200-mm glass-stoppered tube, and transfer 4.0-mL and 5.0-mL portions of the *Standard Preparation* into separate, similar tubes. Place the tubes in a boiling water bath, evaporate to dryness, and then dry in an oven at 90° for 10 min. Remove the tubes from the oven, stopper, and allow to cool to room temperature. Dissolve the residue in each tube in 10.0 mL of 8:10 sulfuric acid, heat in a boiling water bath for 10 min, and then cool in a 10° water bath for 5 min. Using the dilute sulfuric acid as the blank, determine the absorbance of the solutions in 1-cm cells at 535 nm with a suitable spectrophotometer. Record the absorbance of the solution from the *Assay Preparation* as A_U. Note the absorbance of the two solutions prepared from the 4.0-mL and 5.0-mL aliquots of the *Standard Preparation*, and record the absorbance of the final solution giving the value nearest to that of the sample as A_S; record as V the volume of the aliquot used in preparing this solution. Calculate the quantity, in milligrams, of $C_{19}H_{21}KO_6$ in the sample taken by the formula

$$500 \times (C/0.8983) \times (V/5) \times (A_U/A_S),$$

in which C is the exact concentration, in milligrams per milliliter, of the *Standard Preparation*, and 0.8983 is the ratio of the molecular weight of Potassium Gibberellate to that of gibberellic acid.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 100° in vacuum for 4 h.

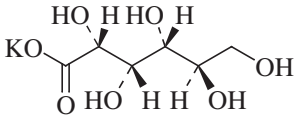
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 50 mg of sample in each milliliter.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight containers protected from light.

Potassium Gluconate

D-Gluconic Acid, Monopotassium Salt; Monopotassium D-Gluconate



$C_6H_{11}KO_7$ Formula wt, anhydrous 234.25
$C_6H_{11}KO_7 \cdot H_2O$ Formula wt, monohydrate 252.26

INS: 577 CAS: anhydrous [299-27-4]
CAS: monohydrate [35398-15-3]

DESCRIPTION

Potassium Gluconate occurs as a white or yellow-white, crystalline powder or granules. It is anhydrous or the monohydrate. It is freely soluble in water and in glycerin, slightly soluble in alcohol, and insoluble in ether.

Function Nutrient; sequestrant.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or the monohydrate.

Identification

A. A sample responds to the flame test for *Potassium*, Appendix IIIA.

B. Dissolve a quantity of sample in water, heating in a water bath at 60° if necessary, to obtain a *Test Solution* containing 10 mg/mL. Similarly, prepare a *Standard Solution* of USP Potassium Gluconate Reference Standard in water, diluting to 10 mg/mL. To prepare a *Spray Reagent*, dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 *N* sulfuric acid to volume, and mix. Apply separate 5-µL portions of the *Test Solution* and the *Standard Solution* on a suitable thin-layer chromatographic plate (see *Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent system consisting of a 50:30:10:10 mixture of alcohol, water, ammonium hydroxide, and ethyl acetate until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray Reagent*. After spraying, heat the plate at 110° for about 10 min. The principal spot obtained from the *Test Solution* corresponds in color, size, and R_f value to that obtained from the *Standard Solution*.

C. The infrared absorption spectrum of a mineral oil dispersion of the sample, previously dried, exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Potassium Gluconate Reference Standard.

Assay Not less than 98.0% of $C_6H_{11}KO_7$, calculated on the dried basis.

Lead Not more than 2 mg/kg.

Loss on Drying *Anhydrous*: Not more than 3.0%; *Monohydrate*: Between 6.0% and 7.5%.

Reducing Substances Not more than 1.0%.

TESTS

Assay Transfer about 175 mg of sample, accurately weighed, into a clean, dry 200-mL Erlenmeyer flask; add 75 mL of glacial acetic acid; and dissolve by heating on a hot plate. Cool, add quinaldine red TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid, using a 10-mL microburet, to a colorless endpoint. Each milliliter of 0.1 *N* perchloric acid is equivalent to 23.42 mg of $C_6H_{11}KO_7$.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in vacuum at 105° for 4 h.

Reducing Substances Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, dissolve it in 10 mL of water, add 25 mL of alkaline cupric citrate TS, and cover the flask with a small beaker. Boil gently for exactly 5 min, and cool rapidly to room temperature. Add 25 mL of a 1:10 solution of acetic acid, 10.0 mL of 0.1 *N* iodine, 10 mL of 2.7 *N* hydrochloric acid, and 3 mL of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in milligrams, of reducing substances (as D-glucose) by the formula

$$27(V_1N_1 - V_2N_2),$$

in which 27 is an empirically determined equivalence factor for D-glucose; V_1 and N_1 are the volume and normality, respectively, of the iodine solution; and V_2 and N_2 are the volume and normality, respectively, of the sodium thiosulfate solution.

Packaging and Storage Store in well-closed containers.

Potassium Glycerophosphate

$C_3H_7K_2O_6P \cdot 3H_2O$ Formula wt 302.30

CAS: [1319-70-6]

DESCRIPTION

Potassium Glycerophosphate occurs as a pale yellow, syrupy liquid containing three molecules of water of hydration, or

as a colorless to pale yellow, syrupy solution having a concentration of 50% to 75%. It is very soluble in water, and its solutions are alkaline to litmus.

Function Nutrient.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Potassium*, Appendix IIIA.
Assay *Trihydrate*: Not less than 80.0% of $C_3H_7K_2O_6P$; *Potassium Glycerophosphate Solutions*: Not less than 95.0% and not more than 105.0% of the labeled concentration of $C_3H_7K_2O_6P$.
Lead Not more than 4 mg/kg.

TESTS

Assay Dissolve a sample equivalent to about 4 g of $C_3H_7K_2O_6P$, accurately weighed, in 30 mL of water, add methyl orange TS, and titrate with 0.5 *N* hydrochloric acid. Each milliliter of 0.5 *N* hydrochloric acid is equivalent to 124.13 mg of $C_3H_7K_2O_6P$.
Lead Determine as directed under *Lead Limit Test*, using a *Sample Solution* prepared as directed for organic compounds, and 4 μg of lead (Pb) ion in the control.

Packaging and Storage Store in tight containers.

Potassium Hydroxide

Caustic Potash

KOH Formula wt 56.11

INS: 525 CAS: [1310-58-3]

DESCRIPTION

Potassium Hydroxide occurs as white or nearly white pellets, flakes, sticks, fused masses, or other forms. Upon exposure to air, it readily absorbs carbon dioxide and moisture, and it deliquesces. One gram dissolves in 1 mL of water, in about 3 mL of alcohol, and in about 2.5 mL of glycerin. It is very soluble in boiling alcohol.

Function pH control.

REQUIREMENTS

Identification A 1:25 aqueous solution gives positive tests for *Potassium*, Appendix IIIA.
Assay Not less than 85.0% and not more than 100.5% of total alkali, calculated as KOH.
Carbonate (as K_2CO_3) Not more than 3.5%.
Insoluble Substances Passes test.
Lead Not more than 2 mg/kg.
Mercury Not more than 0.1 mg/kg.

TESTS

Assay Dissolve about 1.5 g of sample, accurately weighed, in 40 mL of recently boiled and cooled water, cool to 15°, and titrate with 1 *N* sulfuric acid, using phenolphthalein TS as the indicator. When the pink color disappears, record the volume of acid required, then add methyl orange TS as the indicator, and continue the titration until a persistent pink color appears. Record the total volume of acid required for the titration. Each milliliter of the combined amount of 1 *N* sulfuric acid used is equivalent to 56.11 mg of total alkali, calculated as KOH.
Carbonate (as K_2CO_3) Each milliliter of 1 *N* sulfuric acid required between the phenolphthalein and methyl orange endpoints in the *Assay* (above) is equivalent to 138.2 mg of carbonate.
Insoluble Substances A 1:20 aqueous solution is complete, clear, and colorless.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, preparing the *Sample Solution* as follows: Dissolve 1 g of sample in a mixture of 5 mL of water and 11 mL of 2.7 *N* hydrochloric acid, and cool. Use 2 μg of lead (Pb) ion in the control.
Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB, preparing the *Standard Preparation* and the *Sample Preparation* as follows:

Standard Preparation Prepare the stock solution and the dilutions, as directed in the test, to obtain a solution containing 1 μg of mercury per milliliter. Transfer 1.0 mL of the final solution (1 μg of mercury) into a 50-mL beaker, and add 20 mL of water, 1 mL of 1:5 sulfuric acid, and 1 mL of a 1:25 solution of potassium permanganate. Cover the beaker with a watch glass, boil for a few seconds, and cool.

Sample Preparation Transfer 10.0 g of sample into a 100-mL beaker, dissolve in 15 mL of water, add 2 drops of phenolphthalein TS, and while constantly stirring, slowly neutralize with 1:2 hydrochloric acid. Add 1 mL of 1:5 sulfuric acid and 1 mL of a 1:25 solution of potassium permanganate, cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in tight containers.

Potassium Hydroxide Solution

INS: 525

DESCRIPTION

Potassium Hydroxide Solution occurs as a clear or slightly turbid, colorless or slightly colored, strongly caustic, hygro-

scopic solution. It absorbs carbon dioxide when exposed to the air, forming potassium carbonate. It is available as solutions of varying nominal concentrations.

Function pH control.

REQUIREMENTS

Labeling Indicate the percent of Potassium Hydroxide (KOH).
Identification A sample gives positive tests for *Potassium*, Appendix IIIA.
Assay Not less than 97.0% and not more than 103.0%, by weight, of the labeled amount of KOH, calculated as total alkali.
Carbonate (as K_2CO_3) Not more than 3.5%, calculated on the basis of the Potassium Hydroxide (KOH) determined in the *Assay*.
Lead Not more than 2 mg/kg, calculated on the basis of Potassium Hydroxide (KOH) determined in the *Assay*.
Mercury Not more than 0.1 mg/kg, calculated on the basis of Potassium Hydroxide (KOH) determined in the *Assay*.

TESTS

Assay Based on the stated or labeled percentage of Potassium Hydroxide (KOH), accurately weigh a volume of the solution equivalent to about 1.5 g of Potassium Hydroxide, and dilute it to 40 mL with recently boiled and cooled water. Continue as directed under *Assay* in the monograph for *Potassium Hydroxide*, beginning with ''cool to 15°. . . .''
Carbonate (as K_2CO_3) Each milliliter of 1 *N* sulfuric acid required between the phenolphthalein and methyl orange endpoints in the *Assay* is equivalent to 138.2 mg of carbonate.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, preparing the *Sample Solution* as follows: Dilute the equivalent of 1 g of Potassium Hydroxide (KOH), calculated on the basis of the *Assay*, with a mixture of 5 mL of water and 11 mL of 2.7 *N* hydrochloric acid. Use 2 μg of lead (Pb) ion in the control.
Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB, preparing the *Sample Preparation* as follows: Transfer an accurately weighed sample, equivalent to 2.0 g of Potassium Hydroxide (KOH), calculated on the basis of the *Assay*, into a 50-mL beaker, add 10 mL of water and 2 drops of phenolphthalein TS, and while constantly stirring, slowly neutralize the solution with 1:2 hydrochloric acid. Add 1 mL of 1:5 sulfuric acid and 1 mL of a 1:25 solution of potassium permanganate, cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in tight containers.

Potassium Iodate

KIO_3 Formula wt 214.00

INS: 917 CAS: [7758-05-6]

DESCRIPTION

Potassium Iodate occurs as a white, crystalline powder. One gram dissolves in about 15 mL of water. It is insoluble in alcohol. The pH of a 1:20 aqueous solution is between 5 and 8.

Function Maturing agent; oxidizing agent; dough conditioner.

REQUIREMENTS

Identification Add 1 drop of starch TS and a few drops of 20% hypophosphorous acid to 1 mL of a 1:10 aqueous solution. A transient blue color appears.
Assay Not less than 99.0% and not more than 101.0% of KIO_3 after drying.
Chlorate Passes test (limit about 0.01%).
Iodide Passes test (limit about 0.002%).
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 0.5%.

TESTS

Assay Dissolve about 1.2 g of sample, previously dried at 105° for 3 h and accurately weighed, in about 50 mL of water in a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer 10.0 mL into a 250-mL glass-stoppered flask, add 40 mL of water, 3 g of potassium iodide, and 10 mL of 3:10 hydrochloric acid, and stopper the flask. Allow to stand for 5 min, add 100 mL of cold water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 3.567 mg of KIO_3.
Chlorate Add 2 mL of sulfuric acid to 2 g of sample contained in a beaker. The sample remains white, and no odor or gas evolves.
Iodide Dissolve 1 g of sample in 10 mL of water and add 1 mL of 2 *N* sulfuric acid and 1 drop of starch TS. No blue color appears.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.

Packaging and Storage Store in well-closed containers.

Potassium Iodide

KI Formula wt 166.00

CAS: [7681-11-0]

DESCRIPTION

Potassium Iodide occurs as hexahedral crystals, either transparent and colorless or somewhat opaque and white, or as a white, granular powder. It is stable in dry air but slightly hygroscopic in moist air. One gram is soluble in 0.7 mL of water at 25°, in 0.5 mL of boiling water, in 2 mL of glycerin, and in 22 mL of alcohol.

Function Nutrient.

REQUIREMENTS

Identification

A. A 1:10 aqueous solution responds to the tests for *Potassium* and for *Iodide*, Appendix IIIA.

B. The pH of a 1:20 aqueous solution is between 6 and 10.

Assay Not less than 99.0% and not more than 101.5% of KI after drying.

Iodate Not more than 4 mg/kg.

Lead Not more than 4 mg/kg.

Loss on Drying Not more than 1%.

Nitrate, Nitrite, and Ammonia Passes test.

Thiosulfate and Barium Passes test.

TESTS

Assay Dissolve about 500 mg of sample, previously dried at 105° for 4 h and accurately weighed, in about 10 mL of water, and add 35 mL of hydrochloric acid and 5 mL of chloroform. Titrate with 0.05 *M* potassium iodate until the purple color of iodine disappears from the chloroform. Add the last portions of the iodate solution dropwise, agitating vigorously and continuously. After the chloroform is decolorized, allow the mixture to stand for 5 min. If the chloroform develops a purple color, titrate further with the iodate solution. Each milliliter of 0.05 *M* potassium iodate used is equivalent to 16.60 mg of KI.

Iodate Dissolve 1.1 g of sample in sufficient ammonia- and carbon dioxide-free water to make 10 mL of solution, and transfer to a color-comparison tube. Add 1 mL of starch TS and 0.25 mL of 1 *N* sulfuric acid, mix, and compare the color with that of a control containing, in each 10 mL, 100 mg of Potassium Iodide, 1 mL of standard iodate solution (prepared by diluting 1 mL of a 1:2500 solution of potassium iodate to 100 mL with water), 1 mL of starch TS, and 0.25 mL of 1 *N* sulfuric acid. Any color in the sample solution does not exceed that in the control.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

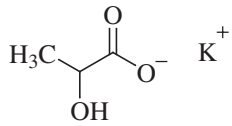
Nitrate, Nitrite, and Ammonia Dissolve 1 g of sample in 5 mL of water in a 40-mL test tube, and add 5 mL of 1 *N* sodium hydroxide and about 200 mg of aluminum wire. Insert a cotton plug in the upper portion of the tube, and place a piece of moistened red litmus paper over the mouth of the tube. Heat in a steam bath for about 15 min. No blue coloration of the paper is discernible.

Thiosulfate and Barium Dissolve 500 mg of sample in 10 mL of ammonia- and carbon dioxide-free water, and add 2 drops of diluted sulfuric acid. No turbidity develops within 1 min.

Packaging and Storage Store in well-closed containers.

Potassium Lactate Solution

2-Hydroxypropanoic Acid, Monopotassium Salt



$C_3H_5KO_3$ Formula wt 128.17

INS: 326 CAS: [996-31-6]

DESCRIPTION

Potassium Lactate Solution occurs as a clear, colorless, or practically colorless, viscous liquid that is odorless or has a slight, not unpleasant, odor. It is miscible with water. It is available in solutions with concentrations ranging from about 50% to 70% by weight.

Function Emulsifier; flavor enhancer; flavoring agent or adjuvant; humectant; pH control agent.

REQUIREMENTS

Labeling Indicate its content, by weight, of Potassium Lactate ($C_3H_5KO_3$).

Identification A sample gives positive tests for *Potassium* and for *Lactate*, Appendix IIIA.

Assay Not less than 50.0%, by weight, and not less than 98.0% and not more than 102.0%, by weight, of the labeled amount of $C_3H_5KO_3$.

Chloride Not more than 0.05%.

Citrate, Oxalate, Phosphate, or Tartrate Passes test.

Cyanide Not more than 0.5 mg/kg.

Lead Not more than 2 mg/kg.

Methanol and Methyl Esters Not more than 0.025%.

pH Between 5.0 and 9.0.

Sodium Not more than 0.1%.

Sugars Passes test.

Sulfate Not more than 0.005%.

TESTS

Assay Transfer a volume of sample equivalent to about 500 mg of potassium lactate and accurately weighed into a suitable flask, Add 60 mL of 1:5 acetic anhydride:glacial acetic acid, mix, and allow to stand for 20 min. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 12.82 mg of $C_3H_5KO_3$.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a quantity of a sample solution containing the equivalent of 40 mg of potassium lactate does not exceed that shown in a control containing 20 µg of chloride (Cl) ion.

Citrate, Oxalate, Phosphate, or Tartrate Dilute 5 mL of sample to 50 mL with recently boiled and cooled water. Add 6 *N* ammonium hydroxide or 3 *N* hydrochloric acid to 4 mL of this solution, if necessary, to bring the pH to between 7.3 and 7.7. Add 1 mL of calcium chloride TS, and heat in a boiling water bath for 5 min. The solution remains clear.

Cyanide (**Caution**: Because of the extremely poisonous nature of potassium cyanide, conduct this test in a fume hood, and exercise great care to prevent skin contact and the inhalation of particles or vapors of solutions of the material. Do not pipet solutions by mouth.)

p-*Phenylenediamine–Pyridine Mixed Reagent* Dissolve 200 mg of *p*-phenylenediamine hydrochloride in 100 mL of water, warming to aid dissolution. Cool, allow the solids to settle, and save the supernatant liquid to make the mixed reagent. Dissolve 128 mL of pyridine in 365 mL of water, add 10 mL of hydrochloric acid, and mix. To prepare the mixed reagent, mix 30 mL of the *p*-phenylenediamine supernatant liquid with all of the pyridine solution, and allow to stand for 24 h before using. The mixed reagent is stable for about 3 weeks when stored in an amber bottle.

Cyanide Standard Solution Dissolve 250 mg of potassium cyanide, accurately weighed, in 10 mL of 0.1 *N* sodium hydroxide contained in a 100-mL volumetric flask, dilute to volume with 0.1 *N* sodium hydroxide, and mix. Transfer a 10-mL aliquot into a 1000-mL volumetric flask, dilute to volume with 0.1 *N* sodium hydroxide, and mix. Each milliliter of this solution contains 10 µg of cyanide.

Sample Solution Transfer a quantity of sample equivalent to 20.0 g of potassium lactate and accurately weighed into a 100-mL volumetric flask, dilute to volume with water, and mix.

Procedure Pipet a 10-mL aliquot of the *Sample Solution* into a 50-mL beaker. Pipet 0.10 mL of the *Cyanide Standard Solution* into a second 50-mL beaker, and add 10 mL of water. Place the beakers in an ice bath, and adjust the pH to between 9 and 10 with 20% sodium hydroxide, stirring slowly and adding the reagent slowly to avoid overheating. Allow the solutions to stand for 3 min, and then slowly add 10% phosphoric acid to a pH between 5 and 6, measured with a pH meter.

Transfer the solutions into 100-mL separators containing 25 mL of cold water, and rinse the beakers and pH meter electrodes with a few milliliters of cold water, collecting the washings in the respective separator. Add 2 mL of bromine TS, stopper, and mix. Add 2 mL of 2% sodium arsenite solution, stopper, and mix. Add 10 mL of *n*-butanol to the clear solutions, stopper, and mix. Finally, add 5 mL of p-*Phenylenediamine–Pyridine Mixed Reagent*, mix, and allow to stand for 15 min. Remove and discard the aqueous phases, and filter the alcohol phases into 1-cm cells. The absorbance of the solution from the *Sample Solution*, determined at 480 nm with a suitable spectrophotometer, is not greater than that from the *Cyanide Standard Solution*.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Methanol and Methyl Esters

Potassium Permanganate and Phosphoric Acid Solution Dissolve 3 g of potassium permanganate in a mixture of 15 mL of phosphoric acid and 70 mL of water. Dilute to 100 mL with water.

Oxalic Acid and Sulfuric Acid Solution Cautiously add 50 mL of sulfuric acid to 50 mL of water, mix, cool, add 5 g of oxalic acid, and mix to dissolve.

Standard Preparation Prepare a solution containing 10.0 mg of methanol in a 100-mL volumetric flask, dilute to volume with 1:10 alcohol, and mix.

Test Preparation Place 40.0 g of sample in a glass-stoppered, round-bottom flask, add 10 mL of water, and cautiously add 30 mL of 5 *N* potassium hydroxide. Connect a condenser to the flask, and steam-distill, collecting the distillate in a suitable 100-mL graduated vessel containing 10 mL of alcohol. Continue the distillation until the volume in the receiver reaches approximately 95 mL, and dilute the distillate to 100.0 mL with water.

Procedure Transfer 10.0 mL each of the *Standard Preparation* and the *Test Preparation* to separate 25-mL volumetric flasks. Add 5.0 mL of *Potassium Permanganate and Phosphoric Acid Solution* to each, and mix. After 15 min, add 2.0 mL of *Oxalic Acid and Sulfuric Acid Solution* to each, stir with a glass rod until the solutions are colorless, add 5.0 mL of fuchsin–sulfurous acid TS (see *Solutions and Indicators*), dilute with water to volume, and mix. After 2 h, using a suitable spectrophotometer, concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 575 nm, using water as the blank. The absorbance of the solution from the *Test Preparation* is not greater than that from the *Standard Preparation*.

pH Determine as directed under *pH Determination*, Appendix IIB.

Sodium

Potassium Chloride Solution Dissolve 100 g of potassium chloride and dilute to 1000 mL with water.

Stock Solution Transfer 127.1 mg of sodium chloride, previously dried at 105° for 2 h and accurately weighed, into a 500-mL volumetric flask, dilute to volume with water, and

mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute to volume with water, and mix to obtain a solution containing 10 μg of sodium per milliliter.

Standard Solutions Pipet 1-, 2-, 5-, and 10-mL aliquots of the *Stock Solution* into separate 100-mL volumetric flasks. Add 1.0 mL of *Potassium Chloride Solution* followed by 1.0 mL of nitric acid to each; dilute to volume with water; and mix to obtain *Standard Solutions* containing 0.1, 0.2, 0.5, and 1.0 μg of sodium per milliliter, respectively.

Test Solution Transfer a quantity of sample equivalent to about 4 g of potassium lactate and accurately weighed into a 50-mL volumetric flask, dilute to volume with water, and mix. Pipet 1 mL of this solution into a 100-mL volumetric flask, add 1.0 mL of *Potassium Chloride Solution* followed by 1.0 mL of nitric acid, dilute to volume with water, and mix.

Blank Solution Transfer 1.0 mL of *Potassium Chloride Solution* into a 100-mL volumetric flask, add 1.0 mL of nitric acid, dilute to volume with water, and mix.

Procedure Use a suitable atomic absorption spectrophotometer equipped with a sodium hollow-cathode lamp and an oxidizing air–acetylene flame. After using the *Blank Solution* to zero the instrument, concomitantly determine the absorbances of the *Standard Solutions* and the *Test Solution* at the sodium emission line of 589 nm. Plot the absorbances of the *Standard Solutions* versus concentration, in micrograms per milliliter, of sodium, and draw the straight line that best fits the plotted points. From the graph so obtained, determine the concentration, *C*, in micrograms per milliliter, of sodium in the *Test Solution*. Calculate the percentage of sodium in the portion of potassium lactate taken by the formula

$$CD/10{,}000W,$$

in which *D* is the dilution factor for the *Test Solution*; and *W* is the quantity, in grams, of potassium lactate taken to prepare the *Test Solution*.

Sugars Add 5 drops of sample to 10 mL of hot alkaline cupric tartrate TS. No red precipitate forms.

Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a quantity of a sample solution containing the equivalent of 4.0 g of potassium lactate does not exceed that shown in a control solution containing 200 μg of sulfate (SO_4) ion.

Packaging and Storage Store in tight containers.

Potassium Metabisulfite

Potassium Pyrosulfite

$K_2S_2O_5$ Formula wt 222.31

INS: 224 CAS: [16731-55-8]

DESCRIPTION

Potassium Metabisulfite occurs as white or colorless, free-flowing crystals, crystalline powder, or granules. It gradually oxidizes in air to the sulfate. It is soluble in water, but it is insoluble in alcohol. Its solutions are acid to litmus.

Function Preservative; antioxidant; bleaching agent.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Potassium* and for *Sulfite*, Appendix IIIA.
Assay Not less than 90.0% of $K_2S_2O_5$.
Lead Not more than 2 mg/kg.
Iron Not more than 10 mg/kg.
Selenium Not more than 5 mg/kg.

TESTS

Assay Add about 250 mg of sample, accurately weighed, to exactly 50 mL of 0.1 *N* iodine contained in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 mL of hydrochloric acid, and back titrate the excess iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. The milliliters of iodine consumed is equivalent to 50 minus the milliliters of 0.1 *N* sodium thiosulfate sulfate used. Each milliliter of 0.1 *N* iodine consumed is equivalent to 5.558 mg of $K_2S_2O_5$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Iron Add 2 mL of hydrochloric acid to 1 g of sample, and evaporate to dryness on a steam bath. Dissolve the residue in 2 mL of hydrochloric acid and 20 mL of water, add a few drops of bromine TS, and boil the solution to remove the bromine. Cool, dilute with water to 25 mL, then add 50 mg of ammonium persulfate and 5 mL of ammonium thiocyanate TS. Any red or pink color does not exceed that produced in a control containing 1.0 mL of *Iron Standard Solution* (10 μg Fe).
Selenium Determine as directed in *Method I* under *Selenium Limit Test*, Appendix IIIB, using 100 mg of sample and 100 mg of magnesium oxide.

Packaging and Storage Store in well-filled, tight containers, and avoid exposure to excessive heat.

Potassium Nitrate

KNO_3 Formula wt 101.10

INS: 252 CAS: [7757-79-1]

DESCRIPTION

Potassium Nitrate occurs as colorless, transparent prisms, as white granules, or as a white, crystalline powder. It is slightly hygroscopic in moist air. One gram dissolves in 3 mL of

water at 25°, in 0.5 mL of boiling water, and in about 620 mL of alcohol. Its solutions are neutral to litmus.

Function Antimicrobial agent; preservative.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Potassium* and *Nitrate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of KNO_3 after drying.
Chlorate Passes test.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 1%.

TESTS

Assay Dissolve about 0.4 g of sample, previously dried at 105° for 4 h and accurately weighed, in about 300 mL of water contained in a 500-mL round-bottom flask. Add 3 g of a powder of Devarda's alloy and 15 mL of a 40% sodium hydroxide solution, and connect with a spray-preventing device and condenser to the flask. Allow to stand for 2 h. Transfer 50 mL of 0.1 *N* sulfuric acid into a receptacle, use this to collect 250 mL of the distillate, and titrate the excess sulfuric acid with 0.1 *N* sodium hydroxide, using 3 drops of methyl red–methylene blue TS as the indicator. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sulfuric acid is equivalent to 10.11 mg of KNO_3.
Chlorate Sprinkle about 100 mg of dry sample on 1 mL of sulfuric acid. The mixture does not turn yellow.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of water, and 4 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Packaging and Storage Store in tight containers.

Potassium Nitrite

KNO_2 Formula wt 85.10

INS: 249 CAS: [7758-09-0]

DESCRIPTION

Potassium Nitrite occurs as small, white or yellow, deliquescent granules or cylindrical sticks. It is very soluble in water, but is sparingly soluble in alcohol.

Function Color fixative in meat and meat products; antimicrobial agent.

REQUIREMENTS

Identification A 1:10 aqueous solution is alkaline to litmus and gives positive tests for *Potassium* and for *Nitrite*, Appendix IIIA.
Assay Not less than 90.0% and not more than 100.5% of KNO_2.
Lead Not more than 4 mg/kg.

TESTS

Assay Transfer about 1.2 g of sample, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Pipet 10 mL of this solution into a mixture of 50.0 mL of 0.1 *N* potassium permanganate, 100 mL of water, and 5 mL of sulfuric acid, keeping the tip of the pipet well below the surface of the liquid. Warm the solution to 40°, allow it to stand for 5 min, and add 25.0 mL of 0.1 *N* oxalic acid. Heat the mixture to about 80°, and titrate with 0.1 *N* potassium permanganate. Each milliliter of 0.1 *N* potassium permanganate is equivalent to 4.255 mg of KNO_2.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 1 g of sample in 10 mL of water, and 4 μg of lead (Pb) ion in the control.

Packaging and Storage Store in tight containers.

Potassium Phosphate, Dibasic

Dipotassium Monophosphate; Dipotassium Phosphate

K_2HPO_4 Formula wt 174.18

INS: 340(ii) CAS: [7758-11-4]

DESCRIPTION

Potassium Phosphate, Dibasic, occurs as a colorless or white, granular salt that is deliquescent when exposed to moist air. One gram is soluble in about 3 mL of water. It is insoluble in alcohol. The pH of a 1% solution is about 9.

Function Buffer; sequestrant; yeast food.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Potassium* and for *Phosphate*, Appendix IIIA.
Assay Not less than 98.0% of K_2HPO_4 after drying.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Insoluble Substances Not more than 0.2%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 2.0%.

TESTS

Assay Transfer about 6.5 g of sample, previously dried at 105° for 4 h and accurately weighed, into a 250-mL beaker, add 50.0 mL of 1 *N* hydrochloric acid and 50 mL of water, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and, stirring constantly, slowly titrate the excess acid with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (*A*), if any, of 1 *N* hydrochloric acid consumed by the sample. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (*B*) of 1 *N* sodium hydroxide required in the titration between the two inflection points (pH 4 to pH 8.8). When *A* is equal to or less than *B*, each milliliter of the volume *A* of 1 *N* hydrochloric acid is equivalent to 174.2 mg of K_2HPO_4. When *A* is greater than *B*, each milliliter of the volume $2B - A$ of 1 *N* sodium hydroxide is equivalent to 174.2 mg of K_2HPO_4.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of water.
Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.
Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Packaging and Storage Store in tight containers.

Potassium Phosphate, Monobasic

Potassium Biphosphate; Potassium Dihydrogen Phosphate; Monopotassium Phosphate

KH_2PO_4 Formula wt 136.09

INS: 340(i) CAS: [7778-77-0]

DESCRIPTION

Potassium Phosphate, Monobasic, occurs as colorless crystals or as a white, granular or crystalline powder. It is stable in air. It is freely soluble in water, but is insoluble in alcohol. The pH of a 1:100 aqueous solution is between 4.2 and 4.7.

Function Buffer; sequestrant; yeast food.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Potassium* and for *Phosphate*, Appendix IIIA.

Assay Not less than 98.0% of KH_2PO_4 after drying.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Insoluble Substances Not more than 0.2%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 1%.

TESTS

Assay Transfer about 5 g of sample, previously dried at 105° for 4 h and accurately weighed, into a 250-mL beaker, add 5.0 mL of 1 *N* hydrochloric acid and 100 mL of water, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and slowly titrate the excess acid, stirring constantly, with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (*A*), if any, of 1 *N* hydrochloric acid consumed by the sample. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (*B*) of 1 *N* sodium hydroxide required in the titration between the two inflection points (pH 4 and pH 8.8). Each milliliter of the volume $B - A$ of 1 *N* sodium hydroxide is equivalent to 136.1 mg of KH_2PO_4.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of water.
Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.
Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Packaging and Storage Store in tight containers.

Potassium Phosphate, Tribasic

Tripotassium Phosphate

K_3PO_4 Formula wt 212.27

INS: 340(iii) CAS: [7778-53-2]

DESCRIPTION

Potassium Phosphate, Tribasic, occurs as white, hygroscopic crystals or granules. It is anhydrous or may contain one molecule of water of hydration. It is freely soluble in water, but

is insoluble in alcohol. The pH of a 1:100 aqueous solution is about 11.5.

Function Emulsifier.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Potassium* and for *Phosphate*, Appendix IIIA.
Assay Not less than 97.0% of K_3PO_4, calculated on the ignited basis.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Insoluble Substances Not more than 0.2%.
Lead Not more than 2 mg/kg.
Loss on Ignition *Anhydrous*: Not more than 5.0%; *Monohydrate*: Between 8.0% and 20.0%.

TESTS

Assay Dissolve an accurately weighed quantity of sample, equivalent to about 8 g of anhydrous K_3PO_4, in 40 mL of water in a 400-mL beaker, and add 100.0 mL of 1 *N* hydrochloric acid. Pass a stream of fine bubbles of carbon dioxide-free air through the solution for 30 min to expel carbon dioxide, covering the beaker loosely to prevent any loss by spraying. Wash the cover and sides of the beaker with a few milliliters of water, and place the electrodes of a suitable pH meter in the solution. Protect the solution from absorbing carbon dioxide. Titrate the solution with 1 *N* sodium hydroxide until the inflection point occurs at about pH 4, then calculate the volume (*A*) of 1 *N* hydrochloric acid consumed. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurs at about pH 8.8. Calculate the volume (*B*) of 1 *N* sodium hydroxide consumed in this titration. When *A* is equal to or greater than 2*B*, each milliliter of the volume *B* of 1 *N* sodium hydroxide is equivalent to 212.3 mg of K_3PO_4. When *A* is less than 2*B*, each milliliter of the volume $A - B$ of 1 *N* sodium hydroxide is equivalent to 212.3 mg of K_3PO_4.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of water.
Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.
Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Ignition Determine as directed under *Loss on Ignition*, Appendix IIC, igniting a sample at 800° for 30 min.

Packaging and Storage Store in tight containers.

Potassium Polymetaphosphate

Potassium Metaphosphate; Potassium Polyphosphates; Potassium Kurrol's Salt

$(KPO_3)_n$

INS: 452(ii) CAS: [7790-53-6]

DESCRIPTION

Potassium Polymetaphosphate occurs as a white powder. It is a straight-chain polyphosphate having a high degree of polymerization. It is insoluble in water, but is soluble in dilute solutions of sodium salts.

Function Emulsifier; moisture-retaining agent.

REQUIREMENTS

Identification

A. Finely powder about 1 g of sample, and while stirring vigorously, add it slowly to 100 mL of a 1:50 sodium chloride solution. A gelatinous mass forms.

B. Mix 500 mg of sample with 10 mL of nitric acid and 50 mL of water, boil for about 30 min, and cool. The resulting solution gives positive tests for *Potassium* and for *Phosphate*, Appendix IIIA.
Assay Not less than 59.0% and not more than 61.0% of P_2O_5.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Lead Not more than 2 mg/kg.
Viscosity Between 6.5 and 15 centipoises.

TESTS

Assay Transfer about 1 g of sample, accurately weighed, into a 400-mL beaker, add 100 mL of water and 25 mL of nitric acid, cover with a watch glass, and boil the solution for 10 min on a hot plate. Rinse any condensate on the watch glass into the beaker, cool the solution to room temperature, transfer it quantitatively to a 500-mL volumetric flask, dilute to volume with water, and mix thoroughly. Pipet 20.0 mL of this solution into a 500-mL Erlenmeyer flask, add 100 mL of water, and heat just to boiling. While stirring, add 50 mL of quimociac TS, then cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared, sintered-glass crucible of medium porosity, and wash with five 25-mL portions of water. Dry at about 225° for 30 min, cool, and weigh. Each milligram of precipitate thus obtained is equivalent to 32.074 μg of P_2O_5.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 15 mL of 2.7 *N* hydrochloric acid.
Fluoride Place 5 g of sample, 25 mL of water, 50 mL of sulfuric acid, 5 drops of a 1:2 silver nitrate solution, and a few glass beads in a 250-mL distilling flask connected to a condenser and carrying a thermometer and a capillary tube,

both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator to the capillary tube. Support the flask on a fireproof mat with a hole that exposes about one-third of the flask to the flame. Distill the mixture into a 250-mL volumetric flask until the temperature reaches 135°. Add water from the funnel or introduce steam through the capillary tube to maintain the temperature between 135° and 140°. Continue the distillation until 225 to 240 mL has been collected, then dilute to 250 mL with water, and mix.

Place a 50-mL aliquot of this solution in a 100-mL Nessler tube. In another, similar Nessler tube, place 50 mL of water as a control. Add 0.1 mL of a filtered solution of 1:1000 sodium alizarinsulfonate and 1 mL of a freshly prepared 1:4000 hydroxylamine hydrochloride solution to each tube, and mix well. While stirring, add, dropwise, 0.05 *N* sodium hydroxide to the tube containing the distillate until its color just matches that of the control, which is faintly pink. Then add exactly 1.0 mL of 0.1 *N* hydrochloric acid to each tube, and mix well. Use a buret, graduated in 0.05-mL units, to add slowly enough a 1:4000 thorium nitrate solution to the tube containing the distillate so that after mixing, the color of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Use a buret to add sodium fluoride TS (10 μg fluoride per milliliter) to the control to make the colors of the two tubes match. Mix well, and allow all air bubbles to escape before making the final color comparison. Check the endpoint by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct color change should take place. Note the volume of sodium fluoride TS added to the control solution. It does not exceed 1.0 mL.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Viscosity

Tetrasodium Pyrophosphate Solution Dissolve 3.5 g of tetrasodium pyrophosphate in 1000 mL of water.

Procedure Using a magnetic stirrer, dissolve 300 mg of sample in 200 mL of *Tetrasodium Pyrophosphate Solution*. When solution is complete, or after 30 min, whichever occurs first, transfer 10 mL of the solution into an Ostwald-Fenske viscometer, and determine the time, *T*, in seconds, required for the liquid to flow from the upper to the lower mark in the capillary tube. Calculate the viscosity, in centipoises, by the formula

$$Tv/dt,$$

in which t is the time, in seconds, required for a glycerin–water mixture of known viscosity, v, and specific gravity, d, to flow from the upper to the lower mark of the capillary tube during calibration of the viscometer under similar conditions.

Packaging and Storage Store in well-closed containers.

Potassium Pyrophosphate

Tetrapotassium Pyrophosphate

$K_4P_2O_7$ Formula wt 330.34

INS: 450v CAS: [7320-34-5]

DESCRIPTION

Potassium Pyrophosphate occurs as colorless or white crystals or as a white, crystalline or granular powder. It is hygroscopic. It is very soluble in water, but is insoluble in alcohol. The pH of a 1:100 aqueous solution is about 10.5.

Function Emulsifier; texturizer.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Potassium*, Appendix IIIA.

B. Dissolve 100 mg of sample in 100 mL of 1.7 *N* nitric acid. Add 0.5 mL of this solution to 30 mL of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and add 0.5 mL of it to 30 mL of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 95.0% of $K_4P_2O_7$, calculated on the ignited basis.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 10 mg/kg.

Insoluble Substances Not more than 0.1%.

Lead Not more than 2 mg/kg.

Loss on Ignition Not more than 0.5%.

TESTS

Assay Dissolve about 600 mg of sample, accurately weighed, in 100 mL of water contained in a 400-mL beaker, and using a pH meter, adjust the pH of the solution to exactly 3.8 with hydrochloric acid. Add 50 mL of a 1:8 solution of zinc sulfate (125 g of $ZnSO_4 \cdot 7H_2O$ dissolved in water, diluted to 1000 mL, filtered, and adjusted to pH 3.8 with hydrochloric acid), and allow the mixture to stand for 2 min. Titrate the liberated acid with 0.1 *N* sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the endpoint, allow time for any precipitated zinc hydroxide to redissolve. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 16.52 mg of $K_4P_2O_7$.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.

Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.

Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter the solution through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Ignition Ignite a sample at about 800° for 30 min.

Packaging and Storage Store in tight containers.

Potassium Sorbate

2,4-Hexadienoic Acid, Potassium Salt

$$CH_3CH{=}CHCH{=}CHCOOK$$

$C_6H_7KO_2$ Formula wt 150.22

INS: 202 CAS: [590-00-1]

DESCRIPTION

Potassium Sorbate occurs as white to off white crystals, crystalline powder, or pellets. It decomposes at about 270°.

Function Antimicrobial agent; preservative.

REQUIREMENTS

Identification

A. A 1:10 aqueous solution responds to the flame test for *Potassium*, Appendix IIIA.

B. Add a few drops of bromine TS to 2 mL of a 1:10 solution. The color disappears.

Assay Not less than 98.0% and not more than 101.0% of $C_6H_7KO_2$, calculated on the dried basis.
Acidity (as sorbic acid) Passes test (about 1%).
Alkalinity (as K_2CO_3) Passes test (about 1%).
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 1.0%.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 40 mL of glacial acetic acid contained in a 250-mL glass-stoppered Erlenmeyer flask, warming if necessary to effect solution. Cool to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to a blue-green endpoint that persists for at least 30 s.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 15.02 mg of $C_6H_7KO_2$.
Acidity (as sorbic acid) Dissolve 1.1 g of sample in 20 mL of water, and add 3 drops of phenolphthalein TS. If the solution is colorless, titrate with 0.1 *N* sodium hydroxide to a pink color that persists for 15 s. Not more than 1.1 mL is required.
Alkalinity (as K_2CO_3) Dissolve 1.1 g of sample in 20 mL of water, and add 3 drops of phenolphthalein TS. If the solution is pink, titrate with 0.1 *N* hydrochloric acid. Not more than 0.8 mL is required to discharge the pink color.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.

Packaging and Storage Store in tight containers.

Potassium Sulfate

K_2SO_4 Formula wt 174.26

INS: 515 CAS: [7778-80-5]

DESCRIPTION

Potassium Sulfate occurs as colorless or white crystals or as a crystalline powder. One gram dissolves in about 8.5 mL of water. It is insoluble in alcohol. The pH of a 1:20 aqueous solution is about 5.5 to 8.5.

Function pH control.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Potassium*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of K_2SO_4.
Lead Not more than 2 mg/kg.
Selenium Not more than 5 mg/kg.

TESTS

Assay Dissolve about 500 mg of sample, accurately weighed, in 200 mL of water, add 1 mL of hydrochloric acid, and heat to boiling. Gradually add, in small portions and while stirring constantly, an excess of hot barium chloride TS (about 8 or 9 mL), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a retentive, ashless filter paper, wash until free from chloride, and place the filter in a suitable tared crucible. Carefully burn away the paper, and ignite at 800° ± 25° to constant weight. The weight of the barium sulfate so obtained, multiplied by 0.7466, indicates its equivalent of K_2SO_4.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 1.2-g sample.

Packaging and Storage Store in well-closed containers.

Potassium Sulfite

K_2SO_3 Formula wt 158.26
INS: 225 CAS: [10117-38-1]

DESCRIPTION

Potassium Sulfite occurs as a white granular powder. It undergoes oxidation in air. One gram dissolves in about 3.5 mL of water. It is slightly soluble in alcohol.

Function Preservative; antioxidant.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Potassium* and for *Sulfite*, Appendix IIIA.
Assay Not less than 90.0% and not more than 100.5% of K_2SO_3.
Alkalinity (as K_2CO_3) Between 0.25% and 0.45%.
Lead Not more than 2 mg/kg.
Selenium Not more than 5 mg/kg.

TESTS

Assay Dissolve about 750 mg of sample, accurately weighed, in a mixture of 100.0 mL of 0.1 *N* iodine and 5 mL of 2.7 *N* hydrochloric acid, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each milliliter of 0.1 *N* iodine is equivalent to 7.912 mg of K_2SO_3.
Alkalinity (as K_2CO_3) Dissolve 1 g of sample in 20 mL of water, add 25 mL of 3% hydrogen peroxide, previously neutralized to methyl red TS, mix thoroughly, cool to room temperature, and titrate with 0.02 *N* hydrochloric acid. Perform a blank determination (see *General Provisions*) using 25 mL of the neutralized hydrogen peroxide solution, and make any necessary correction. Each milliliter of 0.02 *N* hydrochloric acid is equivalent to 1.38 mg of K_2CO_3.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Selenium Determine as directed in *Method I* under *Selenium Limit Test*, Appendix IIIB, using 1.2 g of sample and 600 mg of magnesium oxide.

Packaging and Storage Store in tight containers.

Potassium Tripolyphosphate

Pentapotassium Triphosphate; Potassium Triphosphate
$K_5P_3O_{10}$ Formula wt 448.41
INS: 451(ii) CAS: [13845-36-8]

DESCRIPTION

Potassium Tripolyphosphate occurs as white granules or as a white powder. It is hygroscopic and is very soluble in water. The pH of a 1:100 aqueous solution is between 9.2 and 10.1.

Function Texturizer.

REQUIREMENTS

Identification
A. A 1:20 aqueous solution gives positive tests for *Potassium*, Appendix IIIA.
B. Add a few drops of silver nitrate TS to 1 mL of a 1:100 aqueous solution. A white precipitate forms that is soluble in 1.7 *N* nitric acid.
Assay Not less than 85.0% of $K_5P_3O_{10}$.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Insoluble Substances Not more than 2.0%.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.7%.

TESTS

Assay
Potassium Acetate Buffer (pH 5.0) Dissolve 78.5 g of potassium acetate in 1000 mL of water, and adjust the pH of the solution to 5.0 with glacial acetic acid. Add a few milligrams of mercuric iodide to inhibit mold growth.
0.3 M *Potassium Chloride Solution* Dissolve 22.35 g of potassium chloride in water, add 5 mL of *Potassium Acetate Buffer*, dilute to 1000 mL with water, and mix. Add a few milligrams of mercuric iodide to inhibit mold growth.
0.6 M *Potassium Chloride Solution* Dissolve 44.7 g of potassium chloride in water, add 5 mL of *Potassium Acetate Buffer*, dilute to 1000 mL with water, and mix. Add a few milligrams of mercuric iodide to inhibit mold growth.
1 M *Potassium Chloride Solution* Dissolve 74.5 g of potassium chloride in water, add 5 mL of *Potassium Acetate Buffer*, dilute to 1000 mL with water, and mix. Add a few milligrams of mercuric iodide to inhibit mold growth.
Chromatographic Column Use a standard chromatographic column, 20- to 40-cm long with a 20- to 28-mm id that has a sealed-in, coarse-porosity, fritted disk. If a stopcock is not provided, attach a stopcock having a 3- to 4-mm diameter bore to the outlet of the column with a short length of flexible vinyl tubing.
Procedure Close the column stopcock, fill the space between the fritted disk and the stopcock with water, and connect a vacuum line to the stopcock. Prepare a 1:1 water slurry of Dowex 1 × 8, chloride form, 100- to 200- or 200- to 400-mesh, or a comparable grade of styrene–divinylbenzene ion exchange resin, and decant off any very fine particles and any foam. Do this two or three times or until no more finely suspended material or foaming is observed. Fill the column with the slurry, and open the stopcock to allow the vacuum to pack the resin bed until the water level is slightly above the top of the resin, then immediately close the stopcock. Do not allow the liquid level to fall below the resin level at any time. Repeat this procedure until the packed resin column is 15 cm above the fritted disk. Place one circle of tightly fitting glass-fiber filter paper on top of the resin bed, then place a perforated polyethylene disk on top of the paper. Alternatively, place a loosely packed plug of glass wool on top of the bed.

Close the top of the column with a rubber stopper in which a 7.6-cm length of capillary tubing (1.5-mm id, 7-mm od), or equivalent, has been inserted through the center, so that about 12 mm of the tubing extends through the bottom of the stopper. Connect the top of the capillary tubing to the stem of a 500-mL separator with flexible vinyl tubing, and clamp the separator to a ring stand above the column. Wash the column by adding 100 mL of water to the separator with all stopcocks closed. First open the separator stopcock, then open the column stopcock. The rate of flow should be about 5 mL/min. When the separator is empty, close the column stopcock, then close the separator stopcock.

Transfer about 500 mg of sample, accurately weighed, into a 250-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 10.0 mL of this solution into the separator, open both stopcocks, and allow the solution to drain into the column, rinsing the separator with 20 mL of water. Discard the eluate.

Add 370 mL of *0.3* M *Potassium Chloride Solution* to the separator, and allow this solution to pass through the column, discarding the eluate. Add 250 mL of *0.6* M *Potassium Chloride Solution* to the column, allow the solution to pass through the column, and receive the eluate in a 400-mL beaker. (To ensure a clean column for the next run, pass 100 mL of *1* M *Potassium Chloride Solution* through the column, and then follow with 100 mL of water. Discard all washings.) Add 15 mL of nitric acid to the beaker, mix, and boil for 15 to 20 min. Add methyl orange TS, and neutralize the solution with ammonium hydroxide. Add 1 g of ammonium nitrate crystals, stir to dissolve, and cool. While stirring, add 15 mL of ammonium molybdate TS, and stir vigorously for 3 min, or allow the mixture to stand with occasional stirring for 10 to 15 min. Filter the contents of the beaker by means of suction through a 6- to 7-mm paper-pulp filter pad supported in a 25-mm porcelain disk. The filter pad should be covered with a suspension of infusorial earth. After the contents of the beaker have been transferred to the filter, wash the beaker with five 10-mL portions of a 1:100 solution of sodium or potassium nitrate, passing the washings through the filter, then wash the filter with five 5-mL portions of the wash solution. Return the filter pad and the precipitate to the beaker, wash the funnel thoroughly with water into the beaker, and dilute to about 150 mL. Add 0.1 *N* sodium hydroxide from a buret until the yellow precipitate is dissolved, then add 5 to 8 mL in excess. Add phenolphthalein TS, and titrate the excess alkali with 0.1 *N* nitric acid. Finally, titrate with 0.1 *N* sodium hydroxide to the first appearance of a pink color. The difference between the total volume of 0.1 *N* sodium hydroxide added and the volume of nitric acid required represents the volume, *V*, in milliliters, of 0.1 *N* sodium hydroxide consumed by the phosphomolybdate complex. Calculate the quantity, in milligrams, of $K_5P_3O_{10}$ in the sample taken by the formula

$$0.650 \times 25V.$$

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.

Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.

Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter the solution through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

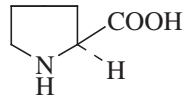
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at about 105° for 1 h.

Packaging and Storage Store in tight containers.

L-Proline

L-2-Pyrrolidinecarboxylic Acid



$C_5H_9NO_2$ Formula wt 115.13

CAS: [147-85-3]

View IR

DESCRIPTION

L-Proline occurs as white crystals or a crystalline powder. It is very soluble in water and in alcohol, but insoluble in ether.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.5% and not more than 101.5% of $C_5H_9NO_2$, calculated on the dried basis.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 0.3%.

Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between −84.0° and −86.3°, calculated on the dried basis.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 220 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 11.51 mg of $C_5H_9NO_2$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and using 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 4 g of a previously dried sample in sufficient water to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Propane

$$CH_3CH_2CH_3$$

C_3H_8 Formula wt 44.10

CAS: [74-98-6]

DESCRIPTION

Propane occurs as a colorless, flammable gas (boiling temperature is about −42°). One hundred volumes of water dissolves 6.5 volumes at 17.8° and 753 mm Hg; 100 volumes of anhydrous alcohol dissolves 790 volumes at 16.6° and 754 mm Hg; 100 volumes of ether dissolves 926 volumes at 16.6° and 757 mm Hg; 100 volumes of chloroform dissolves 1299 volumes at 21.6° and 757 mm Hg. Vapor pressure at 21° is about 10,290 mm Hg (108 psi).

Function Propellant; aerating agent.

REQUIREMENTS

Caution: Propane is highly flammable and explosive. Observe precautions, and perform sampling and analytical operations in a well-ventilated fume hood.

Identification

A. The infrared absorption spectrum of a sample exhibits maxima, among others, at about the following wavelengths, in micrometers: 3.4 (vs), 6.8 (s), and 7.2 (m).

B. The vapor pressure of a sample, obtained as directed under *Sampling Procedure* (below), and determined at 21° by means of a suitable pressure gauge, is between 820 and 875 kPa absolute (119 and 127 psia, respectively).
Assay Not less than 98.0% of Propane (C_3H_8).
Acidity of Residue Passes test.
High-Boiling Residue Not more than 5 mg/kg.
Sulfur Compounds Passes test.
Water Not more than 10 mg/kg.

TESTS

Sampling Procedure Use a stainless steel specimen cylinder equipped with a stainless steel valve and having a capacity of not less than 200 mL and a pressure rating of 240 psi or more. Dry the cylinder at 110° for 2 h with the valve open, and evacuate the hot cylinder to less than 1 mm Hg. Close the valve, and cool and weigh the cylinder. Tightly connect one end of a charging line to the sample cylinder, and loosely connect the other end to the specimen cylinder. Carefully open the sample cylinder, and allow the sample gas to flush out the charging line through the loose connection. Avoid excessive flushing that causes moisture to freeze in the charging line and connections. Tighten the fitting on the specimen cylinder, and open the specimen cylinder valve, allowing the sample gas to flow into the evacuated cylinder. Continue until the desired amount of sample gas is obtained, close the sample cylinder valve, and then close the specimen cylinder valve.

Caution: Do not overload the specimen cylinder.

Weigh the charged specimen cylinder again, and calculate the sample gas weight.

Assay

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a thermal-conductivity detector and a 6-m × 3-mm (id) aluminum column, or equivalent, packed with 10 weight percent tetraethylene glycol dimethyl ether liquid phase, or equivalent, on a support of crushed firebrick (GasChrom R) that has been calcined or burned with a clay binder above 900° and silanized, or equivalent. Use helium as the carrier gas at a flow rate of 50 mL/min, and maintain the temperature of the column at 33°.

System Suitability The peak responses obtained for Propane in the chromatograms from duplicate determinations agree within 1%.

Procedure Connect one sample cylinder to the chromatograph through a suitable sampling valve and a flow-control valve downstream from the sampling valve. Flush the liquid specimen through the sampling valve, taking care to avoid trapping sample gas or air in the valve. Inject a suitable volume, typically 2 μL, of sample gas into the chromatograph, and record the chromatogram. Calculate the percent purity by the following formula:

$$100S/\sum s,$$

in which S is the sample gas response and $\sum s$ is the sum of all of the responses in the chromatogram.

Acidity of Residue Add 10 mL of water to the residue obtained under *High-Boiling Residue* (below), mix by swirling for about 30 s, add 2 drops of methyl orange TS, insert the stopper in the tube, and shake vigorously. No pink or red color appears in the aqueous layer.
High-Boiling Residue Prepare a cooling coil from copper tubing [about 6.1 m × about 6 mm (od)] to fit into a suitable vacuum-jacketed flask. Immerse the cooling coil in a mixture of dry ice and acetone in a vacuum-jacketed flask, and connect one end of the tubing to a sample cylinder (see *Sampling*

Procedure). Carefully open the sample cylinder valve, flush the cooling coil with about 50 mL of the liquified sample, and discard this portion of liquid. Continue delivering liquid from the cooling coil, and collect it in a previously chilled 1000-mL sedimentation cone until the cone is filled to the 1000-mL mark (approximately 600 g). Using a warm water bath maintained at about 40° to reduce evaporating time, allow the liquid to evaporate. When all of the liquid has evaporated, rinse the sedimentation cone with two 50-mL portions of pentane, and combine the rinsings in a tared 150-mL evaporating dish. Transfer 100 mL of the pentane solvent to a second tared 150-mL evaporating dish, place both evaporating dishes on a water bath, evaporate to dryness, and heat the dishes in an oven at 100° for 60 min. Cool the dishes in a desiccator, and weigh. Repeat the heating for 15-min periods until successive weighings are within 0.1 mg. The weight of the residue obtained from the specimen is the difference between the weights of the residues in the two evaporating dishes. Calculate the milligrams per kilogram of high-boiling residue based on a sample weight of 600 g.

Sulfur Compounds Carefully open the sample cylinder valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose. The gas is free from the characteristic odor of sulfur compounds.

Water Determine as directed in the *Karl Fischer Titrimetric Method* under *Water Determination*, Appendix IIB, using the following modifications: (a) Provide the closed-system titrating vessel with an opening through which passes a coarse-porosity gas dispersion tube connected to a sampling cylinder. (b) Dilute the reagent with anhydrous methanol to give a water equivalence factor of between 0.2 and 1.0 mg/mL; age this diluted solution for not less than 16 h before standardization. (c) Obtain a 100-g sample as directed under *Sampling Procedure* (above), and introduce the sample gas into the titration vessel through the gas dispersion tube at a rate of about 100 mL/min; if necessary, heat the specimen cylinder gently to maintain this flow rate.

Packaging and Storage Store in tight cylinders protected from excessive heat.

Propionic Acid

CH_3CH_2COOH

$C_3H_6O_2$ Formula wt 74.08

INS: 280 CAS: [79-09-4]

DESCRIPTION

Propionic Acid occurs as an oily liquid. It is miscible with water, with alcohol, and with various other organic solvents.

Function Preservative; mold inhibitor.

REQUIREMENTS

Assay Not less than 99.5% and not more than 100.5% of $C_3H_6O_2$, calculated on the anhydrous basis.

Aldehydes (as propionaldehyde) Passes test (limit about 0.05%).

Distillation Range Between 138.5° and 142.5°.

Lead Not more than 2 mg/kg.

Nonvolatile Residue Not more than 0.01%.

Readily Oxidizable Substances (as formic acid) Passes test (limit about 0.05%).

Specific Gravity Between 0.993 and 0.997 at 20°/20°.

Water Not more than 0.15%.

TESTS

Assay Mix about 1.5 g of sample, accurately weighed, with 100 mL of recently boiled and cooled water contained in a 250-mL Erlenmeyer flask, add phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a faint-pink endpoint that persists for at least 30 s. Each milliliter of 0.5 *N* sodium hydroxide is equivalent to 37.04 mg of $C_3H_6O_2$.

Aldehydes (as propionaldehyde) Transfer 10.0 mL of sample into a 250-mL glass-stoppered Erlenmeyer flask containing 50 mL of water and 10.0 mL of a 1:80 aqueous solution of sodium bisulfite, stopper the flask, and shake vigorously. Allow the mixture to stand for 30 min, then titrate with 0.1 *N* iodine to the same brown-yellow endpoint obtained with a blank treated with the same quantities of the same reagents (see *General Provisions*). The difference between the volume of 0.1 *N* iodine required for the blank and that required for the sample is not more than 1.75 mL.

Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Nonvolatile Residue Transfer 100 mL of sample into a tared, 125-mL platinum evaporating dish, previously heated at 105° to constant weight, and evaporate the sample to dryness on a steam bath. Heat the dish at 105° for 30 min or to constant weight, cool in a desiccator, and weigh.

Readily Oxidizable Substances (as formic acid) Dissolve 15 g of sodium hydroxide in 50 mL of water, cool, add 6 mL of bromine, stirring to effect complete solution, and dilute to 2000 mL with water. Transfer 25.0 mL of this solution into a 250-mL glass-stoppered Erlenmeyer flask containing 100 mL of water, and add 10 mL of a 1:5 aqueous solution of sodium acetate and 10.0 mL of sample. Allow to stand for 15 min, add 5 mL of a 1:4 aqueous solution of potassium iodide and 10 mL of hydrochloric acid, and titrate with 0.1 *N* sodium thiosulfate just to the disappearance of the brown color. Perform a blank determination (see *General Provisions*), and make any necessary correction. The difference between the volume of 0.1 *N* sodium thiosulfate required for the blank and that required for the sample is not more than 2.2 mL.

Specific Gravity Determine by any reliable method (see *General Provisions*).
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Propylene Glycol

1,2-Propanediol; 1,2-Dihydroxypropane; Methyl Glycol

$CH_3CH(OH)CH_2OH$

$C_3H_8O_2$ Formula wt 76.10

INS: 1520 CAS: [57-55-6]

DESCRIPTION

Propylene Glycol occurs as a clear, colorless, viscous liquid. It absorbs moisture when exposed to moist air. It is miscible with water, with acetone, and with chloroform in all proportions. It is soluble in ether and will dissolve many essential oils, but is immiscible with fixed oils.

Function Solvent; wetting agent; humectant.

REQUIREMENTS

Identification The infrared absorption spectrum of a thin film of the sample exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Propylene Glycol Reference Standard.
Assay Not less than 99.5%, by weight, of $C_3H_8O_2$.
Acidity Passes test.
Distillation Range Between 185° and 189°.
Lead Not more than 1 mg/kg.
Residue on Ignition Not more than 0.007%.
Specific Gravity Between 1.035 and 1.037.
Water Not more than 0.2%.

TESTS

Assay (See *Chromatography*, Appendix IIA.) Inject a 10-μL portion of sample into a suitable gas chromatograph in which the detector is the thermal conductivity type and the column is 1-m × 8-mm (id) stainless steel tubing (Perkin Elmer Instruments, or equivalent) packed with 4% Carbowax compound 20 M on 40- to 60-mesh Chromosorb T, or equivalent materials. The carrier gas is helium flowing at 75 mL/min. The injection port temperature is 240°; the column temperature is 120° to 200°, programmed at a rate of 5°/min; and the block temperature is 250°. Under the conditions described, the approximate retention time for Propylene Glycol is 5.7 min, and 8.2, 9.0, and 10.2 min for the three isomers of dipropylene glycol. Measure the area under all peaks by any convenient means, calculate the normalized area percentage of Propylene Glycol, and report as weight percentage.
Acidity Add 3 to 6 drops of phenol red TS to 50 mL of water, then add 0.01 *N* sodium hydroxide until the solution remains red for 30 s. Add 50 g of sample, accurately weighed, to this solution. Titrate with 0.01 *N* sodium hydroxide until the original red color returns and remains for 15 s. Not more than 1.67 mL of 0.01 *N* sodium hydroxide is required.
Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, using the following procedure: Heat a 50-g sample in a tared, 100-mL shallow dish until it ignites, and allow it to burn without further application of heat in a place free from drafts. Cool, moisten the residue with 5 mL of sulfuric acid, and ignite at about 800° for 15 min.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Propylene Glycol Alginate

Hydroxypropyl Alginate; Algin Derivative

$(C_9H_{14}O_7)_n$ (esterified) Equiv wt, calculated 234.21

INS: 405 CAS: [9005-37-2]

DESCRIPTION

Propylene Glycol Alginate occurs as a white to yellow, fibrous or granular powder. It is the propylene glycol ester of alginic acid (see the monograph for *Alginic Acid*). It varies in composition according to its degree of esterification and the percentages of free and neutralized carboxyl groups in the molecule. It dissolves in water, in solutions of dilute organic acids, and depending upon the degree of esterification, in hydroalcoholic mixtures containing up to 60% by weight of alcohol to form stable, viscous, colloidal solutions at pH 3.

Function Stabilizer; thickener; emulsifier.

REQUIREMENTS

Identification Transfer 20 mL of the saponified solution obtained in the determination of *Esterified Carboxyl Groups* (below) into a 250-mL Erlenmeyer flask, add 50 mL of 0.1 *M* periodic acid, swirl, and allow the mixture to stand for 30 min. Add 2 g of potassium iodide, titrate with 0.1 *N* sodium

thiosulfate to a faint yellow color, and then dilute the mixture to 200 mL with water. Add 5 mL of hydrochloric acid and 10 mL of modified Schiff's reagent to 10 mL of this solution. A blue to blue-violet color appears in about 20 min (*formaldehyde*). Add 1 mL of a saturated solution of piperazine hydrate and 0.5 mL of sodium nitroferricyanide TS to another 10-mL portion of the solution. A green color appears (*acetaldehyde*).

Note: Oxidation of Propylene Glycol Alginate yields formaldehyde and acetaldehyde.

Assay A sample yields not less than 16.0% and not more than 20.0% of carbon dioxide (CO_2), calculated on the dried basis.
Arsenic Not more than 3 mg/kg.
Esterified Carboxyl Groups Not less than 40.0%.
Free Carboxyl Groups Not more than 35.0%, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 20.0%.
Neutralized Carboxyl Groups Not more than 45.0%.

TESTS

Assay Determine as directed under *Alginates Assay*, Appendix IIIC.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Esterified Carboxyl Groups Quantitatively transfer the solution obtained in the determination of *Free Carboxyl Groups* (below) into a 1-L Erlenmeyer flask, add a few drops of phenolphthalein TS and 50.0 mL of 0.1 *N* sodium hydroxide. Stopper the flask, swirl the solution, and then allow it to stand for 30 min at room temperature. Titrate the excess sodium hydroxide to a faint pink endpoint with 0.1 *N* hydrochloric acid. Transfer the solution to a 600-mL beaker, and complete the titration to a pH of 7.0, determining the endpoint potentiometrically. Calculate the percentage of esterified carboxyl groups by the formula

$$(V \times 44)/(\%\ CO_2 \times W),$$

in which V is the volume, in milliliters, of 0.1 *N* sodium hydroxide consumed in this test; $\%\ CO_2$ is the percentage of carbon dioxide in the sample as determined by the *Assay*; and W is the weight, in grams, of the sample taken, calculated on the dried basis, for the *Free Carboxyl Groups* test (below).
Free Carboxyl Groups Transfer about 1 g of sample, accurately weighed, into a 600-mL beaker. Dissolve the sample in 200 mL of water, stirring mechanically for a minimum of 30 min, and titrate with 0.1 *N* sodium hydroxide to pH 7.0, determining the endpoint potentiometrically. Calculate the percentage of free carboxyl groups by the formula

$$(V \times 44)/(\%\ CO_2 \times W),$$

in which V is the volume, in milliliters, of 0.1 *N* sodium hydroxide consumed in this test; $\%\ CO_2$ is the percentage of carbon dioxide in the sample as determined by the *Assay*; and W is the weight, in grams, of the sample taken, calculated on the dried basis.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.
Neutralized Carboxyl Groups Calculate the percentage of *Neutralized Carboxyl Groups* by subtracting the sum of the percentage of *Free Carboxyl Groups* and the percentage of *Esterified Carboxyl Groups* from 100%.

Packaging and Storage Store in well-closed containers.

Propylene Glycol Mono- and Diesters

Propylene Glycol Mono- and Diesters of Fatty Acids; Propylene Glycol Monostearate (or other appropriate ester)

INS: 477

DESCRIPTION

Propylene Glycol Mono- and Diesters occur as a clear liquid or as white to yellow-white beads, flakes, or other solid material. It is a mixture of Propylene Glycol Mono- and Diesters of fats and/or fatty acids. It is insoluble in water, but it is soluble in alcohol, in ethyl acetate, and in chloroform and other chlorinated hydrocarbons.

Function Emulsifier; stabilizer.

REQUIREMENTS

Acid Value Not more than 4.
Free Propylene Glycol Not more than 1.5%.
Lead Not more than 2 mg/kg.
Residue on Ignition Not more than 0.5%.
Soap (as potassium stearate) Not more than 7.0%.
Total Monoester Content Not less than the minimum percentage claimed by the vendor.

The following specifications should conform to the representations of the vendor: *Hydroxyl Value*, *Iodine Value*, and *Saponification Value*.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
Free Propylene Glycol Determine as directed under *Free Glycerin or Propylene Glycol*, Appendix VII.
Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII, using about 2 g of sample, accurately weighed.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using a 4-g sample, accurately weighed.
Soap (as potassium stearate) Determine as directed under *Soap*, Appendix VII, using a 5-g sample, accurately weighed, and using 31.0 as the equivalence factor (*e*) in the calculation.
Total Monoester Content Determine the percentage of free propylene glycol (*G*) in the sample as directed under *Free Glycerin or Propylene Glycol*, Appendix VII, and determine the hydroxyl value (*H*) as directed in *Method II* under *Hydroxyl Value*, Appendix VII. Calculate the hydroxyl equivalent of free propylene glycol (*F*) by the formula

$$561.1G/38.05.$$

Separate the fatty acids as follows: Transfer about 25 g of sample, accurately weighed, into a 500-mL round-bottom boiling flask, add 250 mL of alcohol and 7.5 g of potassium hydroxide, and mix. Connect a suitable condenser to the flask, reflux the mixture for 1 to 2 h, then transfer to an 800-mL beaker, rinsing the flask with about 100 mL of water and adding it to the beaker. Heat on a steam bath to evaporate the alcohol, adding water occasionally to replace the alcohol, and evaporate until the odor of alcohol can no longer be detected. Adjust the final volume to about 250 mL with hot water. Neutralize the soap solution with 1:2 sulfuric acid, add 10% in excess, and heat, while stirring, until the fatty acid layer separates. Determine the acid value (*AV*) as directed in *Method I* under *Acid Value*, Appendix VII.

Calculate the average molecular weight (*mol wt*) of the monoester by the formula

$$(56{,}109/AV) + 76.10 - 18.02.$$

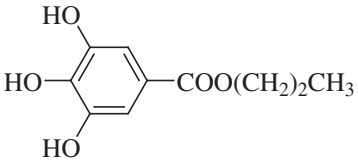
Finally, calculate the percentage of total monoester in the original sample by the formula

$$[(H - F) \times mol\ wt]/561.$$

Packaging and Storage Store in well-closed containers.

Propyl Gallate

Gallic Acid, Propyl Ester



$C_{10}H_{12}O_5$ Formula wt 212.20
INS: 310 CAS: [121-79-9]

DESCRIPTION

Propyl Gallate occurs as a fine, white to nearly white powder. It is slightly soluble in water and freely soluble in alcohol and in ether.

Function Antioxidant.

REQUIREMENTS

Identification Place about 5 g of sample and several boiling chips into a 500-mL round-bottom flask, connect a water-cooled condenser to the flask, and introduce a steady stream of nitrogen into the flask, maintaining the flow of nitrogen at all times during the remainder of the procedure. Pour 100 mL of 1 *N* sodium hydroxide through the top of the condenser, heat the solution to boiling, boil for 30 min, and cool. Place the round-bottom flask in an ice bath, and slowly, with occasional swirling, add dilute sulfuric acid (10%) until a pH of 2 to 3 is obtained (using pH paper). Filter the precipitate through a sintered-glass crucible, wash with a minimum amount of water, and dry at 110° for 2 h. The gallic acid so obtained melts at about 240° with decomposition (see *Melting Range or Temperature*, Appendix IIB).
Assay Not less than 98.0% and not more than 102.0% of $C_{10}H_{12}O_5$ after drying.
Lead Not more than 1 mg/kg.
Loss on Drying Not more than 0.5%.
Melting Range Between 146° and 150°.
Residue on Ignition Not more than 0.1%.

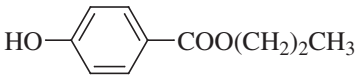
TESTS

Assay Transfer about 200 mg of sample, previously dried at 110° for 4 h and accurately weighed, into a 400-mL beaker, dissolve it in 150 mL of water, and heat to boiling. With constant and vigorous stirring, add 50 mL of bismuth nitrate TS, continue stirring and heating until precipitation is complete, and cool. Filter the yellow precipitate in a tared, sintered-glass crucible, wash it with cold 1:300 nitric acid, and dry at 110° to constant weight. The weight of the precipitate so obtained, multiplied by 0.4866, represents its equivalent of $C_{10}H_{12}O_5$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 3-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 110° for 4 h.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB, immediately using a sample from *Loss on Drying* (above) or after drying a sample at 110° for 4 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed containers.

Propylparaben

Propyl *p*-Hydroxybenzoate



$C_{10}H_{12}O_3$ Formula wt 180.20

INS: 216 CAS: [94-13-3]

DESCRIPTION

Propylparaben occurs as small, colorless crystals or as a white powder. One gram dissolves in about 2500 mL of water at 25°, in about 400 mL of boiling water, in about 1.5 mL of alcohol, and in about 3 mL of ether.

Function Preservative; antimicrobial agent.

REQUIREMENTS

Identification Dissolve about 500 mg of sample in 10 mL of 1 *N* sodium hydroxide, and boil for 30 min, allowing the solution to evaporate to a volume of about 5 mL. Cool the mixture, and carefully acidify it with 2 *N* sulfuric acid. When the precipitate is cool, collect it on a filter, wash it several times with small portions of water, and dry it in a desiccator over silica gel. The liberated *p*-hydroxybenzoic acid melts between 212° and 217° (see *Melting Range or Temperature*, Appendix IIB).
Assay Not less than 99.0% and not more than 100.5% of $C_{10}H_{12}O_3$, calculated on the dried basis.
Acidity Passes test.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.5%.
Melting Range Between 95° and 98°.
Residue on Ignition Not more than 0.05%.

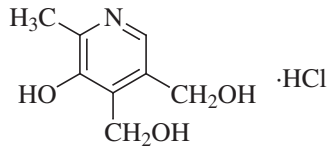
TESTS

Assay Place about 2 g of sample, accurately weighed, into a flask, add 40.0 mL of 1 *N* sodium hydroxide, and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h, cool, and titrate the excess sodium hydroxide with 1 *N* sulfuric acid to pH 6.5. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 1 *N* sodium hydroxide is equivalent to 180.2 mg of $C_{10}H_{12}O_3$, calculated on the dried basis.
Acidity Heat 750 mg of sample with 15 mL of water at 80° for 1 min, cool, and filter. The filtrate is acid or neutral to litmus. Add 0.2 mL of 0.1 *N* sodium hydroxide and 2 drops of methyl red TS to 10 mL of the filtrate. The solution is yellow, without even a light cast of pink.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample over silica gel for 5 h.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 4-g sample.

Packaging and Storage Store in well-closed containers.

Pyridoxine Hydrochloride

5-Hydroxy-6-methyl-3,4-pyridinedimethanol Hydrochloride; Pyridoxol Hydrochloride; Vitamin B_6 Hydrochloride; Vitamin B_6



$C_8H_{11}NO_3 \cdot HCl$ Formula wt 205.64

CAS: [58-56-0]

DESCRIPTION

Pyridoxine Hydrochloride occurs as colorless or white crystals or as a white, crystalline powder. It is stable in air, but is slowly affected by sunlight. Its solutions are acid to litmus, having a pH of about 3. One gram dissolves in 5 mL of water and in about 100 mL of alcohol. It is insoluble in ether. It melts at about 206° with some decomposition.

Function Nutrient.

REQUIREMENTS

Identification

A. Place 1 mL of a solution containing about 100 µg of sample in each milliliter into each of two test tubes marked *A* and *B*, and add 2 mL of a 1:5 sodium acetate solution to each tube. Add 1 mL of water to tube *A*, and add 1 mL of a 1:25 boric acid solution to tube *B*, and mix. Cool both tubes to about 20°, and rapidly add 1 mL of a 1:200 solution of 2,6-dichloroquinonechlorimide in alcohol to each tube. A blue color appears in tube *A*, which fades rapidly and becomes red in a few minutes, but no blue color appears in tube *B*.

B. Add 0.5 mL of phosphotungstic acid TS to 2 mL of a 1:200 aqueous solution. A white precipitate forms.

C. A sample gives positive tests for *Chloride*, Appendix IIIA.

Assay Not less than 98.0% and not more than 100.5% of $C_8H_{11}NO_3 \cdot HCl$, calculated on the dried basis.
Chloride Content Not less than 16.9% and not more than 17.6% of chloride (Cl), calculated on the dried basis.

Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.5%.
Residue on Ignition Not more than 0.1%.

TESTS

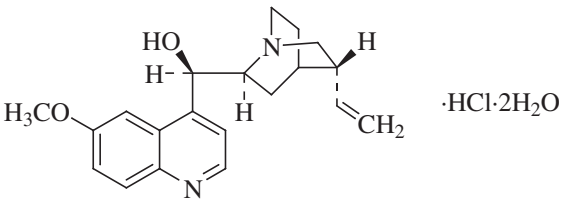
Assay Dissolve about 400 mg of sample, accurately weighed, in a mixture of 10 mL of glacial acetic acid and 10 mL of mercuric acetate TS, warming slightly to effect solution. Cool to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 20.56 mg of $C_8H_{11}NO_3 \cdot HCl$.
Chloride Content Dissolve about 500 mg of sample, accurately weighed, in 50 mL of methanol in a glass-stoppered flask. Add 5 mL of glacial acetic acid and 2 to 3 drops of eosin Y TS, and titrate with 0.1 *N* silver nitrate. Each milliliter of 0.1 *N* silver nitrate is equivalent to 3.545 mg of chloride (Cl).
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, but dry the sample in a vacuum over silica gel for 4 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in tight, light-resistant containers, and avoid exposure to sunlight.

Quinine Hydrochloride



$\cdot HCl \cdot 2H_2O$

$C_{20}H_{24}N_2O_2 \cdot HCl \cdot 2H_2O$ Formula wt 396.91

CAS: [130-89-2]

DESCRIPTION

Quinine Hydrochloride occurs as odorless, white, silky, glistening needle-like crystals with a very bitter taste. It effloresces when exposed to warm air. Its solutions are neutral or alkaline to litmus. One gram dissolves in 16 mL of water, in 1 mL of alcohol, in about 7 mL of glycerin, and in about 1 mL of chloroform. It is very slightly soluble in ether.

Function Flavoring agent.

REQUIREMENTS

Identification

A. Add 1 or 2 drops of bromine TS followed by 1 mL of 6 *N* ammonium hydroxide to 5 mL of a 1:1000 aqueous solution. The liquid turns emerald green, caused by the formation of thalleioquin.

B. A 1:100 aqueous solution is levorotatory [see *Optical (Specific) Rotation*, Appendix IIB].

C. A sample gives positive tests for *Chloride*, Appendix IIIA.

Assay Not less than 99.0% and not more than 101.5% of $C_{20}H_{24}N_2O_2 \cdot HCl$, calculated on the dried basis.
Chloroform–Alcohol Insoluble Substances Passes test.
Loss on Drying Not more than 10.0%.
Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Between −247° and −252°.
Other Cinchona Alkaloids Passes test.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.15%.
Sulfate Not more than 0.05%.

TESTS

Assay Dissolve about 150 mg of sample, accurately weighed, in 20 mL of acetic anhydride; add 2 drops of malachite green TS and 5.5 mL of mercuric acetate TS; and titrate with 0.1 *N* perchloric acid from a microburet to a yellow endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 17.99 mg of $C_{20}H_{24}N_2O_2 \cdot HCl$.
Chloroform–Alcohol Insoluble Substances One gram of sample dissolves completely in 7 mL of a 2:1 (v/v) mixture of chloroform:absolute alcohol.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 120° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 200 mg of sample in 10 mL of 0.1 *N* hydrochloric acid.
Other Cinchona Alkaloids Dissolve about 2.5 g of sample in 60 mL of water contained in a separator, add 10 mL of 6 *N* ammonium hydroxide, extract the mixture successively with 30 mL and 20 mL of chloroform, and evaporate the combined chloroform extracts to dryness on a steam bath. Dissolve 1.5 g of the residue in 25 mL of alcohol; dilute the solution with 50 mL of hot water; add 1 *N* sulfuric acid (about 5 mL) until the solution is acid, using 2 drops of methyl red TS as the indicator; and neutralize the excess acid with 1 *N* sodium hydroxide. Evaporate the solution to dryness on a steam bath,

powder the residue, and agitate it in a test tube with 20 mL of water at 65° for 30 min. Cool the mixture to 15°; macerate it at this temperature for 2 h, shaking it occasionally; and filter it through an 8- to 10-cm filter paper. Transfer 5 mL of the filtrate, at a temperature of 15°, into a test tube, and mix and shake it gently with 6 mL of 6 *N* ammonium hydroxide (which must contain between 10% and 10.2% of NH_3, have a temperature of 15°, and be added all at once). A clear liquid evolves.

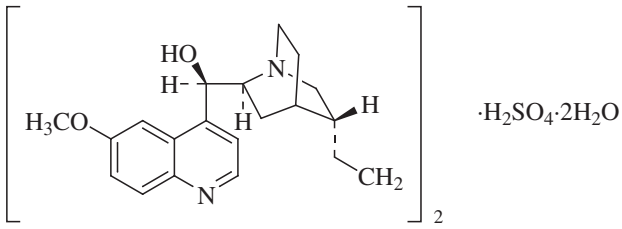
Readily Carbonizable Substances Determine as directed under *Readily Carbonizable Substances*, Appendix IIB, using 100 mg of sample dissolved in 2 mL of 95% sulfuric acid. The solution is no darker than *Matching Fluid M*.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 500-mg sample does not exceed that shown in a control containing 250 µg of sulfate (SO_4).

Packaging and Storage Store in tight, light-resistant containers.

Quinine Sulfate



$(C_{20}H_{24}N_2O_2)_2{\cdot}H_2SO_4{\cdot}2H_2O$ Formula wt 782.96

CAS: anhydrous [804-63-7]

DESCRIPTION

Quinine Sulfate occurs as odorless, fine, white, needle-like crystals, usually lusterless, with a persistent, very bitter taste. It makes a light and readily compressible mass. It darkens on exposure to light. Its saturated solution is neutral or alkaline to litmus. One gram dissolves in about 500 mL of water and in about 120 mL of alcohol at 25°, in about 35 mL of water at 100°, and in about 10 mL of alcohol at 80°. It is slightly soluble in chloroform and in ether, but is freely soluble in a 2:1 (v/v) mixture of chloroform:absolute alcohol.

Function Flavoring agent.

REQUIREMENTS

Identification

A. Add 1 or 2 drops of bromine TS followed by 1 mL of 6 *N* ammonium hydroxide to 5 mL of a 1:1000 aqueous solution. The liquid turns emerald green, caused by the formation of thalleioquin.

B. Acidify a saturated solution of the sample with 2 *N* sulfuric acid. The resulting solution has a vivid blue fluorescence and is levorotatory [see *Optical (Specific) Rotation*, Appendix IIB].

C. A 1:50 aqueous solution made with the aid of a few drops of hydrochloric acid gives positive tests for *Sulfate*, Appendix IIIA.

Assay Not less than 99.0% and not more than 101.0% of $(C_{20}H_{24}N_2O_2)_2{\cdot}H_2SO_4$, calculated on the dried basis.

Chloroform–Alcohol Insoluble Substances Not more than 0.1%.

Loss on Drying Not more than 5.0%.

Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between −240° and −244°.

Other Cinchona Alkaloids Passes test.

Readily Carbonizable Substances Passes test.

Residue on Ignition Not more than 0.05%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 20 mL of acetic anhydride, add 2 drops of malachite green TS, and using a microburet, titrate with 0.1 *N* perchloric acid to a yellow endpoint.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 24.90 mg of $(C_{20}H_{24}N_4O_2)_2{\cdot}H_2SO_4$.

Chloroform–Alcohol Insoluble Substances Warm 2 g of sample with 15 mL of a 2:1 (v/v) mixture of chloroform:absolute alcohol at 50° for 10 min. Filter through a tared, sintered-glass filter, using gentle suction, and wash the filter with five 10-mL portions of the chloroform:alcohol mixture. Dry at 105° for 1 h, cool, and weigh.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 120° for 3 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 200 mg of sample in 10 mL of 0.1 *N* hydrochloric acid.

Other Cinchona Alkaloids Agitate 1.8 g of sample, previously dried at 50° for 2 h, with 20 mL of water at 65° for 30 min. Cool the mixture to 15°; macerate it at this temperature for 2 h, shaking it occasionally; and filter it through an 8- to 10-cm filter paper. Transfer 5 mL of the filtrate, at a temperature of 15°, into a test tube, and mix it gently, without shaking, with 6 mL of 6 *N* ammonium hydroxide (which must contain between 10% and 10.2% of NH_3, have a temperature of 15°, and be added all at once). A clear liquid evolves.

Readily Carbonizable Substances Determine as directed under *Readily Carbonizable Substances*, Appendix IIB, using 200 mg of sample dissolved in 5 mL of 95% sulfuric acid. The solution is no darker than *Matching Fluid M*.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Rapeseed Oil, Fully Hydrogenated

Fully Hydrogenated Rapeseed Oil

INS: 441 CAS: [84681-71-0]

DESCRIPTION

Rapeseed Oil, Fully Hydrogenated, occurs as a white, waxy solid. It is a mixture of triglycerides. The saturated fatty acids are found in the same proportions that result from the full hydrogenation of fatty acids occurring in natural high-erucic acid rapeseed oil. The rapeseed oil is obtained from *Brassica juncea*, *Brassica napus*, and *Brassica rapa* (Fam. Cruciferae). It is made by hydrogenating high-erucic acid rapeseed oil in the presence of a nickel catalyst at temperatures not exceeding 245°.

Function Cooking or salad oil; component of margarine or shortening; coating agent; emulsifying agent; stabilizer; thickener; texturizer.

REQUIREMENTS

Labeling Rapeseed Oil products that have been fully hydrogenated should be labeled as Fully Hydrogenated Rapeseed Oil. Label to indicate the *1-Monoglyceride Content* as well.
Identification Fully Hydrogenated Rapeseed Oil exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII.

Fatty Acid:	14:0	16:0	18:0	18:1	18:2
Weight % (Range):	<1.0	3–5	38–42	1.0	<1.0
Fatty Acid:	20:0	20:1	22:0	22:1	24:0
Weight % (Range):	8–10	<1.0	42–50	<1.0	1.0–2.0

Acid Value Not more than 6.
Color (AOCS-Wesson) Not more than 1.5 red/15 yellow.
Erucic Acid Not more than 1.0%.
Free Fatty Acids (as oleic acid) Not more than 2.0%.
Iodine Value Not more than 4.
Lead Not more than 0.1 mg/kg.
1-Monoglyceride Content Conforms to the representation of the vendor.
Peroxide Value Not more than 2.0 meq/kg.
Residue on Ignition Not more than 0.5%.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.05%.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.
Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII, using a 13.34-cm cell.
Erucic Acid Determine as part of *Fatty Acid Composition*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (e) in the formula given in the procedure:

Free fatty acids as oleic acid, e = 28.2.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
1-Monoglyceride Content Determine as directed under *1-Monoglycerides*, Appendix VII.
Peroxide Value Determine as directed in *Method II* under *Peroxide Value*, Appendix VII.
Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of a 1:1 solution of chloroform:methanol to dissolve the sample.

Packaging and Storage Store in tightly closed containers.

Rapeseed Oil, Superglycerinated

Superglycerinated Fully Hydrogenated Rapeseed Oil

DESCRIPTION

Rapeseed Oil, Superglycerinated, occurs as a white solid. It is a mixture of mono-, di-, and triglycerides, with triglycerides as a minor component. The saturated fatty acids are found in the same proportions that result from the full hydrogenation of fatty acids occurring in natural high-erucic acid rapeseed oil. The rapeseed oil is typically obtained by *n*-hexane extraction from *Brassica juncea*, *Brassica napus*, and *Brassica rapa* (Fam. Cruciferae). It is made by adding excess glycerin to fully hydrogenated rapeseed oil and heating to about 165° in the presence of a sodium hydroxide catalyst under partial vacuum and steam sparging agitation.

Function Cooking or salad oil; component of margarine or shortening; coating agent; emulsifying agent; texturizer.

REQUIREMENTS

Labeling Rapeseed Oil products that have added glycerin (glycerol) and that are fully hydrogenated should be labeled as Fully Hydrogenated and Superglycerinated Rapeseed Oil. The *1-Monoglyceride Content* and *Hydroxyl Value* should be indicated as well.

Identification Superglycerinated Rapeseed Oil exhibits the same fatty acid composition as fully hydrogenated rapeseed oil. It exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	14:0	16:0	18:0	18:1	18:2
Weight % (Range):	<1.0	3–5	38–42	1.0	<1.0
Fatty Acid:	20:0	20:1	22:0	22:1	24:0
Weight % (Range):	8–10	<1.0	42–50	<1.0	1.0–2.0

Acid Value Not more than 6.

Color (AOCS-Wesson) Not more than 1.5 red/15 yellow.

Erucic Acid Not more than 1.0%.

Free Fatty Acids (as oleic acid) Not more than 2.0%.

Free Glycerin Not more than 1%.

Hydroxyl Value Conforms to the representation of the vendor.

Iodine Value Not more than 4.

Lead Not more than 0.1 mg/kg.

1-Monoglyceride Content Conforms to the representation of the vendor.

Peroxide Value Not more than 2.0 meq/kg.

Residue on Ignition Not more than 0.5%.

Unsaponifiable Matter Not more than 1.5%.

Water Not more than 0.05%.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII, using a 13.34-cm cell.

Erucic Acid Determine as part of *Fatty Acid Composition*, Appendix VII.

Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (e) in the formula given in the procedure:

Free fatty acids as oleic acid, e = 28.2.

Free Glycerin Determine as directed under *Free Glycerin or Propylene Glycol*, Appendix VII.

Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

1-Monoglyceride Content Determine as directed under *1-Monoglycerides*, Appendix VII.

Peroxide Value Determine as directed in *Method II* under *Peroxide Value*, Appendix VII.

Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.

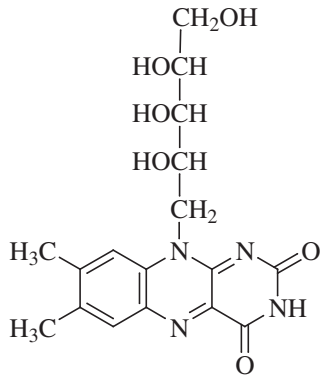
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.

Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of a 1:1 solution of chloroform:methanol to dissolve the sample.

Packaging and Storage Store in tightly closed containers.

Riboflavin

Vitamin B_2



$C_{17}H_{20}N_4O_6$ Formula wt 376.37

INS: 101(i) CAS: [83-88-5]

DESCRIPTION

Riboflavin occurs as a yellow to orange-yellow, crystalline powder. When dry, it is not affected by diffused light, but when in solution, light induces deterioration. It melts at about 280° with decomposition, and its saturated solution is neutral to litmus. One gram dissolves in 3000 to about 20,000 mL of water, the variations being due to differences in the internal crystalline structure. It is less soluble in alcohol than in water. It is insoluble in ether and in chloroform, but it is very soluble in dilute solutions of alkalies.

Function Nutrient.

REQUIREMENTS

Identification A solution of 1 mg of sample in 100 mL of water is pale green-yellow by transmitted light and has an intense yellow-green fluorescence that disappears on the addition of mineral acids or alkalies.

Assay Not less than 98.0% and not more than 102.0% of $C_{17}H_{20}N_4O_6$, calculated on the dried basis.

Loss on Drying Not more than 1.5%.
Lumiflavin Passes test.
Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Between +56.5° and +59.5°, calculated on the dried basis.
Residue on Ignition Not more than 0.3%.

TESTS

Assay (**Note**: Conduct this assay so that the solutions are protected from direct sunlight at all stages.)

Sample Solution Transfer about 50 mg of sample, accurately weighed, into a 1000-mL volumetric flask containing about 50 mL of water. Add 5 mL of 6 *N* acetic acid and sufficient water to make about 800 mL. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to about 25°, add water to volume, and mix. Dilute this solution with water, quantitatively and stepwise, to bring it within the operating sensitivity of the fluorometer used.

Standard Solution In the same manner, prepare a standard solution to contain, in each milliliter, a quantity of USP Riboflavin Reference Standard, accurately weighed, equivalent to that of the *Sample Solution*.

Procedure Measure the intensity of the *Standard Solution*'s fluorescence in a fluorometer at about 530 nm, using an excitation wavelength of about 440 nm. Directly after the reading, add about 10 mg of sodium hydrosulfite to the *Standard Solution*, stirring with a glass rod until dissolved, and immediately measure the fluorescence again. The difference between the two readings represents the intensity of the fluorescence caused by the Reference Standard.

Similarly, measure the intensity of the fluorescence of the *Sample Solution*, both before and after the addition of sodium hydrosulfite. Calculate the quantity of $C_{17}H_{20}N_4O_6$ in the final *Sample Solution* by the formula

$$C(I_U/I_S),$$

in which *C* is the concentration, in gram degrees per milliliter, of USP Riboflavin Reference Standard in the final solution of the *Standard Solution*; and I_U and I_S are the corrected fluorescence values observed for the solutions of the sample and the Reference Standard, respectively.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Lumiflavin

Alcohol-Free Chloroform Shake 20 mL of chloroform gently, but thoroughly, with 20 mL of water for 3 min, draw off the chloroform layer, and wash twice more with 20-mL portions of water. Finally, filter the chloroform through a dry filter paper, shake it well for 5 min with 5 g of powdered anhydrous sodium sulfate, allow the mixture to stand for 2 h, and decant or filter the clear chloroform.

Procedure Shake 25 mg of sample with 10 mL of *Alcohol-Free Chloroform* for 5 min, and filter. The absorbance of the filtrate, determined in a 1-cm cell at 440 nm with a suitable spectrophotometer, using *Alcohol-Free Chloroform* as the blank, does not exceed 0.025.

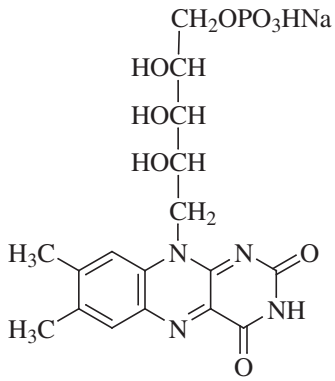
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution of hydrochloric acid containing 50 mg of sample in each 10 mL.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight, light-resistant containers.

Riboflavin 5′-Phosphate Sodium

Flavin Mononucleotide, Sodium Salt; Riboflavin 5′-Phosphate Ester Monosodium Salt; Riboflavin 5′-Phosphate Ester Monosodium Salt, Dihydrate



$C_{17}H_{20}N_4NaO_9P{\cdot}2H_2O$ Formula wt 514.36

INS: 101(ii) CAS: [130-40-5]

DESCRIPTION

Riboflavin 5′-Phosphate Sodium occurs as a fine, orange-yellow, crystalline powder. One gram dissolves in about 30 mL of water. When dry, it is not affected by diffused light, but when in solution, light induces deterioration rapidly. It is hygroscopic.

Function Nutrient.

REQUIREMENTS

Identification A 1.5:100 (w/v) aqueous solution responds to the *Identification Test* in the monograph for *Riboflavin*.
Assay Not less than the equivalent of 73.0% and not more than the equivalent of 79.0% of riboflavin ($C_{17}H_{20}N_4O_6$), calculated on the dried basis.
Free Phosphate Not more than 1.0%, calculated as PO_4.
Free Riboflavin Not more than 6.0%, calculated on the dried basis.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 7.5%.
Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Between +37.0° and +42.0°, calculated on the dried basis.

pH of a 1:100 Solution Between 5.0 and 6.5.
Residue on Ignition Not more than 25.0%.
Riboflavin Diphosphate Not more than 6.0% as riboflavin ($C_{17}H_{20}N_4O_6$), calculated on the dried basis.

TESTS

Assay (**Note**: Use low-actinic glassware, and conduct this assay so that all solutions are protected from direct sunlight at all stages.)

Standard Preparation Transfer about 35 mg of USP Riboflavin Reference Standard, accurately weighed, into a 250-mL Erlenmeyer flask, add 20 mL of pyridine and 75 mL of water, and dissolve the riboflavin by frequent shaking. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. Transfer 10.0 mL of this solution into a second 1000-mL volumetric flask, add sufficient 0.1 *N* sulfuric acid (about 4 mL) so that the final pH of the solution is between 5.9 and 6.1, dilute to volume with water, and mix.

Assay Preparation Transfer about 50 mg of sample, accurately weighed, into a 250-mL Erlenmeyer flask, add 20 mL of pyridine and 75 mL of water, and dissolve the sample by frequent shaking. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. Transfer 10.0 mL of this solution into a second 1000-mL volumetric flask, add sufficient 0.1 *N* sulfuric acid (about 4 mL) so that the final pH of the solution is between 5.9 and 6.1, dilute to volume with water, and mix.

Procedure Using a suitable fluorometer, determine the intensity of the fluorescence of each solution at about 530 nm, using an excitation wavelength of about 440 nm. Record the fluorescence of the *Assay Preparation* as I_U, and that of the *Standard Preparation* as I_S. Calculate the quantity, in milligrams, of $C_{17}H_{20}N_4O_6$ in the sample taken, by the formula

$$100C \times I_U/I_S,$$

in which C is the exact concentration, in micrograms per milliliter, of the *Standard Preparation*, corrected for loss on drying.

Free Phosphate

Acid Molybdate Solution Dilute 25 mL of ammonium molybdate solution [7 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in sufficient water to make 100 mL] to 200 mL with water, and slowly add 25 mL of 7.5 *N* sulfuric acid.

Ferrous Sulfate Solution Just before use, prepare a 10% aqueous ferrous sulfate solution containing 2 mL of 7.5 *N* sulfuric acid per 100 mL of final solution.

Blank Mix 10.0 mL of water, 10.0 mL of *Acid Molybdate Solution*, and 5.0 mL of *Ferrous Sulfate Solution*.

Standard Preparation Transfer 220.0 mg of monobasic potassium phosphate (KH_2PO_4) into a 1000-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 20.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix.

Test Preparation Transfer 300.0 mg of sample into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Procedure Transfer 10.0 mL each of the *Standard Preparation* and of the *Test Preparation* into separate 50-mL Erlenmeyer flasks, add 10.0 mL of *Acid Molybdate Solution* and 5.0 mL of *Ferrous Sulfate Solution* to each flask, and mix. Determine the absorbance of each preparation solution and the *Blank* in a 1-cm cell at 700 nm using a suitable spectrophotometer. The absorbance of the solution from the *Test Preparation* is not greater than that of the *Standard Preparation*.

Free Riboflavin and Riboflavin Diphosphate (**Note**: Conduct this test so that all solutions are protected from actinic light at all stages, preferably by using low-actinic glassware.)

Mobile Phase Mix 850 mL of 0.054 *M* monobasic potassium phosphate with 150 mL of methanol, filter, and degas the solution. Make adjustments if necessary.

Standard Preparation Transfer 60 mg of USP Riboflavin Reference Standard, accurately weighed, into a 250-mL volumetric flask, dissolve carefully in 1 mL of hydrochloric acid, dilute to volume with water, and mix. Pipet a 4-mL aliquot into a 100-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Test Preparation Transfer 100.0 mg of sample into a 100-mL volumetric flask, dissolve in 50 mL of water, dilute to volume with *Mobile Phase*, and mix. Pipet 8 mL of this solution into a 50-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

System Suitability Preparation Dissolve USP Phosphated Riboflavin Reference Standard in water to obtain a solution containing 2 mg/mL. Add an equal volume of *Mobile Phase*, and mix. Dilute 8 mL of this solution to 50 mL with *Mobile Phase*, and mix.

Chromatographic System (See *Chromatography,* Appendix IIA.) Use a liquid chromatograph equipped with a fluorometric detector set at 440-nm excitation wavelength and provided with a 470-nm emission filter or set at about 530 nm for a fluorescence detector that uses a monochromator for emission wavelength selection, and a 30-cm × 3.9-mm (id) column that is packed with μBondapak C_{18}, or equivalent. Set the flow rate at about 2.0 mL/min. Chromatograph the *System Suitability Preparation*, and record the peak responses. The retention time for riboflavin 5′-monophosphate is about 20 to 25 min, and the approximate relative retention times for the components are

Riboflavin 3′4′-diphosphate:	0.23
Riboflavin 3′5′-diphosphate:	0.39
Riboflavin 4′5′-diphosphate:	0.58
Riboflavin 3′-monophosphate:	0.70
Riboflavin 4′-monophosphate:	0.87
Riboflavin 5′-monophosphate:	1.00
Riboflavin	1.63

The resolution, R, between the peaks for riboflavin 4′-monophosphate and riboflavin 5′-monophosphate is not less than 1.0, and the relative standard deviation of the response for riboflavin 5′-monophosphate in replicate injections is not more than 1.5%.

Procedure Separately inject equal volumes (about 100 μL) of the *Standard Preparation*, the *Test Preparation*, and the *System Suitability Preparation* into the chromatograph.

Measure the peak responses obtained from the *Standard Preparation* and the *Test Preparation*, identifying the peaks to be measured in the chromatogram of the *Test Preparation* by comparing retention times with those of the peaks in the chromatogram of the *System Suitability Preparation*. Calculate the percentage of free riboflavin by the formula

$$625C(r_F/r_S),$$

and calculate the percentage of riboflavin in the form of riboflavin diphosphates by the formula

$$625C(r_D/r_S),$$

in which C is the concentration, in milligrams per milliliter, of USP Riboflavin Reference Standard in the *Standard Preparation*; r_F is the riboflavin peak response, if any, obtained from the *Test Preparation*; r_D is the sum of the responses for any of the three riboflavin diphosphate peaks obtained from the *Test Preparation*; and r_S is the riboflavin peak response obtained from the *Standard Preparation*.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 100° in a vacuum over phosphorus pentoxide for 5 h.

Optical (Specific) Rotation Transfer about 750 mg of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in and dilute to volume with 20% hydrochloric acid, and mix. Determine the rotation in a 1-dm tube within 15 min.

pH of a 1:100 Solution Determine as directed under *pH Determination*, Appendix IIB.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight, light-resistant containers.

Rice Bran Wax

INS: 908 CAS: [8016-60-2]

View IR

DESCRIPTION

Rice Bran Wax occurs as a hard, slightly crystalline substance that ranges in color from tan to light brown. It is a refined wax obtained from rice bran. It is soluble in chloroform, but is insoluble in water.

Function Masticatory substance in chewing gum base; coating agent.

REQUIREMENTS

Identification The infrared absorption spectrum of a melted sample on a potassium bromide plate exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Free Fatty Acids Not more than 10.0%.

Iodine Value Not more than 20.

Lead Not more than 3 mg/kg.

Melting Range Between 75° and 80°.

Saponification Value Between 75 and 120.

TESTS

Free Fatty Acids Determine as directed under *Free Fatty Acids*, Appendix VII.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV.

Melting Range Determine as directed in *Procedure for Class II* under *Melting Range or Temperature*, Appendix IIB.

Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Packaging and Storage Store in well-closed containers.

Rosemary Oil

CAS: [8000-25-7]

FEMA: 2992

View IR

DESCRIPTION

Rosemary Oil occurs as a colorless or pale yellow liquid with the characteristic odor of rosemary and a warm, camphoraceous taste. It is the volatile oil obtained by steam distillation from the fresh flowering tops of *Rosemarinus officinalis* L. (Fam. Labiatae). It is soluble in most vegetable oils, but insoluble in alcohol and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay for Esters Not less than 1.5% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).

Assay for Total Borneol Not less than 8.0% of borneol ($C_{10}H_{18}O$).

Angular Rotation Between −5° and +10°.

Refractive Index Between 1.464 and 1.476 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.894 and 0.912.

TESTS

Assay for Esters Determine as directed in *Ester Determination* under *Esters*, Appendix VI, using about 10 mL of sample, accurately weighed, and 98.15 as the equivalence factor (*e*) in the calculation.

Assay for Total Borneol Determine as directed under *Total Alcohols*, Appendix VI, using 5 mL of dried, acetylated sample, accurately weighed, for the saponification. Calculate the percentage of total borneol by the formula

$$7.712A(1 - 0.0021E)/(B - 0.021A),$$

in which *A* is the difference between the number of milliliters of 0.5 *N* hydrochloric acid required for the sample and the number of milliliters of 0.5 *N* hydrochloric acid required for the residual blank titration; *E* is the percentage of esters calculated as bornyl acetate ($C_{12}H_{20}O_2$); and *B* is the weight, in grams, of the acetylated sample taken.

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 90% alcohol. Upon further dilution, the solution may become turbid.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.

Rose Oil

CAS: [8007-01-0]

View IR

DESCRIPTION

Rose Oil occurs as a colorless or yellow liquid with the characteristic odor and taste of rose. It is the volatile oil obtained by steam distillation from the fresh flowers of *Rosa gallica* L., *Rosa damascena* Miller, *Rosa alba* L., *Rosa centifolia* L., and varieties of these species (Fam. Rosaceae). At 25° it is a viscous liquid. Upon gradual cooling it changes to a translucent, crystalline mass, which may be liquefied by warming.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Angular Rotation Between −1° and −4°.

Refractive Index Between 1.457 and 1.463 at 30°.

Solubility Passes test.

Specific Gravity Between 0.848 and 0.863 at 30°/15°.

TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

Solubility One milliliter of sample is miscible with 1 mL of chloroform without turbidity. Add 20 mL of 90% alcohol to this mixture. The resulting liquid is neutral or acid to moistened litmus paper and, on standing at 20°, deposits crystals within 5 min.

Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers.

Rue Oil

CAS: [8014-29-7]

FEMA: 2995

View IR

DESCRIPTION

Rue Oil occurs as a yellow to yellow-amber liquid with a characteristic fatty odor. It is the volatile oil obtained by steam distillation from the fresh blossoming plants *Ruta graveolens* L., *Ruta montana* L., or *Ruta bracteosa* L. (Fam. Rutaceae). It is soluble in most fixed oils and in mineral oil, but it is relatively insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 90.0% of ketones, calculated as methyl nonyl ketone ($C_{11}H_{22}O$).

Angular Rotation Between −1° and +3°.

Refractive Index Between 1.430 and 1.440 at 20°.

Solidification Point Between 7.5° and 10.5°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.826 and 0.838.

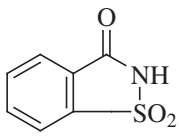
TESTS

Assay Determine as directed in the *Hydroxylamine Method* under *Aldehydes and Ketones*, Appendix VI, using about 1 g of sample, accurately weighed, and 85.10 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solidification Point Determine as directed under *Solidification Point*, Appendix IIB.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 4 mL of 70% alcohol, occasionally with opalescence or precipitation of solids.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Saccharin

o-Benzosulfimide; Gluside; 1,2-Benzisothiazole-3(2H)-one-1,1-dioxide



$C_7H_5NO_3S$ Formula wt 183.18

INS: 954 CAS: [81-07-2]

DESCRIPTION

Saccharin occurs as white crystals or as a white, crystalline powder. Its solutions are acid to litmus. One gram is soluble in 290 mL of water at 25°, in 25 mL of boiling water, and in 30 mL of alcohol. It is slightly soluble in chloroform and in ether, and it is readily dissolved by dilute solutions of ammonia, solutions of alkali hydroxides, or solutions of alkali carbonates with the evolution of carbon dioxide.

Function Nonnutritive sweetener.

REQUIREMENTS

Identification

A. Dissolve about 100 mg of sample in 5 mL of a 1:20 solution of sodium hydroxide, evaporate the mixture to dryness, and gently fuse the residue over a small flame until ammonia no longer evolves. After the residue has cooled, dissolve it in 20 mL of water, neutralize the solution with 2.7 *N* hydrochloric acid, and filter. Add 1 drop of ferric chloride TS to the filtrate. A violet color appears.

B. Mix 20 mg of sample with 40 mg of resorcinol, cautiously add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 mL of water and an excess of 1 *N* sodium hydroxide. A fluorescent green liquid results.
Assay Not less than 98.0% and not more than 101.0% of $C_7H_5NO_3S$ after drying.
Benzoic and Salicylic Acids Passes test.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 1%.
Melting Range Between 226° and 230°.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.2%.
Selenium Not more than 0.003%.
Toluenesulfonamides Not more than 0.0025%.

TESTS

Assay Dissolve about 500 mg of sample, previously dried at 105° for 2 h and accurately weighed, in 75 mL of hot water, cool quickly, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 18.32 mg of $C_7H_5NO_3S$.
Benzoic and Salicylic Acids Dropwise, add ferric chloride TS to 10 mL of a hot, saturated solution. No precipitate or violet color appears in the liquid.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.
Melting Range Determine as directed in the *Procedure for Class Ia* under *Melting Range or Temperature*, Appendix IIB.
Readily Carbonizable Substances Determine as directed under *Readily Carbonizable Substances*, Appendix IIB, using 200 mg of sample dissolved in 5 mL of 95% sulfuric acid and kept at 48° to 50° for 10 min. The color is no darker than that of *Matching Fluid A*.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Selenium Determine as directed in *Method I* under *Selenium Limit Test*, Appendix IIIB, using 200 mg of sample.
Toluenesulfonamides

Methylene Chloride Use a suitable grade (such as that obtainable from Burdick & Jackson Laboratories, Inc.), equivalent to the product obtained by distillation in an all-glass apparatus.

Internal Standard Stock Solution Transfer 100.0 mg of 95% *n*-tricosane (obtainable from Chemical Samples Co.) into a 10-mL volumetric flask, dissolve in and dilute to volume with *n*-heptane, and mix.

Stock Standard Preparation Transfer 20.0 mg each of reagent-grade *o*-toluenesulfonamide and *p*-toluenesulfonamide into a 10-mL volumetric flask, dissolve in and dilute to volume with methylene chloride, and mix.

Diluted Standard Preparations Pipet 0.1, 0.25, 1.0, 2.5, and 5.0 mL, respectively, of the *Stock Standard Preparation* into five 10-mL volumetric flasks. Pipet 0.25 mL of the *Internal Standard Stock Solution* into each flask, dilute each to volume with methylene chloride, and mix. These solutions contain, respectively, 20, 50, 200, 500, and 1000 μg/mL of each toluenesulfonamide, plus 250 μg of *n*-tricosane.

Test Preparation (See *Chromatography*, Appendix IIA.) Dissolve 2.00 g of sample in 8.0 mL of sodium carbonate TS, and mix the solution thoroughly with 10.0 g of chromatographic siliceous earth (Celite 545, Johns-Manville, or equivalent). Transfer the mixture into a 250- × 25-mm chromatographic tube having a fritted-glass disk and a Teflon stopcock at the bottom and a reservoir at the top. Pack the contents of the tube by tapping the column on a padded surface, and then by tamping firmly from the top. Place 100 mL of methylene chloride in the reservoir, and adjust the stopcock so that 50 mL of eluate is collected in 20 to 30 min. Add 25 μL of *Internal Standard Stock Solution* to the eluate, mix, and then concentrate the solution to a volume of 1.0 mL in a suitable concentrator tube fitted with a modified Snyder column, using a Kontes tube heater maintained at 90°.

Procedure Inject 2.5 μL of the *Test Preparation* into a suitable gas chromatograph equipped with a flame-ionization detector and a 3-m × 2-mm (id) glass column, or equivalent, packed with 3% phenyl methyl silicone (OV-17, Applied Science Laboratories, Inc., or equivalent) on 100- to 120-mesh, silanized, calcined, diatomaceous silica (Gas-Chrom Q, Applied Science, or equivalent).

> **Caution**: The glass column should extend into the injector for on-column injection and into the detector base to avoid contact with metal.

Maintain the column at 180°. Set the injection port temperature to 225° and the detector to 250°. Use helium as the carrier gas with a flow rate of 30 mL/min. Adjust the instrument attenuation setting so that 2.5 μL of the *Diluted Standard Preparation* containing 200 μg/mL of each toluenesulfonamide gives a response of 40% to 80% of full-scale deflection. Record the chromatogram, note the peaks for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and the *n*-tricosane internal standard, and calculate the areas for each peak by suitable means. The retention times for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane are about 5, 6, and 15 min, respectively.

In a similar manner, obtain the chromatograms for 2.5-μL portions of each of the five *Diluted Standard Preparations*, and for each solution, determine the areas of the *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane peaks. From the values thus obtained, prepare standard curves by plotting concentration of each toluenesulfonamide, in micrograms per milliliter, versus the ratio of the respective toluenesulfonamide peak area to that of *n*-tricosane. From the standard curve, determine the concentration, in micrograms per milliliter, of each toluenesulfonamide in the *Test Preparation*. Divide each value by 2 to convert the result to milligrams per kilogram of the toluenesulfonamide in the 2-g sample taken for analysis.

> **Note**: If the toluenesulfonamide content of the sample is greater than about 500 mg/kg, the impurity may crystallize out of the methylene chloride concentrate (see *Test Preparation*). Although this level of impurity exceeds that permitted by the specification, the analysis may be completed by diluting the concentrate with methylene chloride containing 250 μg of *n*-tricosane per milliliter, and by applying appropriate dilution factors in the calculation. Care must be taken to redissolve completely any crystalline toluenesulfonamide to give a homogeneous solution.

Packaging and Storage Store in well-closed containers.

Safflower Oil (Unhydrogenated)

CAS: [8001-23-8]

DESCRIPTION

Safflower Oil, Unhydrogenated, occurs as a light yellow oil. It is obtained from the plant *Carthamus tinctorius* (Fam. Asteraceae) by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21° to 27°, but traces of wax may cause the oil to cloud unless removed by winterization. Safflower Oil has the highest linoleic acid [(*Z*),(*Z*)-9,12-octadecadienoic acid] content (typically about 78% of total fatty acids) of any known oil. It is free from visible foreign matter at 21° to 27°.

Function Coating agent; texturizer.

REQUIREMENTS

Identification Safflower Oil exhibits the following composition profile of fatty acids, determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<1.0	2–10	<0.5	1–10	7–16	72–81
Fatty Acid:	18:3	20:0	20:1				
Weight % (Range):	<1.5	<0.5	<0.5				

Cold Test Passes test.
Color (AOCS-Wesson) Not more than 1.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 135 and 150.
Lead Not more than 0.1 mg/kg.
Linoleic Acid Not less than 72% of total fatty acids.
Linolenic Acid Not more than 1.5%.
Peroxide Value Not more than 10 meq/kg.

Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Cold Test Determine as directed under *Cold Test*, Appendix VII.
Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Linoleic Acid Determine as directed under *Fatty Acid Composition*, Appendix VII.
Linolenic Acid Determine as directed under *Fatty Acid Composition*, Appendix VII.
Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed in *Method Ia* under *Water Determination*, Appendix IIB. However, in the *Procedure*, in place of 35 to 40 mL of methanol use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Sage Oil, Dalmatian Type

CAS: [8022-56-8]

View IR

DESCRIPTION

Sage Oil, Dalmatian Type, occurs as a yellow or green-yellow liquid with a warm, camphoraceous and thujone odor and flavor. It is the oil obtained by steam distillation from the partially dried leaves of the plant *Salvia officinalis* L. (Fam. Labiatae). It is soluble in most fixed oils and in mineral oil. Frequently the solutions in mineral oil are opalescent. It is slightly soluble in propylene glycol, but it is practically insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 50.0% of ketones, calculated as thujone ($C_{10}H_{16}O$).
Angular Rotation Between +2° and +29°.
Ester Value after Acetylation Between 25 and 60.
Refractive Index Between 1.457 and 1.469 at 20°.
Saponification Value Between 5 and 20.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.903 and 0.925.

TESTS

Assay Determine as directed in the *Hydroxylamine Method* under *Aldehydes and Ketones*, Appendix VI, using about 1 g of sample, accurately weighed, and 76.12 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Ester Value after Acetylation Determine as directed under *Total Alcohols*, Appendix VI, using about 2.5 g of acetylated oil. Calculate the ester value after acetylation by the formula

$$A \times 28.05/B,$$

in which *A* is the number of milliliters of 0.5 *N* alcoholic potassium hydroxide consumed in the saponification of the acetylated oil; and *B* is the weight, in grams, of the acetylated oil taken as the sample.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 80% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Sage Oil, Spanish Type

CAS: [8016-65-7]

View IR

DESCRIPTION

Sage Oil, Spanish-Type, occurs as a colorless to slightly yellow oil with a camphoraceous odor that has a cineole top note. It is the volatile oil obtained by distillation from the plants of *Salvia lavandulaefolia* Vahl. or *Salvia hispanorium* Lag. (Fam. Labiatae). It is soluble in most fixed oils and in

glycerin. It is soluble, usually with opalescence, in mineral oil and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Angular Rotation Between −3° and +24°.
Refractive Index Between 1.468 and 1.473 at 20°.
Saponification Value Between 14 and 57.
Saponification Value after Acetylation Between 56 and 98.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.909 and 0.932.

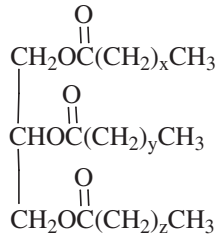
TESTS

Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Saponification Value after Acetylation Acetylate a 10-mL sample as directed under *Total Alcohols*, Appendix VI. Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 2.5 g of the dried, acetylated oil, accurately weighed. Use the weight, in grams, of the acetylated oil for *W* in the calculation formula.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 80% alcohol. The solution may become opalescent on dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Salatrim

Short- and Long-Chain Acyl Triglyceride Molecules



in which (x + y + z) is between 14 and 42

CAS: [177403-56-4]

View IR

DESCRIPTION

Salatrim ranges from a slightly viscous, clear amber liquid to a light-colored, waxy solid. Salatrim is the abbreviated name for short- and long-chain acyl triglyceride molecules. It is prepared by interesterification of triacetin, tripropionin, tributyrin, or of their mixtures with hydrogenated canola, soybean, sunflower, or cottonseed oil. The process removes triglycerides with three short-chain fatty acids. Salatrim triglycerides typically contain 30 to 42 mol-% short-chain fatty acids (SCFA) and 58 to 70 mol-% long-chain fatty acids (LCFA); stearic acid is the predominant LCFA. It is free of particulate matter. It is soluble in hexane, in cyclohexane, in acetone, in ether, in tetrahydrofuran, and in liquid triglyceride oils, but is insoluble in water. It melts at 16° to 71° depending on triglyceride composition.

Function Reduced-energy fat replacement for conventional fats and oils.

REQUIREMENTS

Identification The infrared absorption spectrum of a sample contained in a sodium chloride cell or between salt plates exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 87% of triglycerides, not less than 90% of the triglycerides with an SCFA-to-LCFA mole ratio in the range of 0.5 to 2.0, and not more than 70% by weight of saturated long-chain fatty acids.
Free Fatty Acids (as oleic acid) Not more than 0.5%.
Lead Not more that 0.1 mg/kg.
Monoglycerides Not more than 2%.
Peroxide Value Not more than 2.0.
Residue on Ignition Not more than 0.1%.
Unsaponifiable Matter Not more than 1.0%.
Water Not more than 0.3%.

TESTS

Assay (Excerpted with permission from *J. Agric. Food Chem.* 1994, 42: 453–460. Copyright 1994 American Chemical Society).

Monoglyceride and Triglyceride Content This method permits the quantitation of Monoglycerides (MG) with one long-chain fatty acid (LCFA) and Triglycerides (TG) with the same acyl carbon number (ACN) in Salatrim by high-temperature capillary gas chromatography. The ACN is the sum of the number of carbons of each carboxylic acid side-chain of each TG. For example, the ACN for tristearin is 54 (i.e., 3 × 18), and the ACNs for both dipropionylstearoylglycerol and diacetylarachidoylglycerol are 24, that is, [(2 × 3) + 18] and [(2 × 2) + 20], respectively. MG and TG are identified by comparison with standards. The weight percent of each MG and TG in Salatrim is determined from the peak areas and calibration curves constructed using data from analyses of standard solutions.

MG Standards (2) Obtain monopentadecanoin (mono-C15) and monostearin (mono-C18) with a minimum purity of 99% (available from Nu Check Prep., Inc., Elysian, MN).

TG Standards (13) Obtain tricaproin (tri-C6), triheptanoin (tri-C7), tricaprylin (tri-C8), trinonanoin (tri-C9), tricaprin (tri-C10), triundecanoin (tri-C11), trilaurin (tri-C12), tritridecanoin (tri-C13), trimyristin (tri-C14), tripentadecanoin (tri-C15), tripalmitin (tri-C16), triheptadecanoin (tri-C17), and tristearin (tri-C18) with a minimum purity of 99% (available from Nu Check Prep., Inc., Elysian, MN).

Internal Standard Stock Solution Dissolve 200 mg of tri-C11 in 2 L of spectroscopic-grade undecane:toluene (95:5 v/v).

Standard Solutions (Group 1) Add the two *MG Standards* to each of seven 10-mL volumetric flasks so that each flask, respectively, contains 500, 250, 125, 62.5, 31, 15.6, and 7.8 mg/L of each *MG Standard* when filled to volume with *Internal Standard Stock Solution*.

Standard Solutions (Group 2) Add the 13 *TG Standards* to each of 22 10-mL volumetric flasks, so that each flask, respectively, contains 1600, 800, 400, 200, 100, 50, 32, 25, 20, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, and 2 mg/L of each *TG Standard* when filled to volume with *Internal Standard Stock Solution*.

Salatrim Solution Transfer 2 g of Salatrim, accurately weighed, into a 1-L volumetric flask. Dilute to volume with *Internal Standard Stock Solution*.

Procedure

Equipment Use a Hewlett-Packard 5890 Series II GC equipped with a flame-ionization detector (FID), a pressure-programmable on-column injector, an HP 7673 auto-sampler, and an HP Series II integrator, or equivalent. A Chrompack SIM-DIST CB fused-silica GC column (Chrompack Inc., Raritan, NJ) with a 5-m × 0.32-mm id and a 0.1-μm film thickness, or equivalent. A deactivated fused-silica precolumn (0.5-m × 0.53-mm id) coupled to the analytical column via a butt connector (Quadrex Corp., New Haven, CT), or equivalent.

Set the oven temperature to ramp 140° to 350° at a rate of 10°/min (total run time of 21.0 min). Set the track mode to ''on'' (injector temperature follows the oven temperature conditions). Set the injection mode to on-column injection. Set the FID at 375°. Set the hydrogen carrier gas constant flow mode to ''on'' with a pressure of 5.5 psi (140°).

Quantitation of MG Determine the calibration curve and response factor (*RF*) for Mono-C15 and Mono-C18 by the following procedure: Analyze each of the *Standard Solutions* (Group 1) using a sample injection volume of 0.5 μL. From each chromatogram, establish the response factors (RF_i) for the two MG Standards using the following equation:

$$RF_i = (C_{IS}/C_i) \times (A_i/A_{IS}),$$

in which C_{IS} is the concentration, in milligrams per liter, of the *Internal Standard Stock Solution*; C_i is the concentration, in milligrams per liter, of an MG; A_i is the peak area of an MG; and A_{IS} is the peak area of the *Internal Standard Stock Solution*.

For each MG, construct a calibration curve by plotting the peak area ratios of (A_i/A_{IS}) (x-axis) versus the RF_i (y-axis) for each solution.

Weight Percent of MG Analyze the sample using a sample injection of 0.5 μL. From the chromatograms, obtain the peak area of each MG (A_i) and of the *Internal Standard Stock Solution* (A_{IS}). Calculate the peak area ratio (A_i/A_{IS}), and determine the *RF* for each MG (RF_i) from the calibration curve. Determine the concentration, in micrograms per milliliter, of each MG (C_i) in the sample by the equation

$$C_i = (C_{IS}/RF_i) \times (A_i/A_{IS}),$$

in which C_{IS} is the concentration of the *Internal Standard Stock Solution*. Determine the weight percent [$(W\%)_i$] of each MG in the sample by the equation

$$(W\%)_i = (C_i/C_T) \times 100,$$

in which C_T is the concentration, in milligrams per liter, of the *Salatrim Solution*. Determine the total weight percent of MG in the sample by the formula

$$\sum_i (W\%)_i.$$

Quantitation of TG with the Same ACN To determine the *RF* for *TG Standards*, analyze each of the *Standard Solutions* (Group 2) using a sample injection volume of 0.5 μL. Determine the *RF* for each *TG Standard* (*j*) by the formula

$$RF_{nj} = (C_{IS}/C_{nj}) \times (A_{nj}/A_{IS}),$$

in which C_{IS} is the concentration, in milligrams per liter, of the *Internal Standard Solution*; C_{nj} is the concentration, in milligrams per liter, of each *TG Standard*; A_{nj} is the peak area of each *TG Standard* with an acetyl carbon number equaling *n* (*n* = 18–54 and the acetyl carbon number of the *TG Standard* is as follows: tri-C6, 18; tri-C7, 21; tri-C8, 24; tri-C9, 27; tri-C10, 30; tri-C11, 33; tri-C12, 36; tri-C13, 39; tri-C14, 42; tri-C15, 45; tri-C16, 48; tri-C17, 51; and tri-C18, 54); and A_{IS} is the peak area of the *Internal Standard Stock Solution*.

The relative peak area of each *TG Standard* with ACN = *n* and concentration C_{nj} to that of the *Internal Standard Solution* is given by the equation

$$RA_{nj} = A_{nj}/A_{IS}.$$

Note: The notations ''j + 1,'' ''j,'' and ''j – 1'' denote consecutive concentration values in the series of standard solutions. For example, for a *TG Standard* with ACN = *n* and concentrations of 100, 200, and 400 mg/L, the relative peak areas RA_{nj+1} and RA_{nj-1} correspond to data for standards with concentrations of 400 mg/L and 100 mg/L, respectively.

Calculate the *RF* and relative peak areas for each TG with ACN = *n* + 1 and with ACN = *n* + 2 from the measured *RF* and *RA* values for TG standards with ACN = *n* and *n* + 3 at the same concentration, C_{nj}, according to the following equations:

$$RF_{n+1j} = RF_{nj} + (RF_{n+3j} - RF_{nj}) \times 1/3,$$

$$RA_{n+1j} = RA_{nj} + (RA_{n+3j} - RA_{nj}) \times 1/3,$$

$$RF_{n+2j} = RF_{nj} + (RF_{n+3j} - RF_{nj}) \times 2/3,$$

$$RA_{n+2j} = RA_{nj} + (RA_{n+3j} - RA_{nj}) \times 2/3.$$

For the sample, calculate the *RF* for the TG with a relative peak area RA_{nj} by the equation

$$RF_{nj} = RF_{nj-1} + (RF_{nj+1} - RF_{nj-1}) \times [(RA_{nj} - RA_{nj-1})/(RA_{nj+1} - RA_{nj-1})],$$

in which RF_{nj-1} and RF_{nj+1} are the response factors, and RA_{nj-1} and RA_{nj+1} are the relative peak areas, respectively, of the *TG Standard* with the same ACN value. RA_{nj+1} and RA_{nj-1} must meet the following condition:

$$RA_{nj-1} \le RA_{nj} < RA_{nj+1},$$

in which RA_{nj} is the ratio of the peak area of the TG in Salatrim with ACN = *n* (A_{nj}), to the peak area for the *Internal Standard Solution* (A_{IS}).

To determine the weight percent of Salatrim TG components, the concentration (milligrams per liter) of TG in the sample with ACN = *n* is given by the equation

$$C_{nj} = (C_{IS}/RF_{nj}) \times (A_{nj}/A_{IS}),$$

in which C_{IS} is the concentration, in milligrams per liter, of the *Internal Standard Stock Solution*.

Determine the weight percent of TG with ACN = *n* in the sample by the equation

$$(W\%)_n = (C_{nj}/C_T) \times 100,$$

in which C_T is the concentration, in milligrams per liter, of the *Salatrim Solution*.

Determine the total weight percent of TG in the sample with the formula

$$\sum_n (W\%)_n.$$

Short-Chain Fatty Acids (SCFA)/Long-Chain Fatty Acids (LCFA) Mole Ratio

Apparatus Gas chromatograph with flame ionization detector (Hewlett-Packard 5890 Series II, or equivalent) equipped with a 5-µL syringe for 0.32-mm (id) columns. Automatic sampler (HP 7673, or equivalent). Chromatographic data system or integrator (HP 3365 Series II software, or equivalent). Retention gap, deactivated fused silica, 1-mm × 0.32-mm (id) with capillary column connectors. DB 5-HT, 15-m × 0.32-mm (id) fused silica capillary column (J&W Scientific, Inc., 91 Blue Ravine Road, Folsom, CA 95630-4714, catalog number 123-5711, or equivalent). Crimp caps and vials (HP 5181-3375, or equivalent) for an on-line autosampler.

0.5 N Sodium Butoxide Transfer 9.62 g of sodium butoxide solution (50% in butanol) into a 100-mL volumetric flask, dilute to volume with 1-butanol, and mix.

Butyl Ester Standards Butyl butyrate (98%); *n*-butyl acetate (99%); butyl propionate; butyl palmitate; butyl stearate (93%).

Standard Reference Solution (**Note**: Melt the butyl stearate standard before sampling.) Transfer about 50 mg of each butyl ester standard, accurately weighed, into a single 100-mL volumetric flask. Dilute to volume with HPLC-grade hexane (95%), and mix. More than one *Standard Reference Solution* may be necessary if impurities co-elute with standard peaks.

Sample Solution Transfer approximately 50 mg of sample, previously melted and accurately weighed, into a 100-mL volumetric flask. Dilute to volume with hexane, and mix.

Procedure For each sample (hexane blank, *Standard Reference Solution*, and *Sample Solution*) to be assayed, pipet 5.0 mL of solution into a clean 8-mL clear glass vial. Add 0.5 mL of 0.5 *N* sodium butoxide, seal, and shake vigorously. The solution will turn yellow. For the hexane blank and the *Sample Solutions* only, allow the solution to stand for 2 min. Neutralize the mixture by adding 1.0 mL of 0.5 *N* hydrochloric acid. Seal the vial, and shake well until the solution is clear. Check the pH with pH paper. The solution should be acidic. If it is not, the column will degrade.

Note: 1-Butanol and water may be substituted for 0.5 *N* sodium butoxide and 0.5 *N* hydrochloric acid, respectively, for the *Standard Reference Solution.*

Using helium as the carrier gas, set the chromatograph gas flow at 2.0 psi constant flow. Use a 0.5-µL injection volume. Set the injection temperature in track mode at 3° above oven temperature, and set the oven temperature to 40° for 6 min and raising it to 280° in 15°-per-minute increments over 5 min. Set the detector temperature at 380°.

Allow the butyl ester sample phases to separate (centrifugation may be used to hasten the separation). Transfer approximately 1 mL of the hexane layer into an autosampler vial. Run the gas chromatography program.

Calculation Determine the response factors (RF_i) for butyl ester standards with the equation

$$RF_i = 100\% \times A_i/[W_i \times (\%\ \text{purity})_i],$$

in which A_i is the average peak area counts for the *i*th standard; W_i is the weight, in micrograms, of the *i*th standard in the *Standard Reference Solution*; and $(\%\ \text{purity})_i$ is the purity of the *i*th standard expressed as a percentage.

Determine the weights of butyl esters in the sample with the equation

$$W_i = A_i/RF_i,$$

in which W_i is the weight, in micrograms, of the *i*th ester in the sample; A_i is the peak area counts for the *i*th ester in the

sample; and RF_i is the response factor for the *i*th ester standard (average area counts/microgram).

Determine the weights of fatty acids in the sample with the equation

$$(W_i)_{\text{fatty acid}} = (W_i)_{\text{butyl ester}} \times (MW_i)_{\text{fatty acid}}/(MW_i)_{\text{butyl ester}},$$

in which $(W_i)_{\text{fatty acid}}$ and $(W_i)_{\text{butyl ester}}$ are, respectively, the weights, in micrograms, of the *i*th fatty acid and its butyl ester in the sample, and $(MW_i)_{\text{fatty acid}}$ and $(MW_i)_{\text{butyl ester}}$ are their respective molecular weights.

Calculate the short/long (S/L) mole ratio with the following equations

$$(\text{mmoles}_i)_{\text{fatty acid}} = (W_i)_{\text{fatty acid}}/[1000 \times (MW_i)_{\text{fatty acid}}],$$

$$\text{S/L mole ratio} = \sum_i (\text{mmoles}_i)_{\text{SCFA}}/\sum_i (\text{mmoles}_i)_{\text{LCFA}},$$

in which $(W_i)_{\text{fatty acid}}$ is given in micrograms, $(MW_i)_{\text{fatty acid}}$ is given in milligrams per millimole, and $\sum_i (\text{mmoles}_i)_{\text{SCFA}}$ and $\sum_i (\text{mmoles}_i)_{\text{LCFA}}$ are the respective sums of the millimoles of short-chain fatty acids (C2–C4) and long-chain fatty acids (C14–C18).

Total Saturated LCFAs Using the *SCFA/LCFA Mole Ratio Assay* method above, calculate the weight percent of saturated LCFAs with the formula

$$100 \times [(W_i)_{\text{stearic acid}}) + (W_i)_{\text{palmitic acid}}]/W_S,$$

in which W_S is the weight, in micrograms, of the sample.

Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

$$\text{Free fatty acids as oleic acid, } e = 28.2.$$

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Monoglycerides Determine as directed in the *Assay* (above).

Peroxide Value

Acetic Acid–Chloroform Solution Prepare a 3:2 (v/v) mixture of acetic acid:chloroform.

Saturated Potassium Iodide Solution Dissolve excess potassium iodide in freshly boiled water. Excess solid must remain. Store this solution in the dark. Test it daily by adding 0.5 mL to 30 mL of the *Acetic Acid–Chloroform Solution*, then add 2 drops of starch TS. If the solution turns blue, requiring more than 1 drop of 0.1 *N* sodium thiosulfate to discharge the color, prepare a fresh solution.

Procedure Transfer about 5 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask. Add 30 mL of the *Acetic Acid–Chloroform Solution*, and swirl to dissolve. Add 0.5 mL of the *Saturated Potassium Iodide Solution*, allow the mixture to stand, shaking it occasionally, for 1 min, and add 30 mL of water. Slowly titrate with 0.01 *N* sodium thiosulfate, shaking the flask vigorously until the yellow color is almost gone. Add about 0.5 mL of starch TS, and continue the titration, shaking the flask vigorously to release all the iodine from the chloroform layer until the blue color disappears. Perform a blank determination (see *General Provisions*), and make any necessary correction.

Calculation Determine the *Peroxide Value* (*PV*, milliequivalent peroxide per kilogram of sample) using the following equation:

$$PV = (S \times N \times 1000)/W,$$

in which *S* is the amount, in milliliters, of 0.01 *N* sodium thiosulfate; *N* is the normality of the sodium thiosulfate; and *W* is the weight, in grams, of the sample.

Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Unsaponifiable Matter

Calcium Chloride–Diatomaceous Earth Mixture Using a mortar and pestle, grind 1 part anhydrous calcium chloride with 1 part water, and add 3 parts diatomaceous earth, nonacid washed (Celite 545, or equivalent). Grind to a uniform consistency. This mixture may be stored in a covered amber jar for up to 1 month.

Potassium Hydroxide–Diatomaceous Earth Mixture (**Note**: For multiple analyses, prepare in lots of 75 g or more.) Using a mortar and pestle, grind 2 parts potassium hydroxide pellets with 1 part water.

> **Caution**: This action generates considerable heat; wear eye protection and gloves.

Add 4 parts diatomaceous earth. Grind the mixture to a uniform consistency, and store it in a covered amber jar for up to 10 days.

Procedure

Saponification Place 10 g of *Potassium Hydroxide–Diatomaceous Earth Mixture* in a 400-mL mortar. Transfer 5 g of sample, accurately weighed (W_S), into the mortar. Grind the mixture until the sample is uniformly distributed. Add another 10 g of *Potassium Hydroxide–Diatomaceous Earth Mixture*, and grind to a uniform consistency. Transfer the mixture to a jar. Transfer any residual sample by using the pestle to sweep 5 g of diatomaceous earth along the sides of the mortar and into the jar. Cap the jar securely, and shake until the mixture is uniform. Heat for 20 to 30 min in an oven at 130°.

Gravimetric Extraction Transfer the cooled mixture into the mortar, and regrind it for approximately 30 s to a uniform granular consistency. Loosely fit a plug of glass wool into the tip of a glass chromatography column (30 mm id, 30 cm long overall, with a drip tip 5 cm × 8 mm od, or equivalent). Pack the column with 5 g of *Calcium Chloride–Diatomaceous Earth Mixture*, and transfer the contents of the mortar to the column. Pack to a total height of 50 to 60 mm. Place a 150-mL tared flask under the column. Qualitatively transfer the residue from the mortar to the column with about 25 mL of dichloromethane. Once this solution has percolated into the column bed, add sufficient dichloromethane so that the column bed is wet and a few drops of eluate have been collected in the flask. Charge the column with 150 mL of dichloromethane, and collect the entire volume in the flask (approximately 25 min). While gently heating, remove the solvent under a stream of nitrogen while the eluate is collected. Take the contents of the flask to constant weight under vacuum. Determine the weight of the residue (W_R).

To check for completeness of extraction, add 20 mL of dichloromethane to the column, and collect the eluate in a second tared flask. Evaporate the contents of the second flask to dryness, and examine it for residue. Determine the weight of the residue (W_{R1}), if present. If residue is present, repeat the procedure with an additional 20 mL of dichloromethane.

Calculation Use the total residue weight and the weight of the original sample to calculate the percent of unsaponifiable matter:

$$\% \text{ unsaponifiable matter} = [(W_R + W_{R1} + \ldots)/W_S] \times 100.$$

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Sandalwood Oil, East Indian Type

CAS: [84787-70-2]

View IR

DESCRIPTION

Sandalwood Oil, East Indian Type, occurs as a pale yellow to yellow, somewhat viscous, oily liquid with a strong, persistent, characteristic odor. It is the volatile oil obtained by steam distillation from the dried, ground roots and wood of *Santalum album* L. (Fam. Santalaceae). It is soluble in most fixed oils, in propylene glycol, and in mineral oil, sometimes with haziness. It is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 90.0% of alcohol, calculated as santalol, $C_{15}H_{24}O$.
Angular Rotation Between −15° and −20°.
Refractive Index Between 1.500 and 1.510 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.965 and 0.980.

TESTS

Assay Proceed as directed under *Total Alcohols*, Appendix VI. Accurately weigh about 1.2 g of the acetylated alcohol for the saponification, and use 110.2 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 5 mL of 70% alcohol and remains in solution on dilution to 10 mL.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Savory Oil (Summer Variety)

Summer Savory Oil

CAS: [8016-68-0]

View IR

DESCRIPTION

Savory Oil (Summer Variety) occurs as a light yellow to dark brown liquid with a spicy, aromatic note suggestive of thyme or origanum. It is the volatile oil obtained by steam distillation from the whole dried plant *Satureia hortensis* L. (Fam. Labiatae). It is soluble in most fixed oils and in mineral oil, but it is practically insoluble in glycerin and in propylene glycol.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 20.0% and not more than 57.0% of phenols as carvacrol ($C_{10}H_{14}O$).
Angular Rotation Between −5° and +4°.
Refractive Index Between 1.486 and 1.505 at 20°.
Saponification Value Not more than 6.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.875 and 0.954.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using 5 g of sample, accurately weighed.

Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample usually dissolves in 2 mL of 80% alcohol. Some oils may be slightly hazy in 10 mL of 90% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

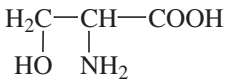
Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and using 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Loss on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

DL-Serine

DL-2-Amino-3-hydroxypropanoic Acid



$C_3H_7NO_3$ Formula wt 105.09

CAS: [302-84-1]

View IR

DESCRIPTION

DL-Serine occurs as white crystals or crystalline powder. It is soluble in water, but insoluble in alcohol and in ether. It melts with decomposition at about 246° using a closed capillary tube and a bath preheated to 225°. It is optically inactive.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_3H_7NO_3$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.

TESTS

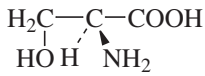
Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 10.51 mg of $C_3H_7NO_3$.

L-Serine

L-2-Amino-3-hydroxypropanoic Acid



$C_3H_7NO_3$ Formula wt 105.09

CAS: [56-45-1]

View IR

DESCRIPTION

L-Serine occurs as a white crystalline powder. It is soluble in water, but is insoluble in alcohol and in ether. It melts with decomposition at about 228°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_3H_7NO_3$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +13.6° and +16.0°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between +13° and +15.6°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 10.51 mg of $C_3H_7NO_3$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 10 g of a previously dried sample in sufficient 2 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIB, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

Sheanut Oil, Refined

Shea Butter; Karite; Galam

DESCRIPTION

Sheanut Oil, Refined, occurs as a pale yellow, viscous liquid. It is obtained from sheanuts derived from the Shea tree *Butyrospermum parkii* (Fam. Sapotaceae). It is composed of triglycerides of primarily stearic and oleic acids.

Function Component of a mixture of oils used as a cocoa butter substitute; as a coating agent; and in margarine and shortening.

REQUIREMENTS

Identification Refined Sheanut Oil exhibits the following typical composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	16:0	18:0	18:1	18:2	20:0
Weight % Range:	3.8–4.1	41.2–56.8	34.0–46.9	3.7–6.5	1.0–2.0

Color (AOCS Wesson) Not more than 4 red/40 yellow.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 28 and 43.
Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.
Saponification Value Between 185 and 195.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.5%.

TESTS

Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.

Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 4 g of sample, accurately weighed.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed for *Method Ia* in the *Karl Fischer Titrimetric Method* under *Water Determination*, Appendix IIB, using a 100-g sample melted on a hot plate at 60°. Use a syringe to apply the oil to the Karl Fisher titrimetric apparatus (usually 1.0 mL is sufficient, but this may vary depending on the water content of the sample).

Packaging and Storage Store in well-closed containers.

Shellac, Bleached

White Shellac; Regular Bleached Shellac

INS: 904 CAS: [9000-59-3]

DESCRIPTION

Shellac, Bleached, occurs as an off white to tan, amorphous, granular resin. Shellac is obtained from lac, the resinous secretion of the insect *Laccifer (Tachardia) lacca* Kerr (Fam. Coccidae). Shellac, Bleached, is obtained by dissolving the lac in aqueous sodium carbonate, followed by bleaching with sodium hypochlorite, precipitation of the bleached lac with a dilute sulfuric acid solution, and drying. It is freely (though very slowly) soluble in alcohol, insoluble in water, and slightly soluble in acetone and in ether. Shellac, Bleached, is usually dissolved in a suitable solvent for application to food products.

Function Coating agent; surface-finishing agent; glaze.

REQUIREMENTS

Identification Create a solution of 1 g of ammonium molybdate in 3 mL of sulfuric acid. Add a few drops of this solution to 50 mg of sample. A green color appears and changes to lilac when the solution is neutralized with 6 *N* ammonium hydroxide.
Acid Value Between 73 and 89.

Lead Not more than 2 mg/kg.
Loss on Drying Not more than 6.0%.
Rosin Passes test.
Wax Not more than 5.5%.

TESTS

Acid Value Dissolve about 2 g of finely ground sample, accurately weighed, in 50 mL of alcohol previously neutralized to phenolphthalein with sodium hydroxide. Add additional phenolphthalein TS, if necessary, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint. Calculate the acid value by the formula

$$56.1V \times N/W,$$

in which V is the exact volume, in milliliters, of 0.1 *N* sodium hydroxide used; N is the exact normality of the sodium hydroxide solution; and W is the weight, in grams, of sample taken, calculated on the dried basis.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 41° ± 2° to constant weight.
Rosin Dissolve 2 g of sample in 10 mL of dehydrated alcohol, and add slowly, with shaking, 50 mL of solvent hexane. Transfer the solution to a separator, wash with two 50-mL portions of water, and discard the washings. Filter the solvent layer, evaporate it to dryness, and add 2 mL of 1:2 (v/v) liquefied phenol:methylene chloride to its residue. Stir, and transfer a portion of the mixture to a cavity of a color-reaction plate. Fill an adjacent cavity with 1:4 (v/v) bromine:methylene chloride, and cover both cavities with an inverted watch glass. No purple or deep indigo blue color appears in or above the liquid containing the sample residue.
Wax Transfer about 10 g of finely ground sample, accurately weighed, and 2.5 g of sodium carbonate into a 200-mL tall-form beaker. Add 150 mL of hot water, immerse the beaker in a boiling water bath, and stir until the sample is dissolved. Cover the beaker with a watch glass, heat for 3 h without agitation, and cool in a cold water bath. When the wax has floated to the surface, filter the mixture through medium-speed, quantitative, ashless filter paper, thus transferring the wax to the paper, and wash the filter with water. Pour 5 to 10 mL of alcohol onto the filter to accelerate drying. Wrap the paper loosely in a larger piece of filter paper, bind with a piece of fine wire, and dry with the aid of gentle heat. Extract with chloroform in a suitable continuous extraction apparatus for 2 h, using a previously dried and accurately weighed flask to receive the extracted wax and solvent. Evaporate the solvent, dry the wax at 105° to constant weight, and calculate the percentage of wax.

Packaging and Storage Store in well-closed containers in a cool place protected from heat.

Shellac, Bleached, Wax-Free

Refined Bleached Shellac

INS: 904 CAS: [9000-59-3]

DESCRIPTION

Shellac, Bleached, Wax-Free, occurs as an amorphous, light yellow, granular resin. It is obtained from lac, the resinous secretion of the insect *Laccifer (Tachardia) lacca* Kerr (Fam. Coccidae). Shellac, Bleached, Wax-Free, is obtained by the same process as that described in the monograph for *Bleached Shellac*, except that, in addition, wax is removed by filtration. Its solubility is the same as that of *Bleached Shellac*. Shellac, Bleached, Wax-Free, is usually dissolved in a suitable solvent for application to food products.

Function Coating agent; surface-finishing agent; glaze.

REQUIREMENTS

Identification Shellac, Bleached, Wax-Free, responds to the *Identification Test* in the monograph for *Bleached Shellac*.
Acid Value Between 75 and 91.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 6.0%.
Rosin Passes test.
Wax Not more than 0.2%.

TESTS

Perform as directed in the monograph for *Bleached Shellac*.

Packaging and Storage Store in well-closed containers in a cool place protected from heat.

Silicon Dioxide

Synthetic Amorphous Silica

SiO_2 Formula wt 60.08

INS: 551 CAS: [7631-86-9]

DESCRIPTION

Silicon Dioxide occurs as an amorphous substance that shows a noncrystalline pattern when examined by X-ray diffraction. It is produced synthetically, either by a vapor-phase hydrolysis process, yielding *fumed silica*, or by a wet process, yielding *precipitated silica*, *silica gel*, *colloidal silica*, or *hydrous silica*. *Fumed silica* is produced in an essentially anhydrous state, whereas the wet-process products are obtained as hydrates or contain surface-adsorbed water.

Fumed silica occurs as a white, fluffy, nongritty powder of extremely fine particle size and is hygroscopic. The wet-process silicas occur as white, fluffy powders or as white, microcellular beads or granules and are hygroscopic or absorb moisture from the air in varying amounts. All of these forms of Silicon Dioxide are insoluble in water and in organic solvents, but they are soluble in hydrofluoric acid and in hot, concentrated solutions of alkalies.

Function Anticaking agent; defoaming agent; carrier; conditioning agent; chillproofing agent in malt beverages; filter aid.

REQUIREMENTS

Identification

A. Place about 5 mg of sample into a platinum crucible, mix with 200 mg of anhydrous potassium carbonate, and ignite over a burner at a red heat for about 10 min. Cool, dissolve the melt in 2 mL of freshly distilled water, warming if necessary, and slowly add 2 mL of ammonium molybdate TS. A deep yellow color appears.

B. Place 1 drop of the solution from *Identification Test A* on a filter paper, and evaporate the solvent. Add 1 drop of a saturated solution of *o*-toluidine in glacial acetic acid, and place the paper over ammonium hydroxide. A green-blue spot develops.

Assay *Fumed Silica*: Not less than 99.0% of SiO_2 after ignition; *Precipitated Silica*, *Silica Gel*, and *Hydrous Silica*: Not less than 94.0% of SiO_2 after ignition.

Lead Not more than 5 mg/kg.

Loss on Drying *Fumed Silica*: Not more than 2.5%; *Precipitated Silica* and *Silica Gel*: Not more than 7.0%; *Hydrous Silica*: Not more than 70.0%; *Colloidal Silica*: Not more than 85.0%.

Loss on Ignition *Fumed Silica*: Not more than 2.0% after drying; *Silica Gel*, *Hydrous Silica*, and *Precipitated Silica*: Not more than 8.5% after drying.

Soluble Ionizable Salts (as Na_2SO_4) *Precipitated Silica*, *Silica Gel*, and *Hydrous Silica*: Not more than 5.0%.

TESTS

Assay Transfer about 1 g of sample, previously dried at 105° for 2 h and accurately weighed, into a tared platinum crucible, ignite as directed under *Loss on Ignition* (below), cool in a desiccator, and weigh to obtain the ignited sample weight (*W*). Moisten the residue with 3 or 4 drops of alcohol, add 2 drops of sulfuric acid, and then add enough hydrofluoric acid to cover the wetted sample.

> **Caution**: Handle hydrofluoric acid in an appropriate fume hood.

Evaporate to dryness on a hot plate, using medium heat (95° to 105°), then add 5 mL of hydrofluoric acid, swirl the dish carefully to wash down the sides, and again evaporate to dryness. Ignite the dried residue over a Meker burner to a red heat, cool in a desiccator, and weigh to obtain the residual weight (*w*). The difference between the ignited sample weight and the residual weight ($W - w$) represents the weight, in grams, of SiO_2 in the ignited sample.

Lead

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL with water, and mix. Each milliliter of this solution contains 100 µg of lead (Pb) ion. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute stepwise and quantitatively an accurately measured volume of *Lead Nitrate Stock Solution* with water to obtain a *Standard Lead Solution* containing 0.25 µg/mL of lead (Pb) ion.

Sample Solution Transfer 5.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 *N* hydrochloric acid, cover with a watch glass, and slowly heat to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through a Whatman No. 3, or equivalent, filter paper into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Procedure Set a suitable atomic absorption spectrophotometer to a wavelength of 217 nm. Adjust the instrument to zero absorbance against water. Read the absorbance of the *Standard Lead Solution*.

Aspirate the *Sample Solution* into the spectrophotometer, and measure the absorbance in the same manner. The absorbance obtained from the *Sample Solution* is not greater than that obtained from the *Standard Lead Solution*.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Loss on Ignition Transfer about 1 g of sample, previously dried at 105° for 2 h and accurately weighed, into a suitable tared crucible. Place the crucible in a cold muffle furnace, and raise the temperature to 900° to 1000° during a 1-h period. Ignite at this temperature for 1 h, cool in a desiccator, and weigh.

Soluble Ionizable Salts (as Na_2SO_4) Stir 5 g of sample, previously dried at 105° for 2 h and accurately weighed, with 150 mL of water for at least 5 min in a high-speed mixer. Filter with the aid of suction, and wash the mixer and filter with 100 mL of water in divided portions, adding the washings to the filtrate. Dilute the filtrate to 250 mL with water, and determine its conductance with a suitable conductance bridge assembly. The conductance is not greater than that produced by a control containing 250 mg of anhydrous sodium sulfate in each 250 mL.

Packaging and Storage Store in well-closed containers.

Sodium Acetate

$C_2H_3NaO_2$ Formula wt, anhydrous 82.03
$C_2H_3NaO_2 \cdot 3H_2O$ Formula wt, trihydrate 136.08
INS: 262(i) CAS: anhydrous [127-09-3]
CAS: trihydrate [6131-90-4]

DESCRIPTION

Sodium Acetate occurs as colorless, transparent crystals or as a granular, crystalline or white powder. The anhydrous form is hygroscopic; the trihydrate effloresces in warm, dry air. One gram of the anhydrous form dissolves in about 2 mL of water; 1 g of the trihydrate dissolves in about 0.8 mL of water and in about 19 mL of alcohol.

Function Buffer.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Sodium* and for *Acetate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 101.0% of $C_2H_3NaO_2$ after drying.
Alkalinity *Anhydrous*: Not more than 0.2%; *Trihydrate*: Not more than 0.05%.
Lead Not more than 2 mg/kg.
Loss on Drying *Anhydrous*: Not more than 1.0%; *Trihydrate*: Between 36.0% and 41.0%.
Potassium Compounds Passes test.

TESTS

Assay Accurately weigh about 400 mg of the dried sample obtained in the test for *Loss on Drying* (below), and dissolve it in 40 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 8.203 mg of $C_2H_3NaO_2$.
Alkalinity Dissolve 2 g of sample in about 20 mL of water, and add 3 drops of phenolphthalein TS. If a pink color appears, not more than 0.4 mL or 0.1 mL of 0.1 *N* sulfuric acid is required to discharge it for the anhydrous and trihydrate, respectively.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 80° overnight, followed by drying it at 120° to constant weight.
Potassium Compounds Mix a few drops of sodium bitartrate TS with 5 mL of a clear, saturated solution of sample. No turbidity develops within 5 min.

Packaging and Storage Store in tight containers.

Sodium Acid Pyrophosphate

Disodium Pyrophosphate; Disodium Dihydrogen Pyrophosphate; Disodium Dihydrogen Diphosphate; Acid Sodium Pyrophosphate

$Na_2H_2P_2O_7$ Formula wt 221.94
INS: 450(i) CAS: [7758-16-9]

DESCRIPTION

Sodium Acid Pyrophosphate occurs as a white, crystalline powder or granules. It is soluble in water. The pH of a 1:100 aqueous solution is about 4. It may contain a suitable aluminum and/or calcium salt to control the rate of reaction in leavening systems.

Function Buffer; emulsifier; leavening agent; sequestrant.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Sodium*, Appendix IIIA.

B. Dissolve 100 mg of sample in 100 mL of 1.7 *N* nitric acid. Add 0.5 mL of this solution to 30 mL of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and add 0.5 mL of it to 30 mL of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 93.0% and not more than 100.5% of $Na_2H_2P_2O_7$.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Insoluble Substances Not more than 1%.
Lead Not more than 2 mg/kg.

TESTS

Assay Dissolve about 500 mg of sample, accurately weighed, in 100 mL of water contained in a 400-mL beaker. Using a pH meter, adjust the pH of the solution to 3.8 with hydrochloric acid, then add 50 mL of a 1:8 solution of zinc sulfate (125 g of $ZnSO_4 \cdot 7H_2O$ dissolved in water, diluted to 1000 mL, filtered, and adjusted to pH 3.8 with hydrochloric acid), and allow the mixture to stand for 2 min. Titrate the liberated acid with 0.1 *N* sodium hydroxide until a pH of 3.8 is again reached. After each addition of 0.1 *N* sodium hydroxide near the endpoint, allow time for any precipitated zinc hydroxide to redissolve. (The 0.1 *N* sodium hydroxide used in this titration must be standardized against the primary standard, potassium biphthalate [$KHC_6H_4(COO)_2$], that has been dried at 105° for 2 h, as described for sodium hydroxide in *Volumetric Solutions* under *Solutions and Indicators*). Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 11.10 mg of $Na_2H_2P_2O_7$.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of water.

Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.
Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter the solution through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Packaging and Storage Store in tight containers.

Sodium Alginate

Algin

$(C_6H_7O_6Na)_n$ Equiv wt, calculated 198.11
Equiv wt, actual (avg.) 222.00

INS: 401 CAS: [9005-38-3]

DESCRIPTION

Sodium Alginate occurs as a white to yellow-brown, fibrous or granular powder. It is the sodium salt of alginic acid (see the monograph for *Alginic Acid*). It dissolves in water to form a viscous, colloidal solution. It is insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater than about 30% by weight. It is insoluble in chloroform, in ether, and in acids having a pH lower than about 3.

Function Stabilizer; thickener; emulsifier; gelling agent.

REQUIREMENTS

Identification

A. Add 1 mL of calcium chloride TS to 5 mL of a 1:100 aqueous solution. A voluminous, gelatinous precipitate forms.

B. Add 1 mL of 2 *N* sulfuric acid to 10 mL of a 1:100 aqueous solution. A heavy, gelatinous precipitate forms.

C. Place about 5 mg of sample into a test tube, add 5 mL of water, 1 mL of a freshly prepared 1:100 naphtholresorcinol:ethanol solution, and 5 mL of hydrochloric acid. Heat the mixture to boiling, boil gently for about 3 min, and then cool to about 15°. Transfer the contents of the test tube to a 30-mL separator with the aid of 5 mL of water, and extract with 15 mL of isopropyl ether. Perform a blank determination (see *General Provisions*), and make any necessary correction. The isopropyl ether extract from the sample exhibits a deeper purple hue than that from the blank.

Assay A sample yields not less than 18.0% and not more than 21.0% of carbon dioxide (CO_2), corresponding to between 90.8% and 106.0% of Sodium Alginate (equiv wt 222.00).
Arsenic Not more than 3 mg/kg.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 15.0%.

TESTS

Assay Determine as directed under *Alginates Assay*, Appendix IIIC. Each milliliter of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 27.75 mg of Sodium Alginate (equiv wt 222.00), calculated on the dried basis.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Packaging and Storage Store in well-closed containers.

Sodium Aluminosilicate

Sodium Silicoaluminate

INS: 554 CAS: [1344-00-9]

DESCRIPTION

Sodium Aluminosilicate occurs as a fine, white, amorphous powder, or as beads. It is a series of hydrated sodium aluminum silicates having Na_2O:Al_2O_3:SiO_2 molar ratios of approximately 1:1:13, respectively. It is insoluble in water and in alcohol and other organic solvents, but at 80° to 100°, it is partially soluble in strong acids and solutions of alkali hydroxides.

Function Anticaking agent.

REQUIREMENTS

Identification

A. Mix 500 mg of sample with 2.5 g of anhydrous potassium carbonate, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, add 5 mL of water, and allow to stand for 3 min. Heat the bottom of the crucible gently, detach the melt, and transfer it into a beaker with the aid of about 50 mL of water. Gradually add hydrochloric acid until no effervescence is observed, add 10 mL more of the acid, and evaporate to dryness on a steam bath. Cool, add 20 mL of water, boil, and filter through ash-free filter paper. An insoluble residue of silica remains. Retain the filtrate for *Identification Test B*.

> **Caution**: Handle hydrofluoric acid in an appropriate fume hood.

Transfer the gelatinous residue to a platinum dish, and cautiously add 5 mL of hydrofluoric acid. The precipitate dissolves. (If it does not dissolve, repeat the treatment with hydrofluoric acid.) Heat, and introduce a glass stirring rod with a drop of water on the tip into the resulting vapors. The drop becomes turbid.

B. Portions of the filtrate obtained in *Identification Test A* give positive tests for *Aluminum* and for *Sodium*, Appendix IIIA.

Assay

Silicon Dioxide Not less than 66.0% and not more than 76.0% of SiO_2 after drying.

Aluminum Oxide Not less than 9.0% and not more than 13.0% of Al_2O_3 after drying.

Sodium Oxide Not less than 4.0% and not more than 7.0% of Na_2O after drying.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 8.0%.

Loss on Ignition Between 8.0% and 13.0% after drying.

pH (20% slurry) Between 6.5 and 10.5.

TESTS

Assay

Silicon Dioxide

Transfer about 500 mg of sample, previously dried at 105° for 2 h and accurately weighed, into a 250-mL beaker, wash the sides of the beaker with a few milliliters of water, and add 30 mL of sulfuric acid and 15 mL of hydrochloric acid. Heat on a hot plate in a hood until dense, white fumes evolve; cool; add 15 mL of hydrochloric acid; and heat again to dense, white fumes. Cool, add 70 mL of water, and filter through Whatman No. 40, or equivalent, filter paper. Wash the filter paper and precipitate thoroughly with hot water to remove the sulfuric acid residue.

Transfer the filter paper and precipitate into a tared platinum crucible, char, and ignite at 900° to constant weight. Moisten the residue with a few drops of water, add 15 mL of hydrofluoric acid and 8 drops of sulfuric acid, and heat on a hot plate in a hood until white fumes of sulfur trioxide evolve. Cool; add 5 mL of water, 10 mL of hydrofluoric acid, and 3 drops of sulfuric acid; and evaporate to dryness on the hot plate. Heat cautiously over an open flame until sulfur trioxide fumes cease to evolve, and ignite at 900° to constant weight. The weight loss after the addition of hydrofluoric acid represents the weight of SiO_2 in the sample taken.

Aluminum Oxide

Sample Solution Transfer about 500 mg of sample, previously dried at 105° for 2 h and accurately weighed, into a tared platinum dish, and moisten with 8 to 10 drops of water. Add 25 mL of 70% perchloric acid and 10 mL of hydrofluoric acid, and heat on a hot plate until dense, white fumes of perchloric acid evolve.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Cool, add 10 mL of hydrofluoric acid, and heat again to dense, white fumes. Cool, dissolve in sufficient water, quantitatively transfer it with the aid of additional water into a 250-mL volumetric flask, and dilute to volume with water. Retain this solution for analysis under *Sodium Oxide*.

Procedure Use a pipet to transfer a 10.0-mL aliquot of *Sample Solution* into a 100-mL volumetric flask, dilute to volume with water, and mix.

Set a suitable atomic absorption spectrophotometer to a wavelength of 309.3 nm. Adjust the instrument to zero absorbance against water. Prepare and read the absorbance of four aqueous solutions containing 5, 10, 20, and 50 μg/mL of aluminum, in the form of the chloride, and plot the standard curve as absorbance versus concentration of aluminum.

Aspirate a 1:10 dilution of *Sample Solution* into the spectrophotometer, read the absorbance in the same manner, and by reference to the standard curve, determine the concentration (C) of aluminum, in micrograms per milliliter, in the *Sample Solution*.

Calculate the quantity, in milligrams, of Al_2O_3 in the sample taken by the formula

$$(250C \times 10 \times 1.8895)/1000.$$

Sodium Oxide

Set a suitable flame photometer to a wavelength of 589 nm. Adjust the instrument to zero transmittance against water, and then adjust it to 100.0% transmittance with an aqueous solution containing 200 μg/mL of sodium, in the form of the chloride. Prepare and read the percent transmittance of three other solutions containing 50, 100, and 150 μg/mL each of sodium, and plot the standard curve as percent transmittance versus concentration of sodium.

Aspirate the *Sample Solution* prepared for the *Aluminum Oxide* determination into the photometer, read the percent transmittance in the same manner, and by reference to the standard curve, determine the concentration (C) of sodium, in micrograms per milliliter, in the *Sample Solution*. Calculate the quantity, in milligrams, of Na_2O in the sample taken by the formula

$$(250C \times 1.348/1000) - F,$$

in which F, as determined below, is the quantity of sodium oxide equivalent to any sodium sulfate present in the sample.

Correction for Sodium Sulfate Content Transfer about 1 g of sample, previously dried at 105° for 2 h and accurately weighed, into a tared platinum dish, and moisten with 8 to 10 drops of water. Add 25 mL of 70% perchloric acid and 10 mL of hydrofluoric acid, and heat on a hot plate in a hood until dense, white fumes of perchloric acid evolve.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Add 10 mL of hydrofluoric acid, and heat again to dense, white fumes. Quantitatively transfer the solution into a 400-mL beaker, add 200 mL of water, and heat to boiling. Gradually add, in small portions and while stirring constantly, an excess of hot barium chloride TS (about 10 mL), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a filter, wash until free from chloride, dry, ignite, and weigh. The weight, in milligrams, of the barium sulfate so obtained, multiplied by 0.6086, indicates its equivalent of Na_2SO_4 (C'). Calculate the correction factor (F) by the formula

$$0.437(C' \times w/W),$$

in which w is the weight, in milligrams, of the sample taken for the *Sodium Oxide* determination, and W is the weight, in milligrams, of the sample taken for the *Sodium Sulfate* determination.

Lead

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL with water, and mix. Each milliliter of this solution contains 100 μg of lead (Pb) ion. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute stepwise and quantitatively an accurately measured volume of *Lead Nitrate Stock Solution* with water to obtain the *Standard Lead Solution*, which contains 0.50 μg/mL of lead (Pb) ion.

Sample Solution Transfer 10.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 4, or equivalent, filter paper into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Procedure Set a suitable atomic absorption spectrophotometer to a wavelength of 217 nm. Adjust the instrument to zero absorbance against water. Read the absorbance of the *Standard Lead Solution*.

Aspirate the *Sample Solution* into the spectrophotometer, and measure the absorbance in the same manner. The absorbance obtained from the *Sample Solution* is not greater than that obtained from the *Standard Lead Solution*.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Loss on Ignition Transfer about 5 g of sample, previously dried at 105° for 2 h and accurately weighed, into a suitable tared crucible, and ignite at 900° to constant weight.

pH (20% slurry) Determine as directed under *pH Determination*, Appendix IIB, using a 1:5 slurry prepared with carbon dioxide-free water.

Packaging and Storage Store in well-closed containers.

Sodium Aluminum Phosphate, Acidic

SALP

$Na_3Al_2H_{15}(PO_4)_8$ Formula wt, anhydrous 897.82
$Na_3Al_3H_{14}(PO_4)_8 \cdot 2H_2O$ Formula wt, dihydrate 959.83
$Na_3Al_3H_{14}(PO_4)_8 \cdot 4H_2O$ Formula wt, tetrahydrate 993.84

INS: 541(i) CAS: anhydrous [7785-88-8]
CAS: dihydrate [15136-87-5]
CAS: tetrahydrate [10305-76-7]

DESCRIPTION

Sodium Aluminum Phosphate, Acidic, occurs as a white powder. It is anhydrous or contains two or four molecules of water of hydration. It is insoluble in water, but is soluble in hydrochloric acid.

Function Leavening agent.

REQUIREMENTS

Identification A 1:10 solution in 1:2 hydrochloric acid gives positive tests for *Aluminum* and for *Phosphate*, Appendix IIIA, and it responds to the flame test for *Sodium*, Appendix IIIA.
Assay *Tetrahydrate*: Not less than 95.0%; *Anhydrous*: Not less than 95.0%.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.0025%.
Lead Not more than 2 mg/kg.
Loss on Ignition *Tetrahydrate*: Between 19.5% and 21.0%; *Anhydrous*: Between 15.0% and 16.0%.

TESTS

Assay Transfer about 800 mg of sample, accurately weighed, into a 400-mL beaker, add 100 mL of water and 25 mL of nitric acid, cover with a watch glass, and boil for 10 min on a hot plate. Rinse any condensate from the watch glass into the beaker, cool the solution to room temperature, transfer it quantitatively to a 500-mL volumetric flask, dilute to volume with water, and mix thoroughly. Pipet 20.0 mL of this solution into a 500-mL Erlenmeyer flask, add 100 mL of water, and heat just to boiling. While stirring, add 50 mL of quimociac TS, cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling; filter through a tared, sintered-glass filter crucible of medium porosity; and wash with five 25-mL portions of water. Dry at about 225° for 30 min, cool, and weigh. Each milligram of precipitate thus obtained is equivalent to 53.66 μg of $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$ or 50.72 μg of $Na_3Al_2H_{15}(PO_4)_8$.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of 1:2 hydrochloric acid.
Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB, using a 2.0-g sample, accurately weighed.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Ignition Ignite a sample at 750° to 800° for 2 h.

Packaging and Storage Store in tightly closed containers.

Sodium Aluminum Phosphate, Basic

KASAL

INS: 541(ii) CAS: [7785-88-8]
CAS: [10279-59-1]

DESCRIPTION

Sodium Aluminum Phosphate, Basic, occurs as a white powder consisting of an autogenous mixture of an alkaline sodium

aluminum phosphate [approximately $Na_8Al_2(OH)_2(PO_4)_4$] with about 30% dibasic sodium phosphate. It is soluble in hydrochloric acid; the sodium phosphate moiety is soluble in water, whereas the sodium aluminum phosphate moiety is only sparingly soluble in water.

Function Emulsifier.

REQUIREMENTS

Identification A 1:10 solution in 1:2 hydrochloric acid gives positive tests for *Aluminum* and for *Phosphate*, Appendix IIIA, and it responds to the flame test for *Sodium*, Appendix IIIA.
Assay Not less than 9.5% and not more than 12.5% of Al_2O_3, calculated on the ignited basis.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.0025%.
Lead Not more than 2 mg/kg.
Loss on Ignition Not more than 9.0%.

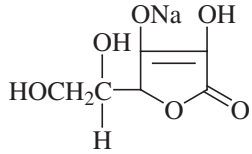
TESTS

Assay Transfer about 2.5 g of sample, accurately weighed, into a 400-mL beaker, add 15 mL of hydrochloric acid and one glass bead, cover with a watch glass, and boil gently for about 5 min. Rinse any condensate on the watch glass into the beaker, cool the solution to room temperature, transfer it quantitatively to a 250-mL volumetric flask, dilute to volume with water, and mix thoroughly. Transfer 10.0 mL of this solution to a 250-mL beaker, add phenolphthalein TS, and neutralize with 6 *N* ammonium hydroxide. Add 1:2 hydrochloric acid until the precipitate just dissolves, then dilute to 100 mL with water and heat to 70° to 80°. Add 10 mL of 8-hydroxyquinoline TS and sufficient ammonium acetate TS until a yellow precipitate forms, then add 30 mL in excess. Digest at 70° for 30 min, filter through a previously dried and weighed sintered glass filter crucible, and wash thoroughly with hot water. Dry at 105° for 2 h, cool, and weigh. Each milligram of the precipitate so obtained corresponds to 0.111 mg of Al_2O_3.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of 1:2 hydrochloric acid.
Fluoride Determine as directed under *Fluoride Limit Test,* Appendix IIIB, using 2.0 g of sample, accurately weighed.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Ignition Ignite at 750° to 800° for 2 h.

Packaging and Storage Store in tightly closed containers.

Sodium Ascorbate

Vitamin C Sodium; Sodium L-Ascorbate



$C_6H_7NaO_6$ Formula wt 198.11

INS: 301 CAS: [134-03-2]

DESCRIPTION

Sodium Ascorbate occurs as a white to yellow, crystalline powder that does not darken on exposure to light. One gram is soluble in 2 mL of water. The pH of a 1:10 aqueous solution is about 7.5.

Function Antioxidant; meat curing aid; nutrient.

REQUIREMENTS

Identification

A. A 1:50 aqueous solution slowly reduces alkaline cupric tartrate TS at 25°, and does so more readily upon heating.

B. Add 4 drops of methylene blue TS to 2 mL of a 1:50 aqueous solution acidified with 0.5 mL of 0.1 *N* hydrochloric acid, and warm to 40°. The deep blue color disappears almost completely within 3 min.

C. Dissolve 15 mg of sample in 15 mL of a 1:20 trichloroacetic acid:water solution, add about 200 mg of activated charcoal, shake vigorously for 1 min, and filter through a small fluted filter, returning the filtrate, if necessary, until clear. Add 1 drop of pyrrole to 5 mL of the filtrate, agitate gently until dissolved, and then heat in a water bath at 50°. A blue color appears.

D. A sample gives positive tests for *Sodium*, Appendix IIIA.
Assay Not less than 99.0% and not more than 101.0% of $C_6H_7NaO_6$ after drying.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.25%.
Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +103° and +108°.

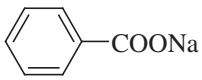
TESTS

Assay Dissolve about 400 mg of sample, previously dried over phosphorus pentoxide for 24 h and accurately weighed, in a mixture of 100 mL of water, recently boiled and cooled, and 25 mL of 2 *N* sulfuric acid, and titrate with 0.1 *N* iodine, adding starch TS near the endpoint. Each milliliter of 0.1 *N* iodine is equivalent to 9.905 mg of $C_6H_7NaO_6$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Dry a sample in vacuum over phosphorus pentoxide at 60° for 4 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 1 g of sample in each 10 mL of water.

Packaging and Storage Store in tight, light-resistant containers.

Sodium Benzoate



$C_7H_5NaO_2$ Formula wt 144.11

INS: 211 CAS: [532-32-1]

DESCRIPTION

Sodium Benzoate occurs as white granules, crystalline powder, or flakes. One gram dissolves in 2 mL of water, in 75 mL of alcohol, and in 50 mL of 90% alcohol.

Function Preservative; antimicrobial agent.

REQUIREMENTS

Identification A sample gives positive tests for *Sodium* and for *Benzoate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of $C_7H_5NaO_2$, calculated on the anhydrous basis.
Alkalinity (as NaOH) Not more than 0.04%.
Lead Not more than 2 mg/kg.
Water Not more than 1.5%.

TESTS

Assay Transfer about 600 mg of sample, accurately weighed, to a 250-mL beaker, add 100 mL of glacial acetic acid, and stir until the sample is completely dissolved. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, using crystal violet TS as the indicator. Each milliliter of 0.1 *N* perchloric acid is equivalent to 14.41 mg of $C_7H_5NaO_2$.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Alkalinity (as NaOH) Dissolve 2 g of sample in 20 mL of hot water, and add 2 drops of phenolphthalein TS. If a pink color appears, not more than 0.2 mL of 0.1 *N* sulfuric acid is required to discharge it.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Sodium Bicarbonate

Sodium Hydrogen Carbonate; Baking Soda

$NaHCO_3$ Formula wt 84.01

INS: 500(ii) CAS: [144-55-8]

DESCRIPTION

Sodium Bicarbonate occurs as a white, crystalline powder. It is stable in dry air, but it slowly decomposes in moist air. One gram dissolves in 10 mL of water. It is insoluble in alcohol. Its solutions, when freshly prepared with cold water without shaking, are alkaline to litmus. The alkalinity increases as the solutions stand, are agitated, or are heated.

Function pH control agent; leavening agent.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Sodium* and for *Bicarbonate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of $NaHCO_3$ after drying.
Ammonia Passes test.
Insoluble Substances Passes test.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.25%.

TESTS

Assay Dissolve about 3 g of sample, previously dried over silica gel for 4 h and accurately weighed, in 100 mL of water. Add 2 drops of methyl red TS, and titrate with 1 *N* hydrochloric acid. Add the acid slowly, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the faint pink color no longer fades after boiling. Each milliliter of 1 *N* hydrochloric acid is equivalent to 84.01 mg of $NaHCO_3$.
Ammonia Heat 1 g of sample in a test tube. No odor of ammonia is detected.
Insoluble Substances One gram dissolves completely in 20 mL of water to give a clear solution.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample over silica gel for 4 h.

Packaging and Storage Store in well-closed containers.

Sodium Bisulfate

Sodium Acid Sulfate; Nitre Cake

$NaHSO_4$ Formula wt 120.06

INS: 514 CAS: [7681-38-1]

DESCRIPTION

Sodium Bisulfate occurs as white crystals or granules. It is soluble in water, and its solutions are strongly acid. It is decomposed by alcohol into sodium sulfate and free sulfuric acid.

Function Acidifier.

REQUIREMENTS

Identification A sample gives positive tests for *Sodium* and for *Sulfate*, Appendix IIIA.
Assay Not less than 35.0% and not more than 39.0% of available H_2SO_4, equivalent to not less than 85.4% and not more than 95.2% of $NaHSO_4$.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.8%.
Selenium Not more than 5 mg/kg.
Water-Insoluble Substances Not more than 0.05%.

TESTS

Assay Dissolve about 5 g of sample, accurately weighed, in about 125 mL of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sodium hydroxide is equivalent to 49.04 mg of H_2SO_4, or to 120.06 mg of $NaHSO_4$.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample in a desiccator over phosphorus pentoxide for 24 h.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 1.2-g sample.
Water-Insoluble Substances Dissolve 50 g of sample in 300 mL of hot water contained in a 600-mL beaker; allow the insoluble matter to settle; and filter by decanting through a tared, sintered-glass filter crucible, washing the insoluble matter into the crucible with additional hot water. Dry at 100° to 110° for 1 h, cool in a desiccator, and weigh.

Packaging and Storage Store in tight containers.

Sodium Bisulfite

Sodium Acid Sulfite; Sodium Hydrogen Sulfite

$NaHSO_3$ Formula wt 104.06

INS: 222 CAS: [7631-90-5]

DESCRIPTION

Sodium Bisulfite occurs as white or yellow-white crystals or as a granular powder. It consists of sodium bisulfite ($NaHSO_3$) and sodium metabisulfite ($Na_2S_2O_5$) in varying proportions and, for all practical purposes, possesses properties of the true bisulfite. It is unstable in air. One gram dissolves in 4 mL of water. It is slightly soluble in alcohol.

Function Preservative.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Sodium* and for *Sulfite*, Appendix IIIA.
Assay Not less than 58.5% and not more than 67.4% of SO_2.
Iron Not more than 0.005%.
Lead Not more than 2 mg/kg.
Selenium Not more than 5 mg/kg.

TESTS

Assay Add about 200 mg of sample, accurately weighed, to 50.0 mL of 0.1 *N* iodine contained in a glass-stoppered flask, and stopper the flask. Allow the mixture to stand for 5 min, add 1 mL of hydrochloric acid, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each milliliter of 0.1 *N* iodine is equivalent to 3.203 mg of SO_2.
Iron Add 2 mL of hydrochloric acid to 500 mg of sample, and evaporate to dryness on a steam bath. Dissolve the residue in 2 mL of hydrochloric acid and 20 mL of water, add a few drops of bromine TS, and boil the solution to remove the bromine. Cool, dilute with water to 25 mL, then add 50 mg of ammonium persulfate and 5 mL of ammonium thiocyanate TS. Any red or pink color does not exceed that produced in a control containing 2.5 mL of *Iron Standard Solution* (25 µg Fe) (see *Solutions and Indicators*).
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, Appendix IIIB, using a 1.2-g sample.

Packaging and Storage Store in well-filled, tight containers, and avoid exposure to excessive heat.

Sodium Carbonate

Soda Ash

Na_2CO_3 Formula wt, anhydrous 105.99
$Na_2CO_3 \cdot H_2O$ Formula wt, monohydrate 124.00
$Na_2CO_3 \cdot 10H_2O$ Formula wt, decahydrate 286.14

INS: 500(i)

CAS: anhydrous [497-19-8]
CAS: monohydrate [5968-11-6]
CAS: decahydrate [6132-02-1]

DESCRIPTION

Sodium Carbonate occurs as colorless crystals or as a white, granular or crystalline powder. It is anhydrous or may contain 1 or 10 molecules of water of hydration. It is freely soluble in water and insoluble in ethanol. Its solutions are alkaline to litmus. The anhydrous salt is hygroscopic, and the two hydrates are efflorescent. The decahydrate melts at about 32°.

Function pH control.

REQUIREMENTS

Identification A sample gives positive tests for *Sodium* and for *Carbonate*, Appendix IIIA.
Assay Not less than 99.5% and not more than 100.5% of Na_2CO_3 after drying.
Lead Not more than 4 mg/kg.
Loss on Drying *Anhydrous*: Not more than 1%; *Monohydrate*: Between 12.0% and 15.0%; *Decahydrate*: Between 55.0% and 65.0%.

TESTS

Assay Accurately weigh about 2 g of the dried sample, obtained as directed under *Loss on Drying* (below), and dissolve it in 50 mL of water. Add 2 drops of methyl red TS, and titrate with 1 *N* hydrochloric acid, adding the acid slowly, while constantly stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool, and continue the titration until the faint pink color no longer fades after boiling. Each milliliter of 1 *N* hydrochloric acid is equivalent to 53.00 mg of Na_2CO_3.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying about 3 g of the anhydrous or monohydrate sample, accurately weighed, at 275° to 300° to constant weight. For the decahydrate, heat about 8 g of sample, accurately weighed, first at 70°, then gradually raise the temperature, and dry the sample at 275° to 300° to constant weight.

Packaging and Storage Store the anhydrous salt and the decahydrate in tight containers; store the monohydrate in well-closed containers.

Sodium Chloride

Salt

NaCl Formula wt 58.44

CAS: [7647-14-5]

DESCRIPTION

Sodium Chloride occurs as a transparent to opaque, white crystalline solid of variable particle size. Salt is a generic term applied to commercially produced Sodium Chloride. It is available in various crystalline forms, referred to as evaporated salt, rock salt (may be white to off white), solar salt, or simply salt. It may contain up to 2% (total) of suitable food-grade anticaking, free-flowing, or conditioning agents, either singly or in combination. It may contain not more than 13 mg/kg of sodium ferrocyanide, or not more than 25 mg/kg of green ferric ammonium citrate as crystal-modifying and anticaking agents. If labeled as iodized, it contains not less than 0.006% and not more than 0.010% of potassium iodide.

Sodium Chloride remains dry in air at a relative humidity below 75%, but becomes deliquescent at higher humidity. One gram is soluble in 2.8 mL of water at 25°, in 2.7 mL of boiling water, and in about 10 mL of glycerin. Sodium Chloride containing water-insoluble anticaking, free-flowing, and conditioning agents may produce cloudy solutions or may dissolve incompletely. A 1:20 aqueous solution usually has a pH between 5.5 and 8.5 (the pH may be higher if alkaline conditioning agents have been added).

Function Nutrient; preservative; flavoring agent and intensifier; curing agent; dough conditioner.

REQUIREMENTS

Labeling Indicate whether the article is iodized.
Identification A sample gives positive tests for *Sodium* and for *Chloride*, Appendix IIIA.
Assay *Evaporated Salt with up to 2% of Suitable Free-Flowing or Conditioning Agents and Anticaking Agents Such as Sodium Ferrocyanide*: Not less than 97.5% or more than 100.5% of NaCl after drying at 625° for 2 h; *Evaporated Salt with Only Anticaking Agents Such as Sodium Ferrocyanide*: Not less than 99.0% or more than 100.5% after drying at 625° for 2 h; *Rock or Solar Salt*: Not less than 97.5% or more than 100.5% of NaCl after drying at 625° for 2 h, the remainder consisting chiefly of minor amounts of naturally occurring components such as alkaline and/or alkaline earth sulfates and chlorides.
Arsenic Not more than 1 mg/kg.
Calcium and Magnesium *Salt Other than Evaporated Salt with Only Anticaking Agents Such as Sodium Ferrocyanide*: Not more than 0.9%; *Evaporated Salt with Only Anticaking Agents Such as Sodium Ferrocyanide*: Not more than 0.35%.
Heavy Metals (as Pb) Not more than 2 mg/kg.

Iodine (**Note**: This specification applies only to iodized salt.) Not less than 0.006% and not more than 0.010% of potassium iodide.
Iron (**Note**: This specification applies only to products to which green ferric ammonium citrate has been added.) Not more than 0.0016% of iron (Fe).
Loss on Drying Not more than 0.5%.
Sodium Ferrocyanide (**Note**: This specification applies only to products to which sodium ferrocyanide has been added.) Not more than 0.0013% of anhydrous sodium ferrocyanide [$Na_4Fe(CN)_6$].

TESTS

Note: In the following procedures, it may be necessary to filter the sample solutions to avoid interference from insoluble or suspended anticaking, free-flowing, or conditioning agents.

Assay Dissolve about 250 mg of sample, previously dried at 625° for 2 h and accurately weighed, in 50 mL of water in a glass-stoppered flask. Add, while agitating, 3 mL of nitric acid, 5 mL of nitrobenzene, 50.0 mL of 0.1 *N* silver nitrate, and 2 mL of ferric ammonium sulfate TS. Shake well, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Each milliliter of 0.1 *N* silver nitrate is equivalent to 5.844 mg of NaCl.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 3 g of sample in 25 mL of water.
Calcium and Magnesium

Standard EDTA Solution Dissolve 4.0 g of disodium EDTA ($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$) in sufficient water to make 1000 mL.

Magnesium Sulfate Solution Dissolve 2.6 g of magnesium sulfate ($MgSO_4{\cdot}7H_2O$) in sufficient water to make 1000 mL.

Buffer Solution

Initial Preparation Transfer 67.5 g of ammonium chloride into a 1000-mL volumetric flask, and dissolve in 570 mL of ammonium hydroxide. Use 2 mL of this solution as directed under *Titer Determination* (below).

Final Preparation Pipet 50.0 mL of *Magnesium Sulfate Solution* into the flask, add exactly the volume, *T*, in milliliters, of *Standard EDTA Solution*, determined as directed under *Titer Determination* (below), then dilute to volume with water, and mix.

Titer Determination Pipet 50.0 mL of *Magnesium Sulfate Solution* into a 400-mL beaker, and add 200 mL of water, 2 mL of *Buffer Solution Initial Preparation*, 1.0 mL of a 1:20 potassium cyanide solution, and 5 drops of eriochrome black TS or another suitable indicator. While stirring with a magnetic stirrer, titrate with the *Standard EDTA Solution* to a true blue endpoint. Record the volume, *T*, in milliliters, of *Standard EDTA Solution* equivalent to 50.0 mL of *Magnesium Sulfate Solution*.

Standardization of EDTA Solution Transfer about 1 g of primary standard calcium carbonate ($CaCO_3$), accurately weighed, into a 1000-mL volumetric flask, dissolve in 800 mL of water containing 5 mL of hydrochloric acid, dilute to volume with water, and mix. Pipet 25.0 mL of this solution into a 400-mL beaker, and add 200 mL of water, 2 mL of *Buffer Solution Final Preparation*, 1.0 mL of a 1:20 potassium cyanide solution, and 20 drops of eriochrome black TS or another suitable indicator. While stirring with a magnetic stirrer, titrate with the *Standard EDTA Solution* to a true blue endpoint. Calculate the factor, *F*, giving the number of milligrams of calcium (Ca) equivalent to 1.0 mL of *Standard EDTA Solution*, by the formula

$$10.011w/v,$$

in which *w* is the exact weight, in grams, of the primary standard calcium carbonate taken, and *v* is the volume, in milliliters, of *Standard EDTA Solution* required in the titration.

Sample Preparation for Rock and Solar Salts Transfer 50.0 g of sample into a 500-mL volumetric flask, dissolve in 400 mL of water containing 2 mL of hydrochloric acid, dilute to volume with water, and mix. Filter a 50-mL aliquot, then pipet 10.0 mL of the filtrate into a 400-mL beaker, and add 190 mL of water.

Sample Preparation for Evaporated Salt Transfer 10.0 g of sample into a 400-mL beaker, and dissolve in 100 mL of water. If free-flowing agents are present, filter and rinse quantitatively. Dilute the solution or filtrate to 200 mL with water.

Procedure Add 5 mL of *Buffer Solution Final Preparation*, 1 mL of a 1:20 potassium cyanide solution, and 5 drops of eriochrome black TS or another suitable indicator to the *Sample Preparation*. Begin stirring with a magnetic stirrer, and titrate with *Standard EDTA Solution* to a true blue endpoint, recording the volume, in milliliters, required as *V*. Calculate the milligrams per kilogram of total calcium and magnesium (both expressed as Ca) in the sample by the formula

$$V \times F \times 1000/W,$$

in which *W* is the weight, in grams, of sample in the final solution titrated.
Heavy Metals (as Pb)

The Committee on Food Chemicals Codex notes that this method may be used only until the First Supplement to this edition is released in 2004. At that time, the committee will set a lead limit as low as practicable for sodium chloride. Manufacturers are encouraged to develop and validate methods for use in industrial settings and that are sensitive enough to detect lead in the amounts typically present in sodium chloride, and to propose such methods to the committee in a timely manner.

This test is designed to limit the content of common metallic impurities colored by sulfide ion (Ag, As, Bi, Cd, Cu, Hg, Pb, Sb, Sn) by comparing the color with a standard containing lead (Pb) ion under the specified test conditions. It demonstrates that the test substance is not grossly contaminated by such heavy metals, and within the precision of the test, that it does not exceed the *Heavy Metals* limit given as determined by concomitant visual comparison with a control solution. In

the specified pH range, the optimum concentration of lead (Pb) ion for matching purposes by this method is 20 μg in 50 mL of solution.

The most common limitation of the *Heavy Metals Test* is that the color the sulfide ion produces in the *Sample Solution* depends on the metals present and may not match the color in the *Lead Solution* used for matching purposes. Lead sulfide is brown, as are Ag, Bi, Cu, Hg, and Sn sulfides. While it is possible that ions not mentioned here may also yield non-matching colors, among the nine common metallic impurities listed above, the sulfides with different colors are those of As and Cd, which are yellow, and that of Sb, which is orange. If a yellow or orange color is observed, the following action is indicated: Because this monograph does not include an arsenic requirement, As should be determined. Any As found should not exceed 3 mg/kg. If these criteria are met, Cd may be a contributor to the yellow color, so the Cd content should be determined. If an orange color is observed, the Sb content should be determined. These additional tests are in accord with the section on *Trace Impurities* in the *General Provisions* of this book, as follows: ''if other possible impurities may be present, additional tests may be required, and should be applied, as necessary, by the manufacturer, vendor, or user to demonstrate that the substance is suitable for its intended application.''

Determine the amount of heavy metals by *Method I* or *Method II* as the following criteria specify: Use *Method I* for samples that yield clear, colorless solutions before adding sulfide ion. Use *Method II* for samples that do not yield clear, colorless solutions under the test conditions specified for *Method I*. Use *Method III*, a wet digestion method, only in those cases where neither *Method I* nor *Method II* can be used.

Special Reagents

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL with water, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* to 100.0 mL with water. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 μg of lead (Pb) ion.

Procedure

(**Note**: In the following procedures, failure to accurately adjust the pH of the solution within the specified limits may result in a significant loss of test sensitivity.)

Method I

Sample Solution Dilute 10 g of sample in 35 mL of water.

Solution A Pipet 2.0 mL of *Standard Lead Solution* (20 μg of Pb) into a 50-mL color-comparison tube, and add water to make 25 mL. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute to 40 mL with water, and mix.

Solution B Place 25 mL of the *Sample Solution* into a 50-mL color-comparison tube that matches the one used for *Solution A*, adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute to 40 mL with water, and mix.

Solution C Place 25 mL of the *Sample Solution* into a third color-comparison tube that matches those used for *Solutions A* and *B*, and add 2.0 mL of *Standard Lead Solution*. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute to 40 mL with water, and mix.

Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*, and the intensity of the color of *Solution C* is equal to or greater than that of *Solution A*. If the color of *Solution C* is lighter than that of *Solution A*, the sample is interfering with the test procedure and *Method II* must be used.

Method II

Solution A Prepare as directed under *Method I*.

Solution B Place a quantity of sample, accurately weighed, into a suitable crucible, add sufficient sulfuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the sample is thoroughly carbonized, add 2 mL of nitric acid and 5 drops of sulfuric acid, cautiously heat until white fumes no longer evolve, then ignite, preferably in a muffle furnace, at 500° to 600° until all of the carbon is burned off. Cool, add 4 mL of 1:2 hydrochloric acid, cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 min. Add 6 *N* ammonia dropwise until the solution is just alkaline to litmus paper, dilute to 25 mL with water, and adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid. Filter if necessary, rinse the crucible and the filter with 10 mL of water, transfer the solution and rinsings into a 50-mL color-comparison tube, dilute to 40 mL with water, and mix.

Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*.

Method III

Solution A Transfer an 8:10 (v/v) mixture of sulfuric acid:nitric acid into a 100-mL Kjeldahl flask, clamp the flask at an angle of 45°, and then add, in small increments, an additional volume of nitric acid equal to that added in the preparation of *Solution B* (below). Heat the solution to dense, white fumes, cool, and cautiously add 10 mL of water. Add a volume of 30% hydrogen peroxide equal to that added in the preparation of *Solution B* (below) then boil gently to dense, white fumes, and cool. Cautiously add 5 mL of water, mix, and boil gently to dense, white fumes. Continue boiling until the volume is reduced to about 2 or 3 mL, then cool, and dilute cautiously with a few milliliters of water. Pipet 2.0 mL of *Standard Lead Solution* into this solution, and mix. Transfer the solution into a 50-mL color-comparison tube, rinse the flask with water, add the rinsings to the tube until the volume

is 25 mL, and mix. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper), initially with ammonium hydroxide and then with 6 *N* ammonia as the desired range is neared, dilute to 40 mL with water, and mix.

Solution B Transfer 4 mg of sample, accurately weighed, into a 100-mL Kjeldahl flask (or into a 300-mL flask if the reaction foams excessively), clamp the flask at an angle of 45°, and add a sufficient amount of an 8:10 (v/v) mixture of sulfuric acid:nitric acid to moisten the sample thoroughly. Warm gently until the reaction begins, allow the reaction to subside, and then add additional portions of the acid mixture, heating after each addition, until all of the 18 mL of acid mixture has been added. Increase the heat, and boil gently until the reaction mixture darkens. Remove the flask from the heat, add 2 mL of nitric acid, and heat to boiling again. Continue the intermittent heating and addition of 2-mL portions of nitric acid until no further darkening occurs, then heat strongly to dense, white fumes, and cool. Cautiously add 5 mL of water, mix, boil gently to dense, white fumes, and continue heating until the volume is reduced to about 2 or 3 mL. Cool, cautiously add 5 mL of water, and examine. If the solution is yellow, cautiously add 1 mL of 30% hydrogen peroxide, and again evaporate to dense, white fumes and to a volume of about 2 or 3 mL. Cool, dilute cautiously with a few milliliters of water, and mix. Transfer into a 50-mL color-comparison tube, rinse the flask with water, add the rinsings to the tube until the volume is 25 mL, and mix. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper), initially with ammonium hydroxide and then with 6 *N* ammonia as the desired range is neared, dilute to 40 mL with water, and mix.

Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*.

Iodine Transfer about 20 g of sample, accurately weighed, into a 600-mL beaker, and dissolve in about 300 mL of water. Add a few drops of methyl orange TS, neutralize the solution with 85% phosphoric acid, and then add 1 mL excess of the acid. Add 25 mL of bromine TS and a few glass beads, boil until the solution is clear, then boil for an additional 5 min. Add about 50 mg of salicylic acid crystals, 1 mL of phosphoric acid, and 10 mL of a 1:20 potassium iodide solution, and titrate to a pale yellow color with 0.01 *N* sodium thiosulfate. Add 1 mL of starch TS, and continue the titration to the disappearance of the blue color. Each milliliter of 0.01 *N* sodium thiosulfate is equivalent to 0.2767 mg of potassium iodide (KI).

Iron

Solution 1 Dissolve 625.0 mg of sample in 10 mL of 2.7 *N* hydrochloric acid, and dilute to 50 mL with water. Add about 40 mg of ammonium persulfate crystals and 10 mL of ammonium thiocyanate TS.

Solution 2 Dissolve 10 µg of iron (Fe) in an equal volume of solution containing 2 mL of hydrochloric acid and the same quantities of ammonium persulfate crystals and ammonium thiocyanate TS used in *Solution 1*.

Any red or pink color from *Solution 1* does not exceed that produced by 1.0 mL of *Solution 2*.

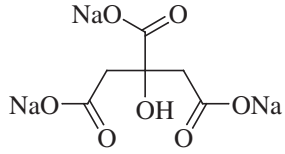
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 110° for 2 h.

Sodium Ferrocyanide Prepare a *Sample Solution* by dissolving 9.62 g of sample in 80 mL of water in a 150-mL glass-stoppered cylinder or flask. Prepare a *Standard Solution* containing 125 µg of sodium ferrocyanide [$Na_4Fe(CN)_6$], in each milliliter by dissolving 99.5 mg of decahydrate ferrocyanide [$Na_4Fe(CN)_6 \cdot 10H_2O$] in 500.0 mL of water. Transfer 1.0 mL of *Standard Solution* into a similar 150-mL container for the control. Add 2 mL of ferrous sulfate TS and 1 mL of 2 *N* sulfuric acid to each container, dilute to 100 mL with water, and mix. Transfer 50-mL portions of the sample and control solutions into matched color-comparison tubes. The *Sample Solution* shows no more blue color than the control.

Packaging and Storage Store in well-closed containers.

Sodium Citrate

Trisodium Citrate



$C_6H_5Na_3O_7$ Formula wt, anhydrous 258.07
$C_6H_5Na_3O_7 \cdot 2H_2O$ Formula wt, dihydrate 294.10

INS: 331(iii) CAS: anhydrous [68-04-2]
CAS: dihydrate [6132-04-3]

DESCRIPTION

Sodium Citrate occurs as colorless crystals or as a white, crystalline powder. It is anhydrous or contains two molecules of water of crystallization. One gram of the dihydrate dissolves in 1.5 mL of water at 25° and in 0.6 mL of boiling water. It is insoluble in alcohol.

Function Buffer; sequestrant; emulsion stabilizer; nutrient for cultured buttermilk.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Sodium* and for *Citrate*, Appendix IIIA.

Assay Not less than 99.0% and not more than 100.5% of $C_6H_5Na_3O_7$, calculated on the anhydrous basis.

Alkalinity Passes test.

Lead Not more than 2 mg/kg.

Water *Anhydrous*: Not more than 1%; *Dihydrate*: Between 10.0% and 13.0%.

TESTS

Assay Transfer about 350 mg of sample, accurately weighed, into a 250-mL beaker. Add 100 mL of glacial acetic acid, stir until completely dissolved, and titrate with 0.1 *N* perchloric acid, using crystal violet TS as the indicator.

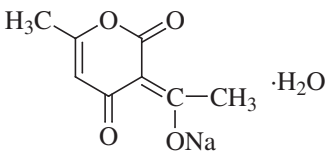
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Each milliliter of 0.1 *N* perchloric acid is equivalent to 8.602 mg of $C_6H_5Na_3O_7$.
Alkalinity A solution of 1 g of sample in 20 mL of water is alkaline to litmus paper. Add 0.2 mL of 0.1 *N* sulfuric acid. No pink color appears when 1 drop of phenolphthalein TS is added.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Sodium Dehydroacetate

Sodium 3-(1-Hydroxyethylidene)-6-methyl-1,2-pyran-2,4(3H)-dione



$C_8H_7NaO_4 \cdot H_2O$ Formula wt 208.15

INS: 266 CAS: [4418-26-2]

DESCRIPTION

Sodium Dehydroacetate occurs as a white or nearly white powder. One gram dissolves in about 3 mL of water, in 2 mL of propylene glycol, and in 7 mL of glycerin.

Function Preservative.

REQUIREMENTS

Identification Dissolve about 1.5 g of sample in 10 mL of water, add 5 mL of 2.7 *N* hydrochloric acid, collect the crystals with suction, wash with 10 mL of water, and dry between 75° and 80° for 4 h. The crystals melt between 109° and 111° (see *Melting Range or Temperature*, Appendix IIB).
Assay Not less than 98.0% and not more than 100.5% of $C_8H_7NaO_4$, calculated on the anhydrous basis.
Lead Not more than 2 mg/kg.
Water Between 8.5% and 10.0%.

TESTS

Assay Transfer about 500 mg of sample, accurately weighed, into a 125-mL Erlenmeyer flask, dissolve it in 25 mL of glacial acetic acid containing 1 drop of a 1:100 *p*-naphtholbenzene:glacial acetic acid solution that has been previously neutralized to a blue color, and titrate with 0.1 *N* perchloric acid to the original blue color. Each milliliter of 0.1 *N* perchloric acid is equivalent to 19.01 mg of $C_8H_7NaO_4$.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Sodium Diacetate

Sodium Hydrogen Diacetate

$CH_3COONa \cdot CH_3COOH \cdot xH_2O$

$C_4H_7NaO_4 \cdot xH_2O$ Formula wt, anhydrous 142.09

INS: 262 CAS: [126-96-5]

DESCRIPTION

Sodium Diacetate occurs as a white, hygroscopic, crystalline solid. It is a molecular compound of sodium acetate and acetic acid. One gram is soluble in about 1 mL of water. The pH of a 1:10 aqueous solution is between 4.5 and 5.0.

Function Sequestrant; preservative; antimicrobial agent; mold inhibitor.

REQUIREMENTS
Identification A 1:10 aqueous solution gives positive tests for *Acetate* and for *Sodium*, Appendix IIIA.
Assay Not less than 39.0% and not more than 41.0% of free acetic acid (CH_3COOH), and not less than 58.0% and not more than 60.0% of sodium acetate (CH_3COONa), calculated on the anhydrous basis.
Lead Not more than 2 mg/kg.
Readily Oxidizable Substances (as formic acid) Not more than 0.2%.
Water Not more than 2.0%.

TESTS

Assay

Free Acetic Acid Dissolve about 4 g of sample, accurately weighed, in 50 mL of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sodium hydroxide is equivalent to 60.05 mg of acetic acid (CH_3COOH).

Sodium Acetate Content Dissolve about 500 mg of sample, accurately weighed, in 50 mL of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Each milliliter of 0.1 *N* perchloric acid is equivalent to 8.203 mg of sodium acetate (CH_3COONa).

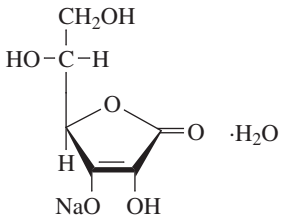
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Readily Oxidizable Substances Dissolve 1.0 g of sample in about 50 mL of water, add 10 mL of 2 *N* sulfuric acid, and heat the solution to between 80° and 90°. Titrate the hot solution with 0.1 *N* potassium permanganate to a faint pink color that persists for at least 15 s. Each milliliter of 0.1 *N* potassium permanganate is equivalent to 2.301 mg of formic acid (CH_2O_2).

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Sodium Erythorbate



$C_6H_7NaO_6 \cdot H_2O$ Formula wt 216.12

CAS: [6381-77-7]

DESCRIPTION

Sodium Erythorbate occurs as a white, crystalline powder or as granules. In the dry state it is reasonably stable in air, but in solution it deteriorates in the presence of air, trace metals, heat, and light. One gram dissolves in about 7 mL of water. The pH of a 1:20 aqueous solution is between 5.5 and 8.0.

Function Preservative; antioxidant.

REQUIREMENTS

Identification

A. A 1:50 aqueous solution slowly reduces alkaline cupric tartrate TS at 25°, but does so more readily upon heating.

B. Add a few drops of sodium nitroferricyanide TS, followed by 1 mL of 0.1 *N* sodium hydroxide to 2 mL of a 1:50 aqueous solution acidified with 0.5 mL of 0.1 *N* hydrochloric acid. A transient blue color immediately appears.

C. It gives positive tests for *Sodium*, Appendix IIIA.

Assay Not less than 98.0% and not more than 100.5% of $C_6H_7NaO_6 \cdot H_2O$.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 0.25%.

Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +95.5° and +98.0°.

Oxalate Passes test.

TESTS

Assay Dissolve about 400 mg of sample, accurately weighed, in a mixture of 100 mL of water, recently boiled and cooled, and 25 mL of 2 *N* sulfuric acid, and immediately titrate with 0.1 *N* iodine, adding starch TS as the indicator near the endpoint. Each milliliter of 0.1 *N* iodine is equivalent to 10.81 mg of $C_6H_7NaO_6 \cdot H_2O$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared from a 2-g sample as directed for organic compounds, and 10 µg of lead (Pb) ion in the control.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at room temperature in a vacuum over silica gel for 24 h.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 1 g of sample in each 10 mL of solute.

Oxalate Add 2 drops of glacial acetic acid and 5 mL of a 1:10 solution of calcium acetate to a solution of 1 g of sample in 10 mL of water. The solution remains clear.

Packaging and Storage Store in tight, light-resistant containers.

Sodium Ferric Pyrophosphate

Sodium Iron Pyrophosphate

$Na_8Fe_4(P_2O_7)_5 \cdot xH_2O$ Formula wt, anhydrous 1277.02

CAS: [1332-96-3]

DESCRIPTION

Sodium Ferric Pyrophosphate occurs as a white to tan powder. It is insoluble in water, but is soluble in hydrochloric acid.

Function Nutrient.

REQUIREMENTS

Identification Dissolve 500 mg of sample in 5 mL of 1:2 hydrochloric acid, and add an excess of 1 *N* sodium hydroxide. A red-brown precipitate forms. Age the solution for several minutes, and then filter, discarding the first few milliliters. Add 1 drop of bromophenol blue TS to 5 mL of the clear filtrate, and titrate with 1 *N* hydrochloric acid to a green color. Add 10 mL of a 1:8 zinc sulfate solution, and readjust the pH to 3.8 (green color). A white precipitate forms (distinction from orthophosphates).

Assay Not less than 14.5% and not more than 16.0% of Fe.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Lead Not more than 4 mg/kg.

Loss on Ignition Not more than 8.0%.

Mercury Not more than 3 mg/kg.

TESTS

Assay Dissolve about 3.5 g of sample, accurately weighed, in 75 mL of 1:2 hydrochloric acid, heat to boiling, and boil for about 5 min. Cool, transfer into a 100-mL volumetric flask, dilute to volume with the 1:2 hydrochloric acid, and mix. Add 100 mL of the 1:2 hydrochloric acid to 25.0 mL of this solution, boil again for 5 min, and add, dropwise and while stirring, stannous chloride TS to the boiling solution, until the iron is just reduced as indicated by the disappearance of the yellow color. Add 2 drops in excess (but no more) of the stannous chloride TS, dilute with about 50 mL of water, and cool to room temperature. While stirring vigorously, add 15 mL of a saturated solution of mercuric chloride, and then allow to stand for 5 min. Add 15 mL of a sulfuric acid–phosphoric acid mixture (prepared by slowly adding 75 mL of sulfuric acid to 300 mL of water, cooling, adding 75 mL of phosphoric acid, and then diluting to 500 mL with water). Mix, add 0.5 mL of barium diphenylamine sulfonate TS, and titrate with 0.1 *N* potassium dichromate to a red-violet endpoint. Each milliliter of 0.1 *N* potassium dichromate is equivalent to 5.585 mg of Fe.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 15 mL of 2.7 *N* hydrochloric acid.

Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB, using 1.0 g of sample, accurately weighed.

Lead (**Note**: In preparing all aqueous solutions and in rinsing glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a lead content as practicable, and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in warm 8 *N* nitric acid for 30 min and by rinsing with deionized water.)

Ascorbic Acid–Sodium Iodide Solution Dissolve 20 g of ascorbic acid and 38.5 g of sodium iodide in water in a 200-mL volumetric flask, dilute with water to volume, and mix.

Trioctylphosphine Oxide Solution (**Caution**: This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.) Dissolve 5.0 g of trioctylphosphine oxide in 4-methyl-2-pentanone in a 100-mL volumetric flask, dilute with the same solvent to volume, and mix.

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute with water to 1000.0 mL, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Preparation and Blank Transfer 5.0 mL of *Lead Nitrate Stock Solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of the resulting solution to a 50-mL volumetric flask. Add 10 mL of 9 *N* hydrochloric acid and about 10 mL of water to this volumetric flask and to a second, empty 50-mL volumetric flask (*Blank*). Add 20 mL of *Ascorbic Acid–Sodium Iodide Solution* and 5.0 mL of *Trioctylphosphine Oxide Solution* to each flask, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of each flask, shake again, and allow the layers to separate. The organic solvent layers are the *Blank* and the *Standard Preparation*, and they contain 0.0 and 2.0 μg of lead per milliliter, respectively.

Test Preparation Add 2.5 g of sample, 10 mL of 9 *N* hydrochloric acid, about 10 mL of water, 20 mL of *Ascorbic Acid–Sodium Iodide Solution*, and 5.0 mL of *Trioctylphosphine Oxide Solution* to a 50-mL volumetric flask, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again and allow the layers to separate. The organic solvent layer is the *Test Preparation.*

Procedure Concomitantly determine the absorbance of the *Blank*, the *Standard Preparation*, and the *Test Preparation* at the lead emission line at 283.3 nm, with a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp and an air–acetylene flame, using 4-methyl-2-pentanone to set the instrument to zero. In a suitable analysis, the absorbance of the *Blank* is not greater than 20% of the difference between the absorbance of the *Standard Preparation* and the absorbance of the *Blank*. The absorbance of the *Test Preparation* does not exceed that of the *Standard Preparation*.

Loss on Ignition Ignite a sample at 800° for 1 h.

Mercury

Standard Preparations Dissolve 338.5 mg of mercuric chloride in about 200 mL of water in a 250-mL volumetric flask, add 14 mL of 1:2 sulfuric acid, dilute to volume with water, and mix. Pipet 10.0 mL of this solution into a 1000-mL volumetric flask containing about 800 mL of water and 56 mL of 1:2 sulfuric acid, dilute to volume with water, and mix. Pipet 10.0 mL of the second solution into a second 1000-mL volumetric flask containing 800 mL of water and 56 mL of 1:2 sulfuric acid, dilute to volume with water, and mix. Each milliliter of this diluted stock solution contains 0.1 μg of mercury. Pipet 1.25, 2.50, 5.00, 7.50, and 10.00 mL of the last solution (equivalent to 0.125, 0.250, 0.500, 0.750, and 1.00 μg of mercury, respectively) into five separate 150-mL beakers. Add 25 mL of aqua regia to each beaker, cover with

watch glasses, heat just to boiling, simmer for about 5 min, and cool to room temperature. Transfer the solutions into separate 250-mL volumetric flasks, dilute to volume with water, and mix. Transfer a 50.0-mL aliquot from each solution into five separate 150-mL beakers, and add 1.0 mL of 1:5 sulfuric acid and 1.0 mL of a 1:25 filtered potassium permanganate solution to each. Heat the solutions just to boiling, simmer for about 5 min, and cool.

Sample Preparation Transfer 5.00 g of sample into a 150-mL beaker, add 25 mL of aqua regia, cover with a watch glass, and allow to stand at room temperature for about 5 min. Heat just to boiling, allow to simmer for about 5 min, and cool. Transfer the solution into a 250-mL volumetric flask, dilute to volume with water, and mix.

Note: Disregard any undissolved material that may be present.

Transfer a 50.0-mL aliquot of this solution into a 150-mL beaker, and add 1.0 mL of 1:5 sulfuric acid and 1.0 mL of a filtered 1:25 potassium permanganate solution. Heat the solution just to boiling, simmer for about 5 min, and cool. Prepare a reagent blank in the same manner.

Apparatus Use a Mercury Detection Instrument as described and an Aeration Apparatus as shown in Fig. 16 under *Mercury Limit Test*, Appendix IIIB. For the purposes of the test described in this monograph, the Techtron AA-1000 atomic absorption spectrophotometer, equipped with a 10-cm silica absorption cell (Beckman Part No. 75144, or equivalent) and coupled with a strip chart recorder (Varian Series A-25, or equivalent), is satisfactory.

Procedure Assemble the *Aeration Apparatus* as shown in Fig. 16 under *Mercury Limit Test*, Appendix IIIB. Use magnesium perchlorate as the absorbent in the absorption cell (*e*), fill gas washing bottle *c* with 60 mL of water, and place stopcock *b* in the bypass position. Connect the assembly to the 10-cm absorption cell (analogous to *f* in the figure) of the spectrophotometer, and adjust the air or nitrogen flow rate so that, in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a baseline reading at 253.7 nm by following the equipment manufacturer's operating instructions. Using the Techtron AA-1000 spectrophotometer, the following conditions are suitable: slit width: 2 Å; lamp current: 3 mA; and scale expansion: × 1. With the strip chart recorder, set the chart speed at 25 in./h and the span at 2 mV. Precondition the apparatus by an appropriate modification of the procedures described below for treatment of the test solutions.

Note: Keep the fritted bubbler in gas washing bottle *c* immersed in water between determinations. After each determination, wash the bubbler with a stream of water.

Treat the blank, each of the *Standard Preparations*, and the *Sample Preparation* as follows: Transfer the solution to be tested into a 125-mL gas-washing bottle (*c*), using a few drops of 1:10 hydroxylamine hydrochloride solution to remove any manganese hydroxide from the beaker. Dilute to about 55 mL with water, and add a magnetic stirring bar. Discharge the permanganate color by adding, dropwise, the 1:10 hydroxylamine hydrochloride solution, swirling after each drop is added. Add 15.0 mL of 10% stannous chloride solution [prepared by dissolving 20 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in 40 mL of warm hydrochloric acid and diluting with 160 mL of water], and immediately connect gas-washing bottle *c* to the aeration apparatus. Switch on the magnetic stirrer, turn stopcock *b* from the bypass to the aerating position, and obtain the absorbance reading. Disconnect bottle *c* from the aeration apparatus, discard the solution just tested, wash bottle *c* and the fritted bubbler with water, and repeat the procedure with the remaining solutions. Correct the sample readings for the reagent blank, and determine the mercury concentration of the *Sample Preparation* from a standard curve prepared by plotting the readings obtained with the *Standard Preparations* against mercury concentration, in milligrams per kilogram, suitable adjustments being made for dilution factors.

Packaging and Storage Store in well-closed containers.

Sodium Ferrocyanide

Yellow Prussiate of Soda

$Na_4Fe(CN)_6 \cdot 10H_2O$ Formula wt 484.07

INS: 535 CAS: [13601-19-9]

DESCRIPTION

Sodium Ferrocyanide occurs as yellow crystals or as a crystalline powder. It is soluble in water, but it is practically insoluble in most organic solvents.

Function Anticaking agent for sodium chloride.

REQUIREMENTS

Identification Add 1 mL of ferric chloride TS to 10 mL of a 1% solution of the sample. A dark blue precipitate forms.
Assay Not less than 99.0% of $Na_4Fe(CN)_6 \cdot 10H_2O$.
Chloride Not more than 0.2%.
Cyanide Passes test.
Ferricyanide Passes test.
Free Moisture Not more than 1%.
Insoluble Matter Not more than 0.03%.
Sulfate Not more than 0.07%.

TESTS

Assay Transfer about 3 g of sample, accurately weighed, into a 400-mL beaker, dissolve it in 225 mL of water, and cautiously add about 25 mL of 95% sulfuric acid. Add, with stirring, 1 drop of orthophenanthroline TS, and titrate with 0.1 *N* ceric sulfate until the color changes sharply from orange to pure yellow. Each milliliter of 0.1 *N* ceric sulfate is equivalent to 96.81 mg of $Na_4Fe(CN)_6 \cdot 10H_2O$.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, using 100 mg of sample dissolved in 100 mL of water. Any turbidity produced by a 10-mL portion of this solution does not exceed that shown in a control containing 20 μg of chloride (Cl) ion.

Cyanide Dissolve 10 mg of copper sulfate in a mixture of 8 mL of water and 2 mL of 6 *N* ammonium hydroxide. Wet a strip of filter paper with this solution, and place the wet paper in a stream of hydrogen sulfide. When 1 drop of a 1% solution of the sample is placed on the brown reagent paper, no white circle appears.

Ferricyanide Dissolve about 10 mg of sample in 10 mL of water, and place 1 drop of this solution on a spot plate. Add 1 drop of a 1% solution of lead nitrate followed by a few drops of a solution prepared by saturating cold 2 *N* acetic acid with benzidine. No blue precipitate or blue color appears.

Free Moisture Heat 20 g of sample at 105° for 6 h, cool in a desiccator, and weigh. Grind the dried sample rapidly, heat 3 g of the powder to constant weight at 105°, and calculate the total water content (*W*). Calculate the percent free moisture in the sample by the formula

$$W - 0.3721A,$$

in which *A* is the percentage of $Na_4Fe(CN)_6 \cdot 10H_2O$ found in the *Assay*.

Insoluble Matter Dissolve 50 g of sample in 300 mL of hot water, and filter off the insoluble matter into a tared, sintered-glass filter crucible. Wash the residue thoroughly with hot water, dry the crucible in an oven at 105° for 1 h, cool in a desiccator, and weigh.

Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 500-mg sample does not exceed that shown in a control containing 350 μg of sulfate (SO_4).

Packaging and Storage Store in tight containers.

Sodium Gluconate

Sodium D-Gluconate

$$CH_2OH(CHOH)_4COONa$$

$C_6H_{11}NaO_7$ Formula wt 218.14

INS: 576 CAS: [527-07-1]

DESCRIPTION

Sodium Gluconate occurs as a white to tan, granular to fine, crystalline powder. It is very soluble in water, and is sparingly soluble in alcohol. It is insoluble in ether.

Function Nutrient; sequestrant.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Sodium*, Appendix IIIA.

B. Dissolve a quantity of sample in water, heating in a water bath at 60° if necessary, to obtain a *Test Solution* containing 10 mg/mL. Similarly, prepare a *Standard Solution* of USP Potassium Gluconate Reference Standard in water, diluting to 10 mg/mL. Apply separate 5-μL portions of the *Test Solution* and the *Standard Solution* on a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent system consisting of a mixture of alcohol, water, ammonium hydroxide, and ethyl acetate (50:30:10:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with a spray reagent prepared as follows: Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute to volume with 2 *N* sulfuric acid, and mix. After spraying, heat the plate at 110° for about 10 min. The principal spot obtained from the *Test Solution* corresponds in color, size, and R_f value to that obtained from the *Standard Solution*.

Assay Not less than 98.0% and not more than 102.0% of $C_6H_{11}NaO_7$.

Lead Not more than 2 mg/kg.

Reducing Substances Not more than 0.5%, calculated as D-glucose.

TESTS

Assay Transfer about 150 mg of sample, accurately weighed, into a clean, dry 200-mL Erlenmeyer flask, add 75 mL of glacial acetic acid, and dissolve by heating on a hot plate. Cool, add quinaldine red TS, and using a 10-mL microburet, titrate with 0.1 *N* perchloric acid in glacial acetic acid to a colorless endpoint. Each milliliter of 0.1 *N* perchloric acid is equivalent to 21.81 milligrams of $C_6H_{11}NaO_7$.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Lead Determine as directed for the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Reducing Substances Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, dissolve it in 10 mL of water, add 25 mL of alkaline cupric citrate TS, and cover the flask with a small beaker. Boil gently for exactly 5 min, and cool rapidly to room temperature. Add 25 mL of a 1:10 solution of acetic acid, 10.0 mL of 0.1 *N* iodine, 10 mL of 2.7 *N* hydrochloric acid, and 3 mL of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in milligrams, of reducing substances (as D-glucose) by the formula

$$27(V_1N_1 - V_2N_2),$$

in which 27 is an empirically determined equivalence factor for D-glucose; V_1 and N_1 are the volume and normality, respectively, of the iodine solution; and V_2 and N_2 are the volume and normality, respectively, of the sodium thiosulfate solution.

Packaging and Storage Store in well-closed containers.

Sodium Hydroxide

Caustic Soda; Lye

NaOH Formula wt 40.00

INS: 524 CAS: [1310-73-2]

DESCRIPTION

Sodium Hydroxide occurs as white or nearly white pellets, flakes, sticks, fused masses, or other forms. Upon exposure to air it readily absorbs carbon dioxide and moisture. One gram dissolves in 1 mL of water. It is freely soluble in alcohol.

Function pH control agent.

REQUIREMENTS

Identification A 1:25 aqueous solution gives positive tests for *Sodium*, Appendix IIIA.
Assay Not less than 95.0% and not more than 100.5% of total alkali, calculated as NaOH.
Arsenic Not more than 3 mg/kg.
Carbonate (as Na_2CO_3) Not more than 3.0%.
Insoluble Substances and Organic Matter Passes test.
Lead Not more than 2 mg/kg.
Mercury Not more than 0.1 mg/kg.

TESTS

Assay Dissolve about 1.5 g of sample, accurately weighed, in 40 mL of recently boiled and cooled water, cool to 15°, add phenolphthalein TS, and titrate with 1 *N* sulfuric acid. When the pink color disappears, record the volume of acid required, then add methyl orange TS, and continue the titration until a persistent pink color appears. Record the total volume of acid required for the titration. Each milliliter of 1 *N* sulfuric acid is equivalent to 40.00 mg of total alkali, calculated as NaOH.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using the following *Sample Solution*: Dissolve 1 g of sample in about 10 mL of water, cautiously neutralize to litmus paper with sulfuric acid, and cool.
Carbonate (as Na_2CO_3) Each milliliter of 1 *N* sulfuric acid required between the phenolphthalein and methyl orange endpoints in the *Assay* (above) is equivalent to 106.0 mg of disodium carbonate.
Insoluble Substances and Organic Matter A 1:20 aqueous solution is complete, clear, and colorless to slightly colored.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 2 μg of lead (Pb) ion in the control and the following as the *Sample Solution*: Dissolve 1 g of sample in a mixture of 5 mL of water and 11 mL of 2.7 *N* hydrochloric acid, and cool.
Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB, preparing the *Standard Preparation* and the *Sample Preparation* as follows:

Standard Preparation Prepare a solution containing 1 μg of mercury per milliliter as directed in *Standard Preparation* under *Mercury Limit Test*, Appendix IIIB. Transfer 1.0 mL of the solution into a 50-mL beaker, and add 20 mL of water, 1 mL of 1:5 sulfuric acid, and 1 mL of a 1:25 solution of potassium permanganate. Cover the beaker with a watch glass, boil for a few seconds, and cool.

Sample Preparation Transfer 10.0 g of sample into a 100-mL beaker, dissolve in 15 mL of water, add 2 drops of phenolphthalein TS, and while constantly stirring, slowly neutralize with 1:2 hydrochloric acid. Add 1 mL of 1:5 sulfuric acid and 1 mL of a 1:25 solution of potassium permanganate, cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in tight containers.

Sodium Hydroxide Solutions

Caustic Soda Solutions; Lye Solutions

DESCRIPTION

Sodium Hydroxide Solutions occur as clear or slightly turbid, colorless or slightly colored liquids. They are usually available in nominal concentrations of 50% and 73% (w/w) of NaOH. These solutions are strongly caustic and hygroscopic. When exposed to air, they absorb carbon dioxide, forming sodium carbonate.

Function pH control agent.

REQUIREMENTS

Labeling Indicate the percent of Sodium Hydroxide (NaOH).
Identification Sample solutions give positive tests for *Sodium*, Appendix IIIA.
Assay Not less than 97.0% and not more than 103.0%, by weight, of the labeled amount of NaOH, calculated as total alkalinity.
Arsenic Not more than 3 mg/kg, calculated on the basis of NaOH determined in the *Assay*.
Carbonate (as Na_2CO_3) Not more than 3.0%, calculated on the basis of NaOH determined in the *Assay*.

Lead Not more than 2 mg/kg, calculated on the basis of NaOH determined in the *Assay*.
Mercury Not more than 0.1 mg/kg, calculated on the basis of NaOH determined in the *Assay*.

TESTS

Caution: Sodium Hydroxide Solutions are a corrosive irritant to skin, eyes, and mucous membranes.

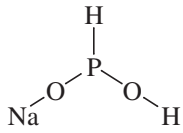
Assay Based on the stated or labeled percentage of NaOH, accurately weigh a volume of the sample solution equivalent to about 1.5 g of sodium hydroxide, and dilute it to 40 mL with recently boiled and cooled water. Continue as directed under *Assay* in the monograph for *Sodium Hydroxide*, beginning with ''. . . cool to 15°. . . .''
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using the following as the *Sample Solution*: Dilute an accurately weighed amount of sample equivalent to 1 g of sodium hydroxide, calculated on the basis of the *Assay* (above), to 10 mL with water, cautiously neutralize to litmus paper with sulfuric acid, and cool.
Carbonate (as Na_2CO_3) Each milliliter of 1 *N* sulfuric acid required between the phenolphthalein and methyl orange endpoints under *Assay* (above) is equivalent to 106.0 mg of disodium carbonate.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 2 µg of lead (Pb) ion in the control and the following as the *Sample Solution*: Dilute an accurately weighed amount of sample equivalent to 1 g of sodium hydroxide, calculated on the basis of the *Assay*, with a mixture of 5 mL of water and 11 mL of 2.7 *N* hydrochloric acid.
Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB, preparing the *Standard Preparation* and the *Sample Preparation* as follows:

Standard Preparation Prepare a solution containing 1 µg of mercury per milliliter as directed in *Standard Preparation* under *Mercury Limit Test*, Appendix IIIB. Transfer 1.0 mL of the solution into a 50-mL beaker, and add 20 mL of water, 1 mL of a 1:5 sulfuric acid solution, and 1 mL of a 1:25 potassium permanganate solution. Cover the beaker with a watch glass, boil for a few seconds, and cool.

Sample Preparation Transfer 10.0 g of sample into a 100-mL beaker, dissolve in 15 mL of water, add 2 drops of phenolphthalein TS, and while constantly stirring, slowly neutralize with 1:2 hydrochloric acid. Add 1 mL of 1:5 sulfuric acid and 1 mL of a 1:25 solution of potassium permanganate, cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in tight containers.

Sodium Hypophosphite



$NaH_2PO_2 \cdot H_2O$ Formula wt 105.99

CAS: [7681-53-0]

DESCRIPTION

Sodium Hypophosphite occurs as a white, crystalline powder; as white granules; or as colorless, pearly crystalline plates. It is very deliquescent. One milliliter of water dissolves about 1 g at 25° and about 6 g at 100°. It is slightly soluble in alcohol.

Caution: Take care in mixing Sodium Hypophosphite with nitrates, chlorates, or other oxidizing agents because an explosion may occur if the mixtures are triturated or heated.

Function Preservative; antioxidant.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Sodium* and for *Hypophosphites*, Appendix IIIA.
Assay Not less than 97.0% and not more than 103.0% of $NaH_2PO_2 \cdot H_2O$.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 10 mg/kg.
Insoluble Substances Not more than 0.1%.
Lead Not more than 4 mg/kg.

TESTS

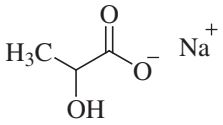
Assay Dissolve about 100 mg of sample, accurately weighed, in 20 mL of water; add 40.0 mL of 0.1 *N* ceric sulfate prepared as directed for *Volumetric Solutions* under *Solutions and Indicators* (or use a commercially available solution); mix well; and add 2 mL of silver sulfate solution (5 g of Ag_2SO_4 dissolved in 95 mL of concentrated sulfuric acid). Cover, heat nearly to boiling, and continue heating for 30 min. Cool to room temperature, and titrate with 0.1 *N* ferrous ammonium sulfate to a pale yellow color. Add 2 drops of orthophenanthroline TS, and continue the titration to a salmon-colored endpoint, recording the volume required, in milliliters, as *S*. Perform a residual blank titration (see *General Provisions*), and record the volume required as *B*. Each milliliter of the volume $B - S$ is equivalent to 2.650 mg of $NaH_2PO_2 \cdot H_2O$.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.
Fluoride Determine as directed under *Fluoride Limit Test*, Appendix IIIB.

Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter through a tared filtering crucible. Wash the residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Packaging and Storage Store in tight containers.

Sodium Lactate Solution

2-Hydroxypropanoic Acid, Monosodium Salt



$C_3H_5NaO_3$ Formula wt, anhydrous 112.06

INS: 325 CAS: [72-17-3]

DESCRIPTION

Sodium Lactate Solution occurs as a clear, colorless or practically colorless, slightly viscous liquid that is odorless or has a slight, not unpleasant odor. It is miscible with water. It is normally available in solutions with concentrations ranging from 60% to about 80%, by weight.

Function Emulsifier; flavor enhancer; flavoring agent or adjuvant; humectant; pH control agent.

REQUIREMENTS

Labeling Indicate the content, by weight, of sodium lactate ($C_3H_5NaO_3$).
Identification A sample gives positive tests for *Sodium* and for *Lactate*, Appendix IIIA.
Assay Not less than 50.0%, by weight, and not less than 98.0% and not more than 102.0%, by weight, of the labeled amount of $C_3H_5NaO_3$.
Chloride Not more than 0.05%.
Citrate, Oxalate, Phosphate, or Tartrate Passes test.
Cyanide Not more than 0.5 mg/kg.
Lead Not more than 2 mg/kg.
Methanol and Methyl Esters Not more than 0.025%.
pH Between 5.0 and 9.0.
Sugars Passes test.
Sulfate Not more than 0.005%.

TESTS

Assay Transfer a volume of sample, equivalent to about 300 mg of sodium lactate and accurately weighed, into a suitable flask. Add 60 mL of 1:5 acetic anhydride:glacial acetic acid, mix, and allow to stand for 20 min. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 11.21 mg of $C_3H_5NaO_3$.
Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a quantity of a sample solution containing the equivalent of 40 mg of sodium lactate does not exceed that shown in a control containing 20 µg of chloride (Cl) ion.
Citrate, Oxalate, Phosphate, or Tartrate Dilute 5 mL of sample to 50 mL with recently boiled and cooled water. Add 6 *N* ammonium hydroxide or 3 *N* hydrochloric acid to 4 mL of this solution, if necessary, to bring the pH to between 7.3 and 7.7. Add 1 mL of calcium chloride TS, and heat in a boiling water bath for 5 min. The solution remains clear.
Cyanide (**Caution**: Because of the extremely poisonous nature of potassium cyanide, conduct this test in a fume hood, and exercise great care to prevent skin contact and the inhalation of particles or vapors of solutions of the material. Under no conditions pipet solutions by mouth.)

p-*Phenylenediamine–Pyridine Mixed Reagent* Dissolve 200 mg of *p*-phenylenediamine hydrochloride in 100 mL of water, warming to aid dissolution. Cool, allow the solids to settle, and save the supernatant liquid to make the mixed reagent. Dissolve 128 mL of pyridine in 365 mL of water, add 10 mL of hydrochloric acid, and mix. To prepare the mixed reagent, mix 30 mL of the *p*-phenylenediamine supernatant liquid with all of the pyridine solution, and allow to stand for 24 h before using. The mixed reagent is stable for about 3 weeks when stored in an amber bottle.

Cyanide Standard Solution Dissolve 250 mg of potassium cyanide, accurately weighed, in 10 mL of 0.1 *N* sodium hydroxide contained in a 100 mL volumetric flask, dilute to volume with 0.1 *N* sodium hydroxide, and mix. Transfer a 10-mL aliquot into a 1000-mL volumetric flask, dilute to volume with 0.1 *N* sodium hydroxide, and mix. Each milliliter of this solution contains 10 µg of cyanide.

Sample Solution Transfer a quantity of sample, equivalent to 20.0 g of sodium lactate and accurately weighed, into a 100-mL volumetric flask, dilute to volume with water, and mix.

Procedure Pipet a 10-mL aliquot of the *Sample Solution* into a 50-mL beaker. Pipet 0.1 mL of the *Cyanide Standard Solution* into a second 50-mL beaker, and add 10 mL of water. Place the beakers in an ice bath, and adjust the pH to between 9 and 10 with 20% sodium hydroxide, stirring slowly and adding the reagent slowly to avoid overheating. Allow the solutions to stand for 3 min, and then slowly add 10% phosphoric acid to a pH between 5 and 6, measured with a pH meter.

Transfer the solutions into 100-mL separators containing 25 mL of cold water, and rinse the beakers and pH meter electrodes with a few milliliters of cold water, collecting the washings in the respective separator. Add 2 mL of bromine

TS, stopper, and mix. Add 2 mL of 2% sodium arsenite solution, stopper, and mix. Add 10 mL of *n*-butanol to the clear solutions, stopper, and mix. Finally, add 5 mL of p-*Phenylenediamine–Pyridine Mixed Reagent*, mix, and allow to stand for 15 min. Remove and discard the aqueous phases, and filter the alcohol phases into 1-cm cells. The absorbance of the solution from the *Sample Solution*, determined at 480 nm with a suitable spectrophotometer, is no greater than that from the *Cyanide Standard Solution*.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Methanol and Methyl Esters

Potassium Permanganate and Phosphoric Acid Solution Dissolve 3 g of potassium permanganate in a mixture of 15 mL of phosphoric acid and 70 mL of water. Dilute to 100 mL with water.

Oxalic Acid and Sulfuric Acid Solution Cautiously add 50 mL of sulfuric acid to 50 mL of water, mix, cool, add 5 g of oxalic acid, and mix to dissolve.

Standard Preparation Prepare a solution containing 10.0 mg of methanol in a 100-mL volumetric flask, dilute to volume with 1:10 alcohol, and mix.

Test Preparation Place 40.0 g of sample in a glass-stoppered, round-bottom flask, add 10 mL of water, and cautiously add 30 mL of 5 *N* potassium hydroxide. Connect a condenser to the flask, and steam-distill, collecting the distillate in a suitable 100-mL graduated vessel containing 10 mL of alcohol. Continue the distillation until the volume in the receiver reaches approximately 95 mL, and dilute the distillate to 100.0 mL with water.

Procedure Transfer 10.0 mL each of the *Standard Preparation* and the *Test Preparation* to 25-mL volumetric flasks. Add 5.0 mL of *Potassium Permanganate and Phosphoric Acid Solution* to each, and mix. After 15 min, add 2.0 mL of *Oxalic Acid and Sulfuric Acid Solution* to each, stir with a glass rod until the solutions are colorless, add 5.0 mL of fuchsin–sulfurous acid TS (prepared as directed under *Solutions and Indicators*), dilute to volume with water, and mix. After 2 h, using a suitable spectrophotometer, concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 575 nm, using water as the blank. The absorbance of the solution from the *Test Preparation* is not greater than that from the *Standard Preparation*.

pH Determine as directed under *pH Determination*, Appendix IIB.

Sugars Add 5 drops of sample to 10 mL of hot alkaline cupric tartrate TS. No red precipitate forms.

Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a quantity of the sample solution containing the equivalent of 4.0 g of sodium lactate does not exceed that shown in a control solution containing 200 µg of sulfate (SO_4) ion.

Packaging and Storage Store in tight containers.

Sodium Lauryl Sulfate

Sodium Dodecyl Sulfate

INS: 487 CAS: [151-21-3]

DESCRIPTION

Sodium Lauryl Sulfate occurs as small, white or light yellow crystals. It is a mixture of sodium alkylsulfates consisting chiefly of Sodium Lauryl Sulfate [$CH_3(CH_2)_{10}CH_2OSO_3Na$]. One gram dissolves in 10 mL of water, forming an opalescent solution.

Function Surface-active agent.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Sodium*, Appendix IIIA. After acidifying with hydrochloric acid and boiling gently for 20 min, it responds to the tests for *Sulfate*, Appendix IIIA.

Assay Not less than 59.0% of total alcohols.

Alkalinity (as NaOH) Passes test (about 0.25%).

Combined Sodium Chloride and Sodium Sulfate Not more than 8.0%.

Lead Not more than 2 mg/kg.

Unsulfated Alcohols Not more than 4.0%.

TESTS

Assay Transfer about 5 g of sample, accurately weighed, to an 800-mL Kjeldahl flask, and add 150 mL of water, 50 mL of hydrochloric acid, and a few boiling chips. Attach a reflux condenser to the flask, heat carefully to avoid excessive frothing, and then boil for about 4 h. Cool the flask, and rinse the condenser with ether, collecting the ether in the flask. Transfer the contents to a 500-mL separator, rinsing the flask twice with ether and adding the washings to the separator. Extract the solution with two 75-mL portions of ether, evaporate the combined ether extracts in a tared beaker on a steam bath, dry the residue at 105° for 30 min, cool, and weigh. The residue represents the total alcohols.

Alkalinity (as NaOH) Dissolve 1 g of sample in 100 mL of water, add phenol red TS, and titrate with 0.1 *N* hydrochloric acid. Not more than 0.5 mL is required for neutralization.

Combined Sodium Chloride and Sodium Sulfate

Sodium Chloride Dissolve about 5 g of sample, accurately weighed, in about 50 mL of water. Neutralize the solution with 1:20 nitric acid, using litmus paper as the indicator; add 2 mL of potassium chromate TS; and titrate with 0.1 *N* silver nitrate. Each milliliter of 0.1 *N* silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).

Sodium Sulfate Transfer about 1 g of sample, accurately weighed, to a 400-mL beaker, add 10 mL of water, heat the mixture, and stir until completely dissolved. Add 100 mL of alcohol to the hot solution, cover, and digest at a temperature just below the boiling point for 2 h. Filter while hot through

a sintered-glass filter crucible, and wash the precipitate with 100 mL of hot alcohol. Dissolve the precipitate in the crucible by washing it with about 150 mL of water, collecting the washings in a beaker. Acidify the beaker's contents with 10 mL of hydrochloric acid, heat to boiling, add 25 mL of barium chloride TS, and allow to stand overnight. Collect the precipitate of barium sulfate on a suitable tared, porous-bottom porcelain filter crucible, wash until free from chloride, dry, and ignite to constant weight at 800° ± 25°. The weight of barium sulfate so obtained, multiplied by 0.6086, represents the weight of sodium sulfate (Na_2SO_4).

Note: Avoid exposing the crucible to sudden temperature changes.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Unsulfated Alcohols Dissolve about 10 g of sample, accurately weighed, in 100 mL of water, and add 100 mL of alcohol. Transfer the solution to a separator, and extract with three 50-mL portions of solvent hexane. If an emulsion forms, add sodium chloride to promote separation of the two layers. Wash the combined solvent hexane extracts with three 50-mL portions of water, and dry with anhydrous sodium sulfate. Filter the solvent hexane extract into a tared beaker, evaporate on a steam bath until the odor of solvent hexane no longer is perceptible, dry the residue at 105° for 30 min, cool, and weigh. The residue represents the unsulfated alcohols.

Packaging and Storage Store in well-closed containers.

Sodium Lignosulfonate

CAS: [8061-51-6]

DESCRIPTION

Sodium Lignosulfonate occurs as a brown, amorphous polymer. It is obtained from the spent sulfite and sulfate pulping liquor of wood or from the sulfate (kraft) pulping process. It may contain up to 30% reducing sugars. It is soluble in water, but not in any of the common organic solvents. The pH of a 1:100 aqueous solution is approximately between 3 and 11.

Function Binder; dispersant; boiler water additive.

REQUIREMENTS

Identification

A. A solution of 0.15 g of sample per liter of water gives positive tests for *Sodium*, Appendix IIIA.

B. Dissolve 100 mg of sample in 50 mL of water. Add 1 mL each of 10% acetic acid and 10% sodium nitrite solutions to this solution. Mix the solution by swirling, and allow it to stand for 15 min at room temperature. A brown color appears.

C. The ultraviolet absorption spectrum of a solution comprising 0.1 g of sample per liter at pH 5 exhibits a peak between 275 and 280 nm.

Assay Not less than 5.0% sulfonate sulfur.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 10.0%.

Reducing Sugars Not more than 30.0%.

Residue on Ignition Not more than 20.0%.

Sodium Not more than 10.0%.

Viscosity of a 50% Solution Not more than 3000 centipoises.

TESTS

Assay (as Sulfonate Sulfur) Dissolve 1.0 g of sample, accurately weighed, in 400 mL of water contained in a beaker. Direct a gentle stream of nitrogen gas over the liquid's surface. Add 10 mL of nitric acid, and swirl the solution thoroughly until the reaction subsides. Add 10 mL of 70% perchloric acid; and swirl thoroughly again.

Caution: Handle perchloric acid in an appropriate fume hood.

Place the uncovered beaker on a hot plate, and heat the contents vigorously until the center of the bottom of the beaker becomes clear. Remove the beaker, and cool to room temperature. Add 5 mL of hydrochloric acid, and heat again until white fumes evolve. After cooling, dilute the solution to approximately 100 mL with water, adjust to pH 6 ± 0.2 with 10% sodium hydroxide, and heat the solution to boiling. Add 15 mL of 10% barium chloride solution, and leave the solution overnight in a fresh beaker in a steam bath at 90° to 95°. Filter through ashless filter paper (Whatman No. 42, or equivalent), and wash the precipitate with 200 mL of warm water. Transfer the paper and precipitate into a tared crucible. Heat the crucible slowly on a Bunsen burner to expel moisture. Place the crucible and contents in a muffle furnace at 850° for 1 h. Let the crucible cool in a desiccator, and then weigh the residue to the nearest 0.0001 g. Calculate the percent sulfonate sulfur by the formula

$$(R/S) \times 13.7,$$

in which R is the weight, in grams, of the residue; S is the weight, in grams, of the sample taken; and 13.7 is the conversion factor.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 24 h.

Reducing Sugars (**Note**: The *Copper Reagent Solution* used in this test must be prepared several days in advance of use.)

Lead Subacetate Solution Dissolve 80 g of lead subacetate in 220 mL of water. Stir overnight, and filter through Whatman No. 42 filter paper, or equivalent. Dilute the supernatant solution to a specific gravity of 1.254 with freshly boiled water.

Copper Reagent Solution Dissolve 28 g of anhydrous dibasic sodium phosphate and 40 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) in 700 mL of water. Add 100

mL of 1 *N* sodium hydroxide and 8 g of copper sulfate pentahydrate, followed by 180 g of anhydrous sodium sulfate. Add 0.7134 g of potassium iodate, and dilute to 1 L. Allow to stand for several days, then filter the clear top part of the solution through a medium-porosity, sintered-glass funnel.

Dextrose Standard Solution Dissolve 140 mg of dried dextrose, accurately weighed, in 500 mL of water.

Dibasic Sodium Phosphate Solution Dissolve 19 g of sodium phosphate, dibasic, heptahydrate in 100 mL of water.

Procedure Dissolve 1 g of sample, accurately weighed, in 150 mL of water, and adjust the pH to between 6.9 and 7.2 with sodium hydroxide solution or acetic acid. Add *Lead Subacetate Solution* in increments until no further precipitation is observed. Bring the volume to 250.0 mL with water, and mix well. Centrifuge the mixture, pipet 10 mL of the supernatant into a 50-mL volumetric flask, and dilute to about 35 mL with water. Add 2 mL or more of *Dibasic Sodium Phosphate Solution* until no further precipitation forms. Dilute to 50 mL with water, and mix. Centrifuge at 2100 × gravity for 10 min. Pipet 5 mL of the supernatant solution into a test tube containing exactly 5 mL of *Copper Reagent Solution*, and mix. Loosely plug the tube, and place it in a boiling water bath for 40 min ± 10 s. At the end of the heating period, cool the tube immediately in cold water. Add 2 mL of 2.5% potassium iodide solution and 1.5 mL of 2 *N* sulfuric acid. Mix well, and titrate with 0.005 *N* sodium thiosulfate, using starch as the indicator, and note the volume of 0.005 *N* sodium thiosulfate consumed as V_S. Run a corresponding blank titration, V_B, using 5 mL of water and 5 mL of *Copper Reagent Solution*.

Repeat the entire procedure with the dextrose standard (5 mL of *Dextrose Standard Solution* and 5 mL of *Copper Reagent Solution*), noting the volume of 0.005 *N* sodium thiosulfate consumed as V_D. Run a corresponding blank titration, V_B, using 5 mL of water and 5 mL of *Copper Reagent Solution*.

Calculate the percent reducing sugars by the formula

$$35(V_B - V_S)/(V_B - V_D),$$

in which $V_B - V_S$ is the number of milliliters of 0.005 *N* sodium thiosulfate consumed by the 5-mL aliquot of sample, and $V_B - V_D$ is the number of milliliters of 0.005 *N* sodium thiosulfate consumed by 5 mL of *Dextrose Standard Solution*.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Sodium

Standard Solution Dilute a certified 1000-ppm Sodium Standard Solution (Mallinckrodt, or equivalent) quantitatively and stepwise with deionized water to obtain a *Standard Solution* containing 2 μg of sodium per milliliter of water. Store the *Standard Solution* in polyethylene bottles because of its instability in glass.

Sample Solution Transfer 1.00 ± 0.05 g of a previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 246° to 260° for 2 to 4 h. Allow the ash to cool, and dissolve it in 5 mL of 20% hydrochloric acid, warming the solution, if necessary, to complete solution of the residue. Filter the solution through acid-washed filter paper into a 500-mL volumetric flask. Wash the filter paper with hot water, dilute to volume with water, and mix. Prepare a 1:100 aqueous dilution of this solution to obtain the final *Sample Solution*.

Procedure Using a suitably calibrated atomic absorption spectrophotometer and following the manufacturer's instructions for optimum operation of the spectrophotometer, determine the absorbance of the *Standard Solution* and of the *Sample Solution* at 589.0 nm. The absorbance of the *Sample Solution* is not greater than that of the *Standard Solution*.

Viscosity of a 50% Solution Dissolve 200 g of sample, calculated on the dried basis and accurately weighed, in 200 mL of water contained in a 500-mL beaker. Equilibrate the solution at 25°, and measure its viscosity with a Brookfield viscometer A (model LVG, or equivalent), using a number 2 spindle at 20 rpm.

Packaging and Storage Store in well-closed containers.

Sodium Magnesium Aluminosilicate

CAS: [12040-43-6]

DESCRIPTION

Sodium Magnesium Aluminosilicate occurs as synthetic, amorphous, food-grade coprecipitates that are fine, white powders or beads. It comprises a series of hydrated sodium magnesium aluminosilicates having Na_2O:MgO:Al_2O_3:SiO_2 molar ratios of approximately 2:1:2:24, respectively. It has a specific gravity of about 2. It is insoluble in water, in alcohol, and in other organic solvents, but it is partially soluble in strongly acidic and alkaline solutions.

Function Anticaking agent.

REQUIREMENTS

Identification

A. Mix 500 mg of sample with 2.5 g of anhydrous potassium carbonate, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, add 5 mL of water, and allow to stand for 3 min. Heat the bottom of the crucible gently, detach the melt, and transfer it into a beaker with the aid of about 50 mL of water. Gradually add hydrochloric acid until no effervescence is observed, add 10 mL more of the acid, and evaporate to dryness on a steam bath. Cool, add 20 mL of water, boil, and filter through ash-free filter paper. An insoluble residue of silica remains. Retain the filtrate for *Identification Test B.*

Transfer the gelatinous residue to a platinum dish, and cautiously add 5 mL of hydrofluoric acid. The precipitate dissolves. (If it does not dissolve, repeat the treatment with hydrofluoric acid.) Heat, and introduce a glass stirring rod with a drop of water on the tip into the resulting vapors. The drop becomes turbid.

B. Portions of the filtrate obtained in *Identification Test A* give positive tests for *Aluminum*, for *Magnesium*, and for *Sodium*, Appendix IIIA.

Assay

Silicon Dioxide Not less than 65.0% and not more than 75.0% of SiO_2 after drying.

Aluminum Oxide Not less than 9.0% and not more than 13.0% of Al_2O_3 after drying.

Magnesium Oxide Not less than 1.0% and not more than 3.0% of MgO after drying.

Sodium Oxide Not less than 3.0% and not more than 9.0% of Na_2O after drying.

Lead Not more than 5 mg/kg.

Loss on Drying Not more than 8.0%.

Loss on Ignition Between 8.0% and 11.0% after drying.

pH (20% slurry) Between 6.5 and 11.0.

Soluble Salt (as Na_2SO_4) Not more than 7.5%.

TESTS

Assay

Silicon Dioxide

Transfer about 500 mg of sample, previously dried at 105° for 2 h and accurately weighed, into a 250-mL beaker, wash the sides of the beaker with a few milliliters of water, and add 30 mL of sulfuric acid and 15 mL of hydrochloric acid. Heat on a hot plate in a hood until dense, white fumes evolve; cool; add 15 mL of hydrochloric acid; and heat again to dense, white fumes. Cool, add 70 mL of water, and filter through Whatman No. 40, or equivalent, filter paper. Wash the filter paper and precipitate thoroughly with hot water to remove the sulfuric acid residue.

Transfer the filter paper and precipitate into a tared platinum crucible, char, and ignite at 900° to constant weight. Moisten the residue with a few drops of water, add 15 mL of hydrofluoric acid and 8 drops of sulfuric acid, and heat on a hot plate in a hood until white fumes of sulfur trioxide evolve. Cool; add 5 mL of water, 10 mL of hydrofluoric acid, and 3 drops of sulfuric acid; and evaporate to dryness on the hot plate. Heat cautiously over an open flame until sulfur trioxide fumes cease to evolve, and ignite at 900° to constant weight. The weight loss after the addition of hydrofluoric acid represents the weight of SiO_2 in the sample taken.

Aluminum Oxide

Sample Solution Transfer about 500 mg of sample, previously dried at 105° for 2 h and accurately weighed, into a tared platinum dish, and moisten with 8 to 10 drops of water. Add 25 mL of 70% perchloric acid and 10 mL of hydrofluoric acid, and heat on a hot plate until dense, white fumes of perchloric acid evolve.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Cool, add 10 mL of hydrofluoric acid, and heat again to dense, white fumes. Cool, dissolve the residue in sufficient water, quantitatively transfer it with the aid of additional water into a 250-mL volumetric flask, and dilute to volume with water. Retain this solution for analysis under *Magnesium Oxide* and *Sodium Oxide*.

Procedure Use a pipet to transfer a 10.0-mL aliquot of *Sample Solution* into a 100-mL volumetric flask, dilute to volume with water, and mix.

Set a suitable atomic absorption spectrophotometer to a wavelength of 309.3 nm. Adjust the instrument to zero absorbance against water. Prepare and read the absorbance of four aqueous solutions containing 5, 10, 20, and 50 μg/mL of aluminum, in the form of the chloride, and plot the standard curve as absorbance versus concentration of aluminum.

Aspirate a 1:10 dilution of *Sample Solution* into the spectrophotometer, read the absorbance in the same manner, and by reference to the standard curve, determine the concentration (C) of aluminum, in micrograms per milliliter, in the *Sample Solution*.

Calculate the quantity, in milligrams, of Al_2O_3 in the sample taken by the formula

$$(250C \times 10 \times 1.8895)/1000.$$

Magnesium Oxide

Set a suitable atomic absorption spectrophotometer to a wavelength of 285.2 nm. Adjust the instrument to zero absorbance against water. Prepare and read the absorbance of four aqueous solutions containing 5, 10, 25, and 50 μg/mL of magnesium, in the form of the chloride, and plot the standard curve as absorbance versus concentration of magnesium.

Aspirate the *Sample Solution* prepared for the *Aluminum Oxide* determination into the spectrophotometer, read the absorbance in the same manner, and by reference to the standard curve, determine the concentration (C) of magnesium, in micrograms per milliliter, in the *Sample Solution*.

Calculate the quantity, in milligrams, of MgO in the sample taken by the formula

$$250C \times 1.6579/1000.$$

Sodium Oxide

Set a suitable flame photometer to a wavelength of 589 nm. Adjust the instrument to zero transmittance against water, and then adjust it to 100.0% transmittance with an aqueous solution containing 200 μg/mL of sodium, in the form of the chloride. Prepare and read the percent transmittance of three other solutions containing 50, 100, and 150 μg/mL each of sodium, and plot the standard curve as percent transmittance versus concentration of sodium.

Aspirate the *Sample Solution* prepared for the *Aluminum Oxide* determination into the photometer, read the percent transmittance in the same manner, and by reference to the standard curve, determine the concentration (C) of sodium, in micrograms per milliliter, in the *Sample Solution*. Calculate the quantity, in milligrams, of Na_2O in the sample taken by the formula

$$(250C \times 1.348/1000) - F,$$

in which F, as determined below, is the quantity of sodium oxide equivalent to any sodium sulfate present in the sample.

Correction for Sodium Sulfate Content Transfer about 1 g of sample, previously dried at 105° for 2 h and accurately weighed, into a tared platinum dish, and moisten with 8 to 10 drops of water. Add 25 mL of 70% perchloric acid and

10 mL of hydrofluoric acid, and heat on a hot plate in a hood until dense, white fumes of perchloric acid evolve.

Caution: Handle perchloric acid in an appropriate fume hood.

Add 10 mL of hydrofluoric acid, and heat again to dense, white fumes. Quantitatively transfer the solution into a 400-mL beaker, add 200 mL of water, and heat to boiling. Gradually add, in small portions and while stirring constantly, an excess of hot barium chloride TS (about 10 mL), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a filter, wash until free from chloride, dry, ignite, and weigh. The weight, in milligrams, of the barium sulfate so obtained, multiplied by 0.6086, indicates its equivalent of Na_2SO_4 (C'). Calculate the correction factor (F) by the formula

$$0.437(C' \times w/W),$$

in which w is the weight, in milligrams, of the sample taken for the *Sodium Oxide* determination, and W is the weight, in milligrams, of the sample taken for the *Sodium Sulfate* determination.

Lead

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid; dilute to 1000.0 mL with water; and mix. Each milliliter of this solution contains 100 μg of lead (Pb) ion. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute, stepwise and quantitatively, an accurately measured volume of *Lead Nitrate Stock Solution* with water to obtain the *Standard Lead Solution*, which contains 0.50 μg/mL of lead (Pb) ion.

Sample Solution Transfer 10.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 4, or equivalent, filter paper into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Procedure Set a suitable atomic absorption spectrophotometer to a wavelength of 217 nm. Adjust the instrument to zero absorbance against water. Read the absorbance of the *Standard Lead Solution*.

Aspirate the *Sample Solution* into the spectrophotometer, and measure the absorbance in the same manner. The absorbance obtained from the *Sample Solution* is not greater than that obtained from the *Standard Lead Solution*.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.

Loss on Ignition Transfer about 5 g of sample, previously dried at 105° for 2 h and accurately weighed, into a suitable tared crucible, and ignite at 900° to constant weight.

pH (20% slurry) Determine as directed under *pH Determination*, Appendix IIB, using a 1:5 slurry prepared with carbon dioxide-free water.

Soluble Salt Calculate the percent sodium sulfate from the weight of barium sulfate obtained in the *Correction for Sodium Sulfate Content* in the *Assay*, by the formula

$$N \times 60.86/W,$$

in which N is the weight, in milligrams, of barium sulfate, and W is the weight, in milligrams, of the sample taken for the *Sodium Sulfate* determination.

Packaging and Storage Store in well-closed containers.

Sodium Metabisulfite

Sodium Pyrosulfite

$Na_2S_2O_5$ Formula wt 190.11

INS: 223 CAS: [7681-57-4]

DESCRIPTION

Sodium Metabisulfite occurs as colorless crystals or as a white to yellow, crystalline powder. It is freely soluble in water and slightly soluble in alcohol. Its solutions are acid to litmus.

Function Preservative; antioxidant.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Sodium* and for *Sulfite*, Appendix IIIA.

Assay Not less than 90.0% and not more than 100.5% of $Na_2S_2O_5$.

Iron Not more than 10 mg/kg.

Lead Not more than 2 mg/kg.

Selenium Not more than 5 mg/kg.

TESTS

Assay Add about 200 mg of sample, accurately weighed, to exactly 50 mL of 0.1 *N* iodine contained in a glass-stoppered flask, and stopper the flask. Allow the solution to stand for 5 min, add 1 mL of hydrochloric acid, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each milliliter of 0.1 *N* iodine is equivalent to 4.752 mg of $Na_2S_2O_5$.

Iron Add 2 mL of hydrochloric acid to 500 mg of sample, and evaporate to dryness on a steam bath. Dissolve the residue in 2 mL of hydrochloric acid and 20 mL of water, add a few drops of bromine TS, and boil the solution to remove the bromine. Cool, dilute with water to 25 mL, then add 50 mg of ammonium persulfate and 5 mL of ammonium thiocyanate TS. Any red or pink color does not exceed that produced in a control containing 1.0 mL of *Iron Standard Solution* (10 μg Fe; see *Solutions and Indicators*).

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Selenium Determine as directed in *Method I* under *Selenium Limit Test*, Appendix IIIB, using 200 mg of sample and 100 mg of magnesium oxide.

Packaging and Storage Store in well-filled, tight containers, and avoid exposure to excessive heat.

Sodium Metaphosphate, Insoluble

Insoluble Sodium Polyphosphate; IMP; Maddrell's Salt

CAS: [50813-16-6]

DESCRIPTION

Sodium Metaphosphate, Insoluble, occurs as a white, crystalline powder. It is a high-molecular-weight sodium polyphosphate composed of two long metaphosphate chains ($NaPO_3$) that spiral in opposite directions about a common axis. The Na_2O/P_2O_5 ratio is about 1.0. It is practically insoluble in water but dissolves in mineral acids and in solutions of potassium and ammonium (but not sodium) chlorides. The pH of a 1:3 slurry in water is about 6.5.

Function Emulsifier; sequestrant; texturizer.

REQUIREMENTS

Identification

A. Finely powder about 1 g of sample, and add it slowly to 100 mL of a 1:20 potassium chloride solution while stirring vigorously. A gelatinous mass forms.

B. Mix 500 mg of sample with 10 mL of nitric acid and 50 mL of water, boil for about 30 min, and cool. The resulting solution gives positive tests for *Sodium* and for *Phosphates*, Appendix IIIA.

Assay Not less than 68.7% and not more than 70.0% of P_2O_5.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Lead Not more than 4 mg/kg.

TESTS

Assay Transfer about 800 mg of sample, accurately weighed, into a 400-mL beaker, add 100 mL of water and 25 mL of nitric acid, cover with a watch glass, and boil for 10 min on a hot plate. Rinse any condensate from the watch glass into the beaker, cool the solution to room temperature, transfer it quantitatively to a 500-mL volumetric flask, dilute to volume with water, and mix thoroughly. Pipet 20.0 mL of this solution into a 500-mL Erlenmeyer flask, add 100 mL of water, and heat just to boiling. While stirring, add 50 mL of quimociac TS, then cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared, sintered-glass filter crucible of medium porosity, and wash with five 25-mL portions of water. Dry at about 225° for 30 min, cool, and weigh. Each milligram of precipitate thus obtained is equivalent to 32.074 μg of P_2O_5.
Arsenic Determine as directed under *Arsenic Limit Test,* Appendix IIIB, using a solution of 1 g in 15 mL of 2.7 *N* hydrochloric acid.
Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, Appendix IIIB.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Packaging and Storage Store in tight containers.

Sodium Metasilicate

$Na_2O{\cdot}SiO_2{\cdot}xH_2O$ Formula wt, anhydrous 122.06

INS: 550 CAS: [6834-92-0]

DESCRIPTION

Sodium Metasilicate occurs as a white, free-flowing, granular material. It is a hydrous (pentahydrate) or anhydrous silicate having a 1:1 molar ratio of SiO_2 to Na_2O. At 30°, the anhydrous Sodium Metasilicate is easily soluble in water (270 g/L) as is its pentahydrate (610 g/L). The pH values of 1% solutions of anhydrous Sodium Metasilicate and of its pentahydrate are about 12.6 and 12.4, respectively.

Function Saponifying agent; boiler water additive.

REQUIREMENTS

Caution: Sodium Metasilicate and its solutions are caustic materials and can cause eye and skin burns. Use proper protective equipment and avoid contact with the eyes, skin, and clothing. Do not inhale vapors from Sodium Metasilicate solutions.

Labeling Indicate the percent, each, of SiO_2 and Na_2O, and whether it is anhydrous or the pentahydrate.
Identification

A. Dissolve about 200 mg of sample in 10 mL of water. Place a drop of this solution on a spot plate. Add 1 drop of 4 *M* sodium hydroxide and 1 drop of a solution prepared by dissolving 0.5 g of ammonium molybdate in 10 mL of water, followed by the addition of 3 mL of sulfuric acid. A deep yellow color indicates the presence of silicate.

B. Dip a clean nichrome wire into the final solution prepared in *Identification Test A*, and place the wire in the flame of a Bunsen burner. A bright yellow color indicates the presence of sodium.

Assay for Silicon Dioxide and Sodium Oxide Not less than 90.0% and not more than 110.0% of the percents claimed on the label.

Lead Not more than 5 mg/kg.
Loss on Ignition *Anhydrous*: Not more than 0.5%; *Pentahydrate*: Between 40.5% and 42.7%.

TESTS

Assay for Silicon Dioxide and Sodium Oxide

Silicon Dioxide In a beaker, acidify 1 g of sample, accurately weighed, with 5 mL of hydrochloric acid, and evaporate to dryness on a steam bath. Repeat the treatment with an additional 5 mL of hydrochloric acid, and mix the residue with a 1:20 (v/v) solution of hydrochloric acid. Digest the residue on the steam bath to dissolve the soluble salts, filter the contents of the beaker through an ashless filter paper, and quantitatively transfer the residue to the paper. Wash the paper and residue thoroughly with hot water, transfer the paper to a platinum crucible, dry for 1 h at 105°, and carefully char it at low heat. Gradually increase the heat to burn away the paper, and finally ignite the crucible and its contents to constant weight at 1000°, cool in a desiccator, and weigh. Moisten the ignited residue with a few drops of water, add 15 mL of hydrofluoric acid and 5 drops of 1:3 sulfuric acid cautiously. Heat the crucible on a hot plate in a fume hood until all of the acid is driven off, and then ignite the residue to constant weight at a temperature of 1000°. Cool the crucible in a desiccator, and weigh. The loss in weight is equivalent to the weight of SiO_2 in the sample taken.

Sodium Oxide Disperse 500 mg of sample, accurately weighed, in 150 mL of water, and heat to ensure its dissolution. Add 2 to 3 drops of phenolphthalein TS and 100.0 mL of 0.1 *N* sulfuric acid. Titrate with 0.1 *N* sodium hydroxide until a permanent pink color first appears. Subtract the volume of 0.1 *N* sodium hydroxide from the volume of 0.1 *N* sulfuric acid. Each milliliter of 0.1 *N* sulfuric acid is equivalent to 3.099 mg of sodium oxide.

Lead

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade Lead Nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid; dilute to 1000.0 mL with water; and mix. Each milliliter of this solution contains 100 μg of lead (Pb) ion. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute stepwise and quantitatively an accurately measured volume of *Lead Nitrate Stock Solution* with water to obtain a *Standard Lead Solution* containing 0.50 μg/mL of lead (Pb) ion.

Sample Solution Transfer 10.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 4, or equivalent, filter paper into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Procedure Set a suitable atomic absorption spectrophotometer to a wavelength of 217 nm. Adjust the instrument to zero absorbance against water. Read the absorbance of the *Standard Lead Solution* containing 0.50 μg/mL of lead (Pb) ion.

Aspirate the *Sample Solution* into the spectrophotometer, and measure the absorbance in the same manner. The absorbance obtained from the *Sample Solution* is not greater than that obtained from the *Standard Lead Solution*.

Loss on Ignition Ignite a sample, accurately weighed, in a suitable tared crucible at 1000° for 20 min.

Packaging and Storage Store in tight containers.

Sodium Methylate

Sodium Methoxide

CH_3ONa Formula wt 54.02

CAS: [124-41-4]

DESCRIPTION

Sodium Methylate occurs as a white, amorphous, hygroscopic, free-flowing powder. It is soluble in fats, in esters, and in alcohols. It decomposes without melting above 127°.

> **Caution**: Sodium Methylate and its solutions are caustic and flammable. Avoid contact with the eyes, skin, and clothing, and do not inhale vapors from Sodium Methylate solutions.

Function Catalyst for the transesterification of fats.

REQUIREMENTS

Identification A sample reacts with oxygen and carbon dioxide and is decomposed by water. The resulting solution gives positive tests for *Sodium*, Appendix IIIA.
Assay Not less than 97.0% of CH_3ONa.
Arsenic Not more than 3 mg/kg.
Lead Not more than 5 mg/kg.
Mercury Not more than 1 mg/kg.
Sodium Carbonate Not more than 0.4%.
Sodium Hydroxide Not more than 1.7%.

TESTS

> **Note**: The tests in the following section must be conducted with a minimum exposure of the sample to air. Preferably conduct the tests in a nitrogen hood.

Assay, **Sodium Carbonate**, and **Sodium Hydroxide**

Sample Preparation Select two tared weighing bottles, each approximately 30 mm × 80 mm, fill each almost to

volume with 12 to 15 g of sample, securely fit the covers, and weigh.

Determination of Alkalinity as CH_3ONa Remove the top from one of the weighing bottles, and immediately place the bottle into a 500-mL Erlenmeyer flask containing 200 mL of ice-cold, carbon dioxide-free water, sliding the weighing bottle gently down the side of the flask to prevent splashing. Immediately stopper the flask with a rubber stopper, and swirl until the sample dissolves. Wash the *Sample Solution* into a 250-mL volumetric flask, and dilute nearly to volume with carbon dioxide-free water. Allow the solution to reach room temperature, then dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a 500-mL glass-stoppered Erlenmeyer flask, add 150 mL of carbon dioxide-free water and 5 mL of barium chloride TS, stopper the flask, mix, and allow to stand for 5 min. Add 3 drops of phenolphthalein TS, and titrate with 1 *N* hydrochloric acid to the disappearance of the pink color. Retain the titrated solution for the *Determination of Sodium Carbonate* (below). Calculate the percentage of alkalinity as CH_3ONa (% *A*) by the formula

$$(V_1 \times N \times 5.403)/(W \times 0.2),$$

in which V_1 is the volume, in milliliters, and N is the exact normality, respectively, of the hydrochloric acid used; and W is the weight, in grams, of the sample.

Determination of Sodium Carbonate Add 2 drops of methyl orange TS to the solution retained from *Determination of Alkalinity* (above), and continue the titration with 1 *N* hydrochloric acid to a permanent pink color. Calculate the percentage of Na_2CO_3 by the formula

$$(V_2 \times N \times 5.30)/(W \times 0.2),$$

in which V_2 is the volume, in milliliters, of 1 *N* hydrochloric acid consumed in the second titration, and N and W are as defined above.

Determination of Sodium Hydroxide Adapt the *Karl Fischer Titrimetric Method* under *Water Determination*, Appendix IIB, for this determination.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using the following as the *Sample Solution*: Cautiously dissolve 1 g of sample in 10 mL of water, neutralize to litmus paper with 2 *N* sulfuric acid, and dilute to 35 mL with water.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 5 µg of lead (Pb) ion in the control and the following as the *Sample Solution*: Cautiously dissolve 1 g of sample in 10 mL of water, add 10 mL of diluted hydrochloric acid, and heat to boiling. Cool, and dilute to 25 mL with water.

Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB, using the following as the *Sample Preparation*: Cautiously dissolve 2 g of sample in 10 mL of water in a small beaker, add 2 drops of phenolphthalein TS, and slowly neutralize, with constant stirring, using 1:5 sulfuric acid. Add 1 mL of the 1:5 sulfuric acid solution and 1 mL of a 1:25 solution of potassium permanganate, and mix.

Packaging and Storage Store in airtight containers, and take all necessary precautions to prevent combustion during handling.

Sodium Nitrate

$NaNO_3$ Formula wt 85.00

INS: 251 CAS: [7631-99-4]

DESCRIPTION

Sodium Nitrate occurs as colorless crystals or white crystalline granules or powder. It is moderately deliquescent in moist air. It is freely soluble in water, and is sparingly soluble in alcohol.

Function Antimicrobial agent; preservative.

REQUIREMENTS

Identification A 1:5 aqueous solution is neutral to litmus and gives positive tests for *Sodium* and for *Nitrate*, Appendix IIIA.

Assay Not less than 99.0% and not more than 100.5% of $NaNO_3$ after drying.

Lead Not more than 4 mg/kg.

Total Chlorine Passes test (approximately 0.2%).

TESTS

Assay Dissolve about 350 mg of sample, previously dried at 105° for 4 h and accurately weighed, in 10 mL of hydrochloric acid in a small beaker or porcelain dish, and evaporate to dryness on a steam bath. Dissolve the residue in 10 mL of hydrochloric acid, and again evaporate to dryness, continuing the heating until the residue, when dissolved in water, is neutral to litmus. Transfer the residue, with the aid of 25 mL of water, into a glass-stoppered flask, add exactly 50 mL of 0.1 *N* silver nitrate, add 3 mL of nitric acid and 3 mL of nitrobenzene, and shake vigorously. Add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Each milliliter of 0.1 *N* silver nitrate is equivalent to 8.50 mg of $NaNO_3$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of water, and 4 µg of lead (Pb) ion in the control.

Total Chlorine Dissolve 1 g of sample in 100 mL of water, add enough 6% sulfurous acid to give the solution a distinct odor of sulfur dioxide, boil gently until the odor of the sulfur dioxide is no longer evident, and adjust the volume to 100 mL with water. Add 1.0 mL of 0.1 *N* silver nitrate followed by 3 mL of nitric acid and 3 mL of nitrobenzene, and shake vigorously. Add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. No more than 0.6 mL of the 0.1 *N* silver nitrate is consumed.

Packaging and Storage Store in tight containers.

Sodium Nitrite

$NaNO_2$ Formula wt 69.00
INS: 250 CAS: [7632-00-0]

DESCRIPTION

Sodium Nitrite occurs as a white to slightly yellow, granular powder, or as white or nearly white, opaque, fused masses or sticks. It is hygroscopic in air. Its solutions are alkaline to litmus. One gram dissolves in 1.5 mL of water, but it is sparingly soluble in alcohol.

Function Color fixative in meat and meat products; antimicrobial agent; preservative.

REQUIREMENTS

Identification An aqueous solution gives positive tests for *Sodium* and for *Nitrite*, Appendix IIIA.
Assay Not less than 97.0% and not more than 100.5% of $NaNO_2$ after drying.
Lead Not more than 4 mg/kg.
Loss on Drying Not more than 0.25%.

TESTS

Assay Dissolve about 3 g of sample, previously dried over silica gel for 4 h and accurately weighed, in water to make 100 mL. Pipet 10 mL of this solution into a mixture of 100.0 mL of 0.1 *N* potassium permanganate, 50 mL of water, and 5 mL of sulfuric acid, keeping the tip of the pipet well below the surface of the liquid. Warm the solution to 40°, allow it to stand for 5 min, and add 25.0 mL of 0.1 *N* oxalic acid. Heat the mixture to about 80°, and titrate with 0.1 *N* potassium permanganate. Each milliliter of 0.1 *N* potassium permanganate is equivalent to 3.450 mg of $NaNO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 10 mL of water, and 4 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample over silica gel for 4 h.

Packaging and Storage Store in tight containers.

Sodium Phosphate, Dibasic

Disodium Monohydrogen Phosphate; Disodium Phosphate

Na_2HPO_4 Formula wt, anhydrous 141.96
$Na_2HPO_4 \cdot 2H_2O$ Formula wt, dihydrate 177.99
INS: 339(ii) CAS: anhydrous [7558-79-4]
CAS: dihydrate [10028-24-7]

DESCRIPTION

Sodium Phosphate, Dibasic, occurs as a white, crystalline powder or as granules. It may be anhydrous or contain two molecules of water of hydration. The anhydrous form is hygroscopic. Both forms are freely soluble in water and insoluble in alcohol.

Function Emulsifier; texturizer; buffer; nutrient.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Phosphate* and for *Sodium*, Appendix IIIA.
Assay Not less than 98.0% of Na_2HPO_4 after drying.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Insoluble Substances Not more than 0.2%.
Lead Not more than 4 mg/kg.
Loss on Drying *Anhydrous*: Not more than 5.0%; *Dihydrate*: Between 18.0% and 22.0%.

TESTS

Assay Transfer about 6.5 g of sample, previously dried at 105° for 4 h and accurately weighed, into a 250-mL beaker, add 50.0 mL of 1 *N* hydrochloric acid and 50 mL of water, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and titrate the excess acid with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (*A*) of 1 *N* hydrochloric acid consumed by the sample. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (*B*) of 1 *N* sodium hydroxide required in the titration between the two inflection points (pH 4 to pH 8.8). When *A* is equal to or less than *B*, each milliliter of the volume *A* of 1 *N* hydrochloric acid is equivalent to 142.0 mg of Na_2HPO_4. When *A* is greater than *B*, each milliliter of the volume $2B + A$ of 1 *N* sodium hydroxide is equivalent to 142.0 mg of Na_2HPO_4.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.
Fluoride Determine as directed in *Method IV* under the *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.
Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter through a tared filtering crucible (not glass). Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying the sample at 120° for 4 h.

Packaging and Storage Store in tightly closed containers.

Sodium Phosphate, Monobasic

Monosodium Phosphate; Sodium Biphosphate; Monosodium Dihydrogen Phosphate

NaH_2PO_4 Formula wt, anhydrous 119.98
$NaH_2PO_4 \cdot H_2O$ Formula wt, monohydrate 137.99

INS: 339(i) CAS: anhydrous [7558-80-7]
CAS: monohydrate [10049-21-5]

DESCRIPTION

Sodium Phosphate, Monobasic, is anhydrous or contains one or two molecules of water of hydration and is slightly hygroscopic. The anhydrous form occurs as a white, crystalline powder or granules. The hydrated forms occur as white or transparent crystals or granules. All forms are freely soluble in water, but are insoluble in alcohol. The pH of a 1:100 solution is between 4.1 and 4.7.

Function Buffer; emulsifier; nutrient.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Phosphate* and for *Sodium*, Appendix IIIA.
Assay Not less than 98.0% and not more than 103.0% of NaH_2PO_4 after drying.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Insoluble Substances Not more than 0.2%.
Lead Not more than 4 mg/kg.
Loss on Drying *Anhydrous*: Not more than 2.0%; *Monohydrate*: Between 10.0% and 15.0%; *Dihydrate*: Between 20.0% and 25.0%.

TESTS

Assay Transfer about 5 g of sample, previously dried at 105° for 4 h and accurately weighed, into a 250-mL beaker, add 100 mL of water and 50.0 mL of 1 *N* hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and slowly titrate the excess acid, stirring constantly, with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (*A*), if any, of 1 *N* hydrochloric acid consumed by the sample. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (*B*) of 1 *N* sodium hydroxide required in the titration between the two inflection points (pH 4 and pH 8.8). Each milliliter of the volume $B - A$ of 1 *N* sodium hydroxide is equivalent to 120.0 mg of NaH_2PO_4.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.
Fluoride Determine as directed in *Method IV* under the *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.
Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter through a tared filtering crucible (not glass). Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample first at 60° for 1 h, then at 105° for 4 h.

Packaging and Storage Store in tightly closed containers.

Sodium Phosphate, Tribasic

Trisodium Phosphate

Na_3PO_4 Formula wt, anhydrous 163.94
$Na_3PO_4 \cdot 12H_2O$ Formula wt, dodecahydrate 380.12

INS: 339(iii) CAS: anhydrous [7601-54-9]
CAS: dodecahydrate [10101-89-0]

DESCRIPTION

Sodium Phosphate, Tribasic, occurs as white crystals or granules or as a crystalline material. It may be anhydrous or contain 1 to 12 molecules of water of hydration. The formula for a crystalline material is approximately $4(Na_3PO_4 \cdot 12H_2O)$-NaOH. It is freely soluble in water, but is insoluble in alcohol. The pH of a 1:100 aqueous solution is between 11.5 and 12.5.

Function Antimicrobial; buffer; emulsifier; nutrient.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Sodium* and for *Phosphate*, Appendix IIIA.
Assay *Anhydrous and Monohydrate*: Not less than 97.0% of Na_3PO_4, calculated on the ignited basis; *Dodecahydrate*: Not less than 90.0% of Na_3PO_4, calculated on the ignited basis.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Insoluble Substances Not more than 0.2%.
Lead Not more than 4 mg/kg.
Loss on Ignition *Anhydrous*: Not more than 2.0%; *Monohydrate*: Between 8.0% and 11.0%; *Dodecahydrate*: Between 45.0% and 57.0%.

TESTS

Assay Transfer an accurately weighed quantity of sample, equivalent to between 5.5 and 6 g of anhydrous Na_3PO_4 to a 400-mL beaker, dissolve in 40 mL of water, and add 100.0 mL of 1 *N* hydrochloric acid. Using flexible latex tubing

ending in a plastic pipet tip having a 0.5-cm hole, pass a stream of fine bubbles of nitrogen, at a rate of about five bubbles per second, through the solution for 15 min to expel carbon dioxide while stirring the solution with a magnetic stirrer. Simultaneously, cover the beaker loosely to prevent any loss by spraying. Wash the cover and sides of the beaker, and wash the plastic tip inside and out, with a few milliliters of water, and place the electrodes of a standard pH meter in the solution. Titrate the solution with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4, then calculate the volume (*A*) of 1 *N* hydrochloric acid consumed. Protect the solution from absorbing carbon dioxide from the air, and continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached. Calculate the volume (*B*) of 1 *N* sodium hydroxide consumed in the titration. When *A* is equal to or greater than 2*B*, each milliliter of the volume *B* of 1 *N* sodium hydroxide is equivalent to 163.9 mg of Na_3PO_4. When *A* is less than 2*B*, each milliliter of the volume *A* + *B* of 1 *N* sodium hydroxide is equivalent to 163.9 mg of Na_3PO_4.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.

Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.

Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter through a tared filtering crucible (not glass). Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

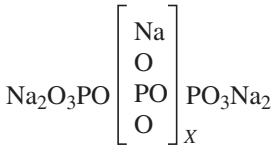
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Ignition Dry at 110° for 5 h, then ignite at about 800° for 30 min.

Packaging and Storage Store in well-closed containers.

Sodium Polyphosphates, Glassy

Sodium Hexametaphosphate; Sodium Tetrapolyphosphate; Graham's Salt



INS: 452(i) CAS: [68915-31-1]
CAS: [10361-03-2]

DESCRIPTION

Sodium Polyphosphates, Glassy, occur as colorless or white, transparent platelets, granules, or powders. They belong to a class consisting of several amorphous, water-soluble polyphosphates composed of linear chains of metaphosphate units $(NaPO_3)_x$ for which $x \geq 2$, terminated by Na_2PO_4— groups. They are usually identified by their Na_2O/P_2O_5 ratio or their P_2O_5 content. The Na_2O/P_2O_5 ratios vary from about 1.3 for sodium tetrapolyphosphate, for which x = approximately 4; through about 1.1 for Graham's salt, commonly called sodium hexametaphosphate, for which x = 13 to 18; to about 1.0 for the higher molecular weight sodium polyphosphates, for which x = 20 to 100 or more. Glassy Sodium Polyphosphates are very soluble in water. The pH of their solutions varies from about 3.0 to 9.0.

Function Emulsifier; sequestrant; texturizer.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Sodium*, Appendix IIIA.

B. Dissolve about 100 mg of sample in 5 mL of hot 1.7 *N* nitric acid, warm on a steam bath for 10 min, and cool. Neutralize to litmus paper with 1 *N* sodium hydroxide, and add silver nitrate TS. A yellow precipitate forms that is soluble in 1.7 *N* nitric acid.

Assay Between 60.0% and 71.0% of P_2O_5.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Insoluble Substances Not more than 0.1%.

Lead Not more than 4 mg/kg.

TESTS

Assay Transfer about 800 mg of sample, accurately weighed, into a 400-mL beaker, add 100 mL of water and 25 mL of nitric acid, cover with a watch glass, and boil for 10 min on a hot plate. Rinse any condensate from the watch glass into the beaker, cool the solution to room temperature, transfer it quantitatively into a 500-mL volumetric flask, dilute to volume with water, and mix thoroughly. Pipet 20.0 mL of this solution into a 500-mL Erlenmeyer flask, add 100 mL of water, and heat just to boiling. While stirring, add 50 mL of quimociac TS, cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared, sintered-glass filter crucible of medium porosity, and wash with five 25-mL portions of water. Dry at about 225° for 30 min, cool, and weigh. Each milligram of precipitate thus obtained is equivalent to 32.074 μg of P_2O_5.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.

Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Packaging and Storage Store in tightly closed containers.

Sodium Potassium Tartrate

Potassium Sodium Tartrate; Rochelle Salt; Seignette Salt

$$KOOCCH(OH)CH(OH)COONa{\cdot}4H_2O$$

$C_4H_4KNaO_6{\cdot}4H_2O$ Formula wt 282.22

INS: 337 CAS: [304-59-6]

DESCRIPTION

Sodium Potassium Tartrate occurs as colorless crystals or as a white, crystalline powder. As it effloresces slightly in warm, dry air, the crystals are often coated with a white powder. It is a salt of L(+)-tartaric acid. One gram dissolves in 1 mL of water. It is practically insoluble in alcohol.

Function Buffer; sequestrant.

REQUIREMENTS

Identification

A. Upon ignition, a sample emits the odor of burning sugar and leaves a residue that is alkaline to litmus and that effervesces with acids.

B. Add 10 mL of acetic acid to 10 mL of a 1:20 aqueous solution. A white, crystalline precipitate forms within 15 min.

C. A 1:10 aqueous solution gives positive tests for *Tartrate*, Appendix IIIA.

Assay Not less than 99.0% and not more than 102.0% of $C_4H_4KNaO_6{\cdot}4H_2O$ after drying.
Alkalinity Passes test.
Lead Not more than 2 mg/kg.
Water Between 21.0% and 26.0%.

TESTS

Assay Mix 0.5 g of sample, accurately weighed, with 50 mL of glacial acetic acid, 30 mL of 96% formic acid, and 45 mL of acetic anhydride. Heat and stir until dissolution is complete, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to a green endpoint with crystal violet indicator.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 14.11 mg of $C_4H_4KNaO_6{\cdot}4H_2O$.
Alkalinity A 1:20 aqueous solution is alkaline to litmus. Add 0.2 mL of 0.1 *N* sulfuric acid to 10 mL of this solution. No pink color appears after 1 drop of phenolphthalein TS is added.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Water Determine as directed under *Water Determination*, Appendix IIB, using 200 mg of sample and 35 mL of methanol in the *Procedure*.

Packaging and Storage Store in tight containers.

Sodium Potassium Tripolyphosphate

Trisodium Dipotassium Tripolyphosphate

$Na_3K_2P_3O_{10}$ Formula wt 400.08

CAS: [24315-83-1]

DESCRIPTION

Sodium Potassium Tripolyphosphate occurs as white, slightly hygroscopic granules or as a powder. It is anhydrous. It is freely soluble in water. The pH of a 1:100 aqueous solution is about 10.

Function Texturizer; sequestrant.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Sodium*, for *Potassium*, and for *Phosphate*, Appendix IIIA.
Assay Not less than 85.0% and not more than 100.5% of $Na_3K_2P_3O_{10}$.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Insoluble Substances Not more than 0.1%.
Lead Not more than 2 mg/kg.

TESTS

Determine as directed in the monograph for *Sodium Tripolyphosphate*.

Packaging and Storage Store in tight containers.

Sodium Propionate

Sodium Propanoate

$$CH_3CH_2COONa$$

$C_3H_5NaO_2$ Formula wt 96.06

INS: 281 CAS: [137-40-6]

DESCRIPTION

Sodium Propionate occurs as white or colorless, transparent crystals or as a granular, crystalline powder. It is hygroscopic

in moist air. One gram is soluble in about 1 mL of water at 25°, in about 0.65 mL of boiling water, and in about 24 mL of alcohol. The pH of a 1:10 aqueous solution is between 8.0 and 10.5.

Function Preservative; mold inhibitor.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Sodium*, Appendix IIIA.

B. Upon ignition, a sample yields an alkaline residue that effervesces with acids.

C. Warm a small sample with sulfuric acid. Propionic acid, recognized by its odor, evolves.

Assay Not less than 99.0% and not more than 100.5% of $C_3H_5NaO_2$ after drying.

Alkalinity (as Na_2CO_3) Passes test (about 0.15%).

Iron Not more than 0.003%.

Lead Not more than 2 mg/kg.

Water Not more than 1%.

TESTS

Assay Dissolve about 250 mg of sample, previously dried at 105° for 1 h and accurately weighed, in 40 mL of glacial acetic acid, warming if necessary to effect solution. Cool to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 9.606 mg of $C_3H_5NaO_2$.

Alkalinity (as Na_2CO_3) Dissolve 4 g of sample in 20 mL of water, and add 3 drops of phenolphthalein TS. If a pink color appears, not more than 0.6 mL of 0.1 *N* sulfuric acid is required to discharge it.

Iron Dissolve 300 mg of sample in 40 mL of water, and add 2 mL of hydrochloric acid, about 40 mg of ammonium persulfate, and 10 mL of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 0.9 mL of *Iron Standard Solution* (9 μg Fe, see *Solutions and Indicators*) in an equal volume of solution containing the quantities of reagents used in the test.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Sodium Pyrophosphate

Tetrasodium Diphosphate; Tetrasodium Pyrophosphate

$Na_4P_2O_7$ Formula wt, anhydrous 265.90

$Na_4P_2O_7 \cdot 10H_2O$ Formula wt, decahydrate 446.06

INS: 450(iii) CAS: anhydrous [7722-88-5]

CAS: decahydrate [13472-36-1]

DESCRIPTION

Sodium Pyrophosphate occurs as colorless or white crystals or as a white, crystalline or granular powder. It is anhydrous or contains 10 molecules of water of hydration. The decahydrate effloresces slightly in dry air. It is soluble in water, but is insoluble in alcohol. The pH of a 1:100 aqueous solution is about 10.

Function Emulsifier; buffer; nutrient; sequestrant; texturizer.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Sodium*, Appendix IIIA.

B. Dissolve 100 mg of sample in 100 mL of 1.7 *N* nitric acid. Add 0.5 mL of this solution to 30 mL of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and then add 0.5 mL of the solution to 30 mL of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 95.0% and not more than 100.5% of $Na_4P_2O_7$, calculated on the ignited basis.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Insoluble Substances Not more than 0.2%.

Lead Not more than 4 mg/kg.

Loss on Ignition *Anhydrous*: Not more than 0.5%; *Decahydrate*: Between 38.0% and 42.0%.

TESTS

Assay Dissolve an accurately weighed quantity of the sample, equivalent to 500 mg of anhydrous $Na_4P_2O_7$, in 100 mL of water contained in a 400-mL beaker. Using a pH meter, adjust the pH of the solution to 3.8 with hydrochloric acid, then add 50 mL of a 1:8 solution of zinc sulfate (125 g of $ZnSO_4 \cdot 7H_2O$ dissolved in water, diluted to 1000 mL, filtered, and adjusted to pH 3.8), and allow the mixture to stand for 2 min. Titrate the liberated acid with 0.1 *N* sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the endpoint, allow time for any precipitated zinc hydroxide to redissolve. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 13.30 mg of $Na_4P_2O_7$.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.

Fluoride (**Note**: Prepare and store all solutions in plastic containers.)

Buffer Solution Dissolve 73.5 g of sodium citrate in water to make 250 mL of solution.

Standard Solution Dissolve an accurately weighed quantity of USP Sodium Fluoride RS quantitatively in water to obtain a solution containing 1.1052 mg/mL. Transfer 20.0 mL of the resulting solution to a 100-mL volumetric flask containing 50 mL of *Buffer Solution*, dilute with water to volume, and mix. Each milliliter of this solution contains 100 μg of fluoride (F) ion.

Sample Solution Transfer 2.0 g of sample into a beaker containing a plastic-coated stirring bar, add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer Solution* and sufficient water to make 100 mL.

Electrode System Use a fluoride-specific, ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV.

Standard Response Line Transfer 50.0 mL of *Buffer Solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in millivolts. Continue stirring, and at 5-min intervals, add 100 μL, 100 μL, 300 μL, and 500 μL of *Standard Solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride (F) ion concentrations (0.1, 0.2, 0.5, and 1.0 μg/mL) versus potential, in millivolts.

Procedure Rinse and dry the electrodes, insert them into the *Sample Solution*, stir for 5 min, and read the potential, in millivolts. From the measured potential and the *Standard Response Line*, determine the concentration, *C*, in micrograms per milliliter, of fluoride (F) ion in the *Sample Solution*. Calculate the percentage of fluoride in the sample taken by the formula

$$C \times 0.005.$$

Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter the solution through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

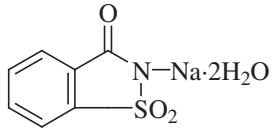
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Loss on Ignition Dry a sample at 110° for 4 h, and then ignite it at about 800° for 30 min.

Packaging and Storage Store in tight containers.

Sodium Saccharin

1,2-Benzisothiazole-3(2H)-one 1,1-Dioxide Sodium Salt; Sodium *o*-Benzosulfimide; Soluble Saccharin



$C_7H_4NNaO_3S \cdot 2H_2O$ Formula wt 241.19

INS: 954 CAS: [128-44-9]

DESCRIPTION

Sodium Saccharin occurs as white crystals or as a white, crystalline powder. In powdered form, it effloresces to the extent that it usually contains only about one-third the amount of water indicated in its molecular formula. One gram is soluble in 1.5 mL of water and in about 50 mL of alcohol.

Function Nonnutritive sweetener.

REQUIREMENTS

Identification

A. Dissolve about 100 mg of sample in 5 mL of a 1:20 solution of sodium hydroxide, evaporate to dryness, and gently fuse the residue over a small flame until ammonia no longer evolves. After the residue has cooled, dissolve it in 20 mL of water, neutralize the solution with 2.7 *N* hydrochloric acid, and filter. Add 1 drop of ferric chloride TS to the filtrate. A violet color appears.

B. Mix 20 mg of sample with 40 mg of resorcinol, cautiously add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 mL of water and an excess of 1 *N* sodium hydroxide. A fluorescent green liquid results.

C. The residue obtained by igniting a 2-g sample gives positive tests for *Sodium*, Appendix IIIA.

D. Add 1 mL of hydrochloric acid to 10 mL of a 1:10 aqueous solution. A crystalline precipitate of saccharin forms. Wash the precipitate well with cold water, and dry at 105° for 2 h. The saccharin thus obtained melts between 226° and 230° (see *Melting Range or Temperature*, Appendix IIB).

Assay Not less than 98.0% and not more than 101.0% of $C_7H_4NNaO_3S$, calculated on the anhydrous basis.

Alkalinity Passes test.

Benzoate and Salicylate Passes test.

Lead Not more than 2 mg/kg.

Readily Carbonizable Substances Passes test.

Selenium Not more than 0.003%.

Toluenesulfonamides Not more than 0.0025%.

Water Not more than 15.0%.

TESTS

Assay With the aid of 10 mL of water, quantitatively transfer about 500 mg of sample, accurately weighed, into a separator. Add 2 mL of 2.7 *N* hydrochloric acid, and extract the precipitated saccharin, first with 30 mL, then with five 20-mL portions of a solvent comprising 9:1 (v/v) chloroform:alcohol. Filter each extract through a small filter paper moistened with the solvent mixture, and evaporate the combined filtrates to dryness on a steam bath with the aid of a current of air. Dissolve the residue in 40 mL of alcohol, add 40 mL of water, mix, add 3 drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*) with a 40:40 alcohol:water (w/w) mixture. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 20.52 mg of $C_7H_4NNaO_3S$.

Alkalinity A 1:10 aqueous solution is neutral or alkaline to litmus, but produces no red color with phenolphthalein TS.

Benzoate and Salicylate Add 3 drops of ferric chloride TS to 10 mL of a 1:20 aqueous solution, previously acidified with 5 drops of acetic acid. No precipitate or violet color appears.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Readily Carbonizable Substances Determine as directed under *Readily Carbonizable Substances*, Appendix IIB, using 200 mg of sample dissolved in 5 mL of 95% sulfuric acid and kept at a temperature of 48° to 50° for 10 min. The color is no darker than that of *Matching Fluid A*.

Selenium Determine as directed in *Method I* under *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Toluenesulfonamides

Methylene Chloride Use a suitable grade (such as that obtainable from Burdick & Jackson Laboratories, Inc.), equivalent to the product obtained by distillation in an all-glass apparatus.

Internal Standard Stock Solution Transfer 100.0 mg of 95% *n*-tricosane (obtainable from Chemical Samples Co.) into a 10-mL volumetric flask, dissolve in and dilute to volume with *n*-heptane, and mix.

Stock Standard Preparation Transfer 20.0 mg each of reagent-grade *o*-toluenesulfonamide and *p*-toluenesulfonamide into a 10-mL volumetric flask, dissolve in and dilute to volume with methylene chloride, and mix.

Diluted Standard Preparations Pipet 0.1, 0.25, 1.0, 2.5, and 5.0 mL, respectively, of the *Stock Standard Preparation* into five 10-mL volumetric flasks. Pipet 0.25 mL of the *Internal Standard Stock Solution* into each flask, dilute each to volume with methylene chloride, and mix. These solutions contain, respectively, 20, 50, 200, 500, and 1000 μg/mL of each toluenesulfonamide, plus 250 μg of *n*-tricosane.

Test Preparation (See *Chromatography*, Appendix IIA.) Dissolve 2.00 g of sample in 8.0 mL of 5% sodium bicarbonate solution, and mix the solution thoroughly with 10.0 g of chromatographic siliceous earth (Celite 545, Johns-Manville, or equivalent). Transfer the mix into a 250- × 25-mm chromatographic tube that has a fritted-glass disk and a Teflon stopcock at the bottom and a reservoir at the top. Pack the contents of the tube by tapping the column on a padded surface, and then by tamping firmly from the top. Place 100 mL of methylene chloride in the reservoir, and adjust the stopcock so that 50 mL of eluate is collected in 20 to 30 min. Add 25 μL of *Internal Standard Stock Solution* to the eluate, mix, and then concentrate the solution to a volume of 1.0 mL in a suitable concentrator tube fitted with a modified Snyder column, using a Kontes tube heater maintained at 90°.

Procedure Inject 2.5 μL of the *Test Preparation* into a suitable gas chromatograph equipped with a flame-ionization detector and a 3-m × 2-mm (id) glass column, or equivalent, packed with 3% phenyl methyl silicone (OV-17, Applied Science Laboratories, Inc., or equivalent) on 100- to 120-mesh silanized, calcined, diatomaceous silica (Gas-Chrom Q, Applied Science, or equivalent).

> **Caution**: The glass column should extend into the injector for on-column injection and into the detector base to avoid contact with metal.

Maintain the column at 180°. Set the injection port temperature to 225° and the detector to 250°. Use helium as the carrier gas, with a flow rate of 30 mL/min. Set the instrument attenuation so that 2.5 μL of the *Diluted Standard Preparation* containing 200 μg/mL of each toluenesulfonamide gives a response of 40% to 80% of full-scale deflection. Record the chromatogram, note the peaks for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and the *n*-tricosane internal standard, and calculate the areas for each peak by suitable means. The retention times for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane are about 5, 6, and 15 min, respectively.

In a similar manner, obtain the chromatograms for 2.5-μL portions of each of the five *Diluted Standard Preparations*, and for each solution, determine the areas of the *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane peaks. From the values thus obtained, prepare standard curves by plotting concentration of each toluenesulfonamide, in micrograms per milliliter, versus the ratio of the respective toluenesulfonamide peak area to that of *n*-tricosane. From the standard curve, determine the concentration, in micrograms per milliliter, of each toluenesulfonamide in the *Test Preparation*. Divide each value by 2 to convert the result to milligrams per kilogram of the toluenesulfonamide in the 2-g sample taken for analysis.

> **Note**: If the toluenesulfonamide content of the sample is greater than about 500 mg/kg, the impurity may crystallize out of the methylene chloride concentrate (see *Test Preparation*). Although this level of impurity exceeds that permitted by the specification, the analysis may be completed by diluting the concentrate with methylene chloride containing 250 μg of *n*-tricosane per milliliter, and by applying appropriate dilution factors in the calculation. Care must be taken to redissolve completely any crystalline toluenesulfonamide to give a homogeneous solution.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Sodium Sesquicarbonate

Sodium Monohydrogendicarbonate; Magadi Soda

$Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$ Formula wt 226.03

INS: 500(iii) CAS: [533-96-0]

DESCRIPTION

Sodium Sesquicarbonate occurs as white crystals or flakes or as a crystalline powder. It is soluble in water, and its solutions are alkaline to litmus.

Function pH control agent; neutralizer in dairy products; buffer.

REQUIREMENTS

Identification A 1:10 aqueous solution gives positive tests for *Sodium* and for *Carbonate*, Appendix IIIA.
Assay *Sodium Bicarbonate*: Not less than 35.0% and not more than 38.6% of $NaHCO_3$; *Sodium Carbonate*: Not less than 46.4% and not more than 50.0% of Na_2CO_3.
Iron Not more than 0.002%.
Lead Not more than 2 mg/kg.
Sodium Chloride Not more than 0.5%.
Water Between 13.8% and 16.7%.

TESTS

Assay for Sodium Bicarbonate Dissolve about 3 g of sample, accurately weighed, in 150 mL of carbon dioxide-free water in a 600-mL beaker containing 50.0 mL of 0.5 *N* sodium hydroxide. While stirring, add 200 mL of 0.48 *M* barium chloride that has been adjusted to a pH of 8.0 with the aid of a pH meter. Using a pH meter that has been standardized to pH 9.0, titrate the solution with 0.5 *N* hydrochloric acid until a pH of 8.8 remains for 1 min, and record the volume, in milliliters, of 0.5 *N* hydrochloric acid required as *S*. Perform a blank determination (see *General Provisions*) using 2.1 g of primary standard sodium carbonate, and record the volume, in milliliters, of 0.5 *N* hydrochloric acid required as *B*. Each milliliter of the volume $B - S$ of 0.5 *N* hydrochloric acid is equivalent to 42.00 mg of sodium bicarbonate ($NaHCO_3$).
Assay for Sodium Carbonate Determine the total alkalinity [as sodium oxide (Na_2O)] of the sample as follows: Dissolve about 4.2 g of sample, accurately weighed, in 100 mL of water contained in a 250-mL beaker, add methyl orange TS, and titrate with 1 *N* sulfuric acid, stirring vigorously near the endpoint to expel carbon dioxide. Each milliliter of 1 *N* sulfuric acid is equivalent to 30.99 mg of sodium oxide. Calculate the percentage of sodium oxide (% Na_2O) in the sample taken.

Calculate the percentage of sodium carbonate in the sample by the formula

$$[\%\ Na_2O - (\%\ NaHCO_3 \times 0.3689)] \times 1.7099,$$

in which % $NaHCO_3$ is the percentage of sodium bicarbonate determined in the *Assay for Sodium Bicarbonate*, 0.3689 is a factor converting sodium bicarbonate to sodium oxide, and 1.7099 is a factor converting sodium oxide to sodium bicarbonate.
Iron Dissolve 500 mg of sample in 10 mL of 2.7 *N* hydrochloric acid, and dilute to 50 mL with water. Add about 40 mg of ammonium persulfate crystals and 10 mL of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 mL of *Iron Standard Solution* (10 μg Fe) (see *Solutions and Indicators*) in an equal volume of solution containing 2 mL of hydrochloric acid and the quantities of ammonium persulfate and ammonium thiocyanate used in the test.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Sodium Chloride Dissolve about 10 g of sample, accurately weighed, in 50 mL of water in a 250-mL beaker. Add sufficient nitric acid to make the solution slightly acid, then add 1 mL of ferric ammonium sulfate TS and 1.00 mL of 0.05 *N* ammonium thiocyanate, and titrate with 0.05 *N* silver nitrate, stirring constantly, until the red color completely disappears. Finally, back titrate with 0.05 *N* ammonium thiocyanate until a faint red color appears. Subtract the total volume of 0.05 *N* ammonium thiocyanate added from the volume of 0.05 *N* silver nitrate required. Each milliliter of 0.05 *N* silver nitrate is equivalent to 2.922 mg of sodium chloride (NaCl). Calculate the percentage of sodium chloride in the sample taken.
Water Calculate the percentage of water by subtracting from 100 the sum of the percentages of *Sodium Bicarbonate*, *Sodium Carbonate*, and *Sodium Chloride* found in the sample.

Packaging and Storage Store in well-closed containers.

Sodium Stearoyl Lactylate

INS: 481(i) CAS: [25383-99-7]

DESCRIPTION

Sodium Stearoyl Lactylate occurs as a cream-colored powder or brittle solid. It is a mixture of sodium salts of stearoyl lactylic acids and minor proportions of other sodium salts of related acids, manufactured by the reaction of stearic acid and lactic acid, neutralized to the sodium salts. It is slightly hygroscopic. It is soluble in ethanol and in hot oil or fat, and is dispersible in warm water.

Function Emulsifier; dough conditioner; stabilizer; whipping agent.

REQUIREMENTS

Identification

A. Heat 1 g of sample with a mixture of 25 mL of water and 5 mL of hydrochloric acid. Fatty acids are liberated,

floating as an oily layer on the surface of the liquid. The water layer gives positive tests for *Sodium*, Appendix IIIA.

B. Mix 25 g of sample with 50 g of a 15% alcoholic potassium hydroxide solution in an Erlenmeyer flask, and reflux for 1 h or until saponification is complete. Cool, add 150 mL of water, and mix. After complete solution of the soap, add 60 mL of 2 *N* sulfuric acid, and while stirring frequently, heat the mixture until the fatty acids separate cleanly as a transparent layer. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, then melt the acids, filter into a dry beaker, and dry at 105° for 20 min. The solidification point of the fatty acids so obtained is not below 54° (see *Melting Range or Temperature*, Appendix IIB).

Acid Value Between 60 and 80.

Ester Value Between 120 and 190.

Lead Not more than 2 mg/kg.

Sodium Content Between 3.5% and 5.0%.

Total Lactic Acid Between 23.0% and 34.0%.

TESTS

Acid Value Transfer about 1 g of sample, accurately weighed, into a 125-mL Erlenmeyer flask, add 25 mL of alcohol, previously neutralized to phenolphthalein TS, and heat on a hot plate until the sample is dissolved. Cool, add 5 drops of phenolphthalein TS, and titrate rapidly with 0.1 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Calculate the acid value by the formula

$$56.1V \times N/W,$$

in which V is the volume, in milliliters, and N is the normality, respectively, of the sodium hydroxide solution, and W is the weight, in grams, of the sample taken. Retain the neutralized solution for the determination of *Ester Value*.

Ester Value Add 10.0 mL of alcoholic potassium hydroxide solution (prepared by dissolving 11.2 g of potassium hydroxide in 250 mL of alcohol and diluting with 25 mL of water) to the neutralized solution retained from the test for *Acid Value*. Add 5 drops of phenolphthalein TS, connect a suitable condenser, and reflux for 2 h. Cool, add 5 additional drops of phenolphthalein TS, and titrate the excess alkali with 0.1 *N* sulfuric acid. Perform a blank determination (see *General Provisions*) using 10.0 mL of the alcoholic potassium hydroxide solution, and make any necessary correction. Calculate the ester value by the formula

$$56.1(B - S)N/W,$$

in which $B - S$ represents the difference between the volumes of 0.1 *N* sulfuric acid required for the blank and the sample, respectively; N is the normality of the sulfuric acid; and W is the weight, in grams, of the sample taken.

Lead Determine as directed for *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Sodium Content [**Note**: Do not use ordinary glassware in this test because of possible contamination by sodium; instead, use suitable plastic (e.g., polyethylene) vessels where necessary.]

Stock Lanthanum Solution Transfer 5.86 g of lanthanum oxide (La_2O_3) into a 100-mL volumetric flask, wet with a few milliliters of water, slowly add 25 mL of hydrochloric acid, and swirl until the material is completely dissolved. Dilute to volume with water, and mix.

Stock Sodium Solution Use a solution containing 1 mg of sodium in each milliliter (1000 mg/kg Na). Obtain the solution commercially or prepare as follows: Transfer 1.271 g of sodium chloride, previously dried at 105° for 2 h and accurately weighed, into a 500-mL volumetric flask, dilute to volume with water, and mix.

Standard Preparations Transfer 10.0 mL of the *Stock Lanthanum Solution* into each of three 100-mL volumetric flasks. Using a microliter syringe, transfer 0.20 mL of the *Stock Sodium Solution* to the first flask, 0.40 mL to the second flask, and 0.50 mL to the third flask. Dilute each flask to volume with water, and mix. The flasks contain 2.0, 4.0, and 5.0 μg of sodium per milliliter, respectively. Prepare these solutions fresh daily.

Sample Preparation Transfer about 250 mg of sample, accurately weighed, into a 30-mL beaker, dissolve with heating in 10 mL of alcohol, and quantitatively transfer the solution into a 25-mL volumetric flask. Wash the beaker with two 5-mL portions of alcohol, adding the washings to the flask, dilute to volume with alcohol, and mix. Transfer 2.5 mL of the *Stock Lanthanum Solution* to a second 25-mL volumetric flask. Using a microliter syringe, transfer 0.25 mL of the alcoholic solution of the sample into the second flask, dilute to volume with water, and mix.

Procedure Concomitantly determine the absorbance of each *Standard Preparation* and of the *Sample Preparation* at 589 nm, with a suitable atomic absorption spectrophotometer, following the operating parameters recommended by the manufacturer. Plot the absorbances of the *Standard Preparations* versus concentration of sodium, in micrograms per milliliter, and from the curve so obtained, determine the concentration, C, in micrograms per milliliter, of sodium in the *Sample Preparation*. Calculate the quantity, in milligrams, of sodium in the sample taken by the formula

$$2.5C.$$

Total Lactic Acid

Diluted Standard Solution Dissolve 1.067 g of lithium lactate in sufficient water to make 1000.0 mL. Transfer 10.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix.

Standard Curve Transfer 1.0, 2.0, 4.0, 6.0, and 8.0 mL of the *Diluted Standard Solution* into separate 100-mL volumetric flasks, dilute each flask to volume with water, and mix. These standards represent 1, 2, 4, 6, and 8 μg of lactic acid per milliliter, respectively. Transfer 1.0 mL of each solution into separate test tubes, and continue as directed in the *Procedure*, beginning with ''Add 1 drop of cupric sulfate TS. . . .'' After color development and reading the absorbance values, construct a *Standard Curve* by plotting absorbance versus micrograms of lactic acid.

Test Preparation Transfer about 200 mg of sample, accurately weighed, into a 125-mL Erlenmeyer flask, add 10 mL of 0.5 *N* alcoholic potassium hydroxide and 10 mL of water. Attach an air condenser, and reflux gently for 45 min. Wash the sides of the flask and the condenser with about 40 mL of water, and heat on a steam bath until no odor of alcohol remains. Add 6 mL of 1:2 sulfuric acid, heat until the fatty acids are melted, then cool to about 60°, and add 25 mL of petroleum ether. Swirl the mixture gently, and quantitatively transfer to a separator. Collect the water layer in a 100-mL volumetric flask, and wash the petroleum ether layer with two 20-mL portions of water, adding the washings to the volumetric flask. Dilute to volume with water, and mix. Transfer 1.0 mL of this solution into a second 100-mL volumetric flask, dilute to volume with water, and mix.

Procedure Transfer 1.0 mL of the *Test Preparation* into a test tube, and transfer 1.0 mL of water into a second test tube to serve as the blank. Treat each tube as follows: Add 1 drop of cupric sulfate TS, swirl gently, and rapidly add 9.0 mL of sulfuric acid from a buret. Loosely stopper the tube, and heat in a water bath at 90° for exactly 5 min. Immediately cool to below 20° in an ice bath for 5 min, add 3 drops of *p*-phenylphenol TS, shake immediately, and heat in a water bath at 30° for 30 min, shaking the tube twice during this time to disperse the reagent. Heat the tube in a water bath at 90° for exactly 90 s, and then cool immediately to room temperature in an ice-water bath. Determine the absorbance of the solution in a 1-cm cell at 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Obtain the weight, in micrograms, of lactic acid in the portion of the *Test Preparation* taken for the *Procedure* by means of the *Standard Curve*.

Packaging and Storage Store in tight containers in a cool, dry place.

Sodium Stearyl Fumarate

$C_{22}H_{39}NaO_4$ Formula wt 390.54

INS: 1169 CAS: [4070-80-8]

DESCRIPTION

Sodium Stearyl Fumarate occurs as a fine, white powder. It is slightly soluble in methanol, but is practically insoluble in water.

Function Dough conditioner.

REQUIREMENTS

Identification The infrared absorption spectrum of a 1:300 potassium bromide dispersion of the sample exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Sodium Stearyl Fumarate Reference Standard.

Assay Not less than 99.0% and not more than 101.5% of $C_{22}H_{39}NaO_4$, calculated on the anhydrous basis.

Lead Not more than 2 mg/kg.

Saponification Value Between 142.2 and 146.0, calculated on the anhydrous basis.

Sodium Stearyl Maleate Not more than 0.25%.

Stearyl Alcohol Not more than 0.5%.

Water Not more than 5.0%.

TESTS

Assay Transfer about 250 mg of sample, accurately weighed, into a 50-mL Erlenmeyer flask, mix with 1 mL of chloroform, and add 20 mL of glacial acetic acid to dissolve the sample. Add quinaldine red TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid. Each milliliter of 0.1 *N* perchloric acid is equivalent to 39.05 mg of $C_{22}H_{39}NaO_4$.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Saponification Value

Ethanolic Potassium Hydroxide Solution Dissolve about 5.5 g of potassium hydroxide in absolute ethanol, heating if necessary to effect solution, and dilute to 1000 mL with absolute ethanol. Prepare fresh daily, and filter if necessary to remove carbonate.

Procedure Transfer about 450 mg of sample, accurately weighed, into a 300-mL Erlenmeyer flask, and add 50.0 mL of *Ethanolic Potassium Hydroxide Solution*, rinsing down the inside of the flask during the addition. Reflux the mixture gently on a steam bath for at least 2 h, occasionally swirling gently but avoiding splashing the mixture up into the condenser. Rinse the condenser with 10 mL of 70% alcohol, followed by three 10-mL portions of water, collecting the rinsings in the flask. Cool, rinse the sides of the flask with two 10-mL portions of 70% alcohol, add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid to the disappearance of any pink color. Perform a blank determination (see *General Provisions*) using the same amount of *Ethanolic Potassium Hydroxide Solution*, and make any necessary correction. Calculate the saponification value by the formula

$$56.1(B - S) \times N/W,$$

in which $B - S$ represents the difference between the volumes of 0.1 *N* hydrochloric acid required for the blank and for the sample, respectively; *N* is the exact normality of the hydrochloric acid; and *W* is the weight, in grams, of the sample taken.

Sodium Stearyl Maleate and **Stearyl Alcohol**

Apparatus Assemble a suitable apparatus for ascending thin-layer chromatography (see *Thin Layer Chromatography* under *Chromatography*, Appendix IIA). Prepare a slurry of 24 g of chromatographic silica gel G in 75 mL of water, apply a uniformly thin layer to 23-cm square glass plates, or other convenient size, and dry in the air at room temperature for 2 h.

Sample Solution Transfer about 200 mg of sample, accurately weighed, into a glass-stoppered, 10-mL volumetric flask; dilute to volume with a solution of 10% acetic acid in chloroform; and mix. If necessary, heat the mixture carefully to dissolve the sample and then cool before diluting to volume with the solvent mixture.

Standard Solution A Transfer about 10 mg of USP Sodium Stearyl Maleate Reference Standard, accurately weighed, into a 100-mL volumetric flask, dilute to volume with 10% acetic acid in chloroform, and shake well.

Standard Solution B Transfer about 20 mg of stearyl alcohol (Aldrich, or equivalent), accurately weighed, into a 100-mL volumetric flask, dilute to volume with 10% acetic acid in chloroform, and shake well.

Standard Solution C Mix 25.0 mL of *Standard Solution A* with 25.0 mL of *Standard Solution B*, and shake well. This mixture represents 0.25% of sodium stearyl maleate and 0.5% of stearyl alcohol, based upon the weight (200 mg) of the sample taken.

Procedure Spot 10 µL each of the *Sample Solution* and of *Standard Solution C* at the bottom of the plate. Allow the spots to dry, then place the plate in a suitable chromatographic chamber containing a 5:5:1 (v/v) mixture of toluene, hexane, and glacial acetic acid, previously equilibrated, and develop by ascending chromatography for 30 min to effect one pass. Remove the plate from the tank, dry in the air for 10 min, and then heat in an oven at 90° for 2 min. After cooling to room temperature, replace the plate in the chamber for a second pass of 30 min. After the second pass, remove the plate from the chamber and dry in the air for 15 to 20 min. Spray evenly with a mixture consisting of 0.5% of potassium permanganate and 0.3% of sodium carbonate in water. Maleate and fumarate appear as yellow spots against a pink background. Spray with sulfuric acid and heat in an oven at 150° to detect the stearyl alcohol.

Visually compare any spots from the sample against the R_f of the spots from the standard. The spots from the sample do not appear to be stronger than the respective spots from the standard.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Sodium Sulfate

Na_2SO_4 Formula wt, anhydrous 142.04
$Na_2SO_4 \cdot 10H_2O$ Formula wt, decahydrate 322.19

INS: 514 CAS: anhydrous [7757-82-6]
CAS: decahydrate [7727-73-3]

DESCRIPTION

Sodium Sulfate occurs as colorless crystals or as a fine, white, crystalline powder. It is anhydrous or contains 10 molecules of water of crystallization. The decahydrate is efflorescent. It is freely soluble in water and practically insoluble in alcohol. A 1:20 aqueous solution is neutral or slightly alkaline to litmus paper.

Function Agent in caramel production.

REQUIREMENTS

Labeling Indicate whether it is anhydrous or the decahydrate.

Identification A 1:20 aqueous solution gives positive tests for *Sodium* and for *Sulfate*, Appendix IIIA.

Assay Not less than 99.0% and not more than 100.5% of Na_2SO_4 after drying.

Lead Not more than 2 mg/kg.

Loss on Drying *Anhydrous*: Not more than 1%; *Decahydrate*: Between 51.0% and 57.0%.

Selenium Not more than 0.003%.

TESTS

Assay Dissolve about 500 mg of sample, previously dried at 105° for 4 h and accurately weighed, in 200 mL of water, add 1 mL of hydrochloric acid, and heat to boiling. Gradually add, in small portions and while constantly stirring, an excess of hot barium chloride TS (about 10 mL), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a retentive, ashless filter paper, wash until free from chloride, and place the filter into a suitable tared crucible. Carefully burn away the paper, and ignite at 800° ± 25° to constant weight. The weight of the barium sulfate so obtained, multiplied by 0.6086, indicates its equivalent of Na_2SO_4.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 4 h.

Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.

Packaging and Storage Store in well-closed containers.

Sodium Sulfite

Na_2SO_3 Formula wt 126.04

INS: 221 CAS: [7757-83-7]

DESCRIPTION

Sodium Sulfite occurs as a white or tan to slightly pink powder. It undergoes oxidation in air. Its solutions are alkaline to

litmus and to phenolphthalein. One gram dissolves in about 4 mL of water. It is sparingly soluble in alcohol.

Function Preservative; antioxidant; bleaching agent.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Sodium* and for *Sulfite*, Appendix IIIA.
Assay Not less than 95.0% of Na_2SO_3.
Lead Not more than 2 mg/kg.
Selenium Not more than 0.003%.

TESTS

Assay Add about 250 mg of sample, accurately weighed, to exactly 50 mL of 0.1 *N* iodine contained in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 mL of hydrochloric acid, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each milliliter of 0.1 *N* iodine is equivalent to 6.302 mg of Na_2SO_3.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Selenium Determine as directed in *Method I* under *Selenium Limit Test*, Appendix IIIB, using 200 mg of sample and 100 mg of magnesium oxide.

Packaging and Storage Store in tight containers.

Sodium Tartrate

Disodium Tartrate; Disodium L-Tartrate

$$NaOOCCH(OH)CH(OH)COONa{\cdot}2H_2O$$

$C_4H_4Na_2O_6{\cdot}2H_2O$ Formula wt 230.08

INS: 335 CAS: [868-18-8]

DESCRIPTION

Sodium Tartrate occurs as transparent, colorless crystals. It is the disodium salt of L(+)-tartaric acid. One gram dissolves in 3 mL of water. It is insoluble in alcohol. The pH of a 1:20 aqueous solution is between 7 and 9. Upon ignition, it emits the odor of burning sugar and leaves a residue that is alkaline to litmus and that effervesces with acids.

Function Sequestrant.

REQUIREMENTS

Identification A sample gives positive tests for *Sodium* and for *Tartrate*, Appendix IIIA.
Assay Not less than 99.0% and not more than 100.5% of $C_4H_4Na_2O_6$ after drying.
Lead Not more than 2 mg/kg.
Loss on Drying Between 14.0% and 17.0%.
Oxalate Passes test (limit about 0.1%).

TESTS

Assay Transfer about 250 mg of sample, previously dried at 150° for 3 h and accurately weighed, to a 250-mL beaker. Add 150 mL of glacial acetic acid, heat to near boiling, stir (preferably with a magnetic stirrer) until the sample is dissolved, and cool to room temperature. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Each milliliter of 0.1 *N* perchloric acid is equivalent to 9.703 mg of $C_4H_4Na_2O_6$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 150° for 3 h.
Oxalate Dissolve 1 g of sample in 10 mL of water, and add 5 drops of 1 *N* acetic acid and 2 mL of calcium chloride TS. No turbidity develops within 1 h.

Packaging and Storage Store in tight containers.

Sodium Thiosulfate

Sodium Hyposulfite

$Na_2S_2O_3{\cdot}5H_2O$ Formula wt 248.19

INS: 539 CAS: [10102-17-7]

DESCRIPTION

Sodium Thiosulfate occurs as large, colorless crystals or as a coarse, crystalline powder. It is deliquescent in moist air and effloresces in dry air at a temperature above 33°. Its solutions are neutral or faintly alkaline to litmus. One gram dissolves in 0.5 mL of water. It is insoluble in alcohol.

Function Sequestrant; antioxidant.

REQUIREMENTS

Identification

A. Add a few drops of iodine TS to a 1:10 aqueous solution. The color disappears.

B. A 1:20 aqueous solution gives positive tests for *Sodium* and for *Thiosulfate*, Appendix IIIA.

Assay Not less than 99.0% and not more than 100.5% of $Na_2S_2O_3$ after drying.
Lead Not more than 2 mg/kg.
Selenium Not more than 0.003%.
Water Between 32.0% and 37.0%.

TESTS

Assay Accurately weigh about 500 mg of the dried sample obtained in the test for *Water* (below), dissolve it in 30 mL of water, and titrate with 0.1 *N* iodine, using starch TS as the indicator. Each milliliter of 0.1 *N* iodine is equivalent to 15.81 mg of $Na_2S_2O_3$.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.
Selenium Determine as directed in *Method I* under *Selenium Limit Test*, Appendix IIIB, using a 200-mg sample.
Water Dry about 1 g of sample, accurately weighed, in a vacuum at 40° to 45° for 16 h, cool, and weigh.

Packaging and Storage Store in tight containers.

Sodium Trimetaphosphate

$(NaPO_3)_3$ Formula wt 305.89

CAS: [7785-84-4]

DESCRIPTION

Sodium Trimetaphosphate occurs as white crystals or as a white, crystalline powder. It is a cyclic polyphosphate composed of three metaphosphate units. It is freely soluble in water. The pH of a 1:100 aqueous solution is about 6.0.

Function Starch-modifying agent.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Sodium*, Appendix IIIA.

B. Dissolve about 100 mg of sample in 5 mL of hot 1.7 *N* nitric acid, warm on a steam bath for 10 min, and cool. Neutralize to litmus paper with 1 *N* sodium hydroxide, and add silver nitrate TS. A yellow precipitate forms that is soluble in 1.7 *N* nitric acid.
Assay Between 68.0% and 70.0% of P_2O_5.
Arsenic Not more than 3 mg/kg.
Fluoride Not more than 0.005%.
Insoluble Substances Not more than 0.1%.
Lead Not more than 4 mg/kg.

TESTS

Assay Transfer about 800 mg of sample, accurately weighed, into a 400-mL beaker, add 100 mL of water and 25 mL of nitric acid, cover with a watch glass, and boil for 10 min on a hot plate. Rinse any condensate from the watch glass into the beaker, cool the solution to room temperature, transfer it quantitatively to a 500-mL volumetric flask, dilute to volume with water, and mix thoroughly. Pipet 20.0 mL of this solution into a 500-mL Erlenmeyer flask, add 100 mL of water, and heat just to boiling. While stirring, add 50 mL of quimociac TS, then cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared, sintered-glass filter crucible of medium porosity, and wash the precipitate with five 25-mL portions of water. Dry the precipitate at about 225° for 30 min, cool, and weigh. Each milligram of precipitate thus obtained is equivalent to 32.074 μg of P_2O_5.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.
Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.
Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter the solution through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Packaging and Storage Store in tight containers.

Sodium Tripolyphosphate

Pentasodium Triphosphate; Triphosphate; Sodium Triphosphate

$Na_5P_3O_{10}$ Formula wt, anhydrous 367.86
$Na_5P_3O_{10}·6H_2O$ Formula wt, hexahydrate 475.96

INS: 451(i)

CAS: anhydrous [7758-29-4]
CAS: hexahydrate [15091-98-2]

DESCRIPTION

Sodium Tripolyphosphate occurs as white, slightly hygroscopic granules, or as a powder. It is anhydrous or contains six molecules of water of hydration. It is freely soluble in water, but insoluble in alcohol. The pH of a 1:100 aqueous solution is about 9.5.

Function Emulsifier; sequestrant.

REQUIREMENTS

Identification

A. A 1:20 aqueous solution gives positive tests for *Sodium*, Appendix IIIA.

B. Add a few drops of silver nitrate TS to 1 mL of a 1:100 aqueous solution. A white precipitate forms that is soluble in 1.7 *N* nitric acid.

Assay *Anhydrous*: Not less than 85.0% of $Na_5P_3O_{10}$; *Hexahydrate*: Not less than 65.0% of $Na_5P_3O_{10}$.

Arsenic Not more than 3 mg/kg.

Fluoride Not more than 0.005%.

Insoluble Substances Not more than 0.1%.

Lead Not more than 2 mg/kg.

TESTS

Assay

Potassium Acetate Buffer (pH 5.0) Dissolve 78.5 g of potassium acetate in 1000 mL of water, and adjust the pH of the solution to 5.0 with glacial acetic acid. Add a few milligrams of mercuric iodide to inhibit mold growth.

0.3 M *Potassium Chloride Solution* Dissolve 22.35 g of potassium chloride in water, add 5 mL of *Potassium Acetate Buffer*, dilute to 1000 mL with water, and mix. Add a few milligrams of mercuric iodide to inhibit mold growth.

0.6 M *Potassium Chloride Solution* Dissolve 44.7 g of potassium chloride in water, add 5 mL of *Potassium Acetate Buffer*, dilute to 1000 mL with water, and mix. Add a few milligrams of mercuric iodide to inhibit mold growth.

1 M *Potassium Chloride Solution* Dissolve 74.5 g of potassium chloride in water, add 5 mL of *Potassium Acetate Buffer*, dilute to 1000 mL with water, and mix. Add a few milligrams of mercuric iodide to inhibit mold growth.

Chromatographic Column Use a standard chromatographic column, 20- to 40-cm long with a 20- to 28-mm id that has a sealed-in, coarse-porosity, fritted disk. If a stopcock is not provided, attach a stopcock having a 3- to 4-mm diameter bore to the outlet of the column with a short length of flexible vinyl tubing.

Procedure Close the column stopcock, fill the space between the fritted disk and the stopcock with water, and connect a vacuum line to the stopcock. Prepare a 1:1 water slurry of Dowex 1 × 8, chloride form, 100- to 200- or 200- to 400-mesh, or a comparable grade of styrene–divinylbenzene ion exchange resin, and decant off any very fine particles and any foam. Do this two or three times or until no more finely suspended material or foaming is observed. Fill the column with the slurry, and open the stopcock to allow the vacuum to pack the resin bed until the water level is slightly above the top of the resin, then immediately close the stopcock. Do not allow the liquid level to fall below the resin level at any time. Repeat this procedure until the packed resin column is 15 cm above the fritted disk. Place one circle of tightly fitting glass-fiber filter paper on top of the resin bed, then place a perforated polyethylene disk on top of the paper. Alternatively, place a loosely packed plug of glass wool on top of the bed. Close the top of the column with a rubber stopper in which a 7.6-cm length of capillary tubing (1.5-mm id, 7-mm od), or equivalent, has been inserted through the center, so that about 12 mm of the tubing extends through the bottom of the stopper. Connect the top of the capillary tubing to the stem of a 500-mL separator with flexible vinyl tubing, and clamp the separator to a ring stand above the column. Wash the column by adding 100 mL of water to the separator with all stopcocks closed. First open the separator stopcock, then open the column stopcock. The rate of flow should be about 5 mL/min. When the separator is empty, close the column stopcock, then close the separator stopcock.

Transfer about 500 mg of sample, accurately weighed, into a 250-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 10.0 mL of this solution into the separator, open both stopcocks, and allow the solution to drain into the column, rinsing the separator with 20 mL of water. Discard the eluate.

Add 370 mL of *0.3* M *Potassium Chloride Solution* to the separator, and allow this solution to pass through the column, discarding the eluate. Add 250 mL of *0.6* M *Potassium Chloride Solution* to the column, allow the solution to pass through the column, and receive the eluate in a 400-mL beaker. (To ensure a clean column for the next run, pass 100 mL of *1* M *Potassium Chloride Solution* through the column, and then follow with 100 mL of water. Discard all washings.) Add 15 mL of nitric acid to the beaker, mix, and boil for 15 to 20 min. Add methyl orange TS, and neutralize the solution with ammonium hydroxide. Add 1 g of ammonium nitrate crystals, stir to dissolve, and cool. While stirring, add 15 mL of ammonium molybdate TS, and stir vigorously for 3 min, or allow to stand with occasional stirring for 10 to 15 min. Filter the contents of the beaker by means of suction through a 6- to 7-mm paper-pulp filter pad supported in a 25-mm porcelain disk. The filter pad should be covered with a suspension of infusorial earth. After the contents of the beaker have been transferred to the filter, wash the beaker with five 10-mL portions of a 1:100 aqueous solution of sodium or potassium nitrate, passing the washings through the filter, then wash the filter with five 5-mL portions of the wash solution. Return the filter pad and the precipitate to the beaker, wash the funnel thoroughly with water into the beaker, and dilute to about 150 mL. Add 0.1 *N* sodium hydroxide from a buret until the yellow precipitate is dissolved, then add 5 to 8 mL in excess. Add phenolphthalein TS, and titrate the excess alkali with 0.1 *N* nitric acid. Finally, titrate with 0.1 *N* sodium hydroxide to the first appearance of a pink color. The difference between the total volume of 0.1 *N* sodium hydroxide added and the volume of nitric acid required represents the volume, *V*, in milliliters, of 0.1 *N* sodium hydroxide consumed by the phosphomolybdate complex. Calculate the quantity, in milligrams, of $Na_5P_3O_{10}$ in the sample taken by the formula

$$0.533 \times 25V.$$

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water.

Fluoride Determine as directed in *Method IV* under *Fluoride Limit Test*, Appendix IIIB, using a 2-g sample.

Insoluble Substances Dissolve 10 g of sample in 100 mL of hot water, and filter the solution through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB.

Packaging and Storage Store in tight containers.

Solin Oil

Low Linolenic Acid Flaxseed Oil (Unhydrogenated); Low Linolenic Acid Linseed Oil

DESCRIPTION

Solin Oil occurs as a light yellow oil. It is obtained from the seed of certain varieties of the flaxseed plant *Linum usitatissimum* L. (Fam. Linaceae) by mechanical expression and/or solvent extraction, differing from linseed oil in having a linolenic acid (C18:3) content of less than 5%. The oil is refined, bleached, and deodorized to remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous non-oil materials. It is liquid and free from visible foreign material at 21° to 27°, but traces of wax may cause the oil to cloud at refrigeration temperatures (2° to 5°) unless removed by winterization.

Function Coating agent; texturizer.

REQUIREMENTS

Identification Solin Oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, Appendix VII.

Fatty Acid:	<14:0	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<0.5	2–9	<0.5	2–5	8–60	40–80
Fatty Acid:	18:3	20:0	20:1	22:0	22:1	24:0	
Weight % (Range):	<5.0	<0.3	<0.3	<0.3	<0.2	<0.2	

Cold Test Passes test.
Color (AOCS-Wesson) Not more than 5.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 100 and 160.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 5.0%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more 0.1%.

TESTS

Cold Test Determine as directed under *Cold Test*, Appendix VII.
Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Linolenic Acid Determine as directed under *Fatty Acid Composition*, Appendix VII.
Peroxide Value Determine as directed in *Method II* under *Peroxide Value*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in tightly closed containers blanketed in an inert gas.

Sorbic Acid

2,4-Hexadienoic Acid

$$CH_3CH{=}CHCH{=}CHCOOH$$

$C_6H_8O_2$ Formula wt 112.13

INS: 200 CAS: [110-44-1]

DESCRIPTION

Sorbic Acid occurs as colorless needles or as a white to off white, free-flowing powder. It is slightly soluble in water. One gram dissolves in about 10 mL of ethanol and in about 20 mL of ether.

Function Preservative; mold inhibitor.

REQUIREMENTS

Identification

A. Add a few drops of bromine TS to 2 mL of a 1:10 solution in alcohol. The color disappears.

B. A 1:400,000 solution in isopropanol exhibits an absorbance maximum at 254 ± 2 nm.

Assay Not less than 99.0% and not more than 101.0% of $C_6H_8O_2$, calculated on the anhydrous basis.
Lead Not more than 2 mg/kg.
Melting Range Between 132° and 135°.
Residue on Ignition Not more than 0.2%.
Water Not more than 0.5%.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 50 mL of anhydrous methanol that previously

has been neutralized with 0.1 *N* sodium hydroxide; add phenolphthalein TS; and titrate with 0.1 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 11.21 mg of $C_6H_8O_2$.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Melting Range Determine as directed in *Procedure for Class Ia* under *Melting Range or Temperature*, Appendix IIB, but increase the heat at a rate of 1°/min until melting is complete.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers protected from light, preferably at a temperature not exceeding 38°.

Sorbitan Monostearate

INS: 491 CAS: [1338-41-6]

DESCRIPTION

Sorbitan Monostearate occurs as an off white to tan colored, hard, waxy solid. It is a mixture of partial stearic and palmitic acid esters of sorbitol and its mono- and dianhydrides. It is manufactured by reacting edible commercial stearic acid (usually containing associated fatty acids, chiefly palmitic) with sorbitol. It is soluble at temperatures above its melting point in toluene, dioxane, ether, ethanol, methanol, and aniline. It is insoluble in cold water, and in mineral spirits and acetone, but is dispersible in warm water and soluble, with haze, above 50° in mineral oil and in ethyl acetate.

Function Emulsifier; stabilizer; defoaming agent.

REQUIREMENTS

Identification

A. The fatty acid residue obtained in the *Assay* (below) has an *Acid Value* between 200 and 215 (see *Method I* under *Acid Value*, Appendix VII) and an *Iodine Value* not greater than 4 (see *Iodine Value*, Appendix VII).

B. *Sample Solution* Transfer 500 mg of the polyols obtained in the *Assay* (below) into a 2-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Standard Solution Transfer 25 mg each of sorbitol, of USP 1,4-Sorbitan Reference Standard, and of USP Isosorbide Reference Standard into a 1-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Procedure Using micropipets, separately spot 2 µL each of the *Sample Solution* and of the *Standard Solution* on a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, then place the plate in a suitable chromatographic chamber containing 100:2 (v/v) acetone:glacial acetic acid as the developing solvent, and develop by ascending chromatography until the solvent front has traveled about 15 cm. Remove the plate from the chamber, dry thoroughly in air, and spray evenly with a 1:2 sulfuric acid solution until the surface is uniformly wet.

Caution: Do not overspray.

Immediately place the sprayed plate on a hot plate maintained at 200° in a hood. Char until white fumes of sulfur trioxide cease, and cool to room temperature. The spots from the sample are located at the same R_f values as those of the polyols from the standard. The approximate R_f values are sorbitol, 0.07; 1,4-sorbitan, 0.40; and isosorbide, 0.77.

Assay Not less than 27.0 g and not more than 34.0 g of polyols (as sorbitol and its mono- and dianhydrides) per 100 g of sample, and not less than 68 g and not more than 76 g of fatty acids per 100 g of sample, calculated on the anhydrous basis.

Acid Value Between 5 and 10.

Hydroxyl Value Between 235 and 260.

Lead Not more than 2 mg/kg.

Saponification Value Between 147 and 157.

Water Not more than 1.5%.

TESTS

Assay Transfer about 25 g of sample, accurately weighed, into a 500-mL round-bottom boiling flask, add 250 mL of alcohol and 7.5 g of potassium hydroxide, and mix. Connect a suitable condenser to the flask, reflux the mixture for 1 to 2 h, then transfer it to an 800-mL beaker, rinsing the flask with about 100 mL of water and adding it to the beaker. Heat on a steam bath to evaporate the alcohol, adding water occasionally to replace the alcohol, and evaporate until the odor of alcohol can no longer be detected. Adjust the final volume to about 250 mL with hot water. Neutralize the soap solution with 1:2 sulfuric acid, add 10% in excess, and heat, while stirring, until the fatty acid layer separates. Transfer the fatty acids to a 500-mL separator, wash with three or four 20-mL portions of hot water to remove polyols, and combine the washings with the original aqueous polyol layer from the saponification. Extract the combined aqueous layer with three 20-mL portions of petroleum ether, add the extracts to the fatty acid layer, evaporate to dryness in a tared dish, cool, and weigh.

Neutralize the polyol solution with a 1:10 potassium hydroxide solution to pH 7 using a suitable pH meter. Evaporate this solution to a moist residue, and separate the polyols from the salts by several extractions with hot alcohol. Evaporate the alcohol extracts to dryness in a tared dish on a steam bath, cool, and weigh. Avoid excessive drying and heating.

Acid Value Determine as directed in *Method II* under *Acid Value*, Appendix VII.

Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

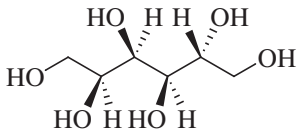
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 4 g of sample, accurately weighed.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Sorbitol

D-Sorbitol; D-Glucitol; D-Sorbite; 1,2,3,4,5,6-Hexanehexol



$C_6H_{14}O_6$ Formula wt 182.17

INS: 420 CAS: [50-70-4]

DESCRIPTION

Sorbitol occurs as a white powder, as granules, or as crystalline masses. It is very soluble in water; slightly soluble in ethanol, in methanol, and in acetic acid; and insoluble in ether. It is hygroscopic.

Function Humectant; texturizing agent; nutritive sweetener.

REQUIREMENTS

Identification

A. Dissolve 1 g of sample in 75 mL of water. Transfer 3 mL of this solution into a 15-cm test tube, add 3 mL of a freshly prepared 1:10 catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for about 30 s. A deep pink or wine red color appears.

B. The retention time of the major peak in the chromatogram of the *Assay Solution* corresponds to that in the chromatogram of the *Standard Solution* obtained in the *Assay*.

Assay Not less than 91.0% and not more than 100.5% of D-Sorbitol ($C_6H_{14}O_6$), calculated on the anhydrous basis.

Lead Not more than 2 mg/kg.

Nickel Not more than 1 mg/kg.

pH of a 10% (w/w) Solution Between 3.5 and 7.0.

Reducing Sugars Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

Water Not more than 1.5%.

TESTS

Assay

Mobile Phase Use degassed water.

Standard Solution Dissolve an accurately weighed quantity of USP Sorbitol Reference Standard in water to obtain a solution with a known concentration of about 4.8 mg/g.

Resolution Solution Dissolve USP Mannitol Reference Standard and USP Sorbitol Reference Standard in water to obtain a solution with concentrations of about 4.8 mg/g of each.

Assay Preparation Dissolve about 0.10 g of sample, accurately weighed, in water, and dilute to about 20 g with water. Accurately record the final solution weight, and mix thoroughly.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with a refractive index detector that is maintained at a constant temperature of about 35° and a 10-cm × 7.8-mm column containing packing L34 (Bio-Rad Laboratories), or equivalent. Maintain the column temperature at about 50° ± 2°. Set the flow rate to about 0.7 mL/min. Chromatograph 10 µL of the *Standard Solution*, and record the peak responses as directed under *Procedure*. The relative standard deviation for three replicate injections is not more than 2.0%. Chromatograph 10 µL of the *Resolution Solution*, and record the peak responses. The resolution, *R*, between the sorbitol and mannitol peaks is not less than 2.0.

Procedure Separately inject equal volumes, about 10 µL each, of the *Assay Preparation* and the *Standard Solution* into the chromatograph, and measure the responses for the major peaks. The relative retention times are about 0.6 for mannitol and 1.0 for Sorbitol. Calculate the percentage of $C_6H_{14}O_6$, on the anhydrous basis, in the sample taken by the formula

$$[(C_S/C_U) \times (r_U/r_S) \times 10{,}000]/(100 - w),$$

in which C_S is the concentration, in milligrams per gram, of sample in the *Assay Preparation;* C_U is the concentration, in milligrams per gram, of USP Sorbitol Reference Standard in the *Standard Solution;* r_U and r_S are the peak responses obtained with the *Assay Solution* and the *Standard Solution*, respectively; and *w* is the percent water as determined by the corresponding test.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Nickel Determine as directed under *Nickel Limit Test*, Appendix IIIB.

pH of a 10% (w/w) Solution Determine as directed under *pH Determination*, Appendix IIB, using a 10% (w/w) solution of sample in carbon dioxide-free water.

Reducing Sugars Dissolve 3.3 g of sample in 3 mL of water with the aid of gentle heat. Cool, and add 20.0 mL of alkaline cupric citrate TS and a few glass beads. Heat so that boiling begins after 4 min, and maintain the boiling for 3 min. Cool rapidly, and add 100 mL of diluted acetic acid TS and 20.0 mL of 0.05 *N* iodine VS, prepared by diluting 0.1 *N* iodine VS 1:1 with water. With continuous shaking, add 25 mL of a mixture of 6 mL of hydrochloric acid and 94 mL of water. When the precipitate has dissolved, titrate the excess iodine with 0.05 *N* sodium thiosulfate VS, prepared by diluting 0.1 *N* sodium thiosulfate VS 1:1 with water. Use 2 mL of starch TS, added toward the end of the titration, as an indicator.

Not less than 12.8 mL of 0.05 *N* sodium thiosulfate VS is required (equivalent to not more than 0.3% of reducing sugars, as glucose).

Residue on Ignition Determine as directed in *Method I* (for solids) under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Sorbitol Solution

INS: 420

DESCRIPTION

Sorbitol Solution occurs as a clear, colorless, syrupy liquid. It is a water solution of sorbitol ($C_6H_{14}O_6$) containing a small amount of mannitol and other isomeric polyhydric alcohols. It is miscible with water, with ethanol, with glycerin, and with propylene glycol. It sometimes separates into crystalline masses.

Function Humectant; texturizing agent; nutritive sweetener.

REQUIREMENTS

Identification

A. Dissolve 1.4 g of sample in 75 mL of water. Transfer 3 mL of this solution into a 15-cm test tube, add 3 mL of a freshly prepared 1:10 catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for about 30 s. A deep pink or wine red color appears.

B. The retention time of the major peak in the chromatogram of the *Assay Solution* corresponds to that in the chromatogram of the *Standard Preparation* obtained in the *Assay*.

Assay Not less than 64.0% of sorbitol ($C_6H_{14}O_6$).

Lead Not more than 2 mg/kg, calculated on the anhydrous basis.

Nickel Not more than 1 mg/kg, calculated on the anhydrous basis.

pH of a 14% (w/w) Solution Between 5.0 and 7.5.

Reducing Sugars Not more than 0.3%, calculated on the anhydrous basis.

Residue on Ignition Not more than 0.1%, calculated on the anhydrous basis.

Water Between 28.5% and 31.5%.

TESTS

Assay

Mobile Phase, *Standard Solution*, *Resolution Solution*, and *Chromatographic System* Proceed as directed under *Assay* in the monograph for *Sorbitol*.

Assay Preparation Dissolve 0.12 g of sample, accurately weighed, in water, and dilute to about 20 g with water. Accurately record the final solution weight.

Procedure Determine as directed for *Procedure* under *Assay* in the monograph for *Sorbitol*, and calculate the percentage of $C_6H_{14}O_6$ in the portion of sample taken by the formula

$$100(C_S/C_U)(r_U/r_S)$$

in which C_S is the concentration, in milligrams per gram, of USP Sorbitol Reference Standard in the *Standard Solution*; C_U is the concentration, in milligrams per gram, of sample in the *Assay Preparation*; and r_U and r_S are the peak responses obtained from the *Assay Preparation* and the *Standard Solution*, respectively.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Nickel Determine as directed under *Nickel Limit Test*, Appendix IIIB.

pH of a 14% (w/w) Solution Determine as directed under *pH Determination*, Appendix IIB, using a 14% (w/w) solution of sample in carbon dioxide-free water.

Reducing Sugars Add 3 mL of water, 20.0 mL of alkaline cupric citrate TS, and a few glass beads to an amount of sample equivalent to 3.3 g on the anhydrous basis. Heat so that boiling begins after 4 min, and maintain the boiling for 3 min. Cool rapidly, and add 100 mL of diluted acetic acid TS and 20.0 mL of 0.05 *N* iodine VS, prepared by diluting 0.1 *N* iodine VS 1:1 with water. With continuous shaking, add 25 mL of a mixture of 6 mL of hydrochloric acid and 94 mL of water. When the precipitate has dissolved, titrate the excess iodine with 0.05 *N* sodium thiosulfate VS, prepared by diluting 0.1 *N* sodium thiosulfate VS 1:1 with water. Use 2 mL of starch TS, added toward the end of the titration, as an indicator. Not less than 12.8 mL of 0.05 *N* sodium thiosulfate VS is required (equivalent to not more than 0.3% of reducing sugars, on the anhydrous basis, as glucose).

Residue on Ignition Determine as directed in *Method II* (for liquids) under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Soybean Oil (Unhydrogenated)

CAS: [8001-22-7]

DESCRIPTION

Soybean Oil (Unhydrogenated) occurs as a light amber colored oil. It is obtained from the seed of the legume *Glycine max* (Fam. Fabaceae), usually by solvent extraction. It is refined,

bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21° to 27° and remains so even at refrigerator temperatures (2° to 4°). It is free from visible foreign matter at 21° to 27°.

Function Coating agent; texturizer.

REQUIREMENTS

Identification Unhydrogenated Soybean Oil exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<0.5	7.0–12	<0.5	2.0–5.5	19–30	48–65
Fatty Acid:	18:3	20:0	20:1	22:0	22:1	24:0	
Weight % (Range):	5–10	<1.0	<1.0	<0.5	<0.1	<0.3	

Cold Test Passes test.
Color (AOCS-Wesson) Not more than 20 yellow/2.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 120 and 143.
Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.
Stability (Active Oxygen Method) Not less than 7 h.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Cold Test Determine as directed under *Cold Test*, Appendix VII.
Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (e) in the formula given in the procedure:

$$\text{Free fatty acids as oleic acid, } e = 28.2.$$

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.
Stability (Active Oxygen Method) Determine as directed under *Stability*, Appendix VII.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Soy Protein Concentrate

CAS: [9010-10-0]

DESCRIPTION

Soy Protein Concentrate occurs as a powder or as granules, textured flakes, or textured chunks, with color ranging from off white to tan. It is derived from soybean (*Glycine max*) (Fam. Leguminosae) by specific processing steps employed to reduce or remove nonprotein constituents (water, oil, and carbohydrates) to achieve a 65% minimum protein content on the dry basis. Good manufacturing practices require that the pH during processing not exceed 9 to avoid formation of lysinoalanine.

Function Protein supplement; water and fat binder; stabilizer and thickener; texturizing agent.

REQUIREMENTS

Note: Calculate all analyses, except those for *Loss on Drying*, on the dried basis.

Identification Soy Protein Concentrate exhibits the compositional profile specified below with respect to *Ash*, *Fat*, *Loss on Drying*, and *Protein*.
Ash (Total) Not more than 9.0%.
Fat Not more than 4.0%.
Lead Not more than 1 mg/kg.
Loss on Drying Not more than 10.0%.
pH of a 10% Suspension Between 5.5 and 8.0.
Protein Not less than 65.0% and not more than 89.9%.

TESTS

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC, to a final gray to white residue.
Fat Determine as directed under *Crude Fat*, Appendix X.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 2-g sample at 65° at a pressure of less than 100 mm Hg for 16 h.
pH of a 10% Suspension Determine as directed under *pH Determination*, Appendix IIB, using a 10% solution.
Protein Determine the percent of nitrogen as directed under *Nitrogen Determination*, Appendix IIIC, or by the Protein Nitrogen Combustion Method, AOAC 992.23 or AOCS Ba 4e-93. The percent protein equals percent N × 6.25 and is calculated to exclude added vitamins, minerals, amino acids, and food additives.

Packaging and Storage Store in tight containers protected from humidity.

Spearmint Oil

CAS: [8008-79-5]

View IR

DESCRIPTION

Spearmint Oil occurs as a colorless, yellow, or green-yellow liquid having the characteristic odor and taste of spearmint. It is the volatile oil obtained by steam distillation from the fresh overground parts of the flowering plant *Mentha spicata* L. (Common Spearmint), or of *Mentha cardiaca* Gerard ex Baker (Scotch Spearmint) (Fam. Labiatae). It may be rectified by distillation.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate whether it is natural or rectified.
Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 55.0%, by volume, of ketones.
Angular Rotation Between −48° and −59°.
Reaction Passes test.
Refractive Index Between 1.484 and 1.491 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.917 and 0.934.

TESTS

Assay Determine as directed in the *Neutral Sulfite Method* under *Aldehydes and Ketones*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Reaction A recently prepared solution of sample in 80% alcohol is neutral or only slightly acid to moistened litmus paper.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 1 mL of 80% alcohol. On further dilution, the solution may become turbid.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers.

Spice Oleoresins

DESCRIPTION

Spice Oleoresins used in foods are derived from spices and contain the total sapid, odorous, and related characterizing principles normally associated with the respective spices. The oleoresins are produced by one of the following processes: (1) by extraction of the spice with any suitable solvent or solvents, in combination or sequence, followed by removal of the solvent or solvents in conformance with applicable residual solvent regulations (see *General Requirements* below); or (2) by removal of the volatile portion of the spice by distillation, followed by extraction of the nonvolatile portion, which after solvent removal, is combined with the total volatile portion.

Spice Oleoresins are frequently used in commerce with added suitable food-grade diluents, preservatives, antioxidants, and other substances consistent with good manufacturing practices, as provided for under *Added Substances* (see *General Provisions*). When added substances are used, they must be declared on the label in accordance with current U.S. regulations or with the regulations of other countries that recognize the *Food Chemicals Codex*.

Oleoresin Angelica Seed Obtained by the solvent extraction of the dried seed of *Angelica archangelica* L. (Fam. Umbelliferae) as a dark brown or green liquid.
Oleoresin Anise Obtained by the solvent extraction of the dried ripe fruit of *anise*, *Pimpinella anisum* L., or star *anise*, *Illicium verum* Hooker (Fam. Umbelliferae) as a dark brown or green liquid.
Oleoresin Basil Obtained by the solvent extraction of the dried plant of *Ocimum basilicum* L. (Fam. Labiatae) as a dark brown or green semisolid.
Oleoresin Black Pepper Obtained by the solvent extraction of the dried fruit of *Piper nigrum* L. (Fam. Piperaceae) as a dark green, olive green, or olive drab extract usually consisting of an upper oily layer and a lower crystalline layer. It may appear as a homogeneous emulsion if examined shortly after the oleoresin has been homogenized, but the product separates on standing. It may be decolorized by partial removal of chlorophyll.
Oleoresin Capsicum Obtained by the solvent extraction of dried pods of *Capsicum frutescens* L. or *Capsicum annum* L. (Fam. Solanaceae) as a clear red to dark red, somewhat viscous liquid of characteristic odor, flavor, and bite. It may be decolorized through good manufacturing practices. It is partly soluble in alcohol (with oily separation and/or sediment) and is soluble in most fixed oils. The bite is usually standardized according to the label declaration.
Oleoresin Caraway Obtained by the solvent extraction of the dried seeds of *Carum carvi* L. (Fam. Umbelliferae) as a green-yellow to brown liquid.
Oleoresin Cardamom Obtained by the solvent extraction of the dried seeds of *Elettaria cardamomum* Maton (Fam. Zingiberaceae) as a dark brown or green liquid.

Oleoresin Celery Obtained by the solvent extraction of the dried ripe seed of *Apium graveolens* L. (Fam. Umbelliferae) as a dark green, somewhat viscous, nonhomogeneous liquid with the characteristic odor and flavor of celery. It may be decolorized by the partial removal of chlorophyll. It is partly soluble in alcohol (with oily separation), and is soluble in most fixed oils.

Oleoresin Coriander Obtained by the solvent extraction of the dried seeds of *Coriandrum sativum* L. (Fam. Umbelliferae) as a brown-yellow to green liquid.

Oleoresin Cubeb Obtained by the solvent extraction of the dried fruit of *Piper cubeba* L. (Fam. Piperaceae) as a green or green-brown liquid.

Oleoresin Cumin Obtained by the solvent extraction of the dried seeds of *Cuminum cyminum* L. (Fam. Umbelliferae) as a brown to yellow-green liquid.

Oleoresin Dillseed Obtained by the solvent extraction of the dried seeds of *Anethum graveolens* L. (Fam. Umbelliferae) as a brown or green liquid.

Oleoresin Fennel Obtained by the solvent extraction of the dried fruit of *Foeniculum vulgare* P. Miller (Fam. Umbelliferae) as a brown-green liquid.

Oleoresin Ginger Obtained by the solvent extraction of the dried rhizomes of *Zingiber officinale* Roscoe (Fam. Zingiberaceae) as a dark brown, viscous to highly viscous liquid with the characteristic odor and flavor of ginger. It is soluble in alcohol (with sediment).

Oleoresin Hop Obtained by the solvent extraction of the dried membranous cones of the female hop plants of *Humulus lupulus* L. or *Humulus americanus* Nutt. (Fam. Moraceae), using a food-grade solvent such as liquid carbon dioxide. It occurs as a light golden to black liquid to semisolid with a characteristic odor. It is soluble in methanol and is slightly soluble in acidified water. It may be reduced with sodium borohydride or with hydrogen and palladium catalyst. It conforms to U.S. Food and Drug Administration regulations pertaining to the specifications for extraction solvents for modified hop extract.

Oleoresin Laurel Leaf Obtained by the solvent extraction of the dried leaves of *Laurus nobilis* L. (Fam. Lauraceae) as a dark brown or green semisolid.

Oleoresin Marjoram Sweet Obtained by the solvent extraction of the dried herb of the marjoram shrub *Majorana hortensis* Moench (Fam. Labiatae) as a dark green to brown viscous liquid or semisolid.

Oleoresin Origanum Obtained by the solvent extraction of the dried flowering herb *Origanum* spp. (Fam. Labiatae) as a dark brown-green semisolid.

Oleoresin Paprika Obtained by the solvent extraction of the pods of *Capsicum annuum* L. (Fam. Solanaceae) as a deep red to deep purple-red, somewhat viscous liquid of characteristic odor and flavor. It frequently occurs as a two-phase mixture. The color is usually standardized according to the label declaration. It is partly soluble in alcohol (with oily separation), and is soluble in most fixed oils.

Oleoresin Parsley Leaf Obtained by the solvent extraction of the dried herb of *Petroselinum crispum* (P. Miller) Nyman ex A.W. Hill (Fam. Umbelliferae) as a brown to green liquid.

Oleoresin Parsley Seed Obtained by the solvent extraction of the dried seeds of *Petroselinum crispum* (P. Miller) Nyman ex A.W. Hill (Fam. Umbelliferae) as a deep green, semiviscous liquid.

Oleoresin Pimenta Berries Obtained by the solvent extraction of the dried fruit of *Pimenta officinalis* Lindl (Fam. Myrtaceae) as a brown-green to dark green liquid.

Oleoresin Rosemary Obtained by the solvent extraction of the dried leaves of *Rosmarinus officinalis* L. (Fam. Labiatae). It is a thick, green paste that can be diluted with food-grade water- or oil-dispersible solvents. It may have a reduced chlorophyll content. The volatile oil content varies depending on its intended effect from a highly camphoraceous note to a subtle herbal note.

Oleoresin Thyme Obtained by the solvent extraction of the dried flowering plant *Thymus vulgaris* L. or *Thymus zygis* L. and its var. *gracelis* Boissier (Fam. Labiatae) as a dark brown to green, viscous semisolid.

Oleoresin Turmeric Obtained by the solvent extraction of the dried rhizomes of *Curcuma longa* L. (Fam. Zingiberaceae) as a yellow-orange to red-brown, viscous liquid with a characteristic odor and flavor. The content of curcumin normally varies, and the product is generally standardized according to the label declaration.

Function Flavoring agent; color (oleoresins paprika and turmeric only).

GENERAL REQUIREMENTS

Identification The volatile oil distilled from an oleoresin is similar in its physical and chemical properties, including its infrared spectrum, to that distilled from the spice of the same origin. To obtain the volatile oil from the oleoresin, proceed as directed under *Volatile Oil Content*, Appendix VIII.

Residual Solvent *Chlorinated Hydrocarbons (total)*: Not more than 0.003%; *Acetone*: Not more than 0.003%; *Isopropanol*: Not more than 0.003%; *Methanol*: Not more than 0.005%; *Hexane*: Not more than 0.0025%.

ADDITIONAL REQUIREMENTS

Oleoresin Angelica Seed *Volatile Oil Content*: Between 2 mL and 7 mL/100 g.

Oleoresin Anise *Volatile Oil Content*: Between 9 mL and 22 mL/100 g.

Oleoresin Basil *Volatile Oil Content*: Between 4 mL and 17 mL/100 g.

Oleoresin Black Pepper *Piperine Content* (Appendix VIII): Not less than 36%; *Volatile Oil Content*: Between 15 mL and 35 mL/100 g.

Oleoresin Capsicum *Scoville Heat Units* (Appendix VIII): Between 100,000 and 2,000,000, as specified on the label.

Oleoresin Caraway *Volatile Oil Content*: Between 10 mL and 20 mL/100 g.

Oleoresin Cardamom *Volatile Oil Content*: Between 50 mL and 80 mL/100 g.

Oleoresin Celery *Volatile Oil Content*: Between 7 mL and 20 mL/100 g.

Oleoresin Coriander *Volatile Oil Content*: Between 2 mL and 12 mL/100 g.
Oleoresin Cubeb *Volatile Oil Content*: Between 50 mL and 80 mL/100 g.
Oleoresin Cumin *Volatile Oil Content*: Between 10 mL and 30 mL/100 g.
Oleoresin Dillseed *Volatile Oil Content*: Between 10 mL and 20 mL/100 g.
Oleoresin Fennel *Volatile Oil Content*: Between 3 mL and 20 mL/100 g.
Oleoresin Ginger *Volatile Oil Content*: Between 18 mL and 35 mL/100 g.
Oleoresin Hop *Volatile Oil Content*: Not more than 30 mL/100 g.
Oleoresin Laurel Leaf *Volatile Oil Content*: Between 5 mL and 25 mL/100 g.
Oleoresin Marjoram Sweet *Volatile Oil Content*: Between 8 mL and 20 mL/100 g.
Oleoresin Origanum *Volatile Oil Content*: Between 20 mL and 45 mL/100 g.
Oleoresin Paprika *Color Value* (Appendix VIII): Between 500 and 4500 units, as specified on the label (according to the method of analysis); *Scoville Heat Units (pungency)*: Not more than 3000.
Oleoresin Parsley Leaf *Volatile Oil Content*: Between 2 mL and 10 mL/100 g.
Oleoresin Parsley Seed *Volatile Oil Content*: Between 2 mL and 7 mL/100 g.
Oleoresin Pimenta Berries *Volatile Oil Content*: Between 20 mL and 50 mL/100 g.
Oleoresin Rosemary *Volatile Oil Content*: Not more than 15 mL/100 g.
Oleoresin Thyme *Volatile Oil Content*: Between 5 mL and 12 mL/100 g.
Oleoresin Turmeric *Curcumin Content (or Color Value equivalent)*: Between 1% and 45%, as specified on the label.

TESTS (GENERAL REQUIREMENTS)

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Residual Solvent Determine as directed under *Residual Solvent*, Appendix VIII.

TESTS (ADDITIONAL REQUIREMENTS)

Color Value Determine as directed under *Color Value*, Appendix VIII.
Curcumin Determine as directed under *Curcumin Content*, Appendix VIII.
Piperine Determine as directed under *Piperine Content*, Appendix VIII.
Scoville Heat Units Determine as directed under *Scoville Heat Units*, Appendix VIII.
Volatile Oil Content Determine as directed under *Volatile Oil Content*, Appendix VIII.

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Spike Lavender Oil

CAS: [84837-04-7]

View IR

DESCRIPTION

Spike Lavender Oil occurs as a pale yellow to yellow liquid with a camphoraceous, lavender odor. It is the volatile oil obtained by steam distillation from the flowers of *Lavandula latifolia*, Vill. (*Lavandula spica*, D.C.) (Fam. Labiatae). It is soluble in most fixed oils and in propylene glycol. It is slightly soluble in glycerin and in mineral oil.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 40.0% and not more than 50.0% of total alcohols, calculated as linalool ($C_{10}H_{18}O$).
Angular Rotation Between −5° and +5°.
Esters Not less than 1.5% and not more than 4.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Refractive Index Between 1.463 and 1.468 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.893 and 0.909.

TESTS

Assay Determine as directed under *Linalool Determination*, Appendix VI, using about 1.5 g of the acetylated oil, accurately weighed, for the ester determination.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Esters Determine as directed for *Ester Determination* under *Esters*, Appendix VI, using about 10 g of sample, accurately weighed, and 98.15 as the equivalence factor (*e*) in the calculation.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 3 mL of 70% alcohol. The solution frequently becomes hazy on further dilution.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Stannous Chloride

Tin Dichloride

$SnCl_2$ Formula wt, anhydrous 189.60
$SnCl_2 \cdot 2H_2O$ Formula wt, dihydrate 225.63

INS: 521 CAS: anhydrous [7772-99-8]
CAS: dihydrate [10025-0969-091]

DESCRIPTION

Stannous Chloride occurs as white or colorless crystals. It is anhydrous or contains two molecules of water of hydration. It is very soluble in water, and it is soluble in alcohol and in glacial acetic acid.

Function Reducing agent; antioxidant.

REQUIREMENTS

Identification

A. Add mercuric chloride TS, dropwise, to a 1:20 solution of sample in 2.7 *N* hydrochloric acid. A white or gray-white precipitate forms.

B. A 1:20 aqueous solution gives positive tests for *Chloride*, Appendix IIIA.

Assay Not less than 99.0% and not more than 101.0% of $SnCl_2$, or not less than 98.0% and not more than 102.2% of $SnCl_2 \cdot 2H_2O$.

Iron Not more than 0.005%.

Lead Not more than 4 mg/kg.

Solubility in Hydrochloric Acid Passes test.

Substances Not Precipitated by Sulfide Not more than 0.05%.

Sulfate Passes test.

TESTS

Assay Transfer about 2 g of sample, accurately weighed, into a 250-mL volumetric flask, dissolve in 15 mL of hydrochloric acid, dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a 500-mL flask, add 5 g of sodium potassium tartrate, and mix. Make the solution alkaline to litmus with a cold saturated solution of sodium bicarbonate, and titrate at once with 0.1 *N* iodine, using starch TS as the indicator. Each milliliter of 0.1 *N* iodine is equivalent to 9.48 mg of $SnCl_2$ or 11.28 mg of $SnCl_2 \cdot 2H_2O$.

Iron Add 3 mL of 1:2 hydrochloric acid to the residue obtained in the test for *Substances Not Precipitated by Sulfide* (below), cover with a watch glass, and digest on a steam bath for 15 min. Remove the cover, and evaporate to dryness on the steam bath. Dissolve the residue in a few milliliters of water and 8 mL of hydrochloric acid, dilute to 100 mL with water, and mix. Add 2 mL of hydrochloric acid, 46 mL of water, 40 mg of ammonium persulfate crystals, and 3 mL of ammonium thiocyanate TS to 2.0 mL of this solution. Any red or pink color does not exceed that produced by 2.0 mL of *Iron Standard Solution* (20 μg of Fe) in an equal volume of solution containing the quantities of the reagents used in the test.

Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Solubility in Hydrochloric Acid Dissolve a 5-g sample in a mixture of 5 mL of hydrochloric acid and 5 mL of water, heating to 40°, if necessary, to effect solution. The sample dissolves completely, and the solution is clear.

Substances Not Precipitated by Sulfide Transfer about 20 g of sample, accurately weighed, into a 250-mL beaker, and add 50 mL of a solution prepared by carefully adding 75 mL of bromine to 425 mL of 48% hydrobromic acid. Add 1 mL of sulfuric acid, and mix to effect complete solution. Place the beaker on a hot plate, and volatilize the tin slowly, with gentle boiling, to fumes of sulfur trioxide. Cool, add 30 mL of water, and pass hydrogen sulfide gas through the solution for about 5 min. Filter through Whatman No. 42 filter paper, or equivalent, into a weighed platinum dish, and wash with three small portions of a 1% solution of sulfuric acid saturated with hydrogen sulfide. Carefully evaporate to dryness on a hot plate, and heat in a furnace at 800° ± 25° for 13 min. Cool in a desiccator for at least 30 min, and weigh. Calculate the percentage of substances not precipitated by sulfide by the formula

$$100A/B,$$

in which A and B are the respective weights, in grams, of the residue and of the sample taken. Retain the residue for the *Iron* test (above).

Sulfate Dissolve 5 g of sample in 5 mL of hydrochloric acid, dilute to 50 mL with water, filter if not clear, and heat the filtrate or clear solution to boiling. Add 5 mL of barium chloride TS, digest in a covered beaker on a steam bath for 2 h, and allow to stand overnight. No precipitate forms.

Packaging and Storage Store in well-closed containers.

Starter Distillate

Butter Starter Distillate

DESCRIPTION

Starter Distillate occurs as a clear, yellow, water-soluble liquid. It is the steam distillate of a culture of one or more species of *Lactococcus lactis* subsp. *diacetylactis* and/or *Leuconostoc cremoris* grown in a medium of skimmed milk that has been

fortified with citric acid. It contains more than 97% water and a mixture of organic flavor compounds, principally diacetyl.

Function Flavoring agent.

REQUIREMENTS

Labeling Indicate the diacetyl content, in milligrams per milliliter.
Assay Not less than 90.0% and not more than 110.0% of the labeled amount of diacetyl.
pH Between 2.8 and 3.8.

TESTS

Assay

Osmic Acid (0.01%) Dissolve 1 g of osmium tetroxide in 1 L of water, and dilute to a 1:10 aqueous solution.

> **Caution**: Osmium tetroxide and its solutions are toxic. Use proper protective equipment, and avoid contact with the eyes, skin, and clothing.

Procedure Transfer an accurately measured volume of sample, equivalent to about 25 mg of diacetyl, into a suitable flask. Add 3 drops of phenolphthalein TS, and neutralize the acidity by titrating with 0.05 *N* sodium hydroxide to a faint pink endpoint. Add 0.25 mL of 30% hydrogen peroxide solution and 3 drops of *0.01% Osmic Acid*. Mix, cover the flask, and allow it to stand in an incubator held at about 38° for not less than 4 h. Cool to room temperature, and titrate with 0.05 *N* sodium hydroxide to a faint pink endpoint. Each milliliter of 0.05 *N* sodium hydroxide is equivalent to 8.6 mg of diacetyl.
pH Determine as directed under *pH Determination*, Appendix IIB.

Packaging and Storage Store in a cool place in tight containers.

Stearic Acid

Octadecanoic Acid

$C_{18}H_{36}O_2$ Formula wt 284.48

CAS: [57-11-4]

DESCRIPTION

Stearic Acid occurs as a hard, white or faintly yellow, somewhat glossy and crystalline solid or as a white or yellow-white powder. It is a mixture of solid organic acids obtained from fats consisting chiefly of Stearic Acid ($C_{18}H_{36}O_2$) and palmitic acid ($C_{16}H_{32}O_2$). Stearic Acid is practically insoluble in water. One gram dissolves in about 20 mL of alcohol, in 2 mL of chloroform, and in about 3 mL of ether.

Function Component in the manufacture of other food-grade additives; lubricant; defoaming agent.

REQUIREMENTS

Acid Value Between 196 and 211.
Iodine Value Not more than 7.
Lead Not more than 2 mg/kg.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 197 and 212.
Titer (Solidification Point) Between 54.5° and 69°.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII, using about 3 g of sample, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, Appendix IIB.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Stearyl Monoglyceridyl Citrate

DESCRIPTION

Stearyl Monoglyceridyl Citrate occurs as a soft, off white to tan, waxy solid having a lardlike consistency. It is prepared by a controlled chemical reaction from citric acid, monoglycerides of fatty acids (obtained by the glycerolysis of edible fats and oils or derived from fatty acids), and stearyl alcohol. It is insoluble in water, but is soluble in chloroform and in ethylene glycol.

Function Emulsion stabilizer.

REQUIREMENTS

Acid Value Between 40 and 52.
Lead Not more than 2 mg/kg.

Residue on Ignition Not more than 0.1%.
Saponification Value Between 215 and 255.
Total Citric Acid Between 15.0% and 18.0%.
Water Not more than 0.25%.

TESTS

Acid Value Determine as directed in *Method II*, under *Acid Value*, Appendix VII.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Saponification Value Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, and add 25 mL of ethylene glycol, 35.0 mL of 0.5 *N* alcoholic potassium hydroxide, and a few glass beads. Reflux for 1 h, using a water condenser, then rinse the condenser with water, and cool. Add 1 mL of phenolphthalein TS, and titrate with 0.5 *N* hydrochloric acid. Perform a blank determination (see *General Provisions*), but do not reflux. The difference between the volumes, in milliliters, of 0.5 *N* hydrochloric acid consumed in the test and in the blank titration, multiplied by 28.05 and divided by the weight, in grams, of the sample taken, is the saponification value.

Total Citric Acid

Brominating Solution Dissolve 19.84 g of potassium bromide, 5.44 g of potassium bromate, and 12 g of sodium metavanadate ($NaVO_3$) in water by warming, and dilute to 1000 mL with water. Filter if necessary.

Ferrous Sulfate Solution Dissolve 44 g of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) in 1 *N* sulfuric acid, dilute to 100 mL with 1 *N* sulfuric acid, and mix. Use within 5 days of preparation.

Sulfide Solution On the day of use, dissolve 4 g of thiourea in 100 mL of a 1:50 solution of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$), and add 2 mL of sodium sulfide TS. Wait 30 min after the addition of the sodium sulfide TS before using.

Standard Solution Transfer about 50 mg of sodium citrate dihydrate, accurately weighed, into a 500-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 15.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix. Calculate the concentration (*C*), in micrograms per milliliter, of citric acid in the final solution by the formula

$$(15 \times 1000 \times 0.6533W)/(100 \times 500),$$

in which 0.6533 is the factor converting sodium citrate dihydrate to citric acid, and *W* is the weight, in milligrams, of the sodium citrate taken.

Sample Solution Transfer about 250 mg of sample, accurately weighed, into a 250-mL extraction flask, and add 15 mL of 0.5 *N* sodium hydroxide, 5 mL of alcohol, and a few glass beads. Connect the flask with a water-cooled condenser, and reflux for 3 h. Immediately cool and neutralize to phenolphthalein TS with 0.5 *N* hydrochloric acid, then place the flask in an ice bath and add 5 mL of 95% sulfuric acid. Transfer the solution to a 125-mL separator, extract with three 40-mL portions of chloroform, and then extract the combined chloroform extracts in a 250-mL separator with three 10-mL portions of 0.5 *N* sulfuric acid, adding the acid extracts to a second 250-mL separator. Wash the combined acid extracts with two 60-mL portions of chloroform, and discard the chloroform washes. Filter the acid solution into a 500-mL volumetric flask, neutralize slowly with 6 *N* sodium carbonate, and dilute to volume with water. Transfer 10.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix. Each milliliter of the final solution contains approximately 10 μg of citric acid.

Procedure Pipet 2 mL each of the *Standard Solution* and of the *Sample Solution* into separate, 40- or 45-mL glass-stoppered centrifuge tubes, and add 3 mL of water to each tube. Place 5 mL of water in a third tube for the reagent blank. Place the tubes in an ice bath, add 5 mL of 95% sulfuric acid, mix thoroughly, and allow to stand for exactly 5 min. Remove the tubes from the ice bath, and allow them to come to room temperature during the next 5 min. Add 5 mL of the *Brominating Solution* to each tube, then insert the stoppers, invert the tubes once or twice, and heat in a water bath at 30° for 20 min. Remove the tubes, add 1.5 mL of *Ferrous Sulfate Solution*, invert again, and allow to stand for 5 min, shaking occasionally to ensure complete reduction of the excess free bromine in the tubes. Add 6.5 mL of petroleum ether, shake for 2 or 3 min, and remove the water layer with a syringe. Wash the ether solutions with 15 mL of water, then remove the water, and filter the ether extracts into the centrifuge tubes, which have been previously rinsed with the *Sulfide Solution*. Filter each ether extract through a tight plug of glass wool containing a sufficient amount of anhydrous sodium sulfate to remove the last traces of water from the ether. Place 5.0 mL of each filtrate into separate clean, dry centrifuge tubes, add 3 mL of *Sulfide Solution*, shake vigorously for 1.5 min, and centrifuge. Decant about 0.5 mL of the supernatant ether layer from each tube, then carefully transfer the ether solutions into 1-cm cells, and determine the absorbance of the extracts obtained from the *Standard Solution* and the *Sample Solution* at 500 nm with a suitable spectrophotometer, using the reagent blank in the reference cell. Calculate the quantity, in milligrams, of citric acid in the sample taken by the formula

$$5C \times A_U/A_S,$$

in which *C* is the exact concentration, in micrograms per milliliter, of citric acid in the *Standard Solution*; A_U is the absorbance of the solution from the *Sample Solution*; and A_S is the absorbance of the solution from the *Standard Solution*.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Succinic Acid

Butanedioic Acid

$$HOOCCH_2CH_2COOH$$

$C_4H_6O_4$ Formula wt 118.09

INS: 363 CAS: [110-15-6]

DESCRIPTION

Succinic Acid occurs as colorless or white crystals. One gram dissolves in 13 mL of water at 25°, in 1 mL of boiling water, in 18.5 mL of alcohol, and in 20 mL of glycerin.

Function Buffer; neutralizing agent.

REQUIREMENTS

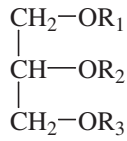
Identification Place a drop of a saturated solution of sample into a micro test tube, and add a drop of a 0.5% solution of ammonium chloride and several milligrams of zinc powder. Cover the mouth of the tube with a disk of filter paper moistened with a solution of 5% *p*-dimethylaminobenzaldehyde and 20% trichloroacetic acid in hexane. Heat with a small flame for about 1 min. A pink to red-violet stain appears on the paper.
Assay Not less than 99.0% and not more than 100.5% of $C_4H_6O_4$.
Lead Not more than 2 mg/kg.
Melting Range Between 185.0° and 190.0°.
Residue on Ignition Not more than 0.025%.

TESTS

Assay Dissolve about 250 mg of sample, accurately weighed, in 25 mL of recently boiled and cooled water, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to the first appearance of a faint pink color that persists for at least 30 s. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 5.905 mg of $C_4H_6O_4$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting an 8-g sample.

Packaging and Storage Store in well-closed containers.

Succinylated Monoglycerides



R_1, R_2, and R_3 may be a fatty acid or succinic acid or hydrogen.

INS: 472g

DESCRIPTION

Succinylated Monoglycerides occur as waxy solids having an off white color. They are a mixture of succinic acid esters of mono- and diglycerides produced by the succinylation of a product obtained by the glycerolysis of edible fats and oils, or by the direct esterification of glycerol with edible fat-forming fatty acids. They melt at about 60°. They are soluble in warm methanol, in ethanol, and in *n*-propanol.

Function Emulsifier; dough conditioner.

REQUIREMENTS

Acid Value Between 70 and 120.
Bound Succinic Acid Not less than 14.8%.
Free Succinic Acid Not more than 3%.
Hydroxyl Value Between 138 and 152.
Iodine Value Not more than 3.
Lead Not more than 2 mg/kg.
Total Succinic Acid Between 14.8% and 25.6%.

TESTS

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Free and Bound Succinic Acid

0.02 N *Sodium Hydroxide in Methanol* Dissolve 4.0 g of sodium hydroxide in 1000 mL of anhydrous methanol. Transfer 200.0 mL of this solution to a 1000-mL volumetric flask, dilute to volume with anhydrous methanol, and mix. Standardize the solution against dried succinic acid, using phenolphthalein TS as the indicator.

Procedure Transfer about 125 mg of sample, accurately weighed, into a 250-mL separator containing 100 mL of benzene, and dissolve the sample by heating the separator with warm water. Treat the sample and a blank, consisting of 100 mL of benzene in another separator, as follows: Cool the contents of the separator, add 50 mL of water, and mix by inverting the separator about 20 times. Allow to stand for about 15 min, and then transfer the aqueous layer into a 125-mL Erlenmeyer flask. Add 10 mL of water to the separator, wash the benzene layer by inverting the separator five times, and add the washings to the 125-mL flask. Add five drops

of phenolphthalein TS to the flask, and titrate with *0.02* N *Sodium Hydroxide in Methanol*. Perform a blank determination (see *General Provisions*), make any necessary correction, and record the net volume of alkali, in milliliters, as V_1.

Transfer the benzene layer into a 500-mL round-bottom flask, and rinse the separator with 10 mL of benzene. Add a few boiling chips to the flask, and evaporate the benzene, preferably on a thin-film evaporator, under partial vacuum at about 60°. Dissolve the residue in the flask in 10 mL of methanol, add 10 mL of water and 5 drops of phenolphthalein TS, and titrate with *0.02* N *Sodium Hydroxide in Methanol*. Perform a blank determination (see *General Provisions*), make any necessary correction, and record the net volume of alkali, in milliliters, as V_2.

Calculate the weight, in milligrams, of free succinic acid in the sample by the formula

$$118.1 \times N \times V_1/2,$$

and calculate the weight, in milligrams, of bound succinic acid in the sample by the formula

$$118.1 \times N \times V_2/2,$$

in which N is the exact normality of the sodium hydroxide solution.

Hydroxyl Value Determine as directed in *Method II* under *Hydroxyl Value*, Appendix VII.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.

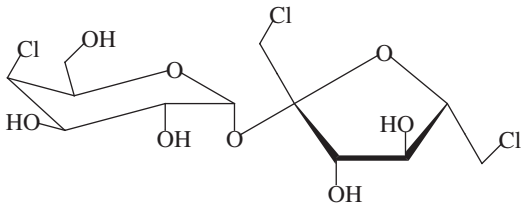
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Total Succinic Acid The sum of the *Free Succinic Acid* and the *Bound Succinic Acid* represents the *Total Succinic Acid*.

Packaging and Storage Store in well-closed containers.

Sucralose

1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-
4-chloro-4-deoxy-α-D-galactopyranoside;
4,1′,6′-Trichlorogalactosucrose



$C_{12}H_{19}Cl_3O_8$ Formula wt 397.64

INS: 955 CAS: [56038-13-2]

DESCRIPTION

Sucralose occurs as a white to off white, crystalline powder. It is freely soluble in water, in methanol, and in alcohol, and is slightly soluble in ethyl acetate.

Function Nonnutritive sweetener; flavor enhancer.

REQUIREMENTS

Identification

A. The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits relative maxima at the same wavelengths as those of a similar preparation of Sucralose Standard for analytical use.[1]

B. The retention time of the major peak (excluding the solvent peak) in the liquid chromatogram of the *Sample Preparation* is the same as that of the *Standard Preparation* obtained in the *Assay* (below).

C. The R_f value of the major spot in the thin-layer chromatogram of the *Test Preparation* is the same as that of the *Standard Preparation* obtained in the test for *Related Substances* (below).

Assay Not less than 98.0% and not more than 102.0% of $C_{12}H_{19}Cl_3O_8$, calculated on the anhydrous basis.

Hydrolysis Products Passes test.

Lead Not more than 1 mg/kg.

Methanol Not more than 0.1%.

Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +84.0° and +87.5°, calculated on the anhydrous basis.

Related Substances Passes test.

Residue on Ignition Not more than 0.7%.

Water Not more than 2.0%.

TESTS

Assay

Mobile Phase Add 150 mL of HPLC-grade acetonitrile that has been filtered through a 0.45-μm filter to 850 mL of glass-distilled (or equivalent) water that also has been filtered through a 0.45-μm filter. Mix, and de-gas thoroughly.

Standard Preparation Transfer about 25 mg of Sucralose Standard for analytical use, accurately weighed, into a 25-mL volumetric flask. Dissolve in and dilute to volume with *Mobile Phase*. Filter the solution through a 0.45-μm filter.

Sample Preparation Transfer about 25 mg of sample, accurately weighed, into a 25-mL volumetric flask. Dissolve in and dilute to volume with *Mobile Phase*. Filter the solution through a 0.45-μm filter.

Chromatographic System (See *Chromatography*, Appendix IIA.) Fit a high-performance liquid chromatographic system, operated at room temperature, with an 8-mm × 10-cm, 5-μm RadPakC18 (or equivalent) reverse-phase column. Maintain the *Mobile Phase* at a pressure and flow rate (typically 1.5 mL/min) capable of giving the required elution time (see the *System Suitability Test*, below). Use a refractive index detector.

System Suitability Test Obtain chromatograms of duplicate 20-μL injections of the *Standard Preparation*. Ensure that the retention time of Sucralose is approximately 9 min. It may be necessary to adjust the *Mobile Phase* composition to obtain the desired retention time. Ensure that the relative

[1]Available from McNeil Specialty Products Company, Regulatory Affairs Department, 501 George Street, New Brunswick, NJ 08903-2400.

standard deviation (100 × standard deviation/mean peak area) does not exceed 2.0%.

Procedure Analyze the *Standard Preparation* and *Sample Preparation* under the conditions described above, making duplicate 20-µL injections, and calculate the mean peak areas.

Calculation Calculate the percent of Sucralose from the peak areas of the *Sample Preparation* (A_U) and *Standard Preparation* (A_S) according to the following formula:

$$100(A_U W_S)/(A_S W_U),$$

in which W_S is the weight, in milligrams, of the Sucralose Standard, and W_U is the weight, in milligrams, of the sample.

Hydrolysis Products

Spray Reagent Dissolve 1.23 g of *p*-anisidine and 1.66 g of phthalic acid in 100 mL of methanol. Store the solution in darkness, and refrigerate to prevent discoloration. Discard if the solution becomes discolored.

> **Caution**: *p*-Anisidine is toxic if inhaled or absorbed through the skin and should be used with due caution.

Standard Solution A Dissolve 10.0 g of mannitol, weighed to 0.001 g, in water in a 100-mL volumetric flask, and dilute to volume with water.

Standard Solution B Dissolve 40 mg of fructose and 10 g of mannitol, accurately weighed, in 25 mL of water in a 100-mL volumetric flask, and dilute to volume with water.

Sample Solution Dissolve 2.5 g of sample in 5 mL of methanol in a 10-mL volumetric flask, and dilute to volume with methanol.

Procedure Use a thin-layer chromatographic (TLC) plate coated with a 0.25-mm layer of Merck-silica gel 60, or equivalent. Spot 5 µL of *Standard Solution A* and of *Standard Solution B* onto the plate, applying the solution slowly in 1-µL aliquots and allowing the plate to dry between applications. Spot 5 µL of the *Sample Solution* onto the plate in a similar manner. The three spots should be of similar size. Spray the plate with the *Spray Reagent*, and heat it at 100° ± 2° for 15 min. Immediately after heating, view the plate against a dark background. The spot from the *Sample Solution* is not more intense in color than the spot from *Standard Solution B* (0.1% limit).

> **Note**: If the spot from *Standard Solution A* darkens, this indicates that the plate has been held too long in the oven, and that a second plate should be prepared.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Methanol

Internal Standard Solution Pipet 1.0 mL of *n*-propanol into a 100-mL volumetric flask, dilute to volume with pyridine, and mix. Transfer 5.0 mL of this solution into a 500-mL volumetric flask, dilute to volume with pyridine, and mix.

Standard Solution Pipet 2.0 mL of methanol into a 100-mL volumetric flask, dilute to volume with *Internal Standard Solution*, and mix. Transfer 1.0 mL of this solution into a 100-mL volumetric flask, dilute to volume with *Internal Standard Solution*, and mix.

Sample Solution Transfer about 2 g of sample, accurately weighed, into a 10-mL volumetric flask, dilute to volume with *Internal Standard Solution*, and mix.

Procedure (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatographic system equipped with a hydrogen flame ionization detector and a 2.1-m × 4-mm (id) glass column, or equivalent, packed with 80- to 100-mesh Porapak PS, or equivalent. Set the column to 150° (isothermal), the inlet to 200°, and the detector to 250°. Use helium as the carrier gas, set at a flow rate of 20 mL/min. Inject a 1-µL portion of the *Standard Solution* into the gas chromatograph, obtain the chromatogram, and measure the area of the peak produced. (The retention time for methanol is about 2 min.) The relative standard deviation for replicate injections is not more than 2.0%. Calculate the mean peak areas for the *Standard Solution*. Similarly, inject a 1-µL portion of the *Sample Solution* into the gas chromatograph, and measure the areas of the peaks produced by methanol. Calculate the mean peak areas, and determine the percent of methanol in the portion of sample taken using the following equation:

$$\% \text{ methanol} = (R_U/R_S)(0.158/W_S),$$

in which R_U is the ratio of the peak areas of methanol to that of the internal standard obtained from the *Sample Solution*; R_S is the ratio of the peak areas of methanol to the internal standard obtained from the *Standard Solution*; the factor 0.158 is equal to the volume of methanol in the standard × dilution factor × density of methanol × 100%; and W_S is the weight, in grams, of the sample.

Related Substances

Chromatographic Plates Use Whatman LKC18 thin-layer chromatographic plates coated with a 0.20-mm layer of silica gel absorbent, or equivalent.

Mobile Phase Mix 70 volumes of 5.0% (w/v) sodium chloride in water, and add 30 volumes of acetonitrile. Prepare fresh before use.

Spray Reagent Use a 15% (v/v) solution of sulfuric acid in methanol.

Standard Solutions Dissolve 500.0 mg of Sucralose Standard for analytical use in 5.0 mL of methanol (*Solution A*). Dilute 0.5 mL of *Solution A* with methanol to 100 mL (*Solution B*).

Test Preparation Dissolve 1.0 g of sample in 10 mL of methanol.

Procedure Apply 5 µL each of *Solution A*, *Solution B*, and *Test Preparation* to the bottom of the chromatographic plate. Place the plate in a suitable chromatographic chamber containing *Mobile Phase*, and allow the solvent front to ascend approximately 15 cm. Remove the plate, allow it to dry, and spray it with the *Spray Reagent*. Heat the plate in an oven at 125° for 10 min. The main spot in the *Test Preparation* is at the same R_f value as the main spot in *Solution A*, and any other single spot in the *Test Preparation* is not more intense than the 0.5% spot in *Solution B*.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1- to 2-g sample.

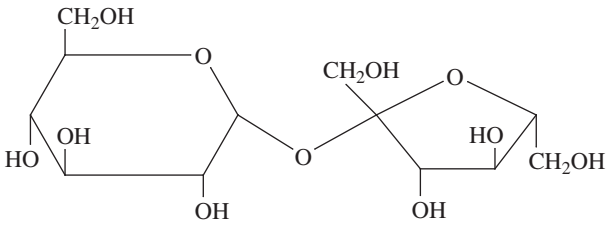
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 1:100 (w/v) aqueous solution, calculated on the anhydrous basis.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers in a cool (lower than 21°), dry place.

Sucrose

Sugar; Granulated Sugar; Cane Sugar; Beet Sugar; β-D-Fructofuranosyl-α-D-glucopyranoside



$C_{12}H_{22}O_{11}$ Formula wt 342.30

CAS: [57-50-1]

DESCRIPTION

Sucrose, in its processed form, occurs as a white, crystalline solid. It is obtained for commercial use from sugar cane and sugar beets. It is very soluble in water, in formamide, and in dimethyl sulfoxide and is slightly soluble in ethanol.

Function Nutritive sweetener; formulation and texturizing aid.

REQUIREMENTS

Identification A sample meets the requirements under *Optical (Specific) Rotation*.
Assay Not less than 99.8 and not more than 100.2 International Sugar Degrees (°Z).
Arsenic Not more than 1 mg/kg.
Color Not more than 75 IU.
Invert Sugar Not more than 0.1%.
Lead Not more than 0.1 mg/kg.
Loss on Drying Not more than 0.1%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +65.9° and +66.7°.
Residue on Ignition Not more than 0.15%.

TESTS

Note: Consult ICUMSA[1] rules for further details applying to *Assay*, *Color*, and *Invert Sugar*.

[1]International Commission for Uniform Methods of Sugar Analysis (ICUMSA), c/o Chemistry Department, University of Ferrara, Via L. Borsari, 46, 44100 Ferrara, Italy.

Assay

Apparatus Use a saccharimeter calibrated with a certified quartz plate according to the directions of the instrument manufacturer and a 20-cm polarimeter tube with cover glasses. The tube and glasses should conform to ICUMSA specifications. Have ready 100-mL flasks accurate to within 0.01 mL. Maintain a water bath at 20° ± 0.1°.

Sample Solution Quantitatively transfer 26.000 g ± 0.002 g of sample to the flask, and add about 80 mL of water. Without heating, dissolve the sample by agitation, and add water to the flask to just below the calibration mark. Place the flask in the water bath to adjust the solution to 20° ± 0.1° (degrees Centigrade unless otherwise specified). Dry the inside wall of the flask neck above the calibration mark with filter paper, and using either a hypodermic syringe or a pipet with a drawn out point, adjust to the exact volume with water. Seal the flask with a clean, dry stopper, and mix the contents thoroughly by shaking.

Procedure Carefully rinse the polarimeter tube twice using two-thirds its volume of *Sample Solution*, and fill it with *Sample Solution* at 20° ± 0.1° in such a way that no air bubbles are trapped. Place the tube in the saccharimeter, and polarize it at 20°. Determine five values to 0.05 °Z, and record the average of these values.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared with 1 g of sample, accurately weighed. Use 1 mL of *Standard Arsenic Solution* in the control (1 μg As).

Color

Apparatus Use a suitable variable wavelength spectrophotometer capable of measuring percent transmittance at 420 nm or a photometer with a 420- ± 10-nm band width filter. The instrument design should permit the use of a 10-cm cell. When using an instrument with a reference cell, the two cells should be identical with water within ±0.2% when the instrument is set at 100% transmittance on one of the cells.

Procedure Prepare a 50% (w/w) sample solution in water. Adjust the pH to 7.0 ± 0.2 with 1% sodium hydroxide or 1% hydrochloric acid. Filter through a 0.45-μm pore-size membrane filter, using a vacuum and a diatomaceous earth filter aid (1% on solids) if necessary. Discard the first portion of the filtrate if it is cloudy. Determine the density and concentration of solids, in grams per milliliters, refractometrically. Rinse the measuring cell three times with the sample solution, and then fill the cell. Measure absorbancy (A_S) at 420 nm. Calculate the color in ICUMSA units (*IU*) as follows:

$$IU = (A_S/bc) \times 1000,$$

in which b is the cell length, in centimeters, and c is the concentration, in grams per milliliter, of total solids determined refractometrically and calculated from density.

Invert Sugar

Apparatus Use a water bath with vigorously boiling water to ensure that the immersion of flasks does not interrupt the boiling. When instructed, place flasks in the water bath so that the water level is 2 cm above the liquid surface in the flasks.

Muller's Solution Dissolve 35 g of cupric sulfate pentahydrate in 400 mL of boiling water. In a separate beaker, dissolve

173 g of potassium sodium tartrate tetrahydrate and 68 g of anhydrous sodium carbonate in 400 mL of boiling water. Cool both solutions, and while stirring, pour the sodium carbonate–potassium sodium tartrate solution into the cupric sulfate solution. Transfer the combined solutions into a 1000-mL volumetric flask, dilute to volume with water, and mix. Add 2 g of activated carbon, shake vigorously, and filter through hardened filter paper under vacuum. If cuprous oxide precipitates during storage, refilter the solution.

Standardized Iodine Solution Dissolve about 4.7 g of iodine in a solution of 6 g of iodate-free potassium iodide in 100 mL of water, add 3 drops of hydrochloric acid, and dilute with water to 1000 mL. Standardize to 0.0333 *N* as directed for *0.1* N *Iodine* (see *Volumetric Solutions* under *Solutions and Indicators*). Adjust the normality repeatedly, if necessary.

Standardized Sodium Thiosulfate Solution Dissolve about 8.7 g of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) and 67 mg of sodium carbonate in 1000 mL of freshly boiled and cooled water. Add 3 mL of 1.0 *N* sodium hydroxide. This solution contains 5.54 g of sodium thiosulfate. Standardize to 0.0333 *N* as directed for *0.1* N *Sodium Thiosulfate* (see *Volumetric Solutions* under *Solutions and Indicators*). Adjust the normality repeatedly, if necessary.

Starch Indicator Solution Dissolve 1 g of soluble starch in 100 mL of saturated sodium chloride solution.

Procedure Transfer about 25 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, and add 100 mL of water. Dissolve, add 10 mL of *Muller's Solution*, and mix well. Place the flask in a boiling water bath for 10 min ± 5 s. Remove the flask, place a small beaker over its neck, and cool rapidly, without agitation, under cold running water. Without agitation (to avoid the oxidation of cuprous oxide with air), acidify the solution with 5 mL of 5 *N* acetic acid, and immediately add an excess (about 20 to 40 mL) of *Standardized Iodine Solution*. Mix well, and when the precipitate is completely dissolved, titrate the excess iodine with *Standardized Sodium Thiosulfate Solution*, adding a few drops of *Starch Indicator Solution* as the endpoint is approached.

Determine a *Water Blank* as well as a *Cold Blank* by the same procedure, but for the *Cold Blank*, allow the flask containing the sample solution to stand at room temperature for 10 min rather than placing it in the boiling water bath. Calculate the percent of invert sugar by the formula

$$[(V_I - V_S - B_W - B_S - 0.2W) \times 100]/W,$$

in which V_I is the volume, in milliliters, of the *Standardized Iodine Solution*; V_S is the volume, in milliliters, of the *Standardized Sodium Thiosulfate Solution*; B_W is the volume, in milliliters, of the *Standardized Iodine Solution* in the *Water Blank*; B_S is the volume, in milliliters, of the *Standardized Iodine Solution* in the *Cold Blank*; 0.2 is a volume correction factor, in milliliters, used to correct for the reducing value of Sucrose; and W is weight, in grams, of sample used in the original test.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine based on *Loss on Drying*, Appendix IIC, drying about 5 g of sample in a forced-draft air oven at 105° for 3 h.

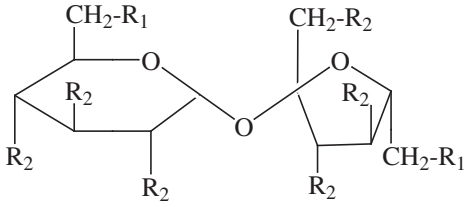
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, dissolving 26 g of sample in water, and diluting to 100 mL at 200 °Z. Use a 20-cm polarimeter tube to determine the specific rotation.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight containers in a dry place.

Sucrose Acetate Isobutyrate

SAIB



in which
R_1 = -OOCCH$_2$CH$_3$, and
R_2 = -OOCCH(CH$_3$)$_2$

$C_{40}H_{62}O_{19}$ Formula wt 846.9 (range 832–856)

INS: 444 CAS: [27216-37-1]
CAS: [123-13-6]

View IR

DESCRIPTION

Sucrose Acetate Isobutyrate occurs as a clear, pale yellow, viscous liquid. It consists of a mixture of sucrose esters of acetic and isobutyric acid, with sucrose diacetate hexaisobutyrate being the predominant sucrose ester. It is produced through the controlled esterification of sucrose with acetic anhydride and isobutyric anhydride. It is very soluble in essential oils such as orange oil, soluble in ethanol and in ethyl acetate, and very slightly soluble in water.

Function Stabilizer.

REQUIREMENTS

Identification Identify Sucrose Acetate Isobutyrate by comparing its infrared absorption spectrum with a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.8% and not more than 101.9% of $C_{40}H_{62}O_{19}$.

Acid Value Not more than 0.2.
Lead Not more than 1 mg/kg.
Saponification Value Not less than 524 and not more than 540.

TESTS

Assay Calculate the percentage of Sucrose Acetate Isobutyrate by the formula

$$[(SV \times 0.10586)/56.1] \times 100,$$

in which *SV* is the saponification value as determined below.
Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Saponification Value Determine as directed under *Saponification Value*, Appendix VII.

Packaging and Storage Store in well-closed containers.

Sucrose Fatty Acid Esters

Sucroesters

INS: 473

DESCRIPTION

Sucrose Fatty Acid Esters occur as stiff gels, soft solids, or white to slightly gray-white powders. They are the mono-, di-, and triesters of sucrose with edible fatty acids. They may be prepared from sucrose; the methyl and ethyl esters of edible fatty acids; or edible, naturally occurring vegetable oils in the presence of food-grade solvents such as ethyl acetate, methyl ethyl ketone, dimethylsulfoxide, or isobutanol. They are soluble in ethanol and, depending on the mono ester percentages, sparingly soluble in water.

Function Emulsifier; stabilizer; texturizer.

REQUIREMENTS

Identification

A. Add 1 mL of alcohol to 0.1 g of sample, dissolve by warming, add 5 mL of 2 *N* sulfuric acid, heat in a water bath for 30 min, and cool. A yellow-white solid or oil forms that is soluble in 3 mL of ether.

B. Take 2 mL of the solution separated from the solid in *Identification Test A*, and add 1 mL of anthrone TS carefully down the inside of the test tube. The boundary surface of the two layers turns to blue or green.

Assay Not less than 80.0% of the combined mono-, di-, and triesters of sucrose.

Acid Value Not more than 6.
Dimethyl Sulfoxide Not more than 2 mg/kg.
Ethyl Acetate Not more than 350 mg/kg.
Free Sucrose Not more than 5.0%.
Isobutanol Not more than 10 mg/kg.
Lead Not more than 2 mg/kg.
Methanol Not more than 10 mg/kg.
Methyl Ethyl Ketone Not more than 10 mg/kg.
Residue on Ignition Not more than 2.0%.

TESTS

Assay

Mobile Phase Use HPLC-grade degassed tetrahydrofuran.

Assay Preparation Transfer about 250 mg of sample, accurately weighed, into a 50-mL volumetric flask. Dilute to volume with *Mobile Phase*, and mix. Filter through a 0.5-μm membrane filter.

Procedure (See *Chromatography*, Appendix IIA.) Inject 100 μL of the *Assay Preparation* into a prestabilized high-performance liquid chromatograph equipped with a styrene–divinylbenzene copolymer column for gel permeation chromatography (TSK-GEL G2000 from Supelco, Inc., or equivalent) and a refractive index detector, both maintained at 38°. The flow rate of the *Mobile Phase* is about 0.7 mL/min. Record the chromatogram for about 90 min. Calculate the percent of sucrose ester content in the sample taken by the formula

$$100A/T,$$

in which *A* is the sum of the peak areas for the three main components, the mono-, di-, and triesters, eluting at about 65, 68, and 73 min, respectively, and *T* is the sum of all peak areas eluting in 90 min.

Acid Value Determine as directed in *Method I* under *Acid Value*, Appendix VII.

Dimethyl Sulfoxide

Standard Solutions Prepare a *Dimethyl Sulfoxide Standard Stock Solution* in tetrahydrofuran containing 0.25 mg/mL. Dilute the *Dimethyl Sulfoxide Standard Stock Solution* quantitatively and stepwise to obtain *Standard Solutions* containing 0.005, 0.001, and 0.0005 mg of dimethyl sulfoxide in each milliliter.

Sample Preparation Transfer 5 g of sample, accurately weighed, into a 25-mL volumetric flask, dilute with tetrahydrofuran to volume, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame photometric detector with a 394-nm sulfur filter and containing a 2-m × 3-mm (id) glass column packed with a 10% PEG 20M and 3% potassium hydroxide on Gas Chrom Z, or equivalent. Condition the column before use by raising the column temperature to 180° at a rate of 1°/min, and letting it stand for 24 to 48 h with nitrogen flowing at 30 to 40 mL/min. Maintain the column temperature at 160° and the injection port at 210°. Use nitrogen as the carrier gas at a flow rate of 50 mL/min.

Procedure Inject 3 µL of each of the *Standard Solutions* into the gas chromatograph. Prepare a calibration curve by plotting the concentration, C_S, in milligrams per milliliter, of each *Standard Solution* versus its peak response, and draw the best straight line. Similarly inject 3 µL of the *Sample Preparation*. Record the peak response for dimethyl sulfoxide for the *Sample Preparation*, and determine its concentration from the standard curve. Calculate the concentration of dimethyl sulfoxide, in milligrams per kilogram, in the sample taken by the formula

$$25000C/W,$$

in which C is the concentration, in milligrams per milliliter of dimethyl sulfoxide obtained from the standard curve; and W is the weight, in grams, of the sample taken.

Ethyl Acetate, Isobutanol, Methanol, and Methyl Ethyl Ketone

Standard Solutions Transfer 1 g each of ethyl acetate, isobutanol, methanol, and methyl ethyl ketone into a 100-mL volumetric flask, dilute to volume with water, and mix. Dilute this solution quantitatively and stepwise to obtain three *Standard Solutions* covering the range of 0.2 to 4 mg/mL.

Sample Preparation Transfer 1 g of powdered sample, accurately weighed, into a sample vial, add 5 µL of water, and seal it immediately with a septum.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph with a headspace sampler, equipped with a flame ionization detector, and containing a 30-m × 0.53-mm (id) silica capillary column coated with 100% methylpolysiloxane (brand name, or equivalent) and preconditioned by heating it to 60° for 2 to 3 h with nitrogen flowing at approximately 10 mL/min. Maintain the temperature of the injection port at 110°, the column at 40°, and the detector at 110°.

Procedure Maintain the headspace sampler at 80° with the syringe temperature at 85°. Prepare a *Calibration Curve* by adding 5 µL of each *Standard Solution* to 1 g of sample, accurately weighed, in separate sample vials. Immediately seal each vial with a septum, and place them in the headspace sampler. Heat each vial at 80° for 40 min, and introduce 400 µL of the headspace volume from each into the gas chromatograph, with nitrogen flowing at 5 mL/min. Draw a *Calibration Curve* for each solvent by plotting the concentration, C_S, in milligrams per milliliter, of each solvent versus its corresponding detector response, r_S. Similarly place the vial containing the *Sample Preparation* in the headspace sampler, proceed as directed above for the *Standard Solutions*, and measure the detector response, r_U, for each solvent at retention times equivalent to those of the standards. Using the detector responses of the *Sample Preparation* and the *Calibration Curve*, calculate the concentration, in milligrams per kilogram, of each solvent in the sample by the formula

$$1000C_S,$$

in which C_S is the concentration, in milligrams per kilogram, of each solvent from its respective *Calibration Curve*.

Free Sucrose

Mobile Phase Use a degassed 85:15 mixture of acetonitrile and water.

Standard Solution Prepare an aqueous solution having known concentrations of 10 mg/mL of sucrose (reference standard material is available from NIST) and 10 mg/mL of maltose (available from Sigma Chemical Co.).

Assay Preparation Transfer 5 g of sample, accurately weighed, into a 100-mL separatory funnel. Add 1.000 g of maltose and 50 mL of a warm 1:2:1 mixture of chloroform:methanol:water, and shake gently to dissolve. Add 10 mL of chloroform, and shake, then add 10 mL of water and 0.2 mL of 2 *N* hydrochloric acid, and shake. Allow the layers to separate, then discard the bottom layer that contains fatty acids and sucrose esters. Quantitatively transfer the upper aqueous layer into a 100-mL volumetric flask that contains 0.3 mL of 2.5% ammonium hydroxide. Wash the separatory funnel with *Mobile Phase*, adding the washings to the volumetric flask. Dilute to volume with the *Mobile Phase*, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a high-performance liquid chromatograph equipped with a 4.6- × 250-mm aminopropyl column (5 µm Spherisorb NH_2, or equivalent) and a refractive index detector and operated at a flow rate of about 1.3 mL/min.

Procedure Introduce equal volumes (20 µL) of the *Standard Solution* and *Assay Preparation* into the chromatograph, and record the chromatograms. Note the retention times of the two major peaks exhibited by the *Standard Solution*, and calculate the ratio of the response of sucrose to that of the internal standard maltose. Calculate the quantity, in milligrams, of sucrose in the portion of sample taken by the formula

$$100C(R_U/R_S),$$

in which C is the concentration, in milligrams per milliliter, of sucrose in the *Standard Preparation*, and R_U and R_S are the ratios of the peak responses for sucrose and maltose obtained from the *Assay Preparation* and the *Standard Preparation*, respectively.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, using a 1-g sample.

Packaging and Storage Store in tight containers, and avoid high temperatures.

Sugar Beet Fiber

Beet Fiber; Dietary Fiber from Beets; Sugar Beet Pulp

DESCRIPTION

Sugar Beet Fiber occurs in various grades, from coarse fibrous flakes to fine, free-flowing powders. It is the natural, light-brown colored fiber of sugar beets remaining after water extraction of the sugar from the mechanically sliced sugar beets.

Function Anticaking agent; binding agent; bulking agent; dispersing agent; source of dietary fiber; stabilizing agent; texturizing agent; thickening agent.

REQUIREMENTS

Identification

A. Boil 10 g of sample with 90 mL of water for 5 min, filter while hot through No. 616 filter paper, and add 5 drops of iodine TS to the filtrate. No change in color from the yellow-red to red-yellow is produced, indicating the absence of starch.

B. Add 95 mL of water to 5 g of sample, and mix (grind coarse grades so a major portion will pass through a 60-mesh screen). Heat to boiling, and filter while hot. Add 1 mL of 1 *N* sodium hydroxide to 5 mL of cooled filtrate, mix, and allow it to stand at room temperature for 15 min. The formation of a thick, yellow gel indicates the presence of soluble fiber, which dissolves upon the addition of a few drops of 6 *N* hydrochloric acid.

C. Mix 1.25 g of α-naphthol with 25 mL of methanol; add a few drops of this solution to a test tube containing 5 mL of cooled filtrate from *Identification Test B*, and mix. Carefully layer sulfuric acid down the side of the test tube. A purple color change occurs at the interface, indicating the presence of residual sucrose.

Assay Not less than 70.0% total fiber, and not less than 20.0% soluble fiber, calculated on the dried basis.

Ash (Total) Not more than 6.0%.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 10.0%.

pH of a 10% Dispersion Between 4.0 and 5.0.

TESTS

Assay (Taken from the *Official Methods of Analysis*, 16th edition, chapter 32, pp. 7–9, method number 991.43. 1995. AOAC INTERNATIONAL.) (**Note**: Verify that all enzymes used in this procedure exhibit not less than 95% of their declared potencies using the tests given in *Enzyme Assays*, Appendix V.)

Mixed 8.2 Buffer Solution Mix equal volumes of 0.1 *M* 2-(*N*-morpholino)ethanesulfonic acid (MES) and 0.1 *M* tris-(hydroxymethyl)aminomethane (TRIS), and carefully adjust the pH to 8.2 at 24°, using 6 *N* sodium hydroxide as necessary. If the buffer temperature differs from 24°, adjust the pH by interpolation from a high of 8.3 at 20° to a low of 8.1 at 28°.

Protease Solution Use a freshly prepared solution containing 50 mg of protease (Sigma Chemical Co. catalog number P 3910, or equivalent) per milliliter of *Mixed 8.2 Buffer Solution*.

Filtering Crucible Prepare six 60-mL filtering crucibles, each with a coarse fritted disk (Corning No. 36060-60C Pyrex Büchner funnel, 40- to 60-μm pore size, or equivalent), as follows: Ignite the crucibles overnight at 525° in a muffle furnace. Allow the temperature to fall below 130° before removing them from the furnace. Treat each crucible as follows: Soak it for 1 h at room temperature in a 2% cleaning solution containing a liquid surfactant-type laboratory cleaner. At the end of the hour, rinse the crucible with water, deionized water, and 15 mL of acetone. Allow to air dry. Add about 1 g of diatomaceous earth (Celite 545 AW, or equivalent), packing it down firmly. Dry at 130° to constant weight. Cool the crucible for about 1 h in a desiccator, then weigh accurately.

Sample Preparation Transfer 1 g of sample, accurately weighed, into a 400-mL beaker, and add 40 mL of *Mixed 8.2 Buffer Solution*. Prepare four samples (two sets of duplicates). Use the two sets to prepare digested samples for the *Determination of Total Fiber* and for the *Determination of Soluble Fiber*.

Procedure (Use two duplicate samples, with a blank for each sample.) Treat each beaker as follows: Stir the *Sample Preparation* magnetically until the sample is totally dispersed. Add 50 μL of heat-stable α-amylase solution (Sigma Chemical Co. catalog number A 3306, or equivalent) to the beaker. Cover the beaker with aluminum foil, place it on a water bath, and while stirring, incubate at 95° to 100° for 15 min. (Start the timing when the temperature reaches 95°.) Remove the beaker from the bath, and cool to 60°. Uncover the beaker. With a spatula, scrape any ring on the inside wall of the beaker, and disperse any gels formed at the bottom of the beaker. Rinse the beaker walls and spatula with 10 mL of water.

Add 100 μL of *Protease Solution* to the beaker, cover with aluminum foil, and while continuously stirring, incubate the mixture at 60° for 30 min. (Start the timing when the temperature of the solution reaches 60°.) Uncover the beaker, continue stirring, and immediately add 5 mL of 0.5 *N* hydrochloric acid. While maintaining the temperature at 60°, adjust the pH to between 4.0 and 4.7 using either 1 *N* hydrochloric acid or sodium hydroxide.

> **Note**: Because the pH is temperature dependent, check and adjust the pH when the solutions are at 60°.

Next, while stirring, add 300 μL of amyloglucosidase solution (Sigma Chemical Co., catalog number AMG A 9913, or equivalent). Cover, and incubate the mixture at 60° for 30 min with constant agitation. (Start the timing when the mixture reaches 60°.)

Determination of Total Fiber Add 225 mL of 78% ethanol to each of the first set of duplicate samples and blanks, still maintained at 60°. Remove them from the bath, cover, and allow to stand at room temperature for 1 h for complete precipitation.

Wet and redistribute the Celite bed in a previously prepared and tared filtering crucible, using 15 mL of 78% ethanol to wash the sides of the crucible. Apply suction to draw the Celite onto the fritted disk as an even mat. Filter an alcohol-treated sample though the crucible, quantitatively transferring all particles to the crucible with the help of a spatula and a wash bottle containing 78% ethanol. If a gum forms, break the film with the spatula.

Wash the residue in the filtering crucible successively with two 15-mL portions of 78% ethanol, 95% ethanol, and acetone, applying a vacuum after each wash. Dry the crucible and its contents at 105° to constant weight.

Use the residue from one sample to determine the nitrogen content as directed under *Nitrogen Determination*, Appendix

IIIC. The weight of nitrogen determined, multiplied by 6.25, gives the weight of protein. Use the second sample of residue for the ash determination [see *Ash* (Total), below]. The weight of the residue, corrected for the blank, protein, and ash, is equal to the weight of the total fiber (see *Calculations*, below).

Determination of Soluble Fiber Using about 3 mL of water, wet and redistribute the Celite bed in a filtering crucible. Apply suction to draw the Celite into an even mat. Treat the second set of duplicate samples and blanks in the following manner: filter through the filtering crucible, collecting the filtrate and subsequent washings. Using water maintained at 70°, rinse the beaker, and wash the residue with two 10-mL portions of the hot water. Discard the residue and transfer the combined filtrate and all washings to a tared 600-mL beaker. Weigh the beaker to obtain an estimate of the volume of the contents. Add four volumes of 95% ethanol maintained at 60°, and allow to stand at room temperature for 1 h for complete precipitation.

Proceed as described above under *Determination of Total Fiber*, beginning with ''Wet and redistribute the Celite bed. . . .'' The weight of this second residue, corrected for the blank, protein, and ash, is equal to the weight of soluble fiber (see *Calculations*, below).

Calculations Determine the blank, B, in milligrams, using the following equation:

$$B = [(BR_1 + BR_2)/2] - P_B - A_B,$$

in which BR_1 and BR_2 are the residue weights, in milligrams, of the duplicate blank determinations; and P_B and A_B are the weights, in milligrams, of protein and ash, respectively, determined in the first and second blank residues.

Determine the percent of fiber (F), which applies to both total fiber and soluble fiber, using the following equation:

$$F = 100\{[(R_1 + R_2)/2] - P - A - B\}/[(M_1 + M_2)/2],$$

in which R_1 and R_2 are the residue weights, in milligrams, for duplicate samples; P and A are the weights, in milligrams, of protein and ash, respectively, determined on the first and second residues; B is the weight, in milligrams, of the blank; and M_1 and M_2 are the weights, in milligrams, of the samples corrected for *Loss on Drying*.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC, using the residue obtained from one of the digested samples obtained under *Determination of Total Fiber* in the *Assay*.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 5 h.

pH of a 10% Dispersion Determine as directed under *pH Determination*, Appendix IIB, using a dispersion prepared by mixing 10 g of sample with 90 mL of water and allowing it to stand at room temperature for 2 h.

Packaging and Storage Store in well-closed containers.

Sulfur Dioxide

SO_2 Formula wt 64.06

INS: 220 CAS: [7446-09-5]

DESCRIPTION

Sulfur Dioxide occurs as a colorless, nonflammable gas under normal conditions of temperature and pressure. It is shipped as a liquid under pressure in containers approved by the U.S. Department of Transportation. Its vapor density is 2.26 times that of air at atmospheric pressure and 0°. The specific gravity of the liquid is about 1.436 at 0°/4°. At 20° the solubility is about 10 g of Sulfur Dioxide per 100 g of solution.

> **Caution**: Sulfur dioxide gas is intensely irritating to the eyes, throat, and upper respiratory system. Liquid sulfur dioxide may cause skin burns, which result from the freezing effect of the liquid on tissue. Safety precautions to be observed in handling the material are specified in ''Pamphlet G-3'' published by the Compressed Gas Association, 4221 Walney Road, Fifth Floor, Chantilly, VA 20151-2923.

Function Antioxidant; bleaching agent; preservative.

REQUIREMENTS

Identification A saturated solution of sample in water gives positive tests for *Sulfite*, Appendix IIIA.
Assay Not less than 99.9% of SO_2 by weight.
Lead Not more than 2 mg/kg by weight.
Nonvolatile Residue Not more than 0.05% by weight.
Selenium Not more than 0.002% by weight.
Water Not more than 0.05% by weight.

TESTS

Sampling Samples of sulfur dioxide may be safely withdrawn from a tank or from transfer lines, either of which should be equipped with a 3/8-in. nozzle and valve. Samples should be placed in sample cylinders constructed of 316 stainless steel, designed to withstand 1000 psig and equipped with 316 stainless-steel needle valves on both ends.

To draw a sample, flush the sample cylinder with dry air to remove any sulfur dioxide remaining from previous sample drawings, and attach it to the tank or transfer lines with a solid pipe connection. Connect a hose to the other end of the sample cylinder, and submerge it in either a weak caustic solution or water. Discharge any gas in the sample cylinder into the caustic solution or water by first opening the valve at the pipe end, followed by slowly opening the valve at the hose end. When all of the gas is dispelled and liquid sulfur dioxide begins to emerge into the solution, block off the valve at the hose end. Tightly close the other valves, and detach the sample cylinder from the pipe connecting it to the tank or transfer line. Discharge approximately 15% of the liquid sulfur dioxide from the sample cylinder into the water or

caustic solution. Cap the sample cylinder at its end, and transfer it to the laboratory for analysis.

> **Caution**: Never store a sample cylinder containing an amount of gas equivalent to more than 85% of the total water capacity of the sample cylinder.

Assay Subtract from 100 the percentages of *Nonvolatile Residue* and of *Water*, as determined below, to obtain the percentage of SO_2.

> **Sample Solution for the Determination of Lead and Selenium** Transfer 100 mL (144 g) of sample into a 125-mL Erlenmeyer flask, and determine the weight of sample taken by the loss in weight of the sample cylinder. Evaporate to dryness on a steam bath, add 3 mL of nitric acid and 10 mL of water to the dry flask, and warm gently on a hot plate for 15 min. Transfer the contents of the flask into a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer a 10.0-mL aliquot into a second 100-mL volumetric flask, dilute to volume with water, and mix.
>
> **Note**: The tests in which this solution is to be used will be accurate assuming a 144-g sample has been taken; if not, the weight of sample actually taken must be considered in the calculations.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 7.0-mL portion of the *Sample Solution* diluted to 40 mL with water, and 2 μg of lead (Pb) ion in the control.
Nonvolatile Residue Transfer 200 mL (288 g) of sample into a 250-mL Erlenmeyer flask, and determine the weight of sample taken by the loss in weight of the sample cylinder. Evaporate to dryness on a steam bath, and displace the residual vapors with dry air. Wipe the flask dry, cool in a desiccator, and weigh.
Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 2.0-mL portion of the *Sample Solution*.
Water Transfer about 50 mL of liquid sulfur dioxide into a Karl Fischer titration jar, determine the weight of sample taken, and determine the water content as directed in the *Karl Fischer Titrimetric Method* under *Water Determination*, Appendix IIB.

Packaging and Storage Store in suitable pressure containers, observing applicable federal regulations pertaining to shipping containers.

Sulfuric Acid

H_2SO_4 Formula wt 98.07

INS: 513 CAS: [7664-93-9]

DESCRIPTION

Sulfuric Acid occurs as a clear, colorless or slightly brown, oily liquid. It is very caustic and corrosive. It is miscible with water and with alcohol with the generation of much heat and contraction in volume. Sulfuric Acid should be added cautiously to the diluent when mixed with other liquids. Some commercially available concentrations of Sulfuric Acid are expressed in degrees Baumé (°Bé) and others (above 93.0%) as a percentage of H_2SO_4. The more common concentrations are 60 °Bé and 66 °Bé, equivalent to 77.67% and 93.19% of H_2SO_4, respectively, and 98.0% of H_2SO_4. Its specific gravity varies with the concentration of H_2SO_4 (see *Sulfuric Acid Table*, Appendix IIC).

Function Acidifier.

REQUIREMENTS

Identification A sample gives positive tests for *Sulfate*, Appendix IIIA.
Assay Not less than the minimum or within the range of °Bé or the percentage of H_2SO_4 claimed or implied by the vendor.
Arsenic Not more than 3 mg/kg.
Chloride Not more than 0.005%.
Iron Not more than 0.02%.
Lead Not more than 5 mg/kg.
Nitrate Not more than 10 mg/kg.
Reducing Substances (as SO_2) Passes test.
Selenium Not more than 0.002%.

TESTS

Assay Transfer 1 mL of sample into a small, tared glass-stoppered Erlenmeyer flask; insert the stopper; accurately weigh; and cautiously add about 30 mL of water. Cool the mixture, add methyl orange TS, and titrate with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sodium hydroxide is equivalent to 49.04 mg of H_2SO_4.

For samples with concentrations below 93.0%, expressed in degrees Baumé, transfer about 200 mL of sample, previously cooled to a temperature below 15°, into a 250-mL hydrometer cylinder. Insert a suitable Baumé hydrometer graduated at 0.1 °Bé intervals, adjust the temperature of the sample to exactly 15.6°, and note the reading at the bottom of the meniscus, estimating it to the nearest 0.05 °Bé. Determine the equivalent percentage of H_2SO_4 from the *Sulfuric Acid Table*, Appendix IIC.
Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a solution of 1 g of sample in 35 mL of water, and a mixture of 3 mL of *Standard Arsenic Solution* (3 μg As) and 1 g of ACS Reagent-Grade Sulfuric Acid as the control.
Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, using the following as the sample: Transfer a volume of sample equivalent to 5 g of Sulfuric Acid into about 25 mL of water contained in a 50-mL volumetric flask, cool, and dilute to volume with water. (Retain the unused portion for the *Iron* and *Lead* tests.) Any turbidity produced by 4 mL of this solution (400-mg sample) does not exceed that shown in a control containing 20 μg of chloride (Cl) ion.

Iron Dilute 1 mL of the solution (100-mg sample) prepared for the *Chloride* test (above) to 40 mL with water. Add about 30 mg of ammonium persulfate crystals and 10 mL of ammonium thiocyanate TS. Any resulting red color does not exceed in intensity that produced by 2.0 mL of *Iron Standard Solution* (20 μg Fe) (see *Solutions and Indicators*) in an equal volume of a solution containing the same quantities of the reagents used in the test.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 10 mL of the solution (1-g sample) prepared for the *Chloride* test (above) diluted to 40 mL with water, and 5 μg of lead (Pb) ion in the control.

Nitrate

Standard Nitrate Solution Transfer 8.022 g of potassium nitrate (KNO_3), previously dried at 105° for 1 h, into a 500-mL volumetric flask, dissolve in and dilute to volume with water, and mix well. Slowly add 5.0 mL of this solution from a buret into 400 mL of ACS Reagent-Grade Sulfuric Acid, previously cooled to 5°, keeping the tip of the buret below the surface of the acid. After the solution has reached room temperature, transfer it into a 500-mL volumetric flask, and dilute to volume with ACS Reagent-Grade Sulfuric Acid. Each milliliter contains 100 μg of nitric acid (HNO_3).

Procedure Transfer 50 mL of ACS Reagent-Grade Sulfuric Acid into each of two 100-mL Nessler tubes, slowly add 5 mL of a freshly prepared 1:10 solution of ferrous sulfate ($FeSO_4 \cdot 7H_2O$), mix with a glass rod, and cool in an ice bath to between 10° and 15°. Add 10 mL of sample, previously cooled to between 10° and 15°, to one tube of the cooled mixture, and dilute to 100 mL with ACS Reagent-Grade Sulfuric Acid chilled to about the same temperature. Add the *Standard Nitrate Solution*, dropwise, from a microburet into the second (control) tube, mixing frequently, until the color of the control solution nearly matches that of the *Sample Solution*. Dilute the control solution to 100 mL with ACS Reagent-Grade sulfuric acid, and continue adding *Standard Nitrate Solution* to as exact a match in color intensity as possible when compared with the *Sample Solution* by looking down through the solutions against a white background illuminated by diffused light. Calculate the weight of Sulfuric Acid in the sample from the specific gravity and the volume taken (see *Sulfuric Acid Table*, Appendix IIC). Not more than 0.1 mL of the *Standard Nitrate Solution* is required for each gram of Sulfuric Acid.

Reducing Substances (as SO_2) Carefully dilute 8 g of sample with about 50 mL of ice-cold water, keeping the solution cool during the addition. Add 0.1 mL of 0.1 *N* potassium permanganate. The solution remains pink for not less than 5 min.

Selenium Determine as directed in *Method II* under *Selenium Limit Test*, Appendix IIIB, using a 300-mg sample. The absorbance of the extract from the *Sample Preparation* is not greater than that from the *Standard Preparation*.

Packaging and Storage Store in tight containers.

Sunflower Oil (Unhydrogenated)

CAS: [8008-31-9]

DESCRIPTION

Sunflower Oil (Unhydrogenated) occurs as a light amber colored oil. It is obtained from the seed of the sunflower plant *Helianthus annuus* (Fam. Asteraceae) by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21° to 27°, but traces of wax may cause the oil to cloud, unless removed by winterization.

Function Coating agent; texturizer.

REQUIREMENTS

Identification Unhydrogenated Sunflower Oil exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range)	<0.1	<0.5	3.0–10	<1.0	1.0–10	14–65	20–75
Fatty Acid:	18:3	20:0	20:1	22:0	22:1	24:0	
Weight % (Range)	<0.5	<1.0	<0.5	<1.0	<0.1	<0.4	

Cold Test Passes test.
Color (AOCS-Wesson) Not more than 1.3 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 110 and 143.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 1.5%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Cold Test Determine as directed under *Cold Test*, Appendix VII.
Color (AOCS-Wesson) Determine as directed under *Color (AOCS-Wesson)*, Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Linolenic Acid Determine as directed under *Fatty Acid Composition*, Appendix VII.
Peroxide Value Determine as directed under *Peroxide Value*, Appendix VII.

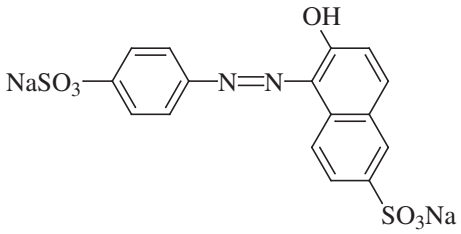
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.

Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Sunset Yellow[1]

Sunset Yellow FCF; CI 15985; Class: Monoazo



$C_{16}H_{10}N_2O_7S_2Na_2$ Formula wt 452.38

INS: 110 CAS: [2783-94-0]

DESCRIPTION

Sunset Yellow occurs as a brown-orange powder or granules. It is principally the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid. The trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonicacid may be added in small amounts. It dissolves in water to give a solution yellow-orange at neutrality or in acid and red-brown in base. When dissolved in concentrated sulfuric acid, it yields an orange solution that turns yellow when diluted with water. It is insoluble in ethanol.

Function Color.

REQUIREMENTS

Identification An aqueous solution (*A*) containing 18.5 mg of sample per liter exhibits absorbance intensities and wavelength maxima as follows: at pH 1, $A = 1.1$ at 480 nm; and at pH 13, $A = 0.46$ at 443 nm, with a shoulder at about 500 nm.

Assay Not less than 87.0% total coloring matters.

[1]To be used or sold for use to color food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Yellow No. 6 can be applied only to FDA-certified batches of this color additive. Sunset Yellow is a common name given to the uncertified colorant. See the monograph entitled FD&C Yellow No. 6 for directions for producing an FDA-certified batch.

Arsenic Not more than 3 mg/kg.

Ether Extracts (combined) Not more than 0.2%.

Lead Not more than 10 mg/kg.

Loss on Drying (Volatile Matter) at 135°, Chlorides, and **Sulfates** (as sodium salts) Not more than 13.0% in combination.

Mercury Not more than 1 mg/kg.

Uncombined Intermediates and Products of Side Reactions

4-Aminoazobenzene Not more than 0.05 mg/kg.

4-Aminobiphenyl Not more than 0.015 mg/kg.

Aniline Not more than 0.25 mg/kg.

Azobenzene Not more than 0.2 mg/kg.

Benzidine Not more than 0.001 mg/kg.

1,3-Diphenyltriazene Not more than 0.04 mg/kg.

1-(Phenylazo)-2-naphthalenol Not more than 10 mg/kg.

Sodium Salt of 4-Aminobenzenesulfonic Acid Not more than 0.2%.

Sodium Salt of 6-Hydroxy-2-naphthalenesulfonic Acid Not more than 0.3%.

Disodium Salt of 6,6′-Oxybis[2-naphthalenesulfonic Acid] Not more than 1%.

Disodium Salt of 4,4′-(1-Triazene-1,3-diyl)bis[benzenesulfonic Acid] Not more than 0.1%.

Sum of the Sodium Salt of 6-Hydroxy-5-(phenylazo)-2-naphthalenesulfonic Acid and the Sodium Salt of 4-[(2-Hydroxy-1-naphthylenyl)azo]benzenesulfonic Acid Not more than 1%.

Sum of the Trisodium Salt of 3-Hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonic Acid and Other, Higher Sulfonated Subsidiaries Not more than 5%.

Water-Insoluble Matter Not more than 0.2%.

TESTS

Assay Determine the total color strength as the weight percent of the sample using *Methods I* and *II* in *Total Color* under *Colors*, Appendix IIIC. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 200 to 225 mg of sample, accurately weighed, into a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (*a*) for Sunset Yellow is 0.054 mg/L/cm at 484 nm.

Method II (Sample Preparation) Transfer approximately 0.2 g of sample, accurately weighed, into the titration flask. The stoichiometric factor (F_s) for Sunset Yellow is 8.84.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Chloride Determine as directed in *Sodium Chloride* under *Colors*, Appendix IIIC.

Ether Extracts Determine as directed in *Ether Extracts* under *Colors*, Appendix IIIC.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 μg of lead (Pb) ion in the control.

Loss on Drying (Volatile Matter) at 135° Determine as directed in *Loss on Drying (Volatile Matter)* under *Colors*, Appendix IIIC.

Mercury Determine as directed in *Mercury* under *Colors*, Appendix IIIC.
Sulfates Determine as directed in *Sodium Sulfate* under *Colors*, Appendix IIIC.
Uncombined Intermediates and Products of Side Reactions Determine as directed for *Method II* in *Uncombined Intermediates and Products of Side Reactions* under *Colors*, Appendix IIIC, except inject 20 µL of the following *Sample Preparation*: Transfer 0.25 g of sample, accurately weighed, into a 100-mL volumetric flask. Dissolve in and dilute to volume with 0.1 *M* disodium tetraborate ($Na_2B_4O_7$).
Water-Insoluble Matter Determine as directed in *Water-Insoluble Matter* under *Colors*, Appendix IIIC.

Packaging and Storage Store in well-closed containers.

Talc

INS: 553(iii) CAS: [14807-96-6]

DESCRIPTION

Talc occurs as a white to gray-white, unctuous powder. It is a naturally occurring form of hydrous magnesium silicate containing varying proportions of such associated minerals as alpha-quartz, calcite, chlorite, dolomite, kaolin, magnesite, and phlogopite. *Talc derived from deposits that are known to contain associated asbestos is not food grade*. It is insoluble in water and in solutions of alkali hydroxides, but is slightly soluble in dilute mineral acids.

Function Anticaking agent; coating agent; lubricating and release agent; surface-finishing agent; texturizing agent.

REQUIREMENTS

Identification

A. The X-ray diffraction pattern of a random powder sample exhibits intense reflections at the following *d* values: 9.34 Å, 4.66 Å, and 3.12 Å.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits major peaks at approximately 1015 cm^{-1} and 450 cm^{-1}.

Acid-Soluble Substances (as SO_4) Not more than 2.5%.
Arsenic Not more than 3 mg/kg.
Free Alkali (as NaOH) Not more than 1%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.5%.
Loss on Ignition Not more than 6.0%.
Soluble Salts Not more than 0.2%.

TESTS

Acid-Soluble Substances (as SO_4) Digest 1.00 g of sample with 20 mL of 3 *N* hydrochloric acid at 50° for 15 min, add water to restore the original volume, mix, and filter. Add 1 mL of 2 *N* sulfuric acid to 10 mL of the filtrate, evaporate to dryness, and ignite to constant weight. The weight of the residue does not exceed 12.5 mg.

> **Sample Solution for the Determination of Arsenic and Lead** Transfer 10.0 g of sample into a 250-mL flask, and add 50 mL of 0.5 *N* hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 3 filter paper, or equivalent, into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a 10-mL portion of the *Sample Solution* (above).
Free Alkali (as NaOH) Add 2 drops of phenolphthalein TS to 20 mL of the diluted filtrate prepared in the test for *Soluble Salts* (below), representing 1 g of sample. If a pink color appears, not more than 2.5 mL of 0.1 *N* hydrochloric acid is required to discharge it.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 10-mL portion of the *Sample Solution* (above), and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 10-g sample at 105° for 1 h.
Loss on Ignition Accurately weigh about 1 g of sample in a tared platinum crucible provided with a cover, initially apply heat gradually, and then ignite to constant weight.
Soluble Salts Boil 10 g of sample with 150 mL of water for 15 min. Cool to room temperature, and add water to restore the original volume. Allow the mixture to stand for 15 min, and filter until clear. Add 25 mL of water to 75 mL of the clear filtrate. Evaporate 50 mL of this solution, representing 2.5 g of sample, in a tared platinum dish on a steam bath to dryness, and ignite gently to constant weight. The weight of the residue does not exceed 5 mg.

Packaging and Storage Store in well-closed containers.

Tallow

DESCRIPTION

Tallow occurs as an off white fat. It is obtained by heat rendering of tissues (cuttings and trimmings) from beef and, to a lesser degree, mutton shortly after slaughter. Rendered Tallow may be alkali refined and bleached, or bleached and deodorized without prior refining. It is a firm fat containing

a high proportion of saturated fatty acids and exhibiting greater flavor stability than lard or unhydrogenated vegetable oils.

Rendered, alkali-refined, and bleached-deodorized Tallows are white to off white solids at 21° to 27°. Alkali-refined and bleached-deodorized Tallows, which are pale yellow to colorless and free of extraneous matter at 54°, differ from rendered Tallow, which is clear to hazy and may contain extraneous matter.

Function Coating agent; texturizer.

REQUIREMENTS

Identification Tallow exhibits the following composition profile of fatty acids determined as directed under *Fatty Acid Composition*, Appendix VII.

Fatty Acid:	<14:0	14:0	14:1	15:0	15:0 iso	16:0
Weight % (Range):	<0.1	1.4–6.3	0.5–1.5	0.5–1.0	<1.5	20–37
Fatty Acid:	16:0 iso	16:1	16:2	17:0	17:1	18:0
Weight % (Range):	<0.5	0.7–8.8	<1.0	0.5–2.0	<1.0	6–40
Fatty Acid:	18:1	18:2	18:3	20:0	20:1	20:4
Weight % (Range):	26–50	0.5–5.0	<2.5	<0.5	<0.5	<0.5

	Rendered Tallow	Alkali-Refined Tallow	Bleached and Deodorized Tallow
Color (AOCS-Wesson)	Not more than 3.0 red	Not more than 1.5 red	Not more than 1.5 red
Free Fatty Acids (as oleic acid)	Not more than 1.5%	Not more than 0.5%	Not more than 0.1%
Hexane-Insoluble Matter	Not more than 0.1%	Not more than 0.01%	Not more than 0.01%
Iodine Value	Between 37 and 50	Between 37 and 50	Between 37 and 50
Unsaponifiable Matter	Not more than 1.5%	Not more than 1.5%	Not more than 1.5%
Water	Not more than 0.5%	Not more than 0.2%	Not more than 0.1%

Arsenic Not more than 0.5 mg/kg.
Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.

TESTS

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared with 2 g of sample, accurately weighed. The absorbance caused by any red color from the solution of the sample does not exceed that produced by 1.0 mL of *Standard Arsenic Solution* (1 µg As) when treated in the same manner and under the same conditions as the sample.
Color (AOCS Wesson) Determine as directed under *Color* (AOCS-Wesson), Appendix VII.
Free Fatty Acids (as oleic acid) Determine as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Hexane-Insoluble Matter If the sample is plastic or semi-solid, soften a portion by warming it at a temperature not exceeding 60°, and then mix it thoroughly. Transfer 100 g of well-mixed sample into a 1500-mL wide-mouth Erlenmeyer flask, add 1000 mL of hexane, and shake until the sample is dissolved. Filter the resulting solution through a 600-mL Corning ''C'' porosity, or equivalent, filtering funnel that previously has been dried at 105° for 1 h, cooled in a desiccator, and weighed. Wash the flask with two successive 250-mL portions of hexane, and pass the washings through the filter. Dry the funnel at 105° for 1 h, cool to room temperature in a desiccator, and weigh. From the gain in weight of the funnel, calculate the percentage of the hexane-insoluble matter in the sample.
Iodine Value Determine as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 3-g sample.
Peroxide Value Accurately weigh about 10 g of sample, add 30 mL of a 3:2 glacial acetic acid:chloroform mixture, and mix. Add 1 mL of a saturated solution of potassium iodide, mix for 1 min, add 100 mL of water, and begin titrating with 0.05 *N* sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value, as milliequivalents of peroxide per kilogram of sample, by the formula

$$[S \times N \times 1000]/W,$$

in which S is the net volume, in milliliters, of sodium thiosulfate solution required for the sample; N is the exact normality of the sodium thiosulfate solution; and W is the weight, in grams, of the sample taken.
Unsaponifiable Matter Determine as directed under *Unsaponifiable Matter*, Appendix VII.
Water Determine as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Tangerine Oil, Coldpressed

Tangerine Oil, Expressed

CAS: [8008-31-9]

FEMA: 3041

View IR

DESCRIPTION

Tangerine Oil, Coldpressed, occurs as a red-orange to brown-orange liquid with a pleasant, orange odor. Oils produced from the unripe fruit are often green. It is the oil obtained by

expression from the peels of the ripe fruit of the Dancy tangerine, *Citrus nobilis* or *reticulata* (Fam. Rutaceae), and from some other closely related varieties. It is soluble in most fixed oils and in mineral oil, slightly soluble in propylene glycol, and relatively insoluble in glycerin. It may contain a suitable antioxidant.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Aldehydes Between 0.8% and 1.9% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{22}O$).
Angular Rotation Between +88° and +96°.
Refractive Index Between 1.473 and 1.476 at 20°.
Residue on Evaporation Between 2.3% and 5.8%.
Specific Gravity Between 0.844 and 0.854.

TESTS

Aldehydes Determine as directed in the *Hydroxylamine/Tert-Butyl Alcohol Method* under *Aldehydes and Ketones*, Appendix VI, using about 10 g of sample, accurately weighed, and 78.13 as the equivalence factor (*e*) in the calculation. Allow the samples and the blank to stand at room temperature for 30 min after adding the hydroxylamine hydrochloride solution.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Residue on Evaporation Determine as directed under *Residue on Evaporation*, Appendix VI, using a 5-g sample, and heating for 5 h.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Tannic Acid

Gallotannic Acid; Hydrolyzable Gallotannin

INS: 181 CAS: [1401-55-4]

FEMA: 3042

DESCRIPTION

Tannic Acid occurs as an amorphous powder, as glistening scales, or as spongy masses, varying in color from yellow-white to light brown. It is odorless or has a faint, characteristic odor and an astringent taste. It is a complex polyphenolic organic structure that yields gallic acid and either glucose or quinic acid as hydrolysis products. It is obtained by solvent extraction from the nutgalls or the excrescences that form on the young twigs of *Quercus infectoria* Olivier and allied species of *Quercus* L. (Fam. Fagaceae); from the seed pods of Tara (*Caesalpinia spinosa*) (Fam. Leguminosa); or from the nutgalls of various sumac species, including *Rhus semialata*, *R. coriaria*, *R. galabra*, and *R. typhia* (Fam. Anacardiaceae). Tannic acid is very soluble in water, in acetone, and in alcohol; slightly soluble in absolute alcohol; and practically insoluble in chloroform, in ether, and in solvent hexane. One gram dissolves in about 1 mL of warm glycerin.

Function Clarifying agent; flavoring agent; flavor enhancer; flavoring adjuvant.

REQUIREMENTS

Identification

A. Add a small quantity of ferric chloride TS to a 1:10 aqueous solution. A blue-black color or precipitate forms.

B. A sample solution, when added to a solution of an alkaloidal salt, albumin, or gelatin, produces a precipitate.

Assay Not less than 96% of Tannic Acid, calculated on the dried basis.
Gums or Dextrin Passes test.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 7.0%.
Residue on Ignition Not more than 1.0%.
Resinous Substances Passes test.

TESTS

Assay

Sample Test Transfer about 2.0 g of sample, accurately weighed, into a 500-mL volumetric flask, and dissolve in and dilute to volume with water. Transfer 100 mL of this solution into a 300-mL Erlenmeyer flask, and add 7.2 g of *Hide Powder* (L. H. Lincoln & Son, Inc., or equivalent). Shake the flask for 20 min, let it stand for 20 min, and filter through a G_4-filter, or equivalent. The filtrate should be clear. Pipet 50 mL of the filtrate into a tared crystallizing dish. Evaporate to dryness on a steam bath, and heat in an oven at 105° for 1 h. Cool in a desiccator, weigh, and calculate the weight difference.

Blank Test Perform a blank test on each lot of *Hide Powder*. Transfer 7.2 g of *Hide Powder EFT*, accurately weighed, into a 300-mL Erlenmeyer flask containing 100 mL of water. Proceed as directed in the *Sample Test*, beginning with ''Shake the flask for 20 min. . . .'' Calculate the weight difference.

Calculation Determine the percent of Tannic Acid by the formula

$$[(A - B) \times 1000]/W,$$

in which A is the weight difference, in grams, found in the *Sample Test*; B is the weight difference, in grams, found in the *Blank Test*; and W is the weight, in grams, of sample, calculated on the dried basis.

Gums or Dextrin Dissolve 1 g of sample in 5 mL of water and filter, then add 10 mL of alcohol to the filtrate. No turbidity forms within 15 min.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 2 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Resinous Substances Dissolve 1 g of sample in 5 mL of water and filter, then dilute the filtrate to 15 mL. No turbidity forms.

Packaging and Storage Store in tight, light-resistant containers.

Tarragon Oil

Estragon Oil

CAS: [8016-88-4]

FEMA: 2412

View IR

DESCRIPTION

Tarragon Oil occurs as a pale yellow to amber liquid having a delicate, spicy odor similar to fennel and sweet basil but characteristic of Tarragon Oil. It is the volatile oil obtained by steam distillation from the leaves, stems, and flowers of the plant *Artemesia dracunculus* L. (Fam. Asteraceae). It is soluble in most fixed oils and in an equal volume of mineral oil, occasionally becoming hazy on further dilution. It is relatively insoluble in propylene glycol, and is insoluble in glycerin.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Acid Value Not more than 2.0.
Angular Rotation Between +1.5° and +6.5°.
Refractive Index Between 1.504 and 1.520 at 20°.
Saponification Value Not more than 18.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.914 and 0.956.

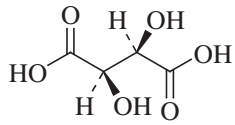
TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VI.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in *Saponification Value* under *Esters*, Appendix VI, using about 5 g of sample, accurately weighed.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample is soluble in 1 mL of 90% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

Tartaric Acid

L(+)-Tartaric Acid



$C_4H_6O_6$ Formula wt 150.09

INS: 334 CAS: [87-69-4]

FEMA: 3044

DESCRIPTION

Tartaric Acid occurs as colorless or translucent crystals or as a white, fine to granular, crystalline powder. It is stable in air. One gram dissolves in 0.8 mL of water at 25°, in about 0.5 mL of boiling water, and in about 3 mL of alcohol. Its solutions are dextrorotatory.

Function Acidifier; sequestrant; flavoring agent.

REQUIREMENTS

Identification A sample solution gives positive tests for *Tartrate*, Appendix IIIA.
Assay Not less than 99.7% and not more than 100.5% of $C_4H_6O_6$ after drying.
Lead Not more than 2 mg/kg.
Loss on Drying Not more than 0.5%.
Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +12.0° and +13.0°.
Oxalate Passes test.
Residue on Ignition Not more than 0.05%.
Sulfate Passes test.

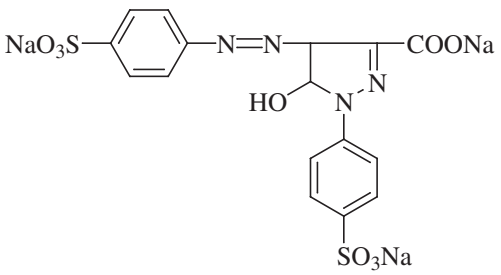
TESTS

Assay Dissolve about 2 g of sample, previously dried over phosphorus pentoxide for 3 h and accurately weighed, in 40 mL of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sodium hydroxide is equivalent to 75.04 mg of $C_4H_6O_6$.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample over phosphorus pentoxide for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 2 g of sample in each 10 mL.
Oxalate Nearly neutralize 10 mL of a 1:10 aqueous solution with 6 *N* ammonium hydroxide, and add 10 mL of calcium sulfate TS. No turbidity forms.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 4-g sample.
Sulfate Add 3 drops of hydrochloric acid and 1 mL of barium chloride TS to 10 mL of a 1:100 aqueous solution. No turbidity forms.

Packaging and Storage Store in well-closed containers.

Tartrazine[1]

CI Food Yellow 4; CI 19140; Class: Pyrazalone



$C_{16}H_9N_4O_9S_2Na_3$ Formula wt 534.37

INS: 102 CAS: [1934-21-0]

DESCRIPTION

Tartrazine occurs as a yellow-orange powder or granules. It is principally the trisodium salt of 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-(4-sulfophenyl-azo)-1*H*-pyrazole-3-carboxylic acid. When dissolved in water, it yields a solution golden yellow at neutrality and in acid. When dissolved in concentrated sulfuric acid, it yields an orange-yellow solution that turns yellow when diluted with water. It is insoluble in ethanol.

Function Color.

REQUIREMENTS

Identification A solution (*A*) containing 19.9 mg of sample per liter of water exhibits absorbance intensities and wavelength maxima as follows: at pH 7, *A* = 1.4 at 425 nm; at pH 1, *A* = 1.1 at 426 nm; and at pH 13, the absorbance maximum is shifted below 400 nm.
Assay Not less than 87.0% total coloring matters.
Arsenic Not more than 3 mg/kg.
Ether Extracts (combined) Not more than 0.2%.
Lead Not more than 10 mg/kg.
Loss on Drying (Volatile Matter) at 135°, **Chlorides**, and **Sulfates** (as sodium salts) Not more than 13.0% in combination.
Uncombined Intermediates and Products of Side Reactions

*4,4′-[4,5-Dihydro-5-oxo-4-[(4-sulfophenyl)-hydrazono]-1*H*-pyrazol-1,3-diyl]bis[benzenesulfonic Acid], Trisodium Salt* Not more than 1%.

*4-[(4′,5-Disulfo[1,1′-biphenyl]-2-yl)hydrazono]-4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic Acid, Tetrasodium Salt* Not more than 1%.

*Ethyl or Methyl 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)hydrazono]-1*H*-pyrazole-3-carboxylate, Disodium Salt* Not more than 1%.

*Sum of 4,5-Dihydro-5-oxo-1-phenyl-4-[(4-sulfophenyl)-azo]-1*H*-pyrazole-3-carboxylic Acid, Disodium Salt, and 4,5-Dihydro-5-oxo-4-(phenylazo)-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic Acid, Disodium Salt* Not more than 0.5%.

4-Aminobenzenesulfonic Acid, Sodium Salt Not more than 0.2%.

*4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic Acid, Disodium Salt* Not more than 0.2%.

*Ethyl or Methyl 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylate, Sodium Salt* Not more than 0.1%.

4,4′-(1-Triazene-1,3-diyl)bis[benzenesulfonic Acid], Disodium Salt Not more than 0.05%.

4-Aminoazobenzene Not more than 0.075 mg/kg.

4-Aminobiphenyl Not more than 0.005 mg/kg.

Aniline Not more than 0.1 mg/kg.

Azobenzene Not more than 0.04 mg/kg.

Benzidine Not more than 0.001 mg/kg.

1,3-Diphenyltriazene Not more than 0.04 mg/kg.

Water-Insoluble Matter Not more than 0.2%.

[1]To be used or sold for use to color food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Yellow No. 5 can be applied only to FDA-certified batches of this color additive. Tartrazine is a common name given to the uncertified colorant. See the monograph entitled FD&C Yellow No. 5 for directions for producing an FDA-certified batch.

TESTS

Assay Determine the total color strength as the weight percent of the sample, using *Methods I* and *II* in *Total Color* under *Colors*, Appendix IIIC. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 175 to 250 mg of sample, accurately weighed, into a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (*a*) for Tartrazine is 0.053 mg/L/cm at 428 nm.

Method II (Sample Preparation) Transfer approximately 0.2 g of sample, accurately weighed, into the titration flask. The stoichiometric factor (F_s) for Tartrazine is 7.49.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds.

Chloride Determine as directed in *Sodium Chloride* under *Colors*, Appendix IIIC.

Ether Extracts Determine as directed in *Ether Extracts* under *Colors*, Appendix IIIC.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 10 µg of lead (Pb) ion in the control.

Loss on Drying (Volatile Matter) at 135° Determine as directed in *Loss on Drying (Volatile Matter)* under *Colors*, Appendix IIIC.

Sulfate Determine as directed in *Sodium Sulfate* under *Colors*, Appendix IIIC.

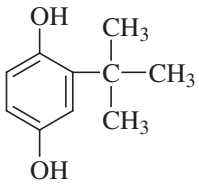
Uncombined Intermediates and Products of Side Reactions Determine as directed for *Method II* in *Uncombined Intermediates and Products of Side Reactions* under *Colors*, Appendix IIIC, injecting 50 µL of the following *Sample Preparation*: Transfer 0.15 g of sample, accurately weighed, into a 100-mL volumetric flask. Dissolve in and dilute to volume with 0.1 *M* disodium tetraborate ($Na_2B_4O_7$).

Water-Insoluble Matter Determine as directed in *Water-Insoluble Matter* under *Colors*, Appendix IIIC.

Packaging and Storage Store in well-closed containers.

TBHQ

tert-Butylhydroquinone; Mono-*tert*-butylhydroquinone



$C_{10}H_{14}O_2$ Formula wt 166.22

INS: 319 CAS: [1948-33-0]

DESCRIPTION

TBHQ occurs as a white, crystalline solid. It is soluble in alcohol and in ether, but it is practically insoluble in water.

Function Antioxidant.

REQUIREMENTS

Identification Dissolve a few milligrams of sample in 1 mL of methanol, and add a few drops of a 25% solution of dimethylamine in water. A pink to red color appears.

Assay Not less than 99.0% of $C_{10}H_{14}O_2$.

***tert*-Butyl-*p*-benzoquinone** Not more than 0.2%.

2,5-Di-*tert*-butylhydroquinone Not more than 0.2%.

Hydroquinone Not more than 0.1%.

Lead Not more than 2 mg/kg.

Melting Range Between 126.5° and 128.5°.

Toluene Not more than 0.0025%.

Ultraviolet Absorbance (polynuclear hydrocarbons) Passes test.

TESTS

Assay Transfer about 170 mg of sample, previously ground to a fine powder and accurately weighed, into a 250-mL wide-mouth Erlenmeyer flask, and dissolve in 10 mL of methanol. Add 150 mL of water, 1 mL of 1 *N* sulfuric acid, and 4 drops of diphenylamine indicator (3 mg of *p*-diphenylaminesulfonic acid sodium salt per milliliter of 0.1 *N* sulfuric acid), and titrate with 0.1 *N* ceric sulfate to the first complete color change from yellow to red-violet. Record the volume, in milliliters, of 0.1 *N* ceric sulfate required as *V*. Calculate the percentage of $C_{10}H_{14}O_2$ in the sample, uncorrected for hydroquinone and 2,5-di-*tert*-butylhydroquinone, by the formula

$$8.311N(V - 0.1\text{ mL})/W,$$

in which *N* is the exact normality of the ceric sulfate solution; 0.1 mL represents the volume of ceric sulfate solution consumed by the primary oxidation products of TBHQ ordinarily present in the sample; and *W* is the weight, in grams, of sample taken. Record the uncorrected percentage thus calculated as *A*. If hydroquinone (HQ) and 2,5-di-*tert*-butylhydroquinone (DTBHQ) are present in the sample, they will be included in the titration. Calculate the corrected percentage of $C_{10}H_{14}O_2$ in the sample by the formula

$$A - (\%\text{ HQ} \times 1.51) - (\%\text{ DTBHQ} \times 0.75),$$

using the respective values for percentage of HQ and percentage of DTBHQ as determined under *2,5-Di-tert-butylhydroquinone and Hydroquinone* (below).

***tert*-Butyl-*p*-benzoquinone**

Standard Preparation Transfer about 10 mg of USP Monotertiary-butyl-*p*-benzoquinone Reference Standard, accurately weighed, into a 10-mL volumetric flask, dissolve in and dilute to volume with chloroform, and mix.

Sample Preparation Transfer about 1 g of sample, previously reduced to a fine powder in a high-speed blender and accurately weighed, into a 10-mL volumetric flask, dilute to volume with chloroform, and shake for 5 min to extract the *tert*-butyl-*p*-benzoquinone. Filter through a Millipore filter (UHWP01300), or equivalent, before use.

Procedure Use a suitable double-beam infrared spectrophotometer and matched 0.4-mm liquid sample cells with

calcium fluoride windows. Fill the reference cell with chloroform and the sample cell with *Standard Preparation*, place the cells in the respective reference and sample beams of the spectrophotometer, and record the infrared spectrum from 1600 to 1775 cm^{-1}. Draw a background line on the spectrum from 1612 to 1750 cm^{-1}, and determine the net absorbance (A_S) of the *Standard Preparation* at 1659 cm^{-1}. Similarly, obtain the spectrum of the *Sample Preparation*, and determine its net absorbance (A_U) at 1659 cm^{-1}. Calculate the percentage of *tert*-butyl-*p*-benzoquinone in the sample taken by the formula

$$100(A_U/A_S)(W_S/W_U),$$

in which W_S is the exact weight, in milligrams, of Reference Standard taken, and W_U is the exact weight, in milligrams, of the sample taken.

2,5-Di-*tert*-butylhydroquinone and Hydroquinone

Stock Solutions Transfer about 50 mg each of hydroquinone (HQ), 2,5-di-*tert*-butylhydroquinone (DTBHQ), and methyl benzoate (internal standard), accurately weighed, into separate 50-mL volumetric flasks, dilute to volume with pyridine, and mix.

Calibration Standards Prepare four HQ calibration standards as follows: Add 0.50, 1.00, 2.00, and 3.00 mL of *HQ Stock Solution* into separate 10-mL volumetric flasks, then add 2.00 mL of *Methyl Benzoate Stock Solution* (internal standard) to each, dilute to volume with pyridine, and mix. In the same manner, prepare four *DTBHQ Stock Solution* calibration standards. Prepare the trimethylsilyl derivative of each standard as follows: Add 9 drops of calibration standard to a 2-mL serum vial, cap the vial, evacuate with a 50-mL gas syringe, add 250 µL of *N,O*-bistrimethylsilylacetamide, and heat at about 80° for 10 min.

Sample Preparation Transfer about 1 g of sample, accurately weighed, into a 10-mL volumetric flask, add 2.00 mL of *Methyl Benzoate Stock Solution*, dilute to volume with pyridine, and mix. Prepare a trimethylsilyl derivative as described under *Calibration Standards* (above).

Procedure (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph equipped with a thermal conductivity detector (F and M Model 810, or equivalent), containing a 0.61-m × 6.35-mm (od) stainless steel column (Perkin Elmer Instruments, or equivalent) packed with 20% Silicone SE-30, by weight, and 80% Diatoport S (60- to 80-mesh), or equivalent materials. Program the column temperature from 100° to 270°, heated at a rate of 15°/min. Set the injection port temperature to 300°; the bridge current at 140 mA; and the sensitivity, 1× for the integrator (Infotronics CRS-100, or equivalent) and 2× for the recorder. Use helium as the carrier gas, with a flow rate of 100 mL/min.

Chromatograph 10 µL portions of each *Calibration Standard* in duplicate, and plot the concentration ratio of *HQ Calibration Standard* to *Methyl Benzoate Stock Solution* (X-axis) against the response ratio of *HQ Calibration Standard* to *Methyl Benzoate Stock Solution* (Y-axis). Plot the same relationships between *DTBHQ Calibration Standard* and the *Methyl Benzoate Stock Solution*.

Chromatograph duplicate 10-µL portions of *Sample Preparation*. The approximate peak times, in minutes, are methyl benzoate, 2.5; TMS derivative of HQ, 5.5; TMS derivative of *tert*-Butylhydroquinone, 7.3; TMS derivative of DTBHQ, 8.4.

Calculation Determine the peak areas (response) of interest by automatic integration or manual triangulation. Calculate the response ratio of *HQ Calibration Standard* and *DTBHQ Calibration Standard* to *Methyl Benzoate Stock Solution*. From the calibration curves, determine the concentration ratio of *HQ Calibration Standard* and *DTBHQ Calibration Standard* to *Methyl Benzoate Stock Solution*, and calculate the percentages of HQ and of DTBHQ in the sample by the formula

$$A = Y \times I \times 10/S,$$

in which A is the percentage of HQ or the percentage of DTBHQ in the sample; Y is the concentration ratio (X-axis on calibration curve); I is the percentage (w/v) of *Methyl Benzoate Stock Solution* in the *Sample Preparation*; and S is the weight, in grams, of the sample taken.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Melting Range Determine as directed under *Melting Range or Temperature*, Appendix IIB.

Toluene

Standard Solution Prepare a solution of toluene in octyl alcohol containing approximately 50 µg/mL, and calculate the exact concentration (C_R) in percent (w/v).

Sample Solution Transfer about 2 g of sample, accurately weighed, into a 10-mL volumetric flask, dissolve in octyl alcohol, dilute to volume with the same solvent, and mix. Calculate the exact concentration of the solution (C_S) in percent (w/v).

Procedure (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph equipped with a flame ionization detector (F and M Model 810, or equivalent), containing a 3.66-m × 3.18-mm (od) stainless steel column, or equivalent, packed with 10% Silicone SE-30, by weight, and 90% Diatoport S (60- to 80-mesh), or equivalent materials. Program the column temperature from 70° to 280°, heated at a rate of 15°/min and held. Set the injection port temperature to 275°; the oven temperature to 300°; the hydrogen and air settings to 20 psi each; and the sensitivity to 1×10^2. Use helium as the carrier gas, with a flow rate of 50 mL/min.

Inject a 5-µL portion of *Standard Solution* into the chromatograph, and measure the height of the toluene peak (H_R) on the chromatogram. The toluene retention time is 3.3 min; other peaks are of no interest in this analysis. Similarly, obtain the chromatogram of a 5-µL portion of the *Sample Solution*, and measure the height of the toluene peak (H_S). Calculate the percentage of toluene in the sample by the formula

$$100(H_S/H_R)(C_R/C_S).$$

Ultraviolet Absorbance (polynuclear hydrocarbons) Dissolve 1 g of L-ascorbic acid in 100 mL of ethanol and 100 mL of water contained in a 500-mL separator (S-1). Transfer about 50 g of sample, accurately weighed, into the separator, shake to dissolve, then add 50 mL of isooctane, and extract for 3 min. After the phases have separated, drain the lower, aqueous phase into a second 500-mL separator (S-2), then

after 1 min of further separating, drain the lower phase into the separator S-2. Add a second 50-mL portion of isooctane to the aqueous solution in S-2, and repeat the extraction procedure as previously described, drawing off the lower, aqueous phase into a third 500-mL separator (S-3). Add a third 50-mL portion of isooctane to the aqueous solution in S-3, and repeat the extraction procedure as previously described, drawing off and discarding the lower, aqueous phase.

Extract the solutions in S-1, -2, and -3 with two 100-mL portions of a 0.5% solution of 25:75 ascorbic acid:ethanol–water. Shake each mixture for 1 min, allow the phases to separate, and discard the lower, aqueous phases. Next, extract each isooctane solution with two 100-mL portions of a 5% solution of ethanol in water, and discard the lower, aqueous phases. Finally, wash each solution twice with 100 mL of water, and discard the washes.

Lightly pack a standard-size chromatographic tube with 100 g of anhydrous sodium sulfate, and wash the packed column with 75 mL of isooctane, discarding the wash. Filter the isooctane solution from S-1 through the column, and collect the filtrate in a 500-mL distillation flask. Wash S-1 with the isooctane solution contained in S-2, and then pour the solution onto the column, collecting the filtrate in the flask. Wash S-2 and S-1, successively, with the isooctane solution in S-3, and filter the solution through the column as before. Wash S-3, S-2, and S-1, in that order and in tandem, with two successive 25-mL portions of isooctane, and pass the washings individually through the column and into the flask. Let the column drain completely.

Add 2 mL of hexadecane and 2 boiling stones to the 500-mL distillation flask containing the combined isooctane extracts, and attach the flask to a suitable vacuum distillation assembly. Evacuate the assembly to about one-third atmosphere, then immerse the flask in a steam bath, and distill the solvent. When isooctane stops dripping into the receiver, turn off the vacuum, wash down the walls of the flask with 5 mL of isooctane added through the top of the distillation head, then replace the thermometer and again evacuate. The isooctane should distill over in about 1 min. At the end of this distillation, add another 5-mL portion of isooctane, and repeat the stripping procedure.

Quantitatively wash the residue from the distillation flask into a 50-mL volumetric flask with isooctane, dilute to volume with isooctane, and mix. Determine the ultraviolet absorption spectrum of the solution in a 5-cm silica cell from 400 nm to 250 nm, with a suitable spectrophotometer, using isooctane as the blank. Determine the absorbance of a solvent control by following the above procedure in every detail, but with the sample omitted. From the sample spectrum determine the maximum absorbance per centimeter pathlength in each of the following wavelength intervals: (a) 280 to 289 nm; (b) 290 to 299 nm; (c) 300 to 359 nm; and (d) 360 to 400 nm. Calculate the maximum net absorbance per centimeter in each interval by subtracting from the sample absorbance the corresponding absorbance per centimeter of the solvent control. The following net absorbance values are not exceeded at the indicated intervals: (a) 0.15; (b) 0.12; (c) 0.08; and (d) 0.02.

Packaging and Storage Store in well-closed containers.

Terpene Resin, Natural

CAS: [9003-74-1]

DESCRIPTION

Terpene Resin, Natural, occurs as a pale yellow to yellow, solid, thermoplastic resin. It is a natural terpene occurring in some coal seams.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Acid Value Less than 8.
Lead Not more than 3 mg/kg.
Melting Point Not less than 155°.

TESTS

Acid Value Dissolve about 3 g of sample, accurately weighed, in 100 mL of a mixture of 75 mL of toluene and 36 mL of alcohol previously neutralized to phenolphthalein TS with sodium hydroxide. Add 25 mL of a saturated sodium chloride solution, 10 g in addition of sodium chloride, and a few drops of phenolphthalein TS, and titrate with 0.1 *N* alcoholic potassium hydroxide to the first pink color that persists for at least 30 s. Calculate the acid value by the formula

$$56.1 \times V \times N/W,$$

in which V is the volume, in milliliters, of 0.1 *N* alcoholic potassium hydroxide, and N is the normality, respectively, of the alcoholic potassium hydroxide solution; and W is the weight, in grams, of the sample.
Lead Prepare a *Sample Solution* as directed under *Sample Solution for Lead Limit Test*, Appendix IV. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 10 μg of lead (Pb) ion in the control.
Melting Point Determine as directed in *Procedure for Class Ib* under *Melting Range or Temperature*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Terpene Resin, Synthetic

DESCRIPTION

Terpene Resin, Synthetic, occurs as a pale yellow to yellow, solid, thermoplastic resin. It is composed essentially of α-pinene, β-pinene, and/or dipentene polymers. Its color is less than 4 on the Gardner scale (measured in 50% mineral spirit solution). It is insoluble in water.

Function Masticatory substance in chewing gum base.

REQUIREMENTS

Acid Value Less than 5.
Lead Not more than 3 mg/kg.
Saponification Value Not more than 5.

TESTS

Acid Value Determine as directed under *Acid Value*, Appendix VII.
Lead Determine as directed under *Sample Solution for Lead Limit Test*, Appendix IV. This solution meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 10 µg of lead (Pb) ion in the control.
Saponification Value Transfer 2 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask. Dissolve in 25 mL of a 2:1 toluene:isopropyl alcohol mixture. Using a volumetric pipet, add exactly 50.0 mL of 0.1 *N* methanolic potassium hydroxide. Connect a condenser to the flask, and reflux gently until the sample is completely saponified (usually 30 min). Cool to room temperature, wash the condenser with a few milliliters of water, add 2 to 3 drops of phenolphthalein TS, titrate the excess potassium hydroxide with 0.1 *N* hydrochloric acid, and record the total volume of acid required. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the saponification value by the formula

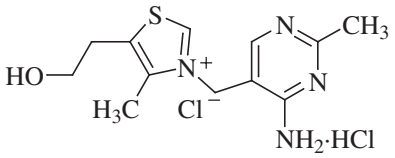
$$56.1(B - S) \times N/W,$$

in which $B - S$ represents the difference between the volumes of 0.1 *N* hydrochloric acid required for the blank and for the sample, respectively; *N* is the normality of the hydrochloric acid; and *W* is the weight, in grams, of the sample taken.

Packaging and Storage Store in well-closed containers.

Thiamine Hydrochloride

Aneurine Hydrochloride; Thiamine Chloride; Vitamin B_1; Vitamin B_1 Hydrochloride



$C_{12}H_{17}ClN_4OS{\cdot}HCl$ Formula wt 337.27

CAS: [67-03-8]

DESCRIPTION

Thiamine Hydrochloride occurs as small, white to yellow-white crystals or crystalline powder. When exposed to air, the anhydrous product rapidly absorbs about 4% of water. It melts at about 248° with some decomposition. One gram dissolves in about 1 mL of water and in about 100 mL of alcohol. It is soluble in glycerin, and is insoluble in ether.

Function Nutrient.

REQUIREMENTS

Identification
A. The infrared absorption spectrum of a potassium bromide dispersion of the sample, previously dried at 105° for 2 h, exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Thiamine Hydrochloride Reference Standard.
B. A 1:50 aqueous solution gives positive tests for *Chloride*, Appendix IIIA.
Assay Not less than 98.0% and not more than 102.0% of $C_{12}H_{17}ClN_4OS{\cdot}HCl$, calculated on the anhydrous basis.
Color of Solution Passes test.
Lead Not more than 2 mg/kg.
Nitrate Passes test.
pH of a 1:100 Solution Between 2.7 and 3.4.
Residue on Ignition Not more than 0.2%.
Water Not more than 5.0%.

TESTS

Assay
Solution A Prepare a 0.005 *M* solution of sodium 1-octanesulfonate in 1:100 glacial acetic acid.
Solution B Prepare a 3:2 mixture of methanol and acetonitrile.
Mobile Phase Prepare a 60:40 mixture of *Solution A* and *Solution B*, filter, and degas. Make adjustments to the *Mobile Phase*, if necessary, to obtain baseline separation of thiamine hydrochloride and methyl benzoate.
Internal Standard Solution Transfer 2.0-mL of methyl benzoate into a 100-mL volumetric flask, dilute with methanol to volume, and mix.
Standard Preparation Dissolve an accurately weighed quantity of USP Thiamine Hydrochloride Reference Standard in *Mobile Phase* to obtain a solution having a known concentration of about 1 mg/mL. Transfer 20.0 mL of this solution into a 50-mL volumetric flask, add 5.0 mL of *Internal Standard Solution*, dilute to volume with *Mobile Phase*, and mix to obtain a *Standard Preparation* having a known concentration of about 400 µg/mL.
Assay Preparation Transfer about 200 mg of sample, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with *Mobile Phase*, and mix. Transfer 10.0 mL of this solution into a 50-mL volumetric flask, add 5.0 mL of *Internal Standard Solution*, dilute to volume with *Mobile Phase*, and mix.
Procedure Use a high-performance liquid chromatograph (see *Chromatography*, Appendix IIA) equipped with an ultraviolet detector that measures at 254 nm. Under typical conditions, the instrument contains a 300- × 4-mm (id) column packed with 10-µm octadecylsilanized silica (µBondapak C 18, or equivalent). Set the flow rate at about

1 mL/min. To obtain accurate results, the resolution between the thiamine and methyl benzoate peaks is not less than 4.0, the tailing factor for the thiamine peak is not more than 2.0, the column efficiency determined from the thiamine peak is not less than 1500 theoretical plates, and three replicate injections of the *Standard Preparation* show a relative standard deviation of not more than 2.0%. Separately inject about 10-μL of the *Standard Preparation* and the *Assay Preparation* into the chromatograph, and record the chromatograms. Measure the peak area responses of the major peaks. Calculate the quantity, in milligrams, of $C_{12}H_{17}ClN_4OS{\cdot}HCl$ in the sample taken by the formula

$$0.5C(R_U/R_S),$$

in which C is the concentration, in micrograms per milliliter, of USP Thiamine Hydrochloride Reference Standard in the *Standard Preparation*, and R_U and R_S are the ratios of the peak area ratios of thiamine to methyl benzoate obtained from the *Assay Preparation* and the *Standard Preparation*, respectively.

Color of Solution Dissolve 1.0 g of sample in sufficient water to make 10 mL. This solution exhibits no more color than does a dilution of 1.5 mL of 0.1 *N* potassium dichromate in sufficient water to make 1000 mL.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Nitrate Add 2 mL of sulfuric acid to 2 mL of a 1:50 aqueous solution, cool, and superimpose 2 mL of ferrous sulfate TS. No brown ring forms at the junction of the two layers.

pH of a 1:100 Solution Determine as directed under *pH Determination*, Appendix IIB.

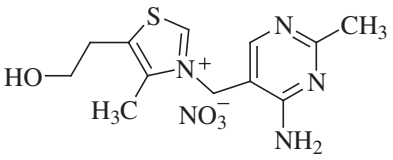
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight, light-resistant containers.

Thiamine Mononitrate

Thiamine Nitrate; Vitamin B_1; Vitamin B_1 Mononitrate



$C_{12}H_{17}N_5O_4S$ Formula wt 327.36

CAS: [532-43-4]

DESCRIPTION

Thiamine Mononitrate occurs as white to yellow-white crystals or crystalline powder. One gram dissolves in about 35 mL of water. It is slightly soluble in alcohol and in chloroform.

Function Nutrient.

REQUIREMENTS

Identification

A. Add 2 mL of sulfuric acid to 2 mL of a 1:50 aqueous solution, cool, and superimpose 2 mL of ferrous sulfate TS. A brown ring forms at the junction of the two liquids.

B. Dissolve about 5 mg of sample in a mixture of 1 mL of lead acetate TS and 1 mL of a 1:10 sodium hydroxide solution. A yellow color appears. Heat the mixture for several minutes on a steam bath. The color changes to brown, and on standing, a precipitate of lead sulfide forms.

C. A 1:10 aqueous solution yields a white precipitate with mercuric chloride TS, and a red-brown precipitate with iodine TS. Mercuric–potassium iodide TS and trinitrophenol TS also cause precipitates to form.

D. Dissolve about 5 mg of sample in 5 mL of 0.5 *N* sodium hydroxide, add 0.5 mL of potassium ferrocyanide TS and 5 mL of isobutyl alcohol, shake vigorously for 2 min, and allow the layers to separate. When illuminated from above by a vertical beam of ultraviolet light and viewed at a right angle to this beam, the air–liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified and reappears when it is again made alkaline.

Assay Not less than 98.0% and not more than 102.0% of $C_{12}H_{17}N_5O_4S$, calculated on the dried basis.

Chloride Not more than 0.06%.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 1.0%.

pH of a 1:50 Solution Between 6.0 and 7.5.

Residue on Ignition Not more than 0.2%.

TESTS

Assay Determine as directed in the monograph for *Thiamine Hydrochloride*. Calculate the quantity, in milligrams, of $C_{12}H_{17}N_5O_4S$ in the sample taken by the formula

$$(327.36/337.27)0.5C(R_U/R_S),$$

in which 327.36 and 337.27 are the formula weights of Thiamine Mononitrate and Thiamine Hydrochloride, respectively; C is the concentration, in micrograms per milliliter, of USP Thiamine Hydrochloride Reference Standard in the *Standard Preparation*; and R_U and R_S are the peak area ratios of thiamine to methyl benzoate obtained from the *Assay Preparation* and the *Standard Preparation*, respectively.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 25-mg sample does not exceed that shown in a control containing 15 μg of chloride (Cl) ion.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying about 500 mg of sample, accurately weighed, at 105° for 2 h.

pH of a 1:50 Solution Determine as directed under *pH Determination*, Appendix IIB.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in tight, light-resistant containers.

L-Threonine

L-2-Amino-3-hydroxybutyric Acid

$C_4H_9NO_3$ Formula wt 119.12

CAS: [72-19-5]

View IR

DESCRIPTION

L-Threonine occurs as a white, crystalline powder. It is freely soluble in water, and insoluble in alcohol, in ether, and in chloroform. It melts with decomposition at about 256°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_4H_9NO_3$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.2%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between −26.5° and −29.0°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between −25.8° and −28.8°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 11.91 mg of $C_4H_9NO_3$.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 6 g of a previously dried sample in sufficient water to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

Thyme Oil

CAS: [8007-46-3]

FEMA: 3064

View IR

DESCRIPTION

Thyme Oil occurs as a colorless, yellow, or red liquid with a characteristic, pleasant odor and a pungent, persistent taste. It is the volatile oil obtained by distillation from the flowering plant *Thymus vulgaris* L., or *Thymus zygis* L., and its var. *gracilis* Boissier (Fam. Labiatae). It is soluble in alcohol, in propylene glycol, and in most vegetable oils.

Function Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 40%, by volume, of phenols.
Angular Rotation Between −3° and −0.1°.
Refractive Index Between 1.495 and 1.505 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.915 and 0.935.
Water-Soluble Phenols Passes test.

TESTS

Assay Determine as directed under *Phenols*, Appendix VI, but allow the mixture to stand overnight, then add sufficient 1 *N* potassium hydroxide to raise the lower limit of the oily layer into the graduated portion of the neck of the flask. After the solution has become clear, adjust the temperature and read the volume of the residual liquid.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 2 mL of 80% alcohol.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Water-Soluble Phenols Shake a 1-mL sample with 10 mL of hot water, and after cooling, pass the water layer through a moistened filter. Add 1 drop of ferric chloride TS to the filtrate. Not even a transient blue or violet color appears.

Packaging and Storage Store in a cool place in full, tight, light-resistant containers.

Titanium Dioxide

TiO_2 Formula wt 79.90

INS: 171 CAS: [13463-67-7]

DESCRIPTION

Titanium Dioxide occurs as a white, amorphous powder. It is prepared synthetically. It is insoluble in water, in hydrochloric acid, in dilute sulfuric acid, and in alcohol and other organic solvents. It dissolves slowly in hydrofluoric acid and in hot sulfuric acid.

Function Color.

REQUIREMENTS

Identification Add 5 mL of sulfuric acid to 500 mg of sample, heat gently until fumes of sulfur trioxide evolve, continue heating for at least 10 s, and cool. Cautiously dilute to about 100 mL with water, and filter. Add a few drops of hydrogen peroxide TS to 5 mL of the clear filtrate. A yellow-red to orange-red color appears immediately.
Assay Not less than 99.0% and not more than 100.5% of TiO_2, after drying and after correcting for any aluminum oxide and/or silicon dioxide found to be present in the sample.
Acid-Soluble Substances Not more than 0.5%.
Aluminum Oxide and/or Silicon Dioxide Not more than 2.0%, either singly or combined.
Antimony Not more than 2 mg/kg.
Arsenic Not more than 1 mg/kg.
Lead Not more than 10 mg/kg.
Loss on Ignition Not more than 0.5% after drying.
Mercury Not more than 1 mg/kg.
Water-Soluble Substances Not more than 0.3%.

TESTS

Assay Transfer about 300 mg of sample, previously dried at 105° for 3 h and accurately weighed, into a 250-mL beaker, add 20 mL of sulfuric acid and 7 to 8 g of ammonium sulfate, and mix. Heat on a hot plate until fumes of sulfur trioxide evolve, and continue heating over a strong flame until the sample dissolves or it is apparent that the undissolved residue is siliceous matter. Cool, cautiously dilute with 100 mL of water, and stir. While stirring, heat carefully to boiling, allow the insoluble matter to settle, and filter. Transfer the entire residue to the filter with the aid of, and wash thoroughly with, cold 2 *N* sulfuric acid. Dilute the filtrate to 200 mL with water, and cautiously add about 10 mL of ammonium hydroxide to reduce the acid concentration to about 5%, by volume, of sulfuric acid.

Prepare zinc amalgam for use as follows: Add 20- to 30-mesh zinc to a 2% mercuric chloride solution, using about 100 mL of the solution for each 100 g of zinc. After about 10 min, decant the solution from the zinc, then wash the zinc with water by decantation. Transfer the zinc amalgam to the reductor tube, and wash the column with 100-mL portions of 2 *N* sulfuric acid until 100 mL of the washing does not decolorize 1 drop of 0.1 *N* potassium permanganate.

Prepare a zinc amalgam column in a 25-cm Jones reductor tube, placing a pledget of glass wool in the bottom of the tube and filling the constricted portion of the tube with prepared zinc amalgam. Place 50 mL of ferric ammonium sulfate TS in a 500-mL suction flask, and add 0.1 *N* potassium permanganate until a faint pink color persists for 5 min. Attach the Jones reductor tube containing the zinc amalgam column to the neck of the flask, and pass 50 mL of 2 *N* sulfuric acid through the tube at a rate of about 30 mL/min. Pass the prepared titanium solution through the column at the same rate, followed by 100 mL each of 2 *N* sulfuric acid and of water. During these operations, keep the tube filled with solution or water above the upper level of the amalgam column. Gradually release the suction, wash down the outlet tube and the sides of the receiver, and titrate immediately with 0.1 *N* potassium permanganate. Perform a blank determination (see *General Provisions*), substituting 200 mL of 1:20 sulfuric acid for the *Sample Solution*, and make any necessary correction. Each milliliter of 0.1 *N* potassium permanganate is equivalent to 7.990 mg of TiO_2.
Acid-Soluble Substances Suspend 5.0 g of sample in 100 mL of 0.5 *N* hydrochloric acid, and heat on a steam bath for 30 min, stirring occasionally. Filter the suspension through a suitable tared, porous-bottom, porcelain filter crucible. Wash the residue with three 10-mL portions of 0.5 *N* hydrochloric acid, evaporate the combined filtrate and washings to dryness, and ignite at 450° ± 25° to constant weight.

> **Caution**: Avoid exposing the crucible to sudden temperature changes.

Calculate the percent acid-soluble substances by the formula

$$100R/1000W,$$

in which 100 is the conversion to percent; R is the weight, in milligrams, of the residue; 1000 is the conversion to milligrams; and W is the starting weight, in grams, of the sample.
Aluminum Oxide

0.01 M *Zinc Sulfate* Dissolve 2.90 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) in sufficient water to make 1000 mL. Standard-

ize the solution as follows: Dissolve 500 mg of high-purity (99.9%) aluminum wire, accurately weighed, in 20 mL of hydrochloric acid, heating gently to effect solution, then transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. Transfer a 10.0-mL aliquot of this solution into a 500-mL Erlenmeyer flask containing 90 mL of water and 3 mL of hydrochloric acid, add 1 drop of methyl orange TS and 25.0 mL of 0.02 *M* disodium EDTA, and continue as directed below under *Sample Solution C*, beginning with ''Add, dropwise, a 1:5 ammonium hydroxide solution. . . .'' Calculate the titer, *T*, of the zinc sulfate solution by the equation

$$T = (18.896 \times W)/V,$$

in which *T* is the number of milligrams of aluminum oxide, Al_2O_3, per milliliter of zinc sulfate solution; *W* is the weight, in grams, of the aluminum wire taken; *V* is the volume, in milliliters, of the zinc sulfate solution consumed in the second titration; and 18.896 is a factor derived as follows:

$$(\text{mol wt } Al_2O_3/\text{mol wt Al}) \times (1000 \text{ mg/g}) \times (10 \text{ mL}/2).$$

Sample Solution A Fuse 1 g of sample, accurately weighed, with 10 g of sodium bisulfate, $NaHSO_4 \cdot H_2O$, contained in a 250-mL high-silica glass Erlenmeyer flask.

> **Caution**: Do not use more sodium bisulfate than specified because an excess concentration of salt will interfere with the EDTA titration later on in this procedure.

Begin heating the fused sample at low heat on a hot plate, and then gradually raise the temperature until full heat is reached. When spattering has stopped and light fumes of sodium trioxide (SO_3) evolve, heat the fused sample in the full flame of a Meker burner, with the flask tilted so that the fusion is concentrated at one end of the flask. Swirl the flask constantly until the melt is clear (except for silica content), but guard against prolonged heating to avoid precipitation of Titanium Dioxide. Cool, add 25 mL of a 1:2 sulfuric acid solution, and heat until the mass has dissolved and a clear solution results. Cool, and dilute to 120 mL with water. Label this *Sample Solution A*.

Sample Solution B Measure 200 mL of approximately 6.25 *M* sodium hydroxide, and add 65 mL of it to *Sample Solution A* while stirring constantly with a magnetic stirrer; pour the remaining 135 mL of the alkali solution into a 500-mL volumetric flask. Slowly and while constantly stirring, add the sample mixture to the alkali solution in the 500-mL volumetric flask, then dilute to volume with water, and mix.

> **Note**: If the procedure is delayed at this point for more than 2 h, store the contents of the volumetric flask in a polyethylene bottle.

Allow most of the precipitate to settle out (or centrifuge for 5 min), and then filter the supernatant liquid through a very fine filter paper. Label the filtrate *Sample Solution B*.

Sample Solution C Transfer 100.0 mL of *Sample Solution B* into a 500-mL Erlenmeyer flask, add 1 drop of methyl orange TS, acidify with 1:2 hydrochloric acid solution, and add about 3 mL in excess. Add 25.0 mL of 0.02 *M* disodium EDTA, and mix.

> **Note**: If the approximate aluminum oxide (Al_2O_3) content is known, calculate the optimum volume of EDTA solution to be added by the formula
>
> $$(4 \times \%\ Al_2O_3) + 5.$$

Prepare an *Ammonium Acetate Buffer Solution* by mixing 77 g of ammonium acetate with 10 mL of glacial acetic acid and diluting to 1000 mL with water. Prepare a *Dibasic Ammonium Phosphate Solution* by dissolving 150 g of dibasic ammonium phosphate in 700 mL of water, adjusting the solution to pH 5.5 with a 1:2 solution of hydrochloric acid, and diluting it to 1000 mL with water.

Add, dropwise, a 1:5 ammonium hydroxide solution to the Erlenmeyer flask until the color of the solution has just completely changed from red to orange-yellow, add 10 mL of *Ammonium Acetate Buffer Solution* and 10 mL of *Dibasic Ammonium Phosphate Solution*. Boil for 5 min, cool quickly to room temperature in a stream of running water, add 3 drops of xylenol orange TS, and mix. If the solution is purple, yellow-brown, or pink, bring the pH to 5.3 to 5.7 by adding acetic acid; at the desired pH, a pink color indicates that not enough of the EDTA solution has been added. In this case, take another 100 mL of *Sample Solution B*, and treat it as directed from the beginning of this paragraph, except use 50.0 mL, rather than 25.0 mL, of 0.02 *M* disodium EDTA.

Procedure Titrate *Sample Solution C* with *0.01* M *Zinc Sulfate* to the first yellow-brown or pink endpoint color that persists for 5 to 10 s.

> **Note**: Perform this titration quickly near the endpoint by rapidly adding 0.2-mL increments of the titrant until the first color change occurs. The color will fade in 5 to 10 s, but it is the true endpoint. Failure to observe the first color change will result in an incorrect titration. Fading does not occur at the second endpoint. The first titration should require more than 8 mL of titrant, but for more accurate work, a titration of 10 to 15 mL is desirable.

Add 2 g of sodium fluoride, boil the mixture for 2 to 5 min, and cool in a stream of running water. Titrate the EDTA (which is released by fluoride from its aluminum complex) with *0.01* M *Zinc Sulfate* to the same transitory yellow-brown or pink endpoint as described above.

Calculation Calculate the percent of aluminum oxide (Al_2O_3) in the sample taken by the formula

$$(V \times T)/(2 \times S),$$

in which *V* is the volume, in milliliters, of *0.01* M *Zinc Sulfate* consumed in the second titration; *T* is the titer of the zinc sulfate solution, determined previously; and *S* is the weight, in grams, of the sample taken to prepare *Sample Solution A*.

Antimony

0.1 N *Ceric Sulfate Solution* Dissolve 3.3 g of ceric sulfate [$Ce(SO_4)_2$] in 100 mL of water containing 3 mL of sulfuric acid.

Stock Standard Solution Transfer 274.28 mg of antimony potassium tartrate ($C_4H_4KO_7Sb \cdot \frac{1}{2}H_2O$) into a 100-mL volumetric flask, dissolve in and dilute to volume with 6 *N* hydrochloric acid, and mix. Each milliliter contains 1 mg of antimony (Sb).

Diluted Standard Solution Pipet 2.00 mL of *Stock Standard Solution* into a 100-mL volumetric flask, dilute to volume with 6 *N* hydrochloric acid, and mix. Each milliliter contains 20 μg of antimony. Prepare this solution fresh weekly.

Standard Preparation Transfer 1.00 mL of *Diluted Standard Solution* into a 250-mL separator, and add 25 mL of mercuric chloride solution (6% in hydrochloric acid) and 25 mL of hydrochloric acid.

Sample Preparation Transfer 10.00 g of sample into a 250-mL beaker, add 50 mL of hot 0.5 *N* hydrochloric acid, cover the beaker, and boil the slurry for 15 min, stirring occasionally. Remove the beaker from the heat, allow the slurry to settle for a few seconds, and decant through a double Whatman No. 42 filter paper plus a No. 12 fluted paper, or equivalent, all previously washed with 0.5 *N* hydrochloric acid. Evaporate the filtrate slowly on a hot plate until the volume is slightly less than 20 mL, then cool, and transfer it to a 25-mL graduate. Rinse the beaker with 5 mL of 0.5 *N* hydrochloric acid, and add this to the graduate. Dilute the solution to volume with 0.5 *N* hydrochloric acid, and transfer it into a 250-mL separator. Rinse the beaker and graduate with a total of 25 mL of mercuric chloride solution (6% in hydrochloric acid) and with a total of 25 mL of hydrochloric acid, adding the washings to the separator.

Procedure Add 1.0 mL of *0.1* N *Ceric Sulfate Solution* to the *Sample Preparation* in the separator, and start a stopwatch at the moment of first addition. Mix the solutions together, and pass a stream of clean air over the mixture. At exactly 1.0 min, add 75 mL of water, mix, and continue passing air over the solution. At 2.0 min, add 8 drops of a 1% solution of hydroxylamine hydrochloride, mix, and continue passing air over the solution. At 3.0 min, add 5.0 mL of a 0.2% solution of rhodamine B. Pipet 50.00 mL of benzene into the separator, shake for 1 min, and allow the layers to separate for 90 s. Discard the aqueous layer and a small portion of the organic phase. Transfer about 15 mL of the organic phase to a centrifuge tube, and centrifuge at high speed for 1 min. Determine the absorbance of the clarified solution in a 1-cm cell at 565 nm with a suitable spectrophotometer, using water as a blank (see *General Provisions*) after having compared benzene in the sample cell to water in the reference cell. The color of the clarified solution is stable for several minutes; measure it within 15 to 20 min after starting the stopwatch.

> **Note**: The colloidal antimony color complex may resist rinsing from the cell with benzene, in which case rinse the cell in succession with dilute nitric acid, hot water, acetone, and benzene. Check the absorbance of the cell with benzene against water contained in the reference cell.

The absorbance produced by the solution from the *Sample Preparation*, after correction for a reagent blank, is not greater than that produced by a solution from the *Standard Preparation*, treated in the same manner in the above procedure as the *Sample Preparation*, beginning with ''Add 1.0 mL of 0.1 *N* ceric sulfate. . . .''

Sample Solution for the Determination of Arsenic and Lead Transfer 10.00 g of sample into a 250-mL beaker, add 50 mL of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat to boiling on a hot plate. Boil gently for 15 min, then pour the slurry into a 100- to 150-mL centrifuge bottle, and centrifuge for 10 to 15 min, or until undissolved material settles. Decant the supernatant extract through a Whatman No. 4 filter paper, or equivalent, collecting the filtrate in a 100-mL volumetric flask and retaining as much as possible of the undissolved material in the centrifuge bottle. Add 10 mL of hot water to the original beaker, washing off the watch glass with the water, and pour the slurry into the centrifuge bottle. Form a slurry, using a glass stirring rod, and centrifuge. Decant through the same filter paper, and collect the washings in the volumetric flask containing the initial extract. Repeat the entire washing process two more times. Finally, wash the filter paper with 10 to 15 mL of hot water. Cool the contents of the flask to room temperature, dilute to volume with water, and mix.

Arsenic Determine as directed under *Arsenic Limit Test*, Appendix IIIB, using a 30-mL portion of the *Sample Solution*.

Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a 20-mL portion of the *Sample Solution*, and 20 μg of lead (Pb) ion in the control.

Loss on Ignition Ignite a 2-g sample, previously dried at 105° for 3 h, at 800° ± 25°, to constant weight.

Mercury

Apparatus Use an apparatus consisting of a source of nitrogen (supplied through a regulator or flowmeter capable of measuring a flow rate of 1 L/min) connected to a suitable quartz combustion tube contained in a hinged furnace (Type 70 T, Arthur H. Thomas Co., or equivalent) in which the sample can be pyrolyzed at 650°. Connect the exit end of the combustion tube to the optical cell of a suitable mercury vapor meter (Beckman Model K-23, or equivalent), the microammeter of which is connected in parallel through an attenuator to a 1-mV strip chart recorder. Fit a 48.3-cm × 18.3-mm (od) quartz combustion tube at each end with Pyrex ball-joint adapters, and pack it near the exit end with 40 g of copper oxide held in place by small wads of quartz wool. Use the inlet end to hold an 88- × 12- × 8-mm combustion boat for the sample.

Standard Mercury Solutions Prepare the *Standard Mercury Stock Solution* by transferring 1.353 g of mercuric chloride to a 1000-mL volumetric flask and dissolving it in and diluting it to volume with water. Prepare the *Standard Mercury Working Solution* by pipetting1.0 mL of the *Standard Mercury Stock Solution* into a 100-mL volumetric flask, diluting to volume with water, and mixing. This solution contains 0.01 μg of mercury per microliter.

Standardization of Mercury Vapor Meter Preheat the combustion tube furnace to 650°, and adjust the nitrogen flow to 1 L/min. Standardize the meter in accordance with the manufacturer's instructions, using the internal standard with which the instrument is equipped. Adjust the attenuator so that the scale on the recorder is 200 mV. Under these conditions, a meter reading of 0.078 mg/m^3, obtained with the internal standard, is 50% of full scale on the recorder. Check the

standardization of the instrument periodically, and adjust as necessary.

Calibration of Mercury Vapor Meter Prepare a set of mercury standards containing 0.01, 0.02, and 0.03 μg of mercury by pipetting the required amount of the *Standard Mercury Working Solution* onto 1- × 0.5- × 0.1-cm asbestos pads, previously ignited at 800° for 1 h, and contained in separate combustion boats. Cover each asbestos pad with 1 to 2 g of fine, granular, anhydrous sodium carbonate, previously checked for absence of mercury by the previous ignition procedure. Place a combustion boat containing a mercury standard in the tube furnace, and close the inlet with a ball joint sealed at one end and held in place by a clamp. After 1 min, start the gas flow by connecting the nitrogen supply tube to the inlet port of the combustion tube. Record the maximum response from either the observed meter deflection or the chart record for each mercury standard, and prepare a standard curve of the response versus the amount of mercury added.

> **Note**: Calibrate the mercury vapor meter each time a series of samples is run, and check the calibration periodically by running a single mercury standard.

Sample Analysis Place 25 mg of sample in a combustion boat, and cover it with 1 to 2 g of the fine, granular, anhydrous sodium carbonate prepared as previously described. Ignite the sample as described for the mercury standards, record the maximum response, and determine the amount of mercury in the sample by referring to the standard curve.

Silicon Dioxide Fuse 1 g of sample, accurately weighed, with 10 g of sodium bisulfate, $NaHSO_4 \cdot H_2O$, contained in a 250-mL high-silica glass Erlenmeyer flask. While swirling the flask, heat it gently over a Meker burner until decomposition and fusion are complete and the melt is clear, except for the silica content, and then cool.

> **Caution**: Do not overheat the contents of the flask at the beginning, and heat cautiously during fusion to avoid spattering.

Add 25 mL of a 1:2 sulfuric acid solution to the cold melt, and heat very carefully and very slowly until the melt is dissolved. Cool, and carefully add 150 mL of water, pouring very small portions down the sides of the flask, swirling frequently to avoid overheating and spattering. Allow the contents of the flask to cool, and filter them through fine ashless filter paper, using a 60° gravity funnel. Use a 1:10 sulfuric acid solution to wash out all of the silica from the flask onto the filter paper. Transfer the filter paper and its contents into a tared platinum crucible, dry in an oven at 120°, and then partly cover the crucible and heat it over a Bunsen burner. To prevent the filter paper from flaming, first heat the cover from above and then the crucible from below. When the filter paper is consumed, transfer the crucible to a muffle furnace, and ignite at 1000° for 30 min, cool the crucible in a desiccator, and weigh. Add 2 drops of 1:2 sulfuric acid and 5 mL of hydrofluoric acid (specific gravity: 1.15) to the contents, and carefully evaporate to dryness, first on a low-heat hot plate to remove the hydrofluoric acid, and then over a Bunsen burner to remove the sulfuric acid. Take precautions to avoid spattering, especially after removing the hydrofluoric acid. Ignite in a muffle furnace at 1000° for 10 min, cool in a desiccator, and weigh again. Record the difference between the two weights as the content of silicon dioxide, SiO_2, in the sample.

Water-Soluble Substances Suspend 4.0 g of sample in 50 mL of water, mix, and allow to stand overnight. Transfer the suspension to a 200-mL volumetric flask; add 2 mL of ammonium chloride TS, and mix. If the sample does not settle, add another 2-mL portion of ammonium chloride TS, then allow the suspension to settle, dilute to volume with water, and mix. Filter the suspension through a double thickness of filter paper, discarding the first 10 mL of filtrate, and collect 100 mL of the subsequent clear filtrate. Transfer the filtrate into a tared platinum dish, evaporate on a hot plate to dryness, and ignite at a dull red heat to constant weight. Calculate the percent water-soluble substances by the formula

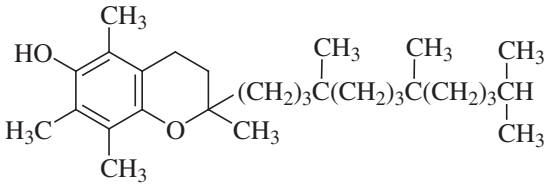
$$100AR/1000VW,$$

in which 100 is the conversion to percent; A is the amount, in milliliters, of filtrate collected; R is the weight, in milligrams, of the residue; 1000 is the conversion to milligrams; V is the initial volume, in milliliters, of the suspension; and W is the starting weight, in grams, of the sample.

Packaging and Storage Store in well-closed containers.

All-rac-α-Tocopherol

DL-α-Tocopherol



$C_{29}H_{50}O_2$ Formula wt 430.71

INS: 307c CAS: [10191-41-0]

DESCRIPTION

All-rac-α-Tocopherol occurs as a yellow to amber, clear, viscous oil. It is a form of vitamin E. It oxidizes and darkens in air and on exposure to light. It is insoluble in water; freely soluble in alcohol; and miscible with acetone, with chloroform, with ether, with fats, and with vegetable oils.

Function Nutrient; antioxidant.

REQUIREMENTS

Labeling All label claims that are in terms of International Units (IU) should be based on the following: 1 mg of all-rac-α-Tocopherol = 1.1 IU.

Identification

A. Dissolve about 10 mg of sample in 10 mL of absolute alcohol; add, with swirling, 2 mL of nitric acid; and heat at about 75° for 15 min. A bright red to orange color develops.

B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the *Internal Standard Solution*, as obtained in the *Assay* (below).

C. If the isomeric form is not otherwise known, determine the optical rotation [see *Optical (Specific) Rotation*, Appendix IIB] of a 1:10 solution in chloroform. The optical rotation is approximately ±0.05°.

Assay Not less than 96.0% and not more than 102.0% of $C_{29}H_{50}O_2$.

Acidity Passes test.

Lead Not more than 2 mg/kg.

TESTS

Note: Use low-actinic glassware for all solutions containing tocopherols.

Assay

Internal Standard Solution Prepare a solution containing about 3 mg of hexadecyl hexadecanoate (Aldrich, or equivalent), accurately weighed, in each milliliter of *n*-hexane.

Standard Preparation Dissolve about 30 mg of USP Alpha Tocopherol Reference Standard, accurately weighed, in 10.0 mL of the *Internal Standard Solution*.

Assay Preparation Dissolve about 30 mg of sample, accurately weighed, in 10.0 mL of the *Internal Standard Solution*.

Chromatographic System Use a gas chromatograph equipped with a flame-ionization detector and a 2-m × 4-mm (id) borosilicate glass column, or equivalent, packed with 2% to 5% methylpolysiloxane gum on 80- to 100-mesh acid-washed, base-washed, silanized, chromatographic diatomaceous earth, or equivalent materials. The column should have a glass-lined sample-introduction system or on-column injection. Maintain the column isothermally between 240° and 260°, the injection port at about 290°, and the detector block at about 300°. Use a dry carrier gas with the flow rate adjusted to obtain a hexadecyl hexadecanoate peak approximately 18 to 20 min after sample introduction when using a 2% column, or 30 to 32 min when using a 5% column.

Note: Cure and condition the column as necessary before use (see *Procedure* in *Gas Chromatography* under *Chromatography*, Appendix IIA).

System Suitability Chromatograph a suitable number of injections of 1 mg each of USP Alpha Tocopherol Reference Standard and USP Alpha Tocopheryl Acetate Reference Standard per milliliter of *n*-hexane, as directed under *Calibration* (below), to ensure that the resolution factor, *R*, is not less than 1.0 (see *System Suitability* in *High-Performance Liquid Chromatography* under *Chromatography*, Appendix IIA).

Calibration Chromatograph successive 2- to 5-μL portions of the *Standard Preparation* until the relative response factor, *F*, is constant (i.e., within a range of approximately 2%) for three consecutive injections. If using graphic integration, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the major peaks occurring at relative retention times of approximately 0.51 (α-tocopherol) and 1.00 (hexadecyl hexadecanoate), and record the values as A_S and A_I, respectively. Calculate *F* by the formula

$$(A_S/A_I) \times (C_I/C_S),$$

in which C_I and C_S are the exact concentrations, in milligrams per milliliter, of hexadecyl hexadecanoate and of USP Alpha Tocopherol Reference Standard in the *Standard Preparation*, respectively.

Procedure Chromatograph 2 to 5 μL of the *Assay Preparation*, and record the chromatogram as described under *Calibration*. Measure the areas under the major peaks occurring at relative retention times of approximately 0.51 (α-tocopherol) and 1.00 (hexadecyl hexadecanoate), and record the values as a_U and a_I, respectively. Calculate the weight, in milligrams, of all-rac-α-Tocopherol in the sample by the formula

$$(10C_I/F) \times (a_U/a_I),$$

in which *F* is the relative response factor (see *Calibration*).

Acidity Dissolve 1.0 g of sample in 25 mL of a 1:1 (v/v) alcohol:ether mixture that has been neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide. Add 0.5 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 30 s. Not more than 1.0 mL of 0.1 *N* sodium hydroxide is required.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Packaging and Storage Store in tight containers blanketed by inert gas and protected from heat and light.

RRR-α-Tocopherol Concentrate

D-α-Tocopherol Concentrate

$C_{29}H_{50}O_2$ Formula wt 430.71

INS: 307 CAS: [59-02-9]

DESCRIPTION

RRR-α-Tocopherol Concentrate occurs as a brown-red to light yellow, clear, viscous oil. It is a form of vitamin E obtained by the vacuum steam distillation of edible vegetable oil products, comprising a concentrated form of RRR-α-tocopherol. It oxidizes and darkens slowly in air and on exposure to light. It may contain an edible vegetable oil added to adjust the required amount of total tocopherols, and the content of RRR-α-tocopherol may be adjusted by suitable physical and chemical means. It is insoluble in water; soluble in alcohol; and miscible with acetone, with chloroform, with ether, and with vegetable oils.

Function Nutrient; antioxidant.

REQUIREMENTS

Labeling Indicate the milligrams per gram of RRR-α-tocopherol present. All label claims that are in terms of International Units (IU) should be based on the following: 1 mg of RRR-α-tocopherol = 1.49 IU.

Identification

A. Dissolve about 50 mg of sample in 10 mL of absolute alcohol; add, with swirling, 2 mL of nitric acid; and heat at about 75° for 15 min. A bright-red to orange color develops.

B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the *Internal Standard Solution*, as obtained in the *Assay* (below).

Assay Not less than 40.0% of total tocopherols, of which not less than 95.0% consists of RRR-α-tocopherol ($C_{29}H_{50}O_2$).

Acidity Passes test.

Lead Not more than 2 mg/kg.

Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: Not less than +24°.

TESTS

Assay, **Acidity**, and **Optical (Specific) Rotation** Determine as directed in the monograph for *all-rac-α-Tocopherol*.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Packaging and Storage Store in tight containers blanketed by inert gas and protected from heat and light.

RRR-Tocopherols Concentrate, Mixed

Tocopherols Concentrate, Mixed

INS: 307b

DESCRIPTION

This monograph establishes specifications for two types of mixed tocopherols concentrate. Both types are obtained by the vacuum steam distillation of edible vegetable oil products, and both contain a specified minimum amount of total tocopherols (see *Requirements*, below), differing only in the levels of the RRR-tocopherol forms.

The *high-alpha* type contains a relatively high proportion of RRR-α-tocopherol and is recognized as a form of vitamin E and also as an antioxidant. The *low-alpha* type contains a relatively high proportion of D-β-, D-γ-, and D-δ-tocopherols, with a minor level of RRR-α-tocopherol, and thus is not considered to be a form of vitamin E, but rather an antioxidant. Both types may contain an edible vegetable oil added to adjust the required amount of total tocopherols, and the tocopherol forms may be adjusted by suitable physical or chemical means.

Mixed RRR-Tocopherols Concentrate occurs as a brown-red to light yellow, clear, viscous oil. It may show a slight separation of waxlike constituents in microcrystalline form. It oxidizes and darkens slowly in air and on exposure to light, particularly when in alkaline media. It is insoluble in water; soluble in alcohol; and miscible with acetone, with chloroform, with ether, and with vegetable oils.

Function *High-Alpha Type*: Nutrient; antioxidant. *Low-Alpha Type*: Antioxidant.

REQUIREMENTS

Labeling *High-Alpha Type*: Indicate the milligrams per gram of total tocopherols and of RRR-α-tocopherol present. All label claims that are in terms of International Units (IU) should be based on the following: 1 mg of RRR-α-tocopherol = 1.49 IU. *Low-Alpha Type*: Indicate the milligrams per gram of total tocopherols and of D-β- plus D-γ- plus D-δ-tocopherols present.

Identification

A. Dissolve about 50 mg of sample in 10 mL of absolute alcohol, add, with swirling, 2 mL of nitric acid, and heat at about 75° for 15 min. A bright-red to orange color develops.

B. *High-Alpha Type*: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the *Internal Standard Solution*, as obtained in the *Assay* (below). *Low-Alpha Type*: The retention time of the third major peak (i.e., the peak occurring just before that of the internal standard) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the *Internal Standard Solution*, as obtained in the *Assay*.

Assay *High-Alpha Type*: Not less than 50.0% of total tocopherols, of which not less than 50.0% consists of RRR-α-tocopherol ($C_{29}H_{50}O_2$) and not less than 20.0% consists of D-β- plus D-γ- ($C_{28}H_{48}O_2$) plus D-δ-tocopherols ($C_{27}H_{46}O_2$). *Low-Alpha Type*: Not less than 50.0% of total tocopherols, of which not less than 80.0% consists of D-β- plus D-γ- plus D-δ-tocopherols.

Acidity Passes test.

Lead Not more than 2 mg/kg.

Optical (Specific) Rotation $[\alpha]_D^{25^\circ}$: *High-Alpha Type*: Not less than +24°. *Low-Alpha Type*: Not less than +20°.

TESTS

Note: In the *Assay* and *Optical (Specific) Rotation* tests, use low-actinic glassware for all solutions containing tocopherols.

Assay

Internal Standard Solution Transfer about 600 mg of hexadecyl hexadecanoate, accurately weighed, into a 200-mL volumetric flask, dissolve in and dilute to volume with a 2:1 pyridine:propionic anhydride solution, and mix.

Standard Preparations Transfer 12-, 25-, 37-, and 50-mg portions of USP Alpha Tocopherol Reference Standard, accurately weighed, into separate 50-mL Erlenmeyer flasks having 19/38 standard-taper, ground-glass necks. Pipet 25.0 mL of the *Internal Standard Solution* into each flask, mix, and reflux for 10 min under water-cooled condensers.

Assay Preparation Transfer about 60 mg of sample, accurately weighed, into another 50-mL Erlenmeyer flask, pipet 10.0 mL of the *Internal Standard Solution* into the flask, mix, and reflux for 10 min under a water-cooled condenser.

Chromatographic System Use a gas chromatograph equipped with a flame-ionization detector and a 2-m × 4-mm (id) borosilicate glass column, or equivalent, packed with 2% to 5% methylpolysiloxane gum on 80- to 100-mesh acid-washed, base-washed, silanized, chromatographic diatomaceous earth, or equivalent materials. The column should have a glass-lined sample-introduction system or on-column injection. Maintain the column isothermally between 240° and 260°, the injection port at about 290°, and the detector block at about 300°. Use a dry carrier gas with the flow rate adjusted to obtain a hexadecyl hexadecanoate peak approximately 18 to 20 min after sample introduction when using a 2% column, or 30 to 32 min when using a 5% column.

Note: Cure and condition the column as necessary before use (see *Procedure* in *Gas Chromatography* under *Chromatography*, Appendix IIA).

System Suitability Chromatograph a suitable number of injections of the *Assay Preparation*, as directed under *Calibration*, to ensure that the resolution factor, R, between the major peaks occurring at retention times of approximately 0.50 (δ-tocopheryl propionate) and 0.63 (β- plus γ-tocopheryl propionates), relative to hexadecyl hexadecanoate at 1.00, is not less than 2.5 (see *System Suitability* in *High-Performance Liquid Chromatography* under *Chromatography*, Appendix IIA).

Calibration Chromatograph successive 2- to 5-μL portions of each *Standard Preparation* until the relative response factor, F, for each is constant (i.e., within a range of approximately 2%) for three consecutive injections. If using graphic integration, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the first (α-tocopheryl propionate) and second (hexadecyl hexadecanoate) major peaks (excluding the solvent peak), and record the values as A_S and A_I, respectively. Calculate F for each concentration of *Standard Preparation* by the formula

$$(A_S/A_I) \times (C_I/C_S),$$

in which C_I and C_S are the exact concentrations, in milligrams per milliliter, of hexadecyl hexadecanoate and of USP Alpha Tocopherol Reference Standard in the *Standard Preparation*, respectively. Prepare a relative response factor curve by plotting the area of α-tocopheryl propionate versus relative response factor.

Procedure Chromatograph 2 to 5 μL of the *Assay Preparation* in the chromatograph, and record the chromatogram as directed under *Calibration* (above). Measure the areas under the four major peaks occurring at relative retention times of approximately 0.50 (δ-tocopheryl propionate), 0.63 (β- plus γ-tocopheryl propionates), 0.76 (α-tocopheryl propionate), and 1.00 (hexadecyl hexadecanoate), and record the values as a_δ, $a_{\beta+\gamma}$, a_α, and a_I. Calculate the weight, in milligrams, of each tocopherol form in the sample by the following equations:

$$\delta\text{-tocopherol} = (10C_I/F) \times (a_\delta/a_I),$$

$$\beta\text{- plus } \gamma\text{-tocopherols} = (10C_I/F) \times (a_{\beta+\gamma}/a_I),$$

$$\alpha\text{-tocopherol} = (10C_I/F) \times (a_\alpha/a_I),$$

in which F is obtained from the relative response factor curve (see *Calibration*) for each of the corresponding areas under the δ-, β- plus γ-, and α-tocopheryl propionate peaks produced by the *Assay Preparation*.

Note: The relative response factor for δ-tocopheryl propionate and for β- plus γ-tocopheryl propionates has been determined empirically to be the same as that for α-tocopheryl propionate.

Acidity Dissolve 1.0 g of sample in 25 mL of a 1:1 (v/v) alcohol:ether mixture that has been neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide. Add 0.5 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 30 s. Not more than 1.0 mL of 0.1 *N* sodium hydroxide is required.

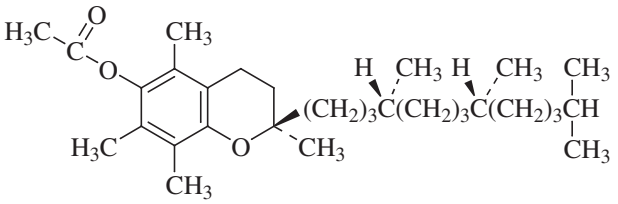
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Optical (Specific) Rotation Transfer an accurately weighed amount of sample, equivalent to about 100 mg of total tocopherols, into a separator, and dissolve it in 50 mL of ether. Add 20 mL of a 10% solution of potassium ferricyanide in a 1:125 sodium hydroxide solution, and shake for 3 min. Wash the ether solution with four 50-mL portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 mL remains, and then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 mL of isooctane, and determine the optical rotation. Calculate the optical rotation [see *Optical (Specific) Rotation*, Appendix IIB], using as c the concentration expressed as the number of grams of total tocopherols, as determined in the *Assay* (above), in 100 mL of the solution.

Packaging and Storage Store in tight containers blanketed by inert gas and protected from heat and light.

RRR-α-Tocopheryl Acetate

D-α-Tocopheryl Acetate



$C_{31}H_{52}O_3$ Formula wt 472.75

CAS: [58-95-7]

DESCRIPTION

RRR-α-Tocopheryl Acetate occurs as a colorless to yellow, clear, viscous oil. It is a form of vitamin E obtained by the vacuum steam distillation and acetylation of edible vegetable oil products. It may solidify on standing, and melts at about 25°. It is unstable in the presence of alkalies. It is insoluble in water; freely soluble in alcohol; and miscible with acetone, with chloroform, with ether, and with vegetable oils.

Function Nutrient.

REQUIREMENTS

Labeling All label claims that are in terms of International Units (IU) should be based on the following: 1 mg of RRR-α-Tocopheryl Acetate = 1.36 IU.

Identification

A. Add, with swirling, 2 mL of nitric acid to 10 mL of the *Test Solution* from *Optical (Specific) Rotation* (below), and heat at about 75° for 15 min. A bright-red to orange color develops.

B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the *Internal Standard Solution*, as obtained in the *Assay* (below).

Assay Not less than 96.0% and not more than 102.0% of $C_{31}H_{52}O_3$.

Acidity Passes test.

Lead Not more than 2 mg/kg.

Optical (Specific) Rotation $[\alpha]_D^{25°}$: Not less than +24°.

TESTS

Note: In the *Assay* and *Optical (Specific) Rotation* tests, use low-actinic glassware for all solutions containing tocopherols.

Assay

Internal Standard Solution Prepare a solution containing about 3 mg of hexadecyl hexadecanoate, accurately weighed, in each milliliter of *n*-hexane.

Standard Preparation Dissolve about 30 mg of USP Alpha Tocopheryl Acetate Reference Standard, accurately weighed, in 10.0 mL of the *Internal Standard Solution*.

Assay Preparation Dissolve about 30 mg of sample, accurately weighed, in 10.0 mL of the *Internal Standard Solution*.

Chromatographic System Use a gas chromatograph equipped with a flame-ionization detector and a 2-m × 4-mm (id) borosilicate glass column, or equivalent, packed with 2% to 5% methylpolysiloxane gum on 80- to 100-mesh acid-washed, base-washed, silanized, chromatographic diatomaceous earth, or equivalent materials. The column should have a glass-lined sample-introduction system or on-column injection. Maintain the column isothermally between 240° and 260°, the injection port at about 290°, and the detector block at about 300°. Use a dry carrier gas with the flow rate adjusted to obtain a hexadecyl hexadecanoate peak approximately 18 to 20 min after sample introduction when using a 2% column, or 30 to 32 min when using a 5% column.

Note: Cure and condition the column as necessary before use (see *Procedure* in *Gas Chromatography* under *Chromatography*, Appendix IIA).

System Suitability Chromatograph a suitable number of injections of 1 mg each of USP Alpha Tocopherol Reference Standard and USP Alpha Tocopheryl Acetate Reference Standard per milliliter of *n*-hexane, as directed under *Calibration* (below), to ensure that the resolution factor, *R*, is not less than 1.0 (see *System Suitability* in *High-Performance Liquid Chromatography* under *Chromatography*, Appendix IIA).

Calibration Chromatograph successive 2- to 5-μL portions of the *Standard Preparation* until the relative response factor, *F*, is constant (i.e., within a range of approximately 2%) for three consecutive injections. If using graphic integration, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the major peaks occurring at relative retention times of approximately 0.60 (α-tocopheryl acetate) and 1.00 (hexadecyl hexadecanoate), and record the values as A_S and A_I, respectively. Calculate *F* by the formula

$$(A_S/A_I) \times (C_I/C_S),$$

in which C_I and C_S are the exact concentrations, in milligrams per milliliter, of hexadecyl hexadecanoate and of USP Alpha Tocopheryl Acetate Reference Standard in the *Standard Preparation*, respectively.

Procedure Chromatograph 2 to 5 μL of the *Assay Preparation* as described under *Calibration*. Measure the areas under the major peaks occurring at relative retention times of approximately 0.60 (α-tocopheryl acetate) and 1.00 (hexadecyl hexadecanoate), and record the values as a_U and a_I, respectively. Calculate the weight, in milligrams, of RRR-α-Tocopheryl Acetate in the sample by the formula

$$(10C_I/F) \times (a_U/a_I),$$

in which *F* is the relative response factor (see *Calibration*).

Acidity Dissolve 1.0 g of sample in 25 mL of a 1:1 (v/v) alcohol:ether mixture that has been neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide. Add 0.5 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide

until the solution remains faintly pink after shaking for 30 s. Not more than 1.0 mL of 0.1 *N* sodium hydroxide is required.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Optical (Specific) Rotation

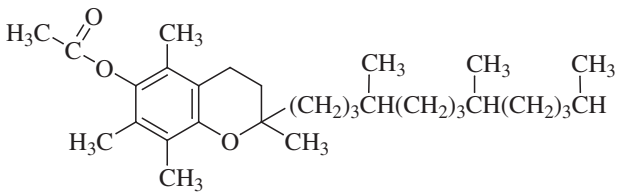
Test Solution Transfer an accurately weighed amount of sample, equivalent to about 200 mg of α-tocopherol, into a 150-mL round-bottom, glass-stoppered flask, and dissolve it in 25 mL of absolute alcohol. Add 20 mL of a 1:7 mixture of 2 *N* sulfuric acid in alcohol, and reflux in an all-glass apparatus for 3 h, protected from sunlight. Cool, transfer into a 200-mL volumetric flask, dilute to volume with a 1:72 mixture of 2 *N* sulfuric acid in alcohol, and mix. Retain 10 mL of this solution for *Identification Test A* (above).

Procedure Transfer an accurately measured volume of the *Test Solution*, equivalent to about 100 mg of α-tocopherol, into a separator, and add 200 mL of water. Extract first with 75 mL, then with two 25-mL portions of ether, and combine the ether extracts in another separator. Add 20 mL of a 10% solution of potassium ferricyanide in a 1:125 sodium hydroxide solution to the ether solution, and shake for 3 min. Wash the ether solution with four 50-mL portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 mL remain, and then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 mL of isooctane, and determine the optical rotation. Calculate the optical rotation [see *Optical (Specific) Rotation*, Appendix IIB], using as *c* the concentration of D-α-Tocopheryl Acetate, as determined in the *Assay* (above), in 100 mL of solution.

Packaging and Storage Store in tight, light-resistant containers.

All-rac-α-Tocopheryl Acetate

DL-α-Tocopheryl Acetate; Vitamin E Acetate; DL-alpha-Tocopherol Acetate



$C_{31}H_{52}O_3$ Formula wt 472.75

CAS: [7695-91-2]

DESCRIPTION

All-rac-α-Tocopheryl Acetate occurs as a colorless to yellow or green-yellow, clear, viscous oil. It is a form of vitamin E. It is unstable in the presence of alkalies. It is insoluble in water; freely soluble in alcohol; and miscible with acetone, with chloroform, with ether, and with vegetable oils.

Function Nutrient.

REQUIREMENTS

Labeling All label claims that are in terms of International Units (IU) should be based on the following: 1 mg of all-rac-α-tocopheryl acetate = 1 IU.

Identification

A. Add, with swirling, 2 mL of nitric acid to 10 mL of the *Test Solution* from *Optical (Specific) Rotation* (below), and heat at about 75° for 15 min. A bright red to orange color develops.

B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the *Internal Standard Solution*, as obtained in the *Assay* (below).

C. If the isomeric form is not otherwise known, determine the optical rotation [see *Optical (Specific) Rotation*, Appendix IIB] of a 1:10 solution in chloroform. The optical rotation is approximately ± 0.05°.

Assay Not less than 96.0% and not more than 102.0% of $C_{31}H_{52}O_3$.

Acidity Passes test.

Lead Not more than 2 mg/kg.

TESTS

Note: Use low-actinic glassware for all solutions containing tocopherols.

Assay Determine as directed in the monograph for *RRR-α-Tocopheryl Acetate*, using the following as the *Assay Preparation*: Dissolve an accurately weighed amount of sample, equivalent to about 30 mg of all-rac-α-tocopheryl acetate, in 10.0 mL of the *Internal Standard Solution*.

Acidity Determine as directed in the monograph for *RRR-α-Tocopheryl Acetate*.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Packaging and Storage Store in tight, light-resistant containers.

RRR-α-Tocopheryl Acetate Concentrate

D-α-Tocopheryl Acetate Concentrate; D-α-Tocopheryl Acetate Preparation

DESCRIPTION

RRR-α-Tocopheryl Acetate Concentrate occurs as a light brown to light yellow, clear, viscous oil. It is a form of vitamin

E obtained by the vacuum steam distillation and acetylation of edible vegetable oil products. The content of RRR-α-tocopheryl acetate may be adjusted by suitable physical or chemical means. It is unstable in the presence of alkalies. It is insoluble in water; soluble in alcohol; and miscible with acetone, with chloroform, with ether, and with vegetable oils.

Function Nutrient.

REQUIREMENTS

Labeling Indicate the milligrams per gram of RRR-α-tocopheryl acetate present. All label claims that are in terms of International Units (IU) should be based on the following: 1 mg of RRR-α-tocopheryl acetate = 1.36 IU.
Identification A sample meets the requirements of *Identification Tests A* and *B* in the monograph for *RRR-α-Tocopheryl Acetate*.
Assay Not less than 40.0% of $C_{31}H_{52}O_3$.
Acidity Passes test.
Lead Not more than 2 mg/kg.
Optical (Specific) Rotation $[\alpha]_D^{25°}$: Not less than +24°.

TESTS

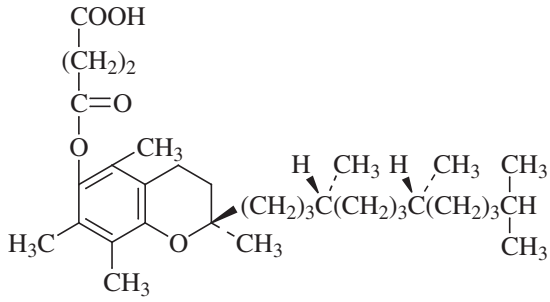
Note: In the *Assay* and *Optical (Specific) Rotation* tests, use low-actinic glassware for all solutions containing tocopherols.

Assay Proceed as directed under *Assay* in the monograph for *all-rac-α-Tocopheryl Acetate*, using the following as the *Assay Preparation*: Dissolve an accurately weighed amount of sample, equivalent to about 30 mg of RRR-α-tocopheryl acetate, in 10.0 mL of the *Internal Standard Solution*.
Acidity Determine as directed in the monograph for *all-rac-α-Tocopheryl Acetate*.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Optical (Specific) Rotation Determine as directed in the monograph for *DL-α-Tocopheryl Acetate*, using an accurately weighed sample equivalent to about 200 mg of α-tocopherol as the *Test Solution*.

Packaging and Storage Store in tight, light-resistant containers.

RRR-α-Tocopheryl Acid Succinate

D-α-Tocopheryl Acid Succinate



$C_{33}H_{54}O_5$ Formula wt 530.79

CAS: [4345-03-3]

DESCRIPTION

RRR-α-Tocopheryl Acid Succinate occurs as a white to off white, crystalline powder. It is a form of vitamin E obtained by the vacuum steam distillation and succinylation of edible vegetable oil products. It is stable in air, but is unstable to alkali and to heat. It is insoluble in water; soluble in acetone, in alcohol, in ether, and in vegetable oils; and very soluble in chloroform. It melts at about 75°.

Function Nutrient.

REQUIREMENTS

Labeling All label claims that are in terms of International Units (IU) should be based on the following: 1 mg of RRR-α-Tocopheryl Acid Succinate = 1.21 IU.
Identification

A. Add, with swirling, 2 mL of nitric acid to 10 mL of the *Test Solution* from *Optical (Specific) Rotation* (below), and heat at about 75° for 15 min. A bright red to orange color develops.

B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the *Internal Standard Solution*, as obtained in the *Assay* (below).

Assay Not less than 96.0% and not more than 102.0% of $C_{33}H_{54}O_5$.
Acidity Passes test.
Lead Not more than 2 mg/kg.
Optical (Specific) Rotation $[\alpha]_D^{25°}$: Not less than +24°.

TESTS

Note: In the *Assay* and *Optical (Specific) Rotation* tests, use low-actinic glassware for all solutions containing tocopherols.

Assay

Internal Standard Solution Prepare a solution containing about 3 mg of hexadecyl hexadecanoate, accurately weighed, in each milliliter of *n*-hexane.

Standard Preparation Transfer about 30 mg of USP Alpha Tocopheryl Acid Succinate Reference Standard, accurately weighed, into a 4-dram (approximately 15-mL) screw-cap vial. Pipet 2.0 mL of absolute methanol, 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of concentrated hydrochloric acid into the vial, cap, mix well, and allow to stand in the dark for 1 h. Evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Pipet 10.0 mL of the *Internal Standard Solution* into the vial, cap, and shake vigorously.

Assay Preparation Prepare as directed for the *Standard Preparation*, using an accurately weighed amount of sample equivalent to about 30 mg of RRR-α-Tocopheryl Acid Succinate.

Chromatographic System Use a gas chromatograph equipped with a flame-ionization detector and a 2-m × 4-mm (id) borosilicate glass column, or equivalent, packed with 2% to 5% methylpolysiloxane gum on 80- to 100-mesh, acid-washed, base-washed, silanized, chromatographic diatomaceous earth, or equivalent materials. The column should have a glass-lined sample-introduction system or on-column injection. Maintain the column isothermally between 260° and 280°, the injection port at about 290°, and the detector block at about 300°. Use a dry carrier gas, with the flow rate adjusted to obtain a hexadecyl hexadecanoate peak 12 to 14 min after sample introduction.

Note: Cure and condition the column as necessary before use (see *Procedure* in *Gas Chromatography* under *Chromatography*, Appendix IIA).

System Suitability Chromatograph a suitable number of injections of 1 mg each of USP Alpha Tocopherol Reference Standard and USP Alpha Tocopheryl Acetate Reference Standard per milliliter of *n*-hexane, as directed under *Calibration* (below), to ensure that the resolution factor, *R*, is not less than 1.0 (see *System Suitability* in *High-Performance Liquid Chromatography* under *Chromatography*, Appendix IIA).

Calibration Chromatograph successive 2- to 5-μL portions of the upper layer of the *Standard Preparation* until the relative response factor, *F*, is constant (i.e., within a range of approximately 2%) for three consecutive injections. If using graphic integration, adjust the instrument to obtain 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the major peaks occurring at relative retention times of approximately 1.00 (hexadecyl hexadecanoate) and 1.99 (methyl α-tocopheryl succinate), and record the values as A_I and A_S, respectively. Calculate *F* by the formula

$$(A_S/A_I) \times (C_I/C_S),$$

in which C_I and C_S are the exact concentrations, in milligrams per milliliter, of hexadecyl hexadecanoate and of USP Alpha Tocopheryl Acid Succinate Reference Standard in the *Standard Preparation*, respectively.

Procedure Chromatograph 2 to 5 μL of the *Assay Preparation* as described under *Calibration*. Measure the areas under the major peaks occurring at relative retention times of approximately 1.00 (hexadecyl hexadecanoate) and 1.99 (methyl α-tocopheryl succinate), and record the values as a_I and a_U, respectively. Calculate the weight, in milligrams, of RRR-α-Tocopheryl Acid Succinate in the sample by the formula

$$(10C_I/F) \times (a_U/a_I),$$

in which *F* is the relative response factor (see *Calibration*)

Acidity Dissolve 1.0 g of sample in 25 mL of a 1:1 (v/v) alcohol:ether mixture that has been neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide, add 0.5 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 30 s. Between 18.0 mL and 19.3 mL of 0.1 *N* sodium hydroxide is required.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Optical (Specific) Rotation

Test Solution Transfer an accurately weighed amount of sample, equivalent to about 200 mg of α-tocopherol, into a 250-mL round-bottom, glass-stoppered flask; dissolve it in 50 mL of absolute alcohol; and reflux for 1 min. While the solution is boiling, add 1 g of potassium hydroxide pellets, one at a time to avoid overheating, through the condenser.

Caution: Wear safety goggles.

Continue refluxing for 20 min, and then, without cooling, add 2 mL of hydrochloric acid, dropwise, through the condenser. (This technique is essential to prevent oxidative action by air while the sample is in an alkaline medium.) Cool, and transfer the contents of the flask into a 500-mL separator, rinsing the flask with 100 mL each of water and of ether and adding the rinsings to the separator. Shake vigorously, allow the layers to separate, and collect each of the two layers in separate separators. Extract the aqueous layer with two 50-mL portions of ether, and add these extracts to the main ether extract. Wash the combined ether extracts with four 100-mL portions of water, and then evaporate the solutions on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 mL remain. Complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in a 1:72 solution of 2 *N* sulfuric acid in alcohol, transfer to a 200-mL volumetric flask, dilute to volume with the same, and mix. Save 10 mL of this solution for the *Identification Test A* (above).

Procedure Transfer an accurately measured volume of the *Test Solution*, equivalent to about 100 mg of α-tocopherol, into a separator, and add 200 mL of water. Extract first with 75 mL, then with two 25-mL portions of ether, and combine the ether extracts in another separator. Add 20 mL of a 10% solution of potassium ferricyanide in a 1:125 sodium hydroxide solution to the ether solution, and shake for 3 min. Wash the ether solution with four 50-mL portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 mL remains, and then complete the evaporation, removing the

last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 mL of isooctane, and determine the specific rotation. Calculate the specific rotation [see *Specific (Optical) Rotation*, Appendix IIB], using as *c* the concentration expressed as the number of grams of RRR-α-Tocopheryl Acid Succinate, as determined in the *Assay* (above), in 100 mL of solution.

Packaging and Storage Store in tight, light-resistant containers.

Tragacanth

Gum Tragacanth; Tragacanth Gum

INS: 413 CAS: [9000-65-1]

DESCRIPTION

Tragacanth is a dried, gummy exudation obtained from the stems and branches of *Astragalus gummifer* Labillardiere, or other Asiatic species of *Astragalus* (Fam. Leguminosae). It consists mainly of high-molecular-weight polysaccharides (arabinogalactans and acidic polysaccharides) that, on hydrolysis, yield galacturonic acid, galactose, arabinose, xylose, and fucose; small amounts of rhammnose and of glucose (derived from traces of starch and/or cellulose) may also be present. Unground Tragacanth occurs as flattened, lamellated, frequently curved fragments or straight or spirally twisted linear pieces from 0.5 to 2.5 mm in thickness. It is white to weak yellow (although some pieces may have a red tinge) and translucent, with a horny texture and a short fracture. It is easier to pulverize if heated to 50°. Powdered Tragacanth is white to yellow-white or pink-brown.

Function Stabilizer; thickener; emulsifier.

REQUIREMENTS

Identification

A. Microscopic examination of an unground sample in water mounts reveals numerous angular fragments with circular or irregular lamellae, and starch grains up to 25 μm in diameter. There should be very few or no fragments of lignified vegetable tissue.

B. Add iodinated zinc chloride solution to a representative sample. Cellular membranes turn violet.

C. One gram of sample in 50 mL of water swells to form a smooth, stiff, opalescent mucilage free from cellular fragments. The same amount of sample does not swell in 60% (w/v) aqueous ethanol.

Ash (Total) Not more than 3.0%.

Ash (Acid-Insoluble) Not more than 0.5%.

Karaya Gum Passes test.

Lead Not more than 2 mg/kg.

Viscosity of a 1% Solution Not less than 250 centipoises.

TESTS

Note: Prepare unground samples by powdering them to pass through a Number 45 sieve, and mix well.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.

Ash (Acid-Insoluble) Determine as directed under *Ash (Acid-Insoluble)*, Appendix IIC.

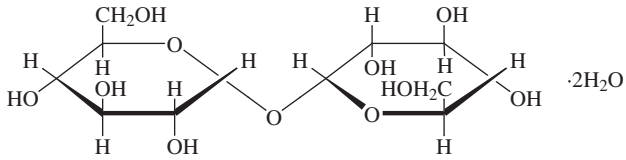
Karaya Gum Boil 1 g of sample with 20 mL of water until they form a mucilage, add 5 mL of hydrochloric acid, and boil the mixture for 5 min. No permanent pink or red color appears.

Lead Determine as directed for the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Viscosity Transfer 4.0 g of finely powdered sample into the container of a stirring apparatus equipped with blades capable of revolving at 10,000 rpm. Add 10 mL of alcohol, swirl to wet the gum uniformly, and then add 390 mL of water, avoiding the formation of lumps. Immediately stir the mixture for 7 min, pour the resulting dispersion into a 500-mL bottle, insert a stopper, and allow it to stand for about 24 h in a water bath at 25°. Determine the apparent viscosity at this temperature with a Model LVF Brookfield, or equivalent, viscometer (see *Viscosity of Cellulose Gum*, Appendix IIB), using Spindle No. 2 at 30 rpm and a factor of 10.

Packaging and Storage Store in well-closed containers.

Trehalose



$C_{12}H_{22}O_{11}\cdot 2H_2O$ Formula wt 378.33

CAS: anhydrous [99-20-7]
CAS: dihydrate [6138-23-4]

DESCRIPTION

Trehalose occurs as a nonhygroscopic, white, crystalline powder. It is obtained through enzymatic conversion of food-grade starch into a stable, nonreducing disaccharide with two glucose molecules linked in an α,α-1,1 configuration. The powder is freely soluble or readily dispersible in water. Trehalose is typically used in its dihydrate form.

Function Humectant; nutritive sweetener; stabilizer; thickener; texturizer.

REQUIREMENTS

Identification Observe a sample with a light microscope at 50×. Trehalose is composed of colorless, rectangular crystals with a prismatic structure.
Assay Not less than 98.0% Trehalose, calculated on a dry basis.
Color in Solution Less than or equal to 0.100.
Lead Not more than 0.1 mg/kg.
Loss on Drying Not more than 1.5%.
pH of a 30% Solution Between 4.5 and 6.5.
Residue on Ignition Less than or equal to 0.05%.
Turbidity of a 30% Solution Less than or equal to 0.050.

TESTS

Assay

Trehalose Standard Preparation Determine the water content of a Trehalose standard (available from Hayashibara International, Inc., Suite 200, 8670 Wolff Court, Westminster, CO 80031-6953) as directed under *Water Determination*, Appendix IIB. Use the calculated dry weight as the value for 100% pure Trehalose. Prepare the *Trehalose Standard Solution* by dissolving 1 g of anhydrous Trehalose in sufficient water to bring the volume to 25 mL.

Sample Solution Preparation Transfer 3 g of sample into a 100-mL volumetric flask, and dilute to volume with water (3% w/v). Filter the solution through a 0.45-μm membrane filter.

Procedure Use a suitable HPLC system (see *General Provisions*) equipped with a differential refractometer and a 300-mm × 10-mm (id) column (Shodex Ionpack KS 801, or equivalent) heated to 35°. Use a 73:27 mixture of chromatographic-grade acetonitrile and water as the mobile phase. Prepare two samples of *Sample Solution* and one of *Trehalose Standard Solution* by adding 10 mL of acetonitrile to each, and mixing. Filter each solution through a 0.45-μm membrane filter, and separately inject 20 μL of each solution into the chromatograph. Operate the instrument as directed by the HPLC manufacturer.

Calculation Calculate the area of the *Trehalose Standard Solution* peak, and use the data to determine the percent Trehalose concentration in the sample using the following formula:

$$100(A/B \times C/D),$$

in which A and B are the areas of the Trehalose peak of the *Sample Solution* and of the *Trehalose Standard Solution*, respectively; and C and D are the concentrations, in grams per milliliter, of the *Trehalose Standard Solution* and of the *Sample Solution* injected, respectively.

Color in Solution

Sample Preparation Dissolve 33 g of sample in 67 g of recently boiled water. Confirm the concentration of the solution as 30% ± 1% with a refractometer.

Procedure Use a suitable spectrophotometer (see *General Provisions*) to measure the absorbance of the *Sample Preparation* at 420 nm and 720 nm in a 1-cm cuvette.

Calculation Determine the color in solution by the following formula:

$$A_{420} - A_{720},$$

in which A_{420} is the absorbance of the *Sample Preparation* at 420 nm, and A_{720} is the absorbance of the *Sample Preparation* at 720 nm.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test,* Appendix IIIB, using a 5-g sample.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 60° for 5 h.
pH of a 30% Solution

Sample Preparation Dissolve 33 g of sample in 67 g of recently boiled water. Confirm the concentration of the solution as 30% ± 1% with a suitable refractometer (see *General Provisions*).

Procedure Determine as directed under *pH Determination,* Appendix IIB.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 5-g sample.
Turbidity of a 30% Solution

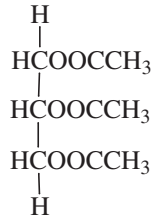
Sample Preparation Dissolve 33 g of sample in 67 g of recently boiled water. Confirm the concentration of the solution as 30% ± 1% with a suitable refractometer (see *General Provisions*).

Procedure Using a suitable spectrophotometer (see *General Provisions*), determine the absorbance of the *Sample Preparation* at 720 nm.

Packaging and Storage Store in tight containers in a dry place.

Triacetin

Glyceryl Triacetate



$C_9H_{14}O_6$ Formula wt 218.21

INS: 1518 CAS: [102-76-1]

View IR

DESCRIPTION

Triacetin occurs as a colorless, somewhat oily liquid. It is soluble in 14 parts water, and is miscible with alcohol, with ether, and with chloroform. It distills between 258° and 270°.

Function Humectant; solvent.

REQUIREMENTS

Identification

A. Heat a few drops of sample in a test tube with about 500 mg of potassium bisulfate. Pungent vapors of acrolein evolve.

B. The solution resulting from the *Assay* (below) gives positive tests for *Acetate*, Appendix IIIA.

Assay Not less than 98.5% of $C_9H_{14}O_6$.

Acidity Passes test.

Lead Not more than 1 mg/kg.

Refractive Index Between 1.429 and 1.431 at 25°.

Specific Gravity Between 1.154 and 1.158.

Unsaturated Compounds Passes test.

Water Not more than 0.2%.

TESTS

Assay Transfer about 1 g of sample, accurately weighed, into a suitable pressure bottle; add 25.0 mL of 1 *N* potassium hydroxide and 15 mL of isopropanol; stopper the bottle; and wrap securely in a canvas bag. Place in a water bath maintained at 98° ± 2°, and heat for 1 h, allowing the water in the bath to just cover the liquid in the bottle. Remove the bottle from the bath, cool in air to room temperature, then loosen the wrapper, uncap the bottle to release any pressure, and remove the wrapper. Add 6 to 8 drops of phenolphthalein TS, and titrate the excess alkali with 0.5 *N* sulfuric acid just to the disappearance of the pink color. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.5 *N* sulfuric acid is equivalent to 36.37 mg of $C_9H_{14}O_6$.

Acidity Transfer about 25 g of sample, accurately weighed, into a 125-mL conical flask; add 50 mL of toluene and 2 drops of thymol blue TS; and titrate rapidly with 0.02 *M* sodium methoxide in toluene. Swirl the flask continuously until the yellow color changes to a dark color, and then continue the titration without stopping but slowing the addition of titrant until a single drop changes the solution to a clear blue color. The endpoint is stable for about 8 to 15 s. Not more than 1.0 mL of 0.02 *M* sodium methoxide is required.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, at 25° using an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see *General Provisions*).

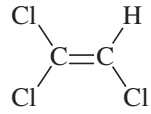
Unsaturated Compounds Add, dropwise, a 1:100 (v/v) solution of bromine in carbon tetrachloride to 10 mL of sample in a glass-stoppered tube until a permanent yellow color appears, and allow to stand in a dark place for 18 h. No turbidity or precipitate forms.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Trichloroethylene

Ethylene Trichloride; Trichloroethene; 1,1,2-Trichloroethylene



C_2HCl_3 Formula wt 131.39

CAS: [79-01-6]

DESCRIPTION

Trichloroethylene occurs as a clear, colorless liquid free from sediment and suspended matter. It is immiscible with water, but is miscible with alcohol, with ether, and with acetone. Its refractive index at 20° is about 1.477. It may contain a suitable stabilizer.

Function Extraction solvent.

REQUIREMENTS

Identification Transfer 5 mL of sample into a glass-stoppered test tube, add 5 mL of bromine TS, and shake vigorously every 15 min for 1 h. The color of the bromine fades, and a white turbidity forms in the lower layer.

Acidity (as HCl) Not more than 10 mg/kg.

Alkalinity (as NaOH) Not more than 10 mg/kg.

Distillation Range Between 86° and 88°.

Free Halogens Passes test.

Lead Not more than 1 mg/kg.

Nonvolatile Residue Not more than 10 mg/kg.

Specific Gravity Between 1.454 and 1.458.

Water Not more than 0.05%.

TESTS

Acidity (as HCl) **or Alkalinity** (as NaOH) Transfer 25 mL of water and 2 drops of phenolphthalein TS into a 250-mL glass-stoppered flask, and add 0.01 *N* sodium hydroxide to the first appearance of a slight pink color. Add 25 mL of sample, and shake for 30 s. If the pink color persists, titrate with 0.01 *N* hydrochloric acid, shaking repeatedly, until the pink color just disappears. Not more than 0.9 mL is required. If the pink color is discharged when the sample is added, titrate with 0.01 *N* sodium hydroxide until the faint pink color is restored. Not more than 1.0 mL is required.

Distillation Range Determine as directed under *Distillation Range*, Appendix IIB.

Free Halogens Combine 10 mL of sample with 10 mL of a 1:10 potassium iodide solution and 1 mL of starch TS, and shake vigorously for 2 min. The water layer does not turn blue.

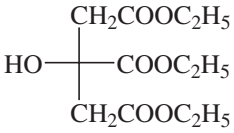
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Nonvolatile Residue (**Caution**: Carry out this procedure in a fume hood.) Evaporate 69 mL (about 100 g) of sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in tight containers.

Triethyl Citrate

Ethyl Citrate



$C_{12}H_{20}O_7$ Formula wt 276.29

INS: 1519 CAS: [77-93-0]

DESCRIPTION

Triethyl Citrate occurs as a practically colorless, oily liquid. It is slightly soluble in water, but is miscible with alcohol and with ether.

Function Solvent.

REQUIREMENTS

Assay Not less than 99.0% and not more than 100.5% of $C_{12}H_{20}O_7$, calculated on the anhydrous basis.
Acidity (as citric acid) Not more than 0.02%.
Lead Not more than 2 mg/kg.
Refractive Index Between 1.439 and 1.443 at 25°; or between 1.440 and 1.444 at 20°.
Specific Gravity Between 1.135 and 1.139 at 25°.
Water Not more than 0.25%.

TESTS

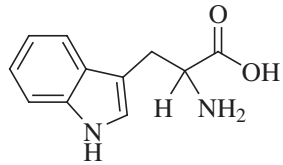
Assay Transfer about 1.5 g of sample, accurately weighed, into a 500-mL flask equipped with a standard-taper ground joint, and add 25 mL of isopropyl alcohol and 25 mL of water. Pipet 50 mL of 0.5 *N* sodium hydroxide into the mixture, add a few boiling chips, and attach a suitable water-cooled condenser. Reflux for 1.5 h, then cool, wash down the condenser with about 20 mL of water, add 5 drops of phenolphthalein TS, and titrate the excess alkali with 0.5 *N* sulfuric acid. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.5 *N* sodium hydroxide is equivalent to 46.05 mg of $C_{12}H_{20}O_7$.

Acidity (as citric acid) Dissolve 32 g of sample, accurately weighed, in 30 mL of alcohol neutralized to bromothymol blue, and titrate with 0.1 *N* sodium hydroxide. Not more than 1.0 mL is required.
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see *General Provisions*).
Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

DL-Tryptophan

DL-α-Amino-3-indolepropionic Acid



$C_{11}H_{12}N_2O_2$ Formula wt 204.22

CAS: [54-12-6]

View IR

DESCRIPTION

DL-Tryptophan occurs as white crystals or as a crystalline powder. It is soluble in water and in dilute acids and alkalies. It is sparingly soluble in alcohol. It is optically inactive.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_{11}H_{12}N_2O_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 300 mg of sample, previously dried at 105° for 3 h and accurately weighed, in 3 mL of formic

acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely.

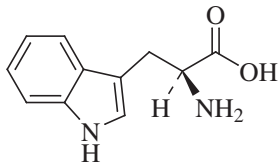
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed containers.

L-Tryptophan

L-α-Amino-3-indolepropionic Acid



$C_{11}H_{12}N_2O_2$ Formula wt 204.22

CAS: [73-22-3]

View IR

DESCRIPTION

L-Tryptophan occurs as white to yellow-white crystals or crystalline powder. One gram dissolves in about 100 mL of water. It is soluble in hot alcohol, in dilute hydrochloric acid, and in alkali hydroxide solutions.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_{11}H_{12}N_2O_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between −30.0° and −33.0°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between −29.7° and −32.7°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 300 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely.

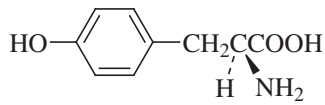
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 1 g of a previously dried sample in sufficient water to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed, light-resistant containers.

L-Tyrosine

L-β-(*p*-Hydroxyphenyl)alanine



$C_9H_{11}NO_3$ Formula wt 181.19

CAS: [60-18-4]

View IR

DESCRIPTION

L-Tyrosine occurs as colorless, silky needles or as a white, crystalline powder. One gram is soluble in about 230 mL of water. It is soluble in dilute mineral acids and in alkaline solutions. It is very slightly soluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 98.5% and not more than 101.5% of $C_9H_{11}NO_3$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between −11.3° and −12.3°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between −10.0° and −11.0°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 400 mg of sample, previously dried at 105° for 3 h and accurately weighed, into a 250-mL flask. Dissolve the sample in about 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a blue-green endpoint.

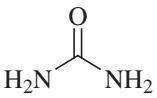
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 18.12 mg of $C_9H_{11}NO_3$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 5 g of a previously dried sample in sufficient 1 *N* hydrochloric acid to make 100 mL.
Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Packaging and Storage Store in well-closed containers.

Urea

Carbamide



CH_4N_2O Formula wt 60.06

CAS: [57-13-6]

DESCRIPTION

Urea occurs as a colorless to white, prismatic, crystalline powder or as small, white pellets. It is commonly produced from CO_2 by ammonolysis or from cyanamide by hydrolysis. It is freely soluble in water and in boiling alcohol, but practically insoluble in chloroform and in ether. It melts at a range of 132° to 135°.

Function Fermentation aid.

REQUIREMENTS

Identification

A. Heat about 500 mg of sample in a test tube until it liquefies. Ammonia vapor is produced. Continue heating until the liquid becomes turbid, and then cool. Dissolve the fused mass in a 1:10 sodium hydroxide solution:water mixture. Add 1 drop of cupric sulfate TS. A red-violet colored solution develops.

B. Dissolve 100 mg of sample in 1 mL of water, and add 1 mL of nitric acid. A white precipitate of urea nitrate forms.
Assay Not less than 99.0% and not more than 100.5% of CH_4N_2O.
Alcohol-Insoluble Matter Not more than 0.04%.
Chloride Not more than 0.007%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 1.0%.
Residue on Ignition Not more than 0.1%.
Sulfate Not more than 0.01%.

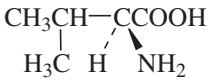
TESTS

Assay Transfer about 500 mg of sample, accurately weighed, into a 200-mL volumetric flask, and dissolve it in 100 mL of water, dilute to volume with water, and mix. Pipet 2 mL of this solution into a semimicro Kjeldahl digestion flask, and proceed as directed in *Method II* under *Nitrogen Determination*, Appendix IIIC. Heat the sample until it begins to fume, and then heat for 1 additional hour. Each milliliter of 0.01 *N* acid is equivalent to 0.3003 mg of CH_4N_2O.
Alcohol-Insoluble Matter Dissolve about 5 g of sample, accurately weighed, in 50 mL of warm alcohol. If any residue remains, filter the solution through a tared filter, wash the residue, and filter with 20 mL of warm alcohol. Dry at 105° for 1 h. Cool in a desiccator, and weigh.
Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, using 0.2 g of sample, and 14 μg of chloride (Cl) ion in the control.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using 2 g of sample, and 10 μg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.
Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB, using 2 g of sample, and 200 μg of sulfate (SO_4) ion in the control.

Packaging and Storage Store in a well-closed container.

L-Valine

L-2-Amino-3-methylbutyric Acid



$C_5H_{11}NO_2$ Formula wt 117.15

CAS: [72-18-4]

View IR

DESCRIPTION

L-Valine occurs as a white, crystalline powder. It is freely soluble in water, and practically insoluble in alcohol and in ether. The pH of a 1:20 aqueous solution is between 5.5 and 7.0. In a closed capillary tube, it melts at about 315°.

Function Nutrient.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.5% and not more than 101.5% of $C_5H_{11}NO_2$, calculated on the dried basis.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 0.3%.
Optical (Specific) Rotation $[\alpha]_D^{20°}$: Between +26.7° and +29.0°, calculated on the dried basis; or $[\alpha]_D^{25°}$: Between +26.6° and +28.9°, calculated on the dried basis.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 200 mg of sample, accurately weighed, in 3 mL of formic acid and 50 mL of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely.

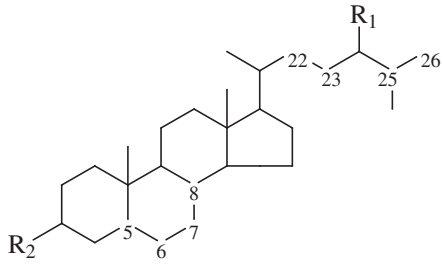
> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination (see *General Provisions*), and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 11.72 mg of $C_5H_{11}NO_2$.
Lead Determine as directed under *Lead Limit Test*, Appendix IIIB, using a *Sample Solution* prepared as directed for organic compounds, and 5 µg of lead (Pb) ion in the control.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 3 h.
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 mL.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 1-g sample.

Packaging and Storage Store in well-closed containers.

Vegetable Oil Phytosterol Esters



R_1 = H, Δ^5 (Cholesterol)

R_1 = CH_3, Δ^5, Δ^{22} (Brassicasterol)

R_1 = CH_3, Δ^5 or = CH_3 (Campesterol)

R_1 = C_2H_5, Δ^5, Δ^{22} (Stigmasterol)

R_1 = C_2H_5, Δ^5 or = C_2H_5 (β-Sitosterol)

R_1 = C_2H_5, Δ^5, Δ^{25} (Δ^5-Avenasterol)

R_1 = C_2H_5, Δ^7 (Δ^7-Stigmastenol = Δ^7-β-Sitosterol)

R_1 = C_2H_5, Δ^7, Δ^{25} (Δ^7-Avenasterol)

R_2 = Vegetable oil derived fatty acid

Δ^5 = double C bond at 5,6 position

Δ^7 = double C bond at 7,8 position

Δ^{22} = double C bond at 22,23 position

Δ^{25} = double C bond at 25,26 position

View IR

DESCRIPTION

Vegetable Oil Phytosterol Esters occur as a light yellow, thick liquid to waxy solid at room temperature and become fully liquid at elevated temperatures. Liquid Vegetable Oil Phytosterol Esters are more viscous than vegetable oils. They are obtained by esterification of free vegetable oil sterols with fatty acids obtained from vegetable oils or fats. (Vegetable oil sterols are commercially obtained by various processes, including vacuum distillation of vegetable oils or fats such as those recovered from deodorizer distillates.) They are freely soluble in edible oils, in chloroform, and in methylene chloride; partly soluble in alcohol, in acetone, in ethyl acetate, and in hexane; and insoluble in water.

Function Source of phytosterols.

REQUIREMENTS

Labeling Label as Vegetable Oil (Plant) Sterol Esters to indicate that the vegetable oil sterols and the fatty acids originate from vegetable oils. Declare the presence of any preservative, antioxidant, or other added substance.

Identification

A. The retention times of the major peaks in the chromatogram of the *Assay Procedure* for *Sterol Content* (below) are the same as that of the *Standard Preparation*, both relative to the *Internal Standard*, as obtained in the *Assay* (below).

B. The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.

Assay Not less than 86.0% of Vegetable Oil Phytosterol Esters and not more than 9.0% of free vegetable oil sterols, the sum not less than 95.0%. Not less than 59.0% of des-methyl-sterols. Not more than 5.0% of acyl-glycerides. The vegetable oil sterols in Vegetable Oil Phytosterol Esters show the following typical distribution:

Vegetable Oil Sterols in Vegetable Oil Phytosterol Esters

Sterol	Min (%)	Max (%)
Cholesterol	0.0	2.0
Brassicasterol	0.0	12.0
Campesterol plus Campestanol	10.0	40.0
Stigmasterol	0.0	30.0
β-Sitosterol plus Sitostanol	30.0	65.0
Δ^5-Avenasterol plus Δ^7-Stigmastenol	0.0	6.0
Δ^7-Avenasterol plus others	0.0	7.0

Acidity Not more than 0.2 expressed as gram of potassium hydroxide (KOH) per kilogram of Vegetable Oil Phytosterol Esters.

Lead Not more than 0.1 mg/kg.

Loss on Drying Not more than 0.1%.

TESTS

Assay

Total Level of Des-methyl-sterols

Internal Standard Solution Transfer about 1 g of β-cholestanol (5α-cholestan-3β-ol), accurately weighed, into a 10-mL volumetric flask, dissolve in and dilute to volume with methyl-tertiary butyl ether to a final concentration of 100 mg/mL.

Saponification Solution Dissolve about 14 g of potassium hydroxide in 10 mL of boiled water in a 100-mL volumetric flask. Dilute to volume with ethanol.

Standard Preparation Transfer about 25 mg of a commercially available soybean sterol mix into a 10-mL volumetric flask. Dissolve in and dilute to volume with heptane to a final concentration of about 2.5 mg/mL. Dilute a suitable portion 1:40 with heptane.

Assay Preparation Melt a sample in an oven to obtain a clear and homogenous liquid. Transfer about 50 mg of melted sample, accurately weighed, into a 10-mL reaction vial with screw cap, and add 200 µL of *Internal Standard Solution* and 1 mL of the *Saponification Solution*. Hydrolyze the sample by heating for 50 min at 70°, shaking at regular intervals to ensure proper mixing. Add 1 mL of water, and extract the free vegetable oil sterols and the *Internal Standard Solution* with 5 mL of heptane. Repeat the extraction three more times using 3 mL of heptane each time. Combine the heptane layers, and use anhydrous sodium sulfate to remove any traces of water. Dilute a suitable portion of the combined, dried heptane layers 1:40 with heptane.

Procedure Use a gas chromatograph equipped with a flame-ionization detector, a cold-on-column injector, a suitable deactivated precolumn, and a 10-m × 0.32-mm (id) capillary column coated with an apolar stationary phase 0.12-µL film thickness. Program the column to heat to 60°, hold for 1 min, heat to 300° at 20°/min, and hold for 3 min. Set the flame-ionization detector to 320°. Chromatograph five injections of the *Standard Preparation*. Measure the responses. The relative standard deviation for each peak should be below 2%. The peaks for brassicasterol and campesterol should be baseline resolved ($R_S > 1.0$) and show no tailing. Measure the response of the *Internal Standard Solution* and all the individual sterols eluting in the relative retention window of 0.98 to 1.13.

Relative Retention Times, Relative to the *Internal Standard Solution*

Substance	Retention Time
Cholesterol	0.98
Internal Standard Solution	1.00
Brassicasterol	1.01
Campesterol, Campestanol	1.03
Stigmasterol	1.04
Δ^7-Campesterol, Clerosterol, β-Sitosterol, Sitostanol	1.06
Δ^5-Avenasterol, Δ^7-Stigmasterol	1.08
Δ^7-Avenasterol	1.11

Calculation Calculate the individual and total percentages of vegetable sterol esters in the sample by the formula

$$[(C_{IS} \times V_{IS} \times A_{sterol})/(A_{IS} \times W_S \times 10)] \times (purity_{IS}/100),$$

in which C_{IS} is the concentration, in milligrams per milliliter, of the *Internal Standard Solution*; V_{IS} is the volume, in microliters, of the *Internal Standard Solution*; A_{sterol} is the peak response of the sterol(s); A_{IS} is the peak response of the *Internal Standard Solution*; W is the weight, in milligrams, of the sample; and $purity_{IS}$ is the purity, in percent, of the *Internal Standard Solution*.

Relative Levels of Vegetable Oil Phytosterol Esters and Vegetable Oil Sterols

Assay Preparation Melt a sample in an oven to obtain a clear and homogeneous liquid. Transfer about 50 mg of melted sample, accurately weighed, into a 10-mL reaction vial with screw cap, and successively add 200 µL of *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA, 99%, silylation reagent) and 800 µL of pyridine (catalyst). Homogenize by means of a vortex, and silylate for 30 min at room temperature. Dilute with 9 mL of hexane.

Procedure (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector, a cold-on-column injector, a suitable deactivated precolumn, and a 10-m × 0.32-mm (id) capillary column coated with an apolar stationary phase of 0.12-μL film thickness, or equivalent. Program the column to heat to 80°, hold for 2 min, heat to 360° at 10°/min, and hold for 15 min. Set the flame-ionization detector to 370°. Chromatograph five injections of the *Standard Preparation* from *Total Level of Des-methyl-sterols* (above). Measure the responses. The relative standard deviation for each peak should be below 2%. The peaks for stigmasterol and campesterol should be baseline resolved (R_S > 1.0) and show no tailing. Measure the response of sterols (sum) and the sterol esters (sum). Inspect the chromatogram for the presence of partial acyl-glycerides and triacyl-glycerides. Derive the relative Vegetable Oil Phytosterol Esters and vegetable oil sterols content of the sample from the areas for each component group relative to the total area of the chromatogram, assuming uniform response.

Acidity Determine as directed under *Acid Value*, Appendix VII.

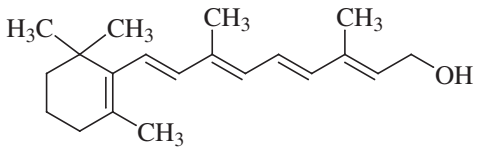
Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC.

Packaging and Storage Store in well-closed, light-resistant containers.

Vitamin A

All-*trans*-Retinol



CAS: [68-26-8]

DESCRIPTION

Vitamin A occurs as a light yellow to red oil that may solidify on refrigeration (liquid form). In solid form it may have the appearance of the diluent that has been added to it. It is a suitable form or derivative of retinol ($C_{20}H_{30}O$; Vitamin A alcohol). It usually consists of retinol or esters of retinol formed from edible fatty acids, principally acetic and palmitic acids, or mixtures of these. It may be diluted with edible oils, or it may be incorporated in solid edible carriers, extenders, or excipients. It may contain suitable preservatives, dispersants, and antioxidants, providing it is not to be used in foods in which such substances are prohibited. In liquid form it is very soluble in chloroform and in ether, it is soluble in absolute alcohol and in vegetable oils, but it is insoluble in glycerin and in water. In solid form it may be dispersible in water. It is unstable in air and light.

Function Nutrient.

REQUIREMENTS

Labeling Indicate the form of the Vitamin A; the presence of any preservative, dispersant, antioxidant, or other added substance; and the Vitamin A activity in terms of the equivalent amount of retinol in milligrams per gram and in International Units.

Identification

A. Dissolve an amount of sample equivalent to about 6 μg of retinol in 1 mL of chloroform, and add 10 mL of antimony trichloride TS. A transient blue color appears at once.

B. Assemble an apparatus for *Thin-Layer Chromatography* (see *Chromatography*, Appendix IIA), using chromatographic silica gel as the adsorbent and a 4:1 mixture of cyclohexane:ether as the solvent system. Prepare a *Standard Solution* by dissolving the contents of 1 capsule of USP Vitamin A Reference Standard in sufficient chloroform to make 25.0 mL.

If the sample is in liquid form, dissolve a volume representing approximately 15,000 International Units in sufficient chloroform to make 10 mL of *Sample Solution*. If the sample is in solid form, weigh a quantity representing approximately 15,000 International Units, place in a separator, add 75 mL of water, heat, if necessary, to dissolve the carrier, and cool. Shake vigorously for 1 min, extract with 10 mL of chloroform by shaking for 1 min, and centrifuge to clarify the chloroform extract.

At the starting point of the chromatogram, apply 0.015 mL of the *Standard Solution* and 0.01 mL of the *Sample Solution*. Develop the chromatogram in the chromatographic chamber lined with filter paper dipping into the solvent mixture. When the solvent has ascended 10 cm, remove the plate, allow it to dry in air, and spray it with antimony trichloride TS. The blue spot formed is indicative of the presence of retinol. The approximate R_f values of the predominant spots, corresponding to the different forms of retinol, are 0.1 for the alcohol, 0.45 for the acetate, and 0.7 for the palmitate.

Assay Not less than 95.0% and not more than 100.5% of the Vitamin A activity declared on the label.

Absorbance Ratio (corrected/observed at 325 nm) Not less than 0.85.

> **Note**: One International Vitamin A Unit is the specific biologic activity of 0.3 μg of the all-*trans* isomer of retinol.

Lead Not more than 2 mg/kg.

TESTS

Assay

If the sample is in the form of an ester (acetate or palmitate), use the following procedure:

Assay for Vitamin A Ester (Acetate or Palmitate) (**Note**: Use low-actinic glassware throughout this procedure.)

Mobile Phase Use *n*-hexane.

Standard Preparation Dissolve an accurately weighed quantity of USP Vitamin A Reference Standard (all-*trans* retinyl acetate) in *Mobile Phase*, and dilute quantitatively (and stepwise, if necessary), to obtain a solution having a known concentration of about 40 μg/mL.

System Suitability Preparation Dissolve an accurately weighed quantity of retinyl palmitate[1] in *Mobile Phase* to obtain a solution having a known concentration of about 40 μg/mL. Mix equal volumes of this solution and the *Standard Preparation* to obtain a solution having a concentration of about 20 μg/mL each of retinyl acetate and retinyl palmitate.

Assay Preparation Dissolve an accurately weighed quantity of sample in *Mobile Phase*, and dilute quantitatively (and stepwise, if necessary) to obtain a solution having a concentration of vitamin A ester (acetate or palmitate) of about 40 μg/mL.

Chromatographic System Use a high-performance liquid chromatograph equipped with a 325-nm detector and a 15-cm × 4.6-mm (id) column that contains 3-μm silica (Supelcosil LC-Si, or equivalent). Maintain the flow rate at about 1 mL/min.

Chromatograph the *System Suitability Preparation*, and measure the peak areas as directed under *Procedure*. The resolution, *R*, between the all-*trans* retinyl acetate and the all-*trans* retinyl palmitate peaks is not less than 10 (see *System Suitability* in *High-Performance Liquid Chromatography* under *Chromatography*, Appendix IIA), and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure Separately inject equal volumes (about 40 μL) of the *Standard Preparation* and the *Assay Preparation* into the chromatograph, record the chromatograms, and measure the peak areas for the all-*trans* retinyl acetate (or palmitate) and the 13-*cis* retinyl acetate (or palmitate), if present, obtained from the *Standard Preparation* and the peak areas for the all-*trans* retinyl acetate (or palmitate) and the 13-*cis* retinyl acetate (or palmitate), if present, in the chromatogram of the *Assay Preparation*. The relative retention times are about 0.7 for 13-*cis* retinyl acetate and 1.0 for all-*trans* retinyl acetate; or the relative retention times are about 0.8 for 13-*cis* retinyl palmitate and 1.0 for all-*trans* retinyl palmitate.

If the sample is in the form of the acetate, calculate the quantity, in milligrams per gram, of vitamin A acetate in the sample taken by the formula

$$(C/D)(r_U/r_S),$$

in which *C* is the concentration, in milligrams per milliliter, of USP Vitamin A Reference Standard in the *Standard Preparation*; *D* is the concentration, in milligrams per milliliter, of sample in the *Assay Preparation*; and r_U and r_S are the summed peak areas of the 13-*cis* and all-*trans* retinyl acetate obtained from the *Assay Preparation* and the *Standard Preparation*, respectively.

If the sample is in the form of the palmitate, calculate the quantity, in milligrams per gram, of vitamin A palmitate in the sample taken by the formula

$$(524.96/328.54)(C/D)(r_U/r_S),$$

in which 524.96 is the formula weight of vitamin A palmitate; 328.54 is the formula weight of vitamin A acetate; *C* is the concentration, in milligrams per milliliter, of USP Vitamin A Reference Standard in the *Standard Preparation*; *D* is the concentration, in milligrams per milliliter, of sample in the *Assay Preparation*; r_U is the summed peak areas of the 13-*cis* and all-*trans* retinyl palmitate obtained from the *Assay Preparation*; and r_S is the summed peak areas of the 13-*cis* and all-*trans* retinyl acetate obtained from the *Standard Preparation*.

Assay for Other Forms of Vitamin A

> **Note**: Complete the assay promptly and exercise care throughout the procedure to keep to a minimum exposure to atmospheric oxygen and other oxidizing agents and to actinic light, preferably by using an atmosphere of an inert gas and nonactinic glassware.

Isopropyl Alcohol Use reagent-grade isopropyl alcohol. Redistill, if necessary, to meet the following requirements for spectral purity: When measured in a 1-cm quartz cell against water, it shows an absorbance not greater than 0.05 at 300 nm and not greater than 0.01 between 320 and 350 nm.

Ether Use freshly redistilled reagent-grade ether, discarding the first and last 10% portions.

Procedure Transfer a portion of sample, accurately weighed and containing the equivalent of not less than 0.15 mg of retinol, but containing not more than 1 g of fat, into a saponification flask. If the sample is in solid form, heat the portion taken in 10 mL of water on a steam bath for about 10 min, crush the remaining solid with a blunt glass rod, and warm for about 5 min.

Add 30 mL of alcohol if the sample is liquid, or 23 mL of alcohol and 7 mL of glycerin if the sample is solid, followed by 3 mL of a 9:10 potassium hydroxide solution. Reflux under an all-glass condenser for 30 min. Cool the solution, add 30 mL of water, and transfer into a separator. Add 2 g of finely powdered sodium sulfate. Extract by shaking for 2 min with one 150-mL portion of ether and if an emulsion forms, with three additional 25-mL portions of ether. Combine the ether extracts, if necessary, and wash by swirling gently with 50 mL of water. Wash more vigorously with three additional 50-mL portions of water. Transfer the washed ether extract into a 250-mL volumetric flask, and add ether to volume.

Evaporate a 25.0-mL portion of the ether extract to about 5 mL. *Without applying heat and with the aid of a stream of inert gas or vacuum*, continue the evaporation to about 3 mL. Dissolve the residue in sufficient isopropyl alcohol to give an expected concentration of the equivalent of 3 to 5 μg of retinol per milliliter or such that it will give an absorbance in the range of 0.5 to 0.8 at 325 nm. Using a suitable spectrophotometer fitted with matched quartz cells, determine the absorbances of the resulting solution at the wavelengths of 310, 325, and 334 nm.

[1] A suitable grade of retinyl palmitate may be obtained from Sigma-Aldrich Company.

Calculation If A_{325} has a value not less than $[A_{325}]/1.030$ and not more than $[A_{325}]/0.970$, in which $[A_{325}]$ is the corrected absorbance at 325 nm and is given by the equation

$$[A_{325}] = 6.815A_{325} - 2.555A_{310} - 4.260A_{334},$$

in which A designates the absorbance at the wavelength indicated by the subscript, calculate the retinol value as follows:

$$\text{Content (in mg)} = 0.549A_{325}/LC,$$

in which L is the length, in centimeters, of the absorption cell; and C is the amount of sample, expressed as grams, in each 100 mL of the final isopropyl alcohol solution.

If $[A_{325}]$ has a value less than $A_{325}/1.030$, apply the following equation:

$$\text{Content (in mg)} = 0.549[A_{325}]/LC.$$

Confidence Interval The range of the limits of error, indicating the extent of discrepancy to be expected in the results of different laboratories at $P = 0.05$, is approximately ±8%.

Absorbance Ratio (corrected/observed at 325 nm) Determine by the formula

$$[A_{325}]/A_{325},$$

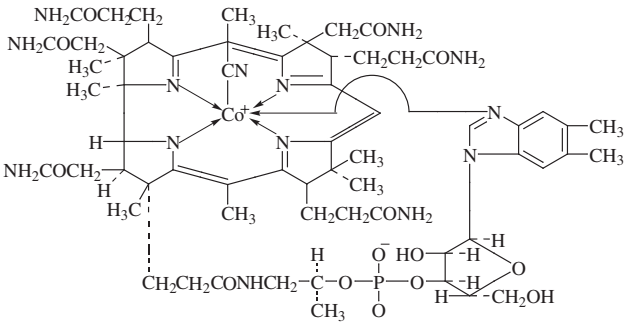
the terms of which are defined under *Calculation* in the *Assay*, as given above.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Packaging and Storage Store in a cool place in tight containers, preferably under an atmosphere of an inert gas, protected from light.

Vitamin B_{12}

Cyanocobalamin



$C_{63}H_{88}CoN_{14}O_{14}P$ Formula wt 1355.38

CAS: [68-19-9]

DESCRIPTION

Vitamin B_{12} occurs as dark red crystals or as an amorphous or crystalline powder. In the anhydrous form, it is very hygroscopic, and when exposed to air, it may absorb about 12% of water. It is sparingly soluble in water; soluble in alcohol; and insoluble in acetone, in chloroform, and in ether.

Function Nutrient.

REQUIREMENTS

Identification

A. The absorption spectrum of the *Sample Solution* used to measure absorbance in the *Assay* (below) exhibits maxima within ±1 nm at 278 nm and 361 nm, and within ±2 nm at 550 nm. The ratio A_{361}/A_{278} is between 1.70 and 1.90, and the ratio A_{361}/A_{550} is between 3.15 and 3.40.

B. Fuse about 1 mg of sample with about 50 mg of potassium pyrosulfate in a porcelain crucible, cool, and break up the mass with a glass rod. Add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, mix, and then add drops of a 1:10 solution of sodium hydroxide until just pink. Add 500 mg of sodium acetate, 0.5 mL of 1 *N* acetic acid, and 0.5 mL of a 1:500 solution of nitroso R salt. A red or orange-red color appears at once. Add 0.5 mL of hydrochloric acid, and boil for 1 min. The red color persists.

C. Dissolve about 5 mg of sample in 5 mL of water contained in a 50-mL distilling flask connected to a short, water-cooled, vertical condenser, the tip of which dips into a test tube containing 1 mL of a 1:50 solution of sodium hydroxide. Add 2.5 mL of hypophosphorous acid to the flask, then close the flask, heat at simmering for 10 min, and distill 1 mL into the test tube. Add 4 drops of cold, saturated ferrous ammonium sulfate solution to the tube, shake gently, then add about 30 mg of sodium fluoride, and bring the contents to a boil. Immediately add drops of 1:7 sulfuric acid until a clear solution results, and then add 3 to 5 drops more of the acid. A blue or blue-green color appears within a few minutes.

Assay Not less than 96.0% and not more than 100.5% of $C_{63}H_{88}CoN_{14}O_{14}P$, calculated on the dried basis.

Loss on Drying Not more than 12.0%.

Pseudo Cyanocobalamin Passes test.

TESTS

Assay With the aid of water, transfer about 30 mg of sample, accurately weighed, into a 1000-mL volumetric flask, dilute to volume with water, and mix to obtain a *Sample Solution*. Dissolve an accurately weighed quantity of USP Cyanocobalamin Reference Standard in water, and dilute quantitatively and stepwise with water to obtain a *Reference Standard Solution* having a known concentration of about 30 µg/mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorption at about 361 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in milligrams, of $C_{63}H_{88}CoN_{14}O_{14}P$ in the sample taken by the formula

$$C \times A_U/A_S,$$

in which C is the concentration of the *Reference Standard Solution*, in micrograms per milliliter, and A_U and A_S are the absorbances of the *Sample Solution* and the *Reference Standard Solution*, respectively.

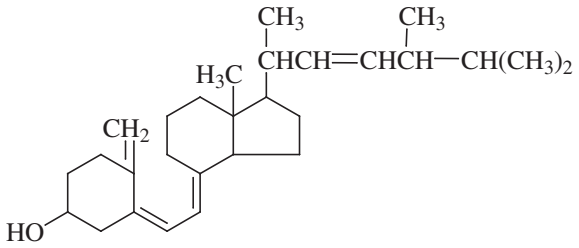
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying about 25 mg of sample at 105° in a vacuum at not more than 5 mm Hg for 2 h.

Pseudo Cyanocobalamin Dissolve 1.0 mg of sample in 20 mL of water contained in a small separator, add 5 mL of a 1:1 (v/v) mixture of carbon tetrachloride:cresol, and shake well for about 1 min. Allow the layers to separate, and draw off the lower layer into a second small separator. Add 5 mL of 1:7 sulfuric acid, shake well, and allow the solution to separate completely, centrifuging if necessary. The separated upper layer is colorless or has no more color than a mixture of 0.15 mL of 0.1 *N* potassium permanganate in 250 mL of water.

Packaging and Storage Store in tight containers.

Vitamin D_2

Ergocalciferol; Vitamin D



$C_{28}H_{44}O$ Formula wt 396.66

CAS: [50-14-6]

DESCRIPTION

Vitamin D_2 occurs as white crystals. It is affected by air and by light. It is insoluble in water, but it is soluble in alcohol, in chloroform, in ether, and in fatty oils.

Function Nutrient.

REQUIREMENTS

Identification

A. Prepare without heating, and handle without delay, (a) a 1:100 solution of squalane:chloroform containing 50 mg of sample per milliliter; (b) a solution of USP Ergocalciferol Reference Standard, using the same solvent and producing a solution with the same concentration; and (c) a 1:100 solution of squalane:chloroform containing 100 μg of USP Ergosterol Reference Standard per milliliter. Spot 10 μL each of the *Sample Solution*, the *Ergocalciferol Standard Solution*, and the *Ergosterol Standard Solution* on a line parallel to and about 2.5 cm from the bottom edge of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel containing a suitable fluorescing substance. Place the plate in a developing chamber containing, and equilibrated with, a mixture of equal volumes of cyclohexane and ether. Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Perform the development and subsequent operations in the dark. Remove the plate from the chamber, allow the solvent to evaporate, and spray with a 1:50 solution of acetyl chloride:antimony trichloride TS. The chromatogram obtained with the *Sample Solution* shows a yellow-orange area (ergocalciferol) having the same R_f value as the area of the *Ergocalciferol Standard Solution*, which may show a violet area underneath (use the chromatogram for the *Ergosterol* test, below).

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample, in the range of 2 to 12 μm, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Ergocalciferol Reference Standard.

C. The ultraviolet absorption spectrum of the sample in alcohol solution exhibits inflections at the same wavelengths as that of USP Ergocalciferol Reference Standard, similarly measured, and the respective absorptivities at 265 nm do not differ by more than 3.0%.

Assay Not less than 97.0% and not more than 103.0% of $C_{28}H_{44}O$.

Ergosterol Passes test.

Melting Range Between 115° and 119°.

Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +103° and +106°.

Reducing Substances Passes test.

TESTS

Assay

Mobile Phase Prepare a 3:1000 mixture of *n*-amyl alcohol:ACS Reagent-Grade Hexanes (The hexanes must be suitable for use in ultraviolet spectrophotometry and be dried by passing through a 60- × 8-cm column containing 500 g of 50- to 250-μm chromatographic siliceous earth. The ratio of components and the flow rate may be varied to meet the requirements of the *System Suitability Test*.)

Standard Preparation (**Note**: Use low-actinic glassware, and prepare solutions fresh daily.) Transfer about 30 mg of USP Ergocalciferol Reference Standard, accurately weighed, into a 50-mL volumetric flask, dissolve in toluene without heating, dilute to volume with toluene, and mix. Pipet 10 mL of this solution into a second 50-mL volumetric flask, dilute to volume with the *Mobile Phase*, and mix.

Assay Preparation (**Note**: Use low-actinic glassware, and prepare solutions fresh daily.) Transfer about 30 mg of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in toluene without heating, dilute to volume with toluene, and mix. Pipet 10 mL of this solution into a second 50-mL volumetric flask, dilute to volume with the *Mobile Phase*, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a suitable high-performance liquid chromatograph, operated at room temperature, and fitted with an ultraviolet detector that monitors absorption at 254 nm and with a 25-cm × 4.6-mm (id) stainless steel column, or equivalent,

packed with porous silica microparticles, 5 to 10 μm in diameter, or equivalent.

System Suitability Preparation Dissolve about 250 mg of USP Vitamin D Assay System Suitability Reference Standard in 10 mL of a 1:1 (v/v) mixture of toluene:*Mobile Phase*. Reflux this solution at 90° for 45 min, and cool. (This solution contains cholecalciferol, pre-cholecalciferol, and *trans*-cholecalciferol.)

System Suitability Test Chromatograph five injections of the *System Suitability Preparation*, and measure the peak responses as directed under *Procedure* (below). The relative standard deviation for the peak response does not exceed 2.0%, and the resolution between *trans*-cholecalciferol and pre-cholecalciferol is not less than 1.0 (see *System Suitability* under *Chromatography*, Appendix IIA). The chromatograms obtained in this test exhibit relative retention times of approximately 0.4, 0.5, and 1.0, for pre-cholecalciferol, *trans*-cholecalciferol, and cholecalciferol, respectively.

Procedure By means of a suitable sampling valve, introduce equal volumes (5 to 10 μL) of the *Standard Preparation* and the *Assay Preparation* into the chromatograph. Measure the peak responses for the major peaks, at corresponding retention times, obtained with the *Assay Preparation* and the *Standard Preparation*. Calculate the quantity, in milligrams, of $C_{28}H_{44}O$ in the sample taken by the formula

$$0.25C \times A_U/A_S,$$

in which C is the exact concentration, in micrograms per milliliter, of USP Ergocalciferol Reference Standard in the *Standard Preparation*, and A_U and A_S are the peak responses for ergocalciferol obtained with the *Assay Preparation* and the *Standard Preparation*, respectively.

Ergosterol The color of any violet area in the chromatogram of the *Sample Solution*, obtained as directed under *Identification Test B*, is not more intense than that of the violet area in the chromatogram of the *Ergosterol Standard Solution*.

Melting Range Determine as directed in the *Procedure for Class Ib* under *Melting Range or Temperature*, Appendix IIB.

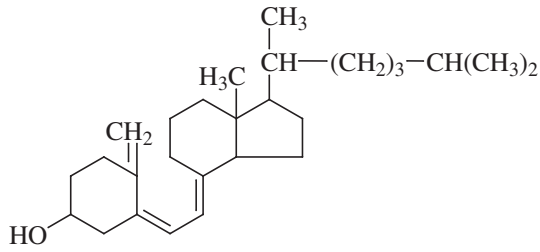
Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution in alcohol containing 50 mg of sample in each 10 mL. Prepare the solution without delay, using a sample from a container opened not longer than 30 min, and determine the rotation within 30 min after the solution has been prepared.

Reducing Substances Add 0.5 mL of a 1:200 solution of blue tetrazolium:absolute alcohol to 10 mL of a 1:100 *Sample Solution* in absolute alcohol. Add 0.5 mL of a 1:9 (v/v) solution of 10% tetramethylammonium hydroxide:absolute alcohol. Allow the mixture to stand for 5 min, then add 1 mL of glacial acetic acid, and mix. Prepare a blank by treating 10 mL of absolute alcohol in the same manner. The absorbance of the *Sample Solution*, determined at 525 nm with a suitable spectrophotometer against the blank, is not greater than that obtained with a solution containing 0.2 μg/mL of hydroquinone in absolute alcohol, similarly treated.

Packaging and Storage Store in hermetically sealed containers under nitrogen in a cool place protected from light.

Vitamin D_3

Cholecalciferol; Vitamin D



$C_{27}H_{44}O$ Formula wt 384.65

CAS: [67-97-0]

DESCRIPTION

Vitamin D_3 occurs as white crystals. It is affected by air and by light. It is insoluble in water. It is soluble in alcohol, in chloroform, and in fatty oils.

Function Nutrient.

REQUIREMENTS

Identification

A. Prepare without heating, and handle without delay, (a) a 1:100 solution of squalane:chloroform containing 50 mg of sample per milliliter; and (b) a solution of USP Cholecalciferol Reference Standard using the same solvent and producing a solution with the same concentration. Spot 10 μL each of the *Sample Solution* and of the *Cholecalciferol Standard Solution* on a line parallel to and about 2.5 cm from the bottom edge of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel containing a suitable fluorescing substance. Place the plate in a developing chamber containing, and equilibrated with, a mixture of equal volumes of cyclohexane and ether. Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Perform the development and subsequent operations in the dark. Remove the plate from the chamber, allow the solvent to evaporate, and spray with a 1:50 solution of acetyl chloride:antimony trichloride TS. The chromatogram obtained with the *Sample Solution* shows a yellow-orange area (cholecalciferol) having the same R_f value as the area of the *Cholecalciferol Standard Solution* and may show a violet area, attributed to 7-dehydrocholesterol, below the cholecalciferol area.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample, in the range of 2 to 12 μm, exhibits maxima only at the same wavelengths as those of a similar preparation of USP Cholecalciferol Reference Standard.

C. The ultraviolet absorption spectrum of the sample in a 1:100,000 solution in ethanol exhibits inflections at the same wavelengths as that of USP Cholecalciferol Reference Standard, similarly prepared, and the respective absorptivities at

the point of maximum absorbance occurring at about 265 nm do not differ by more than 3.0%.

Assay Not less than 97.0% and not more than 103.0% of $C_{27}H_{44}O$.

Melting Range Between 84° and 89°.

Optical (Specific) Rotation $[\alpha]_D^{25°}$: Between +105° and +112°.

TESTS

Assay Determine as directed in the monograph for *Vitamin D_2* but use USP Cholecalciferol Reference Standard instead.

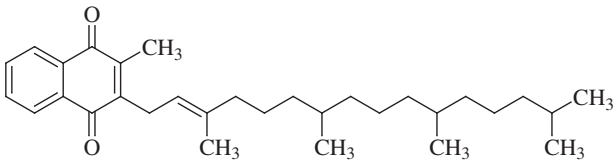
Melting Range Determine as directed in the *Procedure for Class Ib* under *Melting Range or Temperature*, Appendix IIB.

Optical (Specific) Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a solution in alcohol containing 50 mg of sample in each 10 mL. Prepare the solution without delay, using a sample from a container opened not longer than 30 min, and determine the rotation within 30 min after the solution has been prepared.

Packaging and Storage Store in hermetically sealed containers under nitrogen in a cool place protected from light.

Vitamin K

1,4-Naphthalenedione; 2-Methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl); Phylloquinone; Phytonadione



$C_{31}H_{46}O_2$ Formula wt 450.71

CAS: [84-80-0]

DESCRIPTION

Vitamin K occurs as a clear, yellow to amber, very viscous liquid. It has a specific gravity of about 0.967. It is stable in air, but decomposes on exposure to sunlight. It is insoluble in water; soluble in dehydrated alcohol, in chloroform, in ether, and in vegetable oils; and slightly soluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification

A. The infrared absorption spectrum of a thin film of the sample, formed between salt plates, exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Phytonadione Reference Standard.

B. The ultraviolet absorption spectrum of the sample in a solution containing 10 μg/mL in *n*-hexane exhibits maxima and minima at the same wavelengths as those of a similar solution of USP Phytonadione Reference Standard, concomitantly measured, and the respective absorptivities at 248 nm do not differ by more than 3.0%.

Assay Not less than 97.0% and not more than 103.0% of $C_{31}H_{46}O_2$.

Lead Not more than 2 mg/kg.

Menadione Passes test.

Refractive Index Between 1.523 and 1.526.

(Z) Isomer Content Not more than 21.0%.

TESTS

Assay (**Note**: Protect solutions containing Vitamin K from exposure to light.)

Mobile Phase Prepare a filtered and degassed 2000:1.5 solution of *n*-hexane:*n*-amyl alcohol.

Internal Standard Dissolve cholesteryl benzoate in *Mobile Phase* to obtain a solution having a concentration of 2.5 mg/mL.

Standard Preparation Transfer about 60 mg of USP Phytonadione Reference Standard, accurately weighed, into a 50-mL volumetric flask, add 20 mL of *Mobile Phase*, mix, dilute to volume with *Mobile Phase*, and mix again. Pipet 4 mL of the resulting solution into a 50-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix. Pipet 10 mL of this solution and 7 mL of *Internal Standard* into a 25-mL volumetric flask, dilute to volume with *Mobile Phase*, and mix.

Assay Preparation Prepare as directed under *Standard Preparation*, except use a portion of sample in place of the reference standard.

Chromatographic System Use a liquid chromatograph equipped with a 254-nm detector and a 25-cm × 4.6-mm (id) column that contains 5- to 10-μm porous silica microparticles (μPorasil, or equivalent). Set the flow rate to about 1 mL/min. Chromatograph replicate injections of the *Standard Preparation*, and record the peak responses as directed under *Procedure*. The relative standard deviation is not more than 2.0%, and the resolution, *R*, between (*Z*)-phytonadione and (*E*)-phytonadione is not less than 1.5 (see *System Suitability* in *High-Performance Liquid Chromatography* under *Chromatography*, Appendix IIA).

Procedure Separately inject equal volumes (about 50 μL) of the *Standard Preparation* and the *Assay Preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for the *Internal Standard*, 0.9 for (*Z*)-phytonadione, and 1.0 for (*E*)-phytonadione. Calculate the quantity, in milligrams, of $C_{31}H_{46}O_2$ in the portion of sample taken by the formula

$$1.56C(R_U/R_S),$$

in which *C* is the concentration, in micrograms per milliliter, of USP Phytonadione Reference Standard in the *Standard*

Preparation, and R_U and R_S are the relative peak response ratios for the *Assay Preparation* and the *Standard Preparation*, respectively. Calculate R_U and R_S by the formula

$$(r_Z + r_E)/r_I,$$

in which r_Z is the response for the (*Z*)-phytonadione peak, r_E is the response for the (*E*)-phytonadione peak, and r_I is the response for cholesteryl benzoate in the *Internal Standard* peak.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Menadione Mix about 20 mg of sample with 0.5 mL of a 1:1 (v/v) mixture of 6 *N* ammonium hydroxide:alcohol, add 1 drop of ethyl cyanoacetate, and shake gently. No purple or blue color appears.

Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.

(*Z*) Isomer Content (**Note**: Protect solutions containing Vitamin K from exposure to light.)

Mobile Phase, *Internal Standard*, *Assay Preparation*, *Chromatographic System*, and *Procedure* Determine as directed in the *Assay*, except calculate the percentage of (*Z*) isomer taken by the formula

$$100r_Z/(r_Z + r_E),$$

in which r_Z is the peak area of the (*Z*)-phytonadione isomer peak and r_E is the peak area of the (*E*)-phytonadione isomer peak obtained from the *Assay Preparation*.

Packaging and Storage Store in tight, light-resistant containers.

Wheat Gluten

Vital Wheat Gluten; Devitalized Wheat Gluten

CAS: [8002-80-0]

DESCRIPTION

Wheat Gluten occurs as a cream to light tan, free-flowing powder. It is the water-insoluble complex protein obtained by water extraction of wheat or wheat flour. It is soluble in alkalies, and partly soluble in alcohol and dilute acids. *Vital Wheat Gluten* is characterized by high viscoelasticity when hydrated, while *Devitalized Wheat Gluten* has lost this character because of denaturation by heat.

Function Dough strengthener; nutrient; stabilizer and thickener; surface-finishing agent; and texturizing agent.

REQUIREMENTS

Identification Add 40 mL of room-temperature water to 20 g of sample, and stir. *Vital Wheat Gluten* will form a cohesive, viscoelastic mass, which can be lifted with the stirring rod without breaking apart. *Devitalized Wheat Gluten* will not form such a mass.

Assay Not less than 71.0% protein, calculated on the dried basis.

Ash (Total) Not more than 2.0%, calculated on the dried basis.

Crude Fat Not more than 2.0%.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 10.0%.

Starch Not more than 21.0%.

TESTS

Assay Determine as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the percent protein by the formula

$$5.7 \times N,$$

in which *N* is the percent of nitrogen.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.

Crude Fat Determine as directed under *Crude Fat*, Appendix X.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 1-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 2-g sample at 105° for 2 h.

Starch The remainder, after subtracting from 100.0% the sum of the percents of *Ash (Total)*, *Loss on Drying*, and *Protein* (see *Assay*, above), represents the percent of starch in the sample.

Packaging and Storage Store in well-closed containers.

Wheat Protein Isolate

DESCRIPTION

Wheat Protein Isolate occurs as a powder. It is produced by acidic deamidation of gluten that converts glutamine and asparagine to their nonamidated derivatives, glutamic acid and aspartic acid, followed by several purification measures. Alternatively, gluten can be solubilized in an acidic or alkaline medium, and the dissolved protein is then separated and purified by filtration or centrifugation.

Function Texturizer; nutrient; emulsifier; water-binding aid; gelling agent; foaming agent.

REQUIREMENTS

Note: Calculate all analyses, except those for *Loss on Drying*, on the dried basis, drying to constant weight at 130°.

Labeling Indicate the protein content.
Identification Wheat Protein Isolate exhibits the compositional profile specified below with respect to *Ash*, *Fat*, *Loss on Drying*, and *Protein*.
Assay (as Protein) Not less than 75% protein.
Ash (Total) Not more than 8%.
Fat Not more than 6%.
Lead Not more than 0.5 mg/kg.
Loss on Drying Not more than 10%.
pH of a 10% Solution Between 4.3 and 7.5.

TESTS

Assay (as Protein) Determine the percent nitrogen as directed under *Nitrogen Determination*, Appendix IIIC, or by the Protein Nitrogen Combustion Method, AOAC 992.23 or AOCS Ba 4e-93. The percent protein equals percent $N \times 5.7$ and is calculated to exclude added vitamins, minerals, amino acids, and food additives.
Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC, to a final gray to white residue.
Fat Determine as directed under *Crude Fat*, Appendix X.
Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.
Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 2-g sample at 65° at a pressure of less than 100 mm Hg for 16 h.
pH of a 10% Solution Determine as directed under *pH Determination*, Appendix IIB, using a 10% aqueous solution.

Packaging and Storage Store in tight containers protected from humidity.

Whey

DESCRIPTION

Whey is the liquid obtained by separating the coagulum from milk, cream, and/or skim milk in cheese making. Whey obtained from the process in which a significant amount of lactose is converted to lactic acid or obtained from the curd formed by direct acidification of milk is known as acid-type Whey. Whey obtained from the process in which there is insignificant conversion of lactose to lactic acid is known as sweet-type Whey. The acidity of Whey may be adjusted by the addition of safe and suitable pH-adjusting ingredients. The final product is pasteurized and is available as a liquid or dry product.

Function Texturizer; nutrient.

REQUIREMENTS

Note: Calculate all analyses, except those for *Loss on Drying*, on the dry basis.

Labeling State whether the product is sweet or acid, if the product is liquid, and the concentration, as total solids based on the value obtained under *Loss on Drying* (below).
Identification Whey exhibits the compositional profile specified below with respect to *Ash, Fat, Lactose, Loss on Drying,* and *Protein*.
Ash (Total) Between 7.0% and 14.0%.
Fat Between 0.2% and 2.0%.
Lactose Between 61.0% and 75.0%.
Lead Not more than 0.5 mg/kg.
Loss on Drying *Dry Product*: Not more than 5.0%; *Liquid Product*: Not more than 95.0%.
Protein Between 10.0% and 15.0%.
Titratable Acidity (as Lactic Acid) *Sweet-Type Whey*: Not more than 0.16%; *Acid-Type Whey*: Not less than 0.35%.

TESTS

Ash (Total) Proceed as directed under *Ash (Total)*, Appendix IIC, to a final gray to white residue.
Fat Transfer 1 g of sample, accurately weighed, into a fat-extraction flask, add 10 mL of water, and shake until homogeneous (warm if necessary). Add approximately 1 mL of ammonium hydroxide, and heat in a water bath for 15 min at 60° to 70°, shaking occasionally. Add 10 mL of alcohol, and mix well. Add 25 mL of peroxide-free ether, stopper, and shake vigorously for 1 min; allow to cool if necessary; add 25 mL of petroleum ether; and shake vigorously. Allow the layers to separate and clarify, or centrifuge at 600 rpm to expedite the process. Decant the organic layer into a suitable flask or dish, and repeat the extraction twice with 15 mL each of ether and petroleum ether for each extraction. Evaporate the combined ether extractions on a steam bath, and dry the residue to a constant weight at 102°, or 70° to 75° at less than 50 mm Hg. Calculate the percent of fat in the sample taken by the formula

$$(R \times 100)/S,$$

in which R is the weight, in milligrams, of the residue; and S is the weight, in milligrams, of the sample taken.
Lactose

Mobile Phase Use a filtered and degassed 80:20 acetonitrile:water mixture at a flow rate of 2 mL/min.

Internal Standard Solution Prepare an aqueous solution of USP Fructose Reference Standard having a concentration of 100 mg/mL.

Standard Solution Using an accurately weighed quantity of USP Lactose Reference Standard, prepare a solution in water having a concentration of 20 mg/mL. Dilute 9 volumes of this solution with 1 volume of the *Internal Standard Solution* to obtain a *Standard Solution* having a known concentration of 18 mg of USP Lactose Reference Standard per milliliter. Prepare fresh daily.

Assay Preparation Transfer an accurately weighed quantity of sample containing about 180 mg of lactose into a 10-mL volumetric flask, add 1 mL of the *Internal Standard Solution*, dilute with water to volume, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a suitable high-performance liquid chromato-

graphic system operated at room temperature and equipped with a differential refractometer detector and a 250-mm × 4.6-mm (id) microparticle silica gel with siloxane bonded cyano-amino moieties (Whatman P-10 carbohydrate, or equivalent) column. Inject a 25-µL portion of the *Standard Solution* and record the peak responses. Replicate injections show a relative standard deviation of not more than 2.0% for the ratio of the response of lactose to that of the internal standard.

Procedure Separately inject 25-µL portions of the *Assay Preparation* and the *Standard Preparation* into the chromatograph and record the responses. Calculate the percent lactose in the sample taken by the formula

$$(10C_S/W)(R_U/R_S),$$

in which C_S is the concentration, in milligrams per milliliter, of USP Lactose Reference Standard in the *Standard Solution*; W is the weight, in milligrams, of sample taken; and R_U and R_S are the response ratios of lactose to the *Internal Standard Solution* obtained with the *Assay Preparation* and the *Standard Solution*, respectively. Correct to the dried basis using the value obtained under *Loss on Drying* (below).

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying *Dry Product*: Dry a 2-g sample at 65° at a pressure of less than 100 mm Hg for 16 h. *Liquid Product*: Evaporate to dryness on a steam bath, and continue as directed for the dry product.

Protein Determine the percent of nitrogen as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the percent protein by the formula

$$N \times 6.38,$$

in which N is the percent nitrogen.

Titratable Acidity (as Lactic Acid) Accurately weigh a portion of the finely ground dry sample or liquid equivalent to 10.0 g of total solids based on the value obtained under *Loss on Drying* (above), and transfer into a 500-mL conical flask. Add 100 mL of carbon dioxide-free water, and stir for 1 min. Allow to stand for 1 h at room temperature. Add 0.5 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for 30 s. Each milliliter of 0.1 *N* sodium hydroxide is equivalent to 9.008 mg of lactic acid.

Packaging and Storage Store in tight containers protected from humidity.

Whey Protein Concentrate

DESCRIPTION

Whey Protein Concentrate occurs as either a liquid or a dry product. It is the substance obtained by the removal of sufficient nonprotein constituents from whey so that the finished dry product contains not less than 25.0% protein. Whey Protein Concentrate is produced by physical separation techniques such as precipitation, filtration, or dialysis. The acidity of the Whey Protein Concentrate may be adjusted by the addition of safe and suitable pH-adjusting ingredients. The final product is pasteurized.

Function Texturizer; nutrient; emulsifier; water-binding aid; gelling agent.

REQUIREMENTS

Note: Calculate all analyses on a dry basis. Evaporate liquid samples to dryness on a steam bath, then as for the dry product, dry to constant weight at 65° under vacuum.

Labeling Indicate the concentration of protein and, if the product is liquid, the concentration, as total solids, based on the value obtained under *Loss on Drying* (below).

Identification Whey Protein Concentrate exhibits the compositional profile specified below with respect to *Ash (Total)*, *Fat*, *Lactose*, *Loss on Drying*, and *Protein*.

Ash (Total) Between 2.0% and 15.0%.

Fat Between 0.2% and 10.0%.

Lactose Not more than 60.0%.

Lead Not more than 0.5 mg/kg.

Loss on Drying *Dry Product*: Not more than 6.0%.

pH Between 6.0 and 7.2.

Protein Not less than 25.0% and not more than 89.9%.

TESTS

Ash (Total), **Fat**, **Lactose**, **Lead**, **Loss on Drying**, and **Protein** Proceed as directed in the monograph for *Whey*.

pH Determine as directed under *pH Determination*, Appendix IIB, using a 10% aqueous solution if the product is in the dry form.

Packaging and Storage Store in tight containers protected from humidity.

Whey Protein Isolate

DESCRIPTION

Whey Protein Isolate occurs either as a liquid or as a dry product. It is the substance obtained by the removal of sufficient nonprotein constituents from whey so that the finished dry product contains not less than 90% protein. Removal of nonprotein constituents is accomplished by separation techniques such as precipitation, membrane filtration, and/or ion exchange. The acidity of Whey Protein Isolate may be adjusted by the addition of safe and suitable pH-adjusting ingredients. The final product is pasteurized.

Function Source of high-quality protein; gelling agent; water-binding aid; foaming or whipping aid; emulsifier; edible coating used as a moisture barrier.

REQUIREMENTS

Note: Perform all analyses, except that for *Loss on Drying*, on the dry basis. Evaporate liquid samples to dryness on a steam bath; then as for dry samples, dry to constant weight at 65° under vacuum.

Labeling Indicate the concentration of protein and, if the product is liquid, state the concentration, as total solids, based on the value obtained under *Loss on Drying* (below).
Identification Whey Protein Isolate exhibits the compositional profile specified below with respect to *Ash (Total)*, *Fat*, *Lactose*, *Loss on Drying*, and *Protein*.
Ash Less than 6.0%.
Fat Less than 6.0%.
Lactose Not more than 6.0%.
Lead Not more than 0.5 mg/kg.
Loss on Drying *Dry Product*: Not more than 6.0%.
pH Between 6.0 and 7.2.
Protein Not less than 90.0%.

TESTS

Ash (Total), **Fat**, **Lactose**, **Lead, Loss on Drying**, and **Protein** Determine as directed in the monograph for *Whey*.
pH Determine as directed under *pH Determination*, Appendix IIB, using a 10% aqueous solution if the sample is in the dry form.

Packaging and Storage Store in tight containers protected from humidity.

Whey, Reduced Lactose

DESCRIPTION

Whey, Reduced Lactose, occurs as either a liquid or a dry product. It is the substance obtained by the selective removal of lactose from whey. Removal of lactose is accomplished by physical separation techniques such as precipitation, filtration, or dialysis. The acidity of Reduced Lactose Whey may be adjusted by the addition of safe and suitable pH-adjusting ingredients. The final product is pasteurized.

Function Texturizer; nutrient; emulsifier.

REQUIREMENTS

Note: Perform all analyses on a dry basis. Evaporate liquid samples to dryness on a steam bath, then as for the dry product, dry to constant weight at 65° under vacuum.

Labeling If the product is liquid, indicate the concentration, as total solids, based on the value obtained under *Loss on Drying*.
Identification Reduced Lactose Whey exhibits the compositional profile specified below with respect to *Ash* (Total), *Fat*, *Lactose*, *Loss on Drying*, and *Protein*.
Ash (Total) Between 11.0% and 27.0%.
Fat Between 0.2% and 4.0%.
Lactose Not more than 60.0%.
Lead Not more than 0.5 mg/kg.
Loss on Drying *Dry Product*: Not more than 4.0%.
pH Between 5.5 and 7.2.
Protein Between 16.0% and 24.0%.

TESTS

Ash (Total), **Fat**, **Lactose**, **Lead**, **Loss on Drying**, and **Protein** Determine as directed in the monograph for *Whey*.
pH Determine as directed under *pH Determination*, Appendix IIB, using a 10% aqueous solution if the product is in the dry form.

Packaging and Storage Store in tight containers protected from humidity.

Whey, Reduced Minerals

DESCRIPTION

Whey, Reduced Minerals, occurs as either a liquid or a dry product. It is the substance obtained by the removal of a portion of the minerals from whey. Reduced Minerals Whey is produced by physical separation techniques such as precipitation, filtration, ion exchange, or dialysis. The acidity of the Reduced Minerals Whey may be adjusted by the addition of safe and suitable pH-adjusting ingredients. The final product is pasteurized.

Function Texturizer; nutrient.

REQUIREMENTS

Note: Perform all analyses on a dry basis. Evaporate liquid samples to dryness on a steam bath, then as for the dry product, dry to constant weight at 65° under vacuum.

Labeling If the product is liquid, indicate the concentration as total solids based on the value obtained under *Loss on Drying*.
Identification Whey, Reduced Minerals, exhibits the compositional profile specified below with respect to *Ash* (Total), *Fat*, *Lactose*, *Loss on Drying*, and *Protein*.
Ash (Total) Not more than 7.0%.
Fat Between 0.2% and 4.0%.

Lactose Not more than 85.0%.
Lead Not more than 0.5 mg/kg.
Loss on Drying *Dry product*: Not more than 4.0%.
pH Between 6.2 and 7.2.
Protein Between 10.0% and 24.0%.

TESTS

Ash (Total), **Fat**, **Lactose**, **Lead**, **Loss on Drying**, and **Protein** Determine as directed in the monograph for *Whey*.
pH Determine as directed under *pH Determination*, Appendix IIB, using a 10% aqueous solution if the product is in the dry form.

Packaging and Storage Store in tight containers protected from humidity.

Wintergreen Oil

Gaultheria Oil

CAS: [68917-75-9]

View IR

DESCRIPTION

Wintergreen Oil occurs as a colorless to yellow liquid with the characteristic odor and taste of wintergreen. It is obtained by maceration and subsequent distillation with steam from the leaves of *Gualtheria procumbens* L. (Fam. Ericaceae) or from the bark of *Betula lenta* L. (Fam. Betulaceae). It boils, with decomposition, between 219° and 224°. It is soluble in alcohol and in glacial acetic acid, and it is very slightly soluble in water.

Function Flavoring agent.

REQUIREMENTS

Identification
A. Shake 1 drop of sample with about 5 mL of water, and add 1 drop of ferric chloride TS. A deep violet color appears.
B. The infrared absorption spectrum of the sample exhibits relative maxima at the same wavelengths as those of a typical spectrum as shown in the section on *Infrared Spectra*, using the same test conditions as specified therein.
Assay Not less than 98.0% and not more than 100.5% of methyl salicylate ($C_8H_8O_3$).
Acid Value Not more than 1.0.
Angular Rotation Slightly levorotatory, exhibiting a rotation of not more than −1.5°.
Refractive Index Between 1.535 and 1.538 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.176 and 1.182.

TESTS

Assay Determine as directed in *Ester Determination* under *Esters*, Appendix VI, using about 2 g of sample, accurately weighed, and 76.08 as the equivalence factor (*e*) in the calculation. Modify the procedure by using 50.0 mL of 0.5 *N* alcoholic potassium hydroxide and by refluxing on the steam bath for 2 h.
Acid Value Determine as directed under *Acid Value*, Appendix VI, using bromocresol purple TS as the indicator instead of phenolphthalein TS.
Angular Rotation Determine as directed under *Optical (Specific) Rotation*, Appendix IIB, using a 100-mm tube.
Refractive Index Determine as directed under *Refractive Index*, Appendix IIB, using an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Determine as directed under *Solubility in Alcohol*, Appendix VI. One milliliter of sample dissolves in 7 mL of 70% alcohol. The solution may have not more than a slight cloudiness.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in a cool place protected from light in full, tight containers.

Xanthan Gum

INS: 415 CAS: [11138-66-2]

DESCRIPTION

Xanthan Gum occurs as a cream colored powder. It is a high-molecular-weight polysaccharide gum produced by a pure-culture fermentation of a carbohydrate with *Xanthomonas campestris*, purified by recovery with isopropyl alcohol, dried, and milled. It contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid and pyruvic acid, and it is prepared as the sodium, potassium, or calcium salt. It is readily soluble in hot or cold water, but it is insoluble in alcohol. Its solutions are neutral.

Function Stabilizer; thickener; emulsifier; suspending agent; bodying agent; foam enhancer.

REQUIREMENTS

Identification Transfer 300 mL of water, previously heated to 80°, into a 400-mL beaker and stir rapidly with a mechanical stirrer. At the point of maximum agitation, add a dry blend of 1.5 g of sample and 1.5 g of locust bean gum. Stir until the mixture goes into solution, and then continue stirring for 30 min longer. Do not allow the water temperature to drop below 60°. Discontinue stirring, and allow the mixture to cool

at room temperature for at least 2 h. A firm, rubbery gel forms after the temperature of the mixture drops below 40°. No such gel forms in a 1% control solution of sample prepared in the same manner but omitting the locust bean gum.

Assay A sample yields, on the dried basis, not less than 4.2% and not more than 5.4% of carbon dioxide (CO_2), corresponding to between 91.0% and 117.0% of Xanthan Gum.

Isopropyl Alcohol Not more than 0.075%.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 15.0%.

Pyruvic Acid Not less than 1.5%.

Viscosity Passes test.

TESTS

Assay Determine as directed under *Alginates Assay*, Appendix IIIC, but use about 1.2 g of undried sample, accurately weighed.

Isopropyl Alcohol

IPA Standard Solution Transfer 500.0 mg of chromatographic-quality isopropyl alcohol into a 50-mL volumetric flask, dilute to volume with water, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix.

TBA Standard Solution Transfer 500.0 mg of chromatographic-quality *tert*-butyl alcohol into a 50-mL volumetric flask, dilute to volume with water, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute to volume with water, and mix.

Mixed Standard Solution Pipet 4 mL each of the *IPA Standard Solution* and of the *TBA Standard Solution* into a 125-mL graduated Erlenmeyer flask, dilute to about 100 mL with water, and mix. This solution contains approximately 40 µg each of isopropyl alcohol and of *tert*-butyl alcohol per milliliter.

Sample Preparation Disperse 1 mL of a suitable antifoam emulsion, such as Dow-Corning G-10, or equivalent, in 200 mL of water contained in a 1000-mL 24/40 round-bottom distilling flask. Add about 5 g of sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distill about 100 mL, adjusting the heat so that foam does not enter the column. Add 4.0 mL of *TBA Standard Solution* to the distillate to obtain the *Sample Preparation*.

Procedure (See *Chromatography*, Appendix II A.) Inject about 5 µL of the *Mixed Standard Solution* into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m × 3.2-mm (id) stainless steel column, or equivalent, packed with 80- to 100-mesh Porapak QS, or equivalent. Maintain the column at 165°. Set the temperature of both the injection port and the detector to 200°. Use helium as the carrier gas, with a flow rate of 80 mL/min. The retention time of isopropyl alcohol is about 2 min, and that of *tert*-butyl alcohol is about 3 min.

Determine the areas of the IPA and TBA peaks, and calculate the response factor, *f*, by the formula

$$A_{IPA}/A_{TBA},$$

in which A_{IPA} is the area of the isopropyl alcohol peak, and A_{TBA} is the area of the *tert*-butyl alcohol peak.

Similarly, inject about 5 µL of the *Sample Preparation*, and determine the peak areas, recording the area of the isopropyl alcohol peak as S_{IPA}, and that of the *tert*-butyl alcohol peak as S_{TBA}. Calculate the isopropyl alcohol content, in milligrams per kilogram, in the sample taken by the formula

$$(S_{IPA} \times 4000)/(f \times S_{TBA} \times W),$$

in which *W* is the weight, in grams, of the sample taken.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a sample at 105° for 2.5 h.

Pyruvic Acid

Standard Preparation Transfer 45.0 mg of pyruvic acid, accurately weighed, into a 500-mL volumetric flask, dilute to volume with water, and mix. Transfer 10.0 mL of this solution into a 50-mL glass-stoppered flask. Pipet 20 mL of 1 *N* hydrochloric acid into the flask, weigh the flask, and reflux for 3 h, taking precautions to prevent loss of vapors. Cool to room temperature, and add water to make up for any weight loss during refluxing. Pipet 1.0 mL of a 1:200 solution of 2,4-dinitrophenylhydrazine:2 *N* hydrochloric acid into a 30-mL separator, add 2.0 mL of the sample solution, mix, and allow the mixture to stand at room temperature for 5 min. Extract the mixture with 5 mL of ethyl acetate, and discard the aqueous layer. Extract the hydrazone from the ethyl acetate with three 5-mL portions of sodium carbonate TS, collecting the extracts in a 50-mL volumetric flask. Dilute to volume with sodium carbonate TS, and mix.

Sample Preparation Dissolve 600.0 mg of sample, accurately weighed, in sufficient water to make 100.0 mL, transfer 10.0 mL of the solution into a 50-mL glass-stoppered flask, and continue as directed under *Standard Preparation*, beginning with "Pipet 20 mL of 1 *N* hydrochloric acid into the flask. . . ."

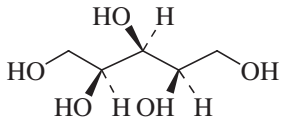
Procedure Determine the absorbance of each solution with a suitable spectrophotometer and in 1-cm cells at the maximum at about 375 nm, using sodium carbonate TS as the blank. The absorbance of the *Sample Preparation* is equal to or greater than that of the *Standard Preparation*.

Viscosity Prepare two identical solutions, each containing 1% of sample and 1% of potassium chloride in water, and stir for 2 h. Determine the viscosity (V_1) of one solution at 23.9° as directed in the *Procedure* under *Viscosity of Cellulose Gum*, Appendix IIB, using a No. 3 spindle rotating at 60 rpm (Brookfield, or equivalent). The viscosity thus determined is not less than 600 cp. Determine the viscosity (V_2) of the other solution in the same manner, but maintain the temperature at 65.6°. The ratio of the viscosities, V_1/V_2, is between 1.02 and 1.45.

Packaging and Storage Store in well-closed containers.

Xylitol

1,2,3,4,5-Pentahydroxypentane



$C_5H_{12}O_5$ Formula wt 152.15

INS: 967 CAS: [87-99-0]

DESCRIPTION

Xylitol occurs as white crystals or as a crystalline powder. One gram dissolves in about 0.65 mL of water. It is sparingly soluble in ethanol.

Function Nutritive sweetener.

REQUIREMENTS

Identification The infrared absorption spectrum of a potassium bromide dispersion of sample between two sodium chloride plates exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Xylitol Reference Standard. If a difference appears, dissolve portions of both the sample and the reference standard in a suitable solvent, evaporate the solutions to dryness, and repeat the test on the residues.

Assay Not less than 98.5% and not more than 101.0% of $C_5H_{12}O_5$, calculated on the dried basis.

Lead Not more than 1 mg/kg.

Nickel Not more than 1 mg/kg.

Other Polyols Not more than 1.0%.

Reducing Sugars (as glucose) Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

Water Not more than 0.5%.

TESTS

Assay

Internal Standard Solution Transfer about 500 mg of erythritol, accurately weighed, into a 25-mL volumetric flask, dilute to volume with water, and mix.

Standard Solution Transfer about 25 mg each of L-arabinitol, galactitol, mannitol, and sorbitol, accurately weighed, into a 100-mL volumetric flask, dilute to volume with water, and mix. Add an accurately weighed amount of USP Xylitol Reference Standard to an accurately measured volume of this solution to obtain a solution with a known concentration of Xylitol of about 49 mg/mL.

Assay Preparation Transfer about 5 g of sample, accurately weighed, into a 100-mL volumetric flask, dilute to volume with water, and mix.

Chromatographic System (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector and a 2-m × 2-mm (id) glass column, or equivalent, packed with 3% liquid phase of 25% phenyl–25% cyanopropylmethylsilicone (OV-225, or equivalent) on silanized, siliceous earth support (Chromosorb W-HP, or equivalent). Maintain the column temperature at 200°. Set the injector port temperature to 250° and the detector to 250°. Use nitrogen as the carrier gas, with a flow rate of about 30 mL/ min. Obtain a chromatogram of the derivatized *Standard Solution*, prepared as directed under *Procedure*, and record the peak responses. The relative retention times corresponding to erythritol, L-arabinitol, Xylitol, galactitol, mannitol, and sorbitol are usually about 1.0, 2.77, 3.90, 6.96, 7.63, and 8.43, respectively. The relative standard deviation of the response ratios of the derivatized Xylitol to the derivatized erythritol from three replicate injections does not exceed 2.0%.

Procedure Pipet 1-mL portions of the *Standard Solution* and of the *Assay Preparation* into separate 100-mL, round-bottom boiling flasks. Add 1.0 mL of *Internal Standard Solution* to each flask, and evaporate each mixture to dryness on a water bath at 60° with the aid of a rotary evaporator. Dissolve each dry residue in 1 mL of pyridine, and add 1 mL of acetic anhydride to each flask. Boil each solution under reflux for 1 h to complete the acetylation. Separately inject 1-µL portions of the derivatized solutions from the *Assay Preparation* and the *Standard Solution* into the gas chromatograph and measure the peak responses. Calculate the percent of Xylitol, on the as-is basis, by the formula

$$100(W_S/W_U)(R_U/R_S),$$

in which W_S is the weight, in milligrams, of USP Xylitol Reference Standard used for the *Standard Solution*; W_U is the weight, in milligrams, of the sample taken for the *Assay Preparation*; and R_U and R_S are the ratios of peak responses of the derivatized analyte to the derivatized erythritol from the *Internal Standard Solution* obtained from the *Assay Preparation* and the *Standard Solution*, respectively. Using the value obtained in the *Water* test, correct the percent to the anhydrous basis.

Lead Determine as directed for *Method I* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Nickel Determine as directed under *Nickel Limit Test*, Appendix IIIB, using a 20.0-g sample.

Other Polyols

Internal Standard Solution, *Standard Solution*, *Assay Preparation*, and *Chromatographic System* Proceed as directed under *Assay* (above).

Procedure Determine as described under *Assay*. Calculate the percentage of each polyol—L-arabinitol, galactitol, mannitol, and sorbitol—by the formula given therein, in which W_S is the weight, in milligrams, of the respective polyol taken for the *Standard Solution*; R_S is the peak response ratio of the corresponding polyol obtained from the *Standard Solution*; and R_U is the peak response ratio of the corresponding polyol obtained from the *Assay Preparation*. Add the four individual polyol percentages to obtain the total.

Reducing Sugars (as glucose) Dissolve about 500 mg of sample, accurately weighed, in 2 mL of water in a 10-mL conical flask. Add 2 mL of a dextrose solution containing

0.75 mg/mL to another conical flask. Add 1 mL of *Fehling's Solution A* and of *Fehling's Solution B* (see *Cupric Tartrate TS, Alkaline*, under *Solutions and Indicators*) to each flask, heat to boiling, and cool. The sample solution is less turbid than the dextrose solution, which forms a red-brown precipitate.

Residue on Ignition Determine as directed under *Residue on Ignition*, Appendix IIC, igniting a 2-g sample.

Water Determine as directed under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers in a dry place.

Yeast, Autolyzed

Autolyzed Yeast

DESCRIPTION

Yeast, Autolyzed, occurs in granular, powdered, flake, or paste form. It is the concentrated, nonextracted, partially soluble digest obtained from food-grade yeasts. Solubilization is accomplished by enzyme hydrolysis or autolysis of yeast cells. Food-grade salts and enzymes may be added. Yeast, Autolyzed contains both soluble and insoluble components derived from the whole yeast cell. It is composed primarily of amino acids, peptides, proteins, carbohydrates, fats, and salts.

Function Flavoring agent; flavor enhancer; protein source; binder.

REQUIREMENTS

> **Note**: Perform all analyses after drying. Liquid and paste samples should be evaporated to dryness on a steam bath, then, as for the powdered and granular forms, dried to constant weight at 65° (see *General Provisions*).

Assay Not less than 6.1% total nitrogen, which is equivalent to not less than 38.1% protein (%N × 6.25), calculated on the sodium chloride-free basis.

α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Not less than 5.0%.

Ammonia Nitrogen Not more than 1.0%, calculated on the sodium chloride-free basis.

Glutamic Acid Not more than 13.0% of glutamic acid ($C_4H_7NO_4$), calculated on the sodium chloride-free basis, and not more than 24.0% of the total amino acids.

Insoluble Matter Between 20.0% and 60.0%.

Lead Not more than 2 mg/kg.

Mercury Not more than 3 mg/kg.

Microbial Limits:

Aerobic Plate Count Not more than 50,000 CFU per gram.

Coliforms Not more than 10 CFU per gram.

Salmonella Negative in 25 g.

Yeasts and Molds Not more than 50 CFU per gram.

Potassium Not more than 13.0%.

Sodium Chloride Not more than 43.0%.

TESTS

Assay Determine as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the percent protein (*P*) by the formula

$$P = 6.25N,$$

in which *N* is the percent nitrogen.

α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Determine *α-Amino Nitrogen* as directed under *α-Amino Nitrogen Determination*, Appendix IIIC. Determine *Total Nitrogen* as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the AN/TN percent ratio by dividing the percent α-amino nitrogen (*AN*) by the percent total nitrogen (*TN*) as corrected for ammonia nitrogen (NH_3-*N*) according to the formula

$$100[(AN - NH_3\text{-}N)/(TN - NH_3\text{-}N)].$$

Ammonia Nitrogen Determine as directed under *Ammonia Nitrogen*, Appendix IIIC.

Glutamic Acid Determine as directed under *Glutamic Acid*, Appendix IIIC.

Insoluble Matter Transfer about 5 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask. Add 75 mL of water, cover the flask with a watch glass, and boil gently for 2 min. Filter the solution through a tared filtering crucible, dry at 105° for 1 h, cool, and weigh.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB.

Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

Aerobic Plate Count

Coliforms

Salmonella

Yeasts and Molds

Potassium

Spectrophotometer Use any suitable atomic absorption spectrophotometer.

Standard Solution Transfer 38.20 mg of reagent-grade potassium chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with deionized water, and mix. Transfer 5.0 mL of this solution to a 1000-mL volumetric flask, dilute to volume with deionized water, and mix. Each milliliter contains 1.0 μg of potassium (K).

Sample Solution Transfer 2.33 g of a previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve it in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 1000-mL volumetric flask. Wash the filter paper with hot water, dilute the solution to volume, and mix. Prepare a 1:300 dilution of this solution in water to obtain the final *Sample Solution*.

Procedure Determine the absorbance of each solution at 766.5 nm, following the manufacturer's instructions for optimum operation of the spectrophotometer. The absorbance of the *Sample Solution* does not exceed that of the *Standard Solution*.

Sodium Chloride

Spectrophotometer Use any suitable atomic absorption spectrophotometer.

Standard Solution Transfer 43.0 mg of reagent-grade sodium chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with deionized water, and mix. Using water as the solvent, prepare a 1:100 dilution of this solution to obtain the final working *Standard Solution*. Each milliliter contains 4.3 μg of sodium chloride (NaCl).

Sample Solution Transfer 1.00 ± 0.05 g of a previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve it in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 100-mL volumetric flask. Wash the filter paper with hot water, dilute the solution to volume, and mix. Using water as the solvent, prepare a 1:100 dilution of this solution to obtain the final *Sample Solution*.

Procedure Determine the absorbance of each solution at 589.0 nm, following the manufacturer's instructions for optimum operation of the spectrophotometer. The absorbance produced by the *Sample Solution* does not exceed that of the *Standard Solution*.

Packaging and Storage Store in well-closed containers.

Yeast, Dried

Brewer's Yeast; Dried Yeast; Torula Yeast

DESCRIPTION

Yeast, Dried, occurs as a light brown to buff powder, granules, or flakes. It is the comminuted, washed, dried, and pasteurized cell walls from *Saccharomyces cerevisiae*, *Saccharomyces fragilis*, or *Torula utilis*. It contains no added substances.

Function Carrier; flavor enhancer.

REQUIREMENTS

Identification When examined under a microscope, a sample exhibits numerous irregular masses and isolated yeast cells—the latter ovate, elliptical, spheroidal, or elliptic-elongate in shape, some with one or more attached buds—up to 12 μm in length and up to 7.5 μm in width. Each has a wall of cellulose surrounding a protoplast containing refractile glycogen vacuoles and oil globules.

Assay Not less than 45.0% protein.

Ash (Total) Not more than 8.0%.

Folic Acid Not more than 0.04 mg/g.

Lead Not more than 1 mg/kg.

Loss on Drying Not more than 7.0%.

Microbial Limits:

Aerobic Plate Count Not more than 7500 CFU per gram.

Coliforms Not more than 10 CFU per gram.

Salmonella Negative in 25 g.

TESTS

Assay Determine the percent nitrogen as directed under *Nitrogen Determination*, Appendix IIIC, and multiply by 6.25 to obtain the percent protein.

Ash (Total) Determine as directed under *Ash (Total)*, Appendix IIC.

Folic Acid (**Note**: In the microbiological assay of folic acid, the microorganism is highly sensitive to minute amounts of growth factors and to many cleansing agents. Meticulously cleanse 20- × 150-mm test tubes and other necessary glassware with a suitable detergent, sodium lauryl sulfate, or an equivalent substitute. Follow cleansing by heating for 1 to 2 h at approximately 250°.) This method is based on AOAC method 960.46.

Vitamin-Free, Acid-Hydrolyzed Casein Solution Prepare the solution by mixing 400 g of vitamin-free casein with 2 L of boiling 5 *N* hydrochloric acid. Autoclave for 10 h at 121°. Concentrate the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the paste in water, adjust the solution to pH 3.5 ± 0.1 with a 10% solution of sodium hydroxide, and dilute with water to a final volume of 4 L. Add 80 g of activated charcoal, stir for 1 h, and filter. Repeat the treatment with activated charcoal. Filter the solution if a precipitate forms on storage.

Adenine–Guanine–Uracil Solution Dissolve 1.0 g each of adenine sulfate, guanine hydrochloride, and uracil in 50 mL of warm 1:2 hydrochloric acid, cool, and dilute with water to 1 L.

Asparagine Solution Dissolve 10 g of L-asparagine monohydrate in approximately 500 mL of water, and dilute with water to 1 L.

Manganese Sulfate Solution Dissolve 2.0 g of manganese sulfate monohydrate in water, and dilute with water to 200 mL.

Polysorbate 80 Solution Dissolve 25 g of polysorbate 80 (polyoxyethylene sorbitan monooleate) in ethyl alcohol, and dilute with ethyl alcohol to make 250 mL.

Salt Solution Dissolve 20 g of magnesium sulfate heptahydrate, 1 g of sodium chloride, 1 g of ferrous sulfate heptahydrate, and 1 g of manganese sulfate monohydrate in water, dilute with water to 1 L, add 10 drops of hydrochloric acid, and mix.

Tryptophan Solution Suspend 2.0 g of L-tryptophan in 800 mL of water, heat to 80°, and add, dropwise and while stirring, 1:2 hydrochloric acid until the suspension dissolves. Cool, and dilute with water to 1 L.

Vitamin Solution Dissolve 10 mg of *p*-aminobenzoic acid, 8 mg of calcium pantothenate, 40 mg of pyridoxine hydrochloride, 4 mg of thiamine hydrochloride, 8 mg of niacin, and 0.2 mg of biotin in approximately 300 mL of water. Add 10 mg of riboflavin dissolved in approximately 200 mL of 0.02 *N* acetic acid. Add a solution containing 1.9 g of anhydrous sodium acetate and 1.6 mL of glacial acetic acid in approximately 40 mL of water. Dilute the solution with water to a final volume of 2 L.

Xanthine Solution Suspend 1.0 g of xanthine in 200 mL of water, heat to approximately 70°, add 30 mL of 2:5 ammonium hydroxide, and stir until the suspension dissolves. Cool, and dilute with water to 1 L.

Basal Medium Stock Solution Prepare the solution by adding, with mixing, in the following order, 25 mL of the *Vitamin-Free, Acid-Hydrolyzed Casein Solution*, 25 mL of the *Tryptophan Solution*, 2.5 mL of the *Adenine–Guanine–Uracil Solution*, 5 mL of the *Xanthine Solution*, 15 mL of the *Asparagine Solution*, 50 mL of the *Vitamin Solution*, and 5 mL of the *Salt Solution*. Add approximately 50 mL of water, and add, with mixing, 0.19 g of L-cysteine monohydrochloride monohydrate, 10 g of anhydrous glucose, 13 g of sodium citrate dihydrate, 1.6 g of anhydrous dipotassium hydrogen phosphate, and 0.0013 g of glutathione. When solution is complete, adjust to pH 6.8 with 10% sodium hydroxide solution, and add, with mixing, 0.25 mL of the *Polysorbate 80 Solution* and 5 mL of the *Manganese Sulfate Solution*. Dilute to a final volume of 250 mL with water.

Liquid Culture Medium Dissolve 15 g of peptonized milk, 5 g of water-soluble yeast extract, 10 g of anhydrous glucose, and 2 g of anhydrous potassium dihydrogen phosphate in about 600 mL of water. Add 100 mL of filtered tomato juice (filtered through Whatman No. 1 filter paper, or equivalent), and adjust to pH 6.5 by the dropwise addition of 1.0 *N* sodium hydroxide. Add, with mixing, 10 mL of the *Polysorbate 80 Solution*. Dilute with water to a final volume of 1000 mL. Add 10-mL portions of this *Liquid Culture Medium* to test tubes, cover to prevent contamination, and sterilize by heating in an autoclave at 121° for 15 min. Cool the tubes rapidly to keep color formation to a minimum, and store at 10° in the dark.

Agar Culture Medium Add 6.0 g of agar to 500 mL of *Liquid Culture Medium*, and heat with stirring on a steam bath until the agar dissolves. Add approximately 10-mL portions of the hot solution to test tubes, cover to prevent contamination, sterilize by heating in an autoclave at 121° for 15 min, and cool tubes in an upright position to keep color formation to a minimum. Store at 10° in the dark.

Suspension Medium Dilute an appropriate volume of the *Basal Medium Stock Solution* with an equal volume of water. Distribute 10-mL portions of this *Suspension Medium* to test tubes, cover to prevent contamination, sterilize by heating in an autoclave at 121° for 15 min, and cool tubes rapidly to keep color formation to a minimum. Store at 10° in the dark.

Assay Organism Maintain *Enterococcus* (*Streptococcus*) *faecalis* ATCC 8043 by subculturing in stab cultures of *Agar Culture Medium* and incubating at 37° for 24 h. Stab cultures may be stored in the dark at 10° for a maximum of 7 days until use. Prepare fresh stab cultures at least on a weekly basis. Before using a new culture in the assay, make several successive transfers of the culture over a 1- to 2-week period. Transfer cells from the stab culture of *Assay Organism* to a sterile tube containing 10 mL of *Liquid Culture Medium*. Incubate for 18 h at 37°. Under aseptic conditions, centrifuge the culture, and decant the supernate. Wash the cells with three 10-mL portions of sterile *Suspension Medium*. Resuspend cells in 10 mL of sterile *Suspension Medium*—these cells serve as the inoculum.

Folic Acid Stock Solutions Accurately weigh, in a closed system, 50 to 60 mg of USP Folic Acid Reference Standard that has been dried to constant weight and stored in the dark over phosphorus pentoxide in a desiccator. Dissolve in approximately 30 mL of 0.01 *N* sodium hydroxide, add approximately 300 mL of water, adjust to pH 7.5 with 1:2 hydrochloric acid, and dilute with additional water to a final folic acid concentration of exactly 100 µg/mL. Store under toluene in the dark at 10°.

Prepare an intermediate *Folic Acid Stock Solution* containing 1 µg/mL by placing 10 mL of the 100 µg/mL *Folic Acid Stock Solution* in a flask, adding approximately 500 mL of water, adjusting to pH 7.5 with dilute hydrochloric acid or sodium hydroxide as necessary, and diluting with additional water to a final volume of 1 L. Store under toluene in the dark at 10°.

Prepare the final *Folic Acid Stock Solution* by taking 100 mL of the intermediate *Folic Acid Stock Solution*, adding approximately 500 mL of water, adjusting to pH 7.5 with dilute hydrochloric acid or sodium hydroxide as necessary, and diluting with additional water to a final volume of 1 L. Store under toluene in the dark at 10°. This final *Folic Acid Stock Solution* has a concentration of 100 ng/mL.

Preparation of the Standard Curve Dilute the *Folic Acid Stock Solution* with water to a measured volume such that after incubation, as described below, response at the 5.0-mL level of this solution is equivalent to a titration volume of 8 to 12 mL. This concentration is usually 1 to 4 ng of folic acid per mL but can vary with the culture used in the assay. Designate this solution as the *Folic Acid Working Standard Solution*. To duplicate test tubes, add 0.0 (for uninoculated blanks), 0.0 (for inoculated blanks), 1.0, 2.0, 3.0, 4.0, and 5.0 mL, respectively, of the *Folic Acid Working Standard Solution*. Add water to each tube to make a final volume of 5.0 mL. Add 5.0 mL of the *Basal Medium Stock Solution* to each tube, and mix. Cover the tubes suitably to prevent bacterial contamination, and sterilize by heating in an autoclave at 121° for 10 min. Cool tubes rapidly to keep color formation to a minimum.

Note: Sterilizing and cooling conditions must be kept uniform to obtain reproducible results.

Aseptically inoculate each tube with 1 drop of the *Assay Organism* inoculum, except for one set of duplicate tubes containing 0.0 mL of the *Folic Acid Working Standard Solution*, which serve as the uninoculated blanks. Incubate the tubes for 72 h at 37°.

> **Note**: Contamination of assay tubes with any foreign organism invalidates the assay.

Titrate the contents of each tube with 0.1 *N* sodium hydroxide, using bromothymol blue as the indicator. Disregard the results of the assay if the titration volume for the inoculated blank is more than 1.5 mL greater than that for the uninoculated blank. The titration volume for the 5.0-mL level of the *Folic Acid Working Standard Solution* should be approximately 8 to 12 mL. Prepare a standard curve by plotting the titration values, expressed in milliliters of 0.1 *N* sodium hydroxide for each level of the *Folic Acid Working Standard Solution* used, against the amount of folic acid contained in that tube.

Assay Solution Weigh and suspend 1.0 g of sample in 100 mL of water. Add 2 mL of 2:5 ammonium hydroxide. If the sample is not readily soluble, comminute to disperse it evenly in the liquid, then agitate vigorously and wash down the sides of the flask with 0.1 *N* ammonium hydroxide. Heat the mixture in an autoclave at 121° for 15 min. If lumping occurs, agitate the sample until the particles are evenly dispersed. Dilute the mixture with water to 200 mL. Filter through Whatman No. 1 filter paper, or equivalent, if necessary, to remove any undissolved particles. Adjust the filtered mixture to pH 6.8 and dilute to 1000 mL with water. Prepare the final *Assay Solution* by diluting 1.0 mL of the intermediate solution with water to a final volume of 50.0 mL.

Procedure To duplicate test tubes, add 0.0 (for uninoculated blanks), 0.0 (for inoculated blanks), 1.0, 2.0, 3.0, 4.0, and 5.0 mL, respectively, of the *Assay Solution*. Add water to each tube to make a final volume of 5.0 mL. Proceed as directed above for *Preparation of the Standard Curve*. Determine the amount of folic acid for each level of the *Assay Solution* by interpolation from the standard curve. Discard any observed titration values equivalent to less than 0.5 mL or more than 4.5 mL of the *Folic Acid Working Standard Solution*. If necessary, the *Assay Solution* can be diluted to achieve the ideal concentration range of folic acid. For each level of *Assay Solution* used, calculate the vitamin content per milliliter of *Assay Solution*. Calculate the average vitamin content of values obtained from tubes that do not vary by greater than 10% from this average. More than two-thirds of the original number of tubes must be within 10% of the average folic acid value, or the data cannot be used to calculate the folic acid concentration in the sample. If the data are acceptable, determine the folic acid concentration in the sample by multiplying the average folic acid concentration, in nanograms per milliliter, of the *Assay Solution* by 0.025 to give the milligrams of folic acid per gram of sample.

Lead Determine as directed for *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 1-g sample at 105° for 4 h.

Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

Aerobic Plate Count
Coliforms
Salmonella

Packaging and Storage Store in tight containers in a cool, dry place.

Yeast Extract

Autolyzed Yeast Extract

DESCRIPTION

Yeast Extract occurs as a liquid, paste, powder, or granular substance. It comprises the water-soluble components of the yeast cell, the composition of which is primarily amino acids, peptides, carbohydrates, and salts. Yeast Extract is produced through the hydrolysis of peptide bonds by the naturally occurring enzymes present in edible yeasts or by the addition of food-grade enzymes. Food-grade salts may be added during processing.

Function Flavoring agent; flavor enhancer.

REQUIREMENTS

> **Note**: Perform all calculations on the dried basis. In a suitable tared container, evaporate liquid and paste samples to dryness on a steam bath, then, as for the powdered and granular forms, dry to constant weight at 105° (see *General Provisions*).

Assay (Protein) Not less than 42.0% protein.

α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Not less than 15.0% or more than 55.0%.

Ammonia Nitrogen Not more than 2.0%, calculated on a dry, sodium chloride-free basis.

Glutamic Acid Not more than 12.0% as $C_5H_9NO_4$ and not more than 28.0% of the total amino acids.

Insoluble Matter Not more than 2%.

Lead Not more than 2 mg/kg.

Mercury Not more than 3 mg/kg.

Microbial Limits:

Aerobic Plate Count Not more than 50,000 CFU per gram.

Coliforms Not more than 10 CFU per gram.

Salmonella Negative in 25 g.

Yeasts and Molds Not more than 50 CFU per gram.

Potassium Not more than 13.0%.

Sodium Chloride Not more than 50.0%.

TESTS

Assay (Protein) Determine as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the percent protein (*P*) by the equation

$$P = 6.25N,$$

in which *N* is the percent nitrogen.

α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Determine *α-Amino Nitrogen* as directed under *α-Amino Nitrogen Determination*, Appendix IIIC. Determine *Total Nitrogen* as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the *AN/TN* percent ratio, in which *AN* is the percent of α-amino nitrogen and *TN* is the percent of total nitrogen.

Ammonia Nitrogen Determine as directed under *Ammonia Nitrogen*, Appendix IIIC.

Glutamic Acid Determine as directed under *Glutamic Acid*, Appendix IIIC.

Insoluble Matter Transfer about 5 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, add 75 mL of water, cover the flask with a watch glass, and boil gently for 2 min. Filter the solution through a tared filtering crucible, dry at 105° for 1 h, cool, and weigh.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB.

Microbial Limits (**Note:** Current methods for the following tests may be found online at <www.cfsan.fda.gov/~ebam/bam-toc.html>):

Aerobic Plate Count
Coliforms
Salmonella
Yeasts and Molds

Potassium Proceed as directed in the monograph for *Yeast, Autolyzed*.

Sodium Chloride Proceed as directed in the monograph for *Yeast, Autolyzed*, except to use 50.0 mg of reagent-grade sodium chloride to prepare the *Standard Solution*.

Packaging and Storage Store in well-closed containers.

Zein

CAS: [9010-66-6]

DESCRIPTION

Zein occurs as a very light yellow to tan colored, granular or fine powder. It comprises the prolamine protein component of corn (*Zea mays* Linne'). It is produced commercially by extraction from corn gluten with alkaline aqueous isopropyl alcohol. The extract is then cooled, which causes the Zein to precipitate. It is insoluble in water.

Function Surface-finishing agent; texturizing agent.

REQUIREMENTS

Identification

A. Dissolve about 0.1 g of sample in 10 mL of 0.1 *N* sodium hydroxide, and add a few drops of cupric sulfate TS. Warm in a water bath. A purple color appears.

B. Add 1 mL of nitric acid to a test tube containing 25 mg of sample. Agitate vigorously. The solution turns light yellow. Further addition of about 10 mL of 6 *N* ammonium hydroxide produces an orange color.

Assay Not less than 88.0% and not more than 96.0% protein, calculated on the dried basis.

Lead Not more than 2 mg/kg.

Loss on Drying Not more than 8.0%.

Loss on Ignition Not more than 2%.

TESTS

Assay Determine as directed under *Nitrogen Determination*, Appendix IIIC. Calculate the percent protein (*P*) by the equation

$$P = 6.25N,$$

in which *N* is the percent nitrogen.

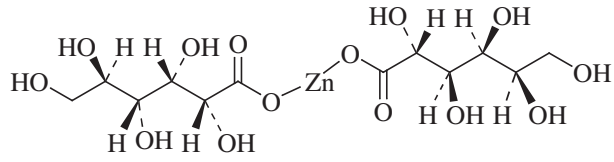
Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 10-g sample.

Loss on Drying Determine as directed under *Loss on Drying*, Appendix IIC, drying a 2-g sample in an air oven at 105° for 2 h.

Loss on Ignition Determine as directed under *Ash (Total)*, Appendix IIC, using a 2-g sample.

Packaging and Storage Store in well-closed containers.

Zinc Gluconate



$C_{12}H_{22}O_{14}Zn$ Formula wt 455.68

CAS: [4468-02-4]

DESCRIPTION

Zinc Gluconate occurs as a white or nearly white, granular or crystalline powder and as a mixture of various states of

hydration, up to the trihydrate, depending on the method of isolation. It is freely soluble in water and very slightly soluble in alcohol.

Function Nutrient

REQUIREMENTS

Labeling Indicate the powder or granular form of the product.

Identification

A. A 1:10 aqueous solution gives positive tests for *Zinc*, Appendix IIIA.

B. Dissolve a quantity of sample in water, heating in a water bath at 60° if necessary, to obtain a *Test Solution* containing 10 mg/mL. Similarly, prepare a *Standard Solution* of USP Potassium Gluconate Reference Standard in water, diluting to 10 mg/mL. Apply separate 5-µL portions of the *Test Solution* and the *Standard Solution* on a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography*, Appendix IIA) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a solvent system consisting of a mixture of alcohol, water, ammonium hydroxide, and ethyl acetate (50:30:10:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with a spray reagent prepared as follows: Dissolve 2.5 g of ammonium molybdate in about 50 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 *N* sulfuric acid to volume, and mix. After spraying, heat the plate at 110° for about 10 min: The principal spot obtained from the *Test Solution* corresponds in color, size, and R_f value to that obtained from the *Standard Solution*.

Assay Not less than 97.0% and not more than 102.0% of $C_{12}H_{22}O_{14}Zn$, calculated on the anhydrous basis.

Cadmium Not more than 2 mg/kg.

Chloride Not more than 0.05%.

Lead Not more than 2 mg/kg.

Reducing Substances Not more than 1.0%.

Sulfate Not more than 0.05%.

Water Not more than 11.6%.

TESTS

Assay Dissolve about 700 mg of sample, accurately weighed, in 100 mL of water, warming if necessary to aid dissolution, and add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with 0.05 *M* disodium EDTA until the solution turns blue. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 22.78 mg of $C_{12}H_{22}O_{14}Zn$.

Cadmium Determine as directed under *Cadmium Limit Test*, Appendix IIIB.

Chloride Determine as directed in the *Chloride Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 40-mg sample does not exceed that shown in a control containing 20 µg of chloride (Cl) ion.

Lead Determine as directed in the *Flame Atomic Absorption Spectrophotometric Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.

Reducing Substances Transfer about 1 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask, dissolve in 10 mL of water, and add 25 mL of alkaline cupric citrate TS. Cover the flask with a small beaker, boil gently for exactly 5 min, and cool rapidly to room temperature. Add 25 mL of a 1:10 acetic acid solution, 10.0 mL of 0.1 *N* iodine, 10 mL of 2.7 *N* hydrochloric acid, and 3 mL of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in milligrams, of reducing substances (as D-glucose) by the formula

$$27 \times (V_1N_1 - V_2N_2),$$

in which 27 is an empirically determined equivalence factor for D-glucose; V_1 and N_1 are the volume, in milliliters, and the normality of the iodine solution, respectively; and V_2 and N_2 are the volume, in milliliters, and the normality of the sodium thiosulfate solution, respectively.

Sulfate Determine as directed in the *Sulfate Limit Test* under *Chloride and Sulfate Limit Tests*, Appendix IIIB. Any turbidity produced by a 500-mg sample does not exceed that in a control containing 250 µg of sulfate (SO_4) ion.

Water Determine as directed for *Method Ib (Residual Titration)* in *Method I (Karl Fischer Titrimetric Method)* under *Water Determination*, Appendix IIB.

Packaging and Storage Store in well-closed containers.

Zinc Oxide

ZnO Formula wt 81.38

CAS: [1314-13-2]

DESCRIPTION

Zinc Oxide occurs as a fine, white, amorphous powder. It gradually absorbs carbon dioxide from the air. It is insoluble in water and in alcohol, and is soluble in dilute acids and in strong bases.

Function Nutrient.

REQUIREMENTS

Identification

A. When strongly heated, a sample assumes a yellow color that disappears on cooling.

B. A solution of sample in a slight excess of 3 *N* hydrochloric acid gives positive tests for *Zinc*, Appendix IIIA.

Assay Not less than 99.0% of ZnO after ignition.

Alkalinity Passes test.

Cadmium Not more than 3 mg/kg.

Lead Not more than 10 mg/kg.
Loss on Ignition Not more than 1.0%.
Substances Not Precipitated by Sulfide Not more than 0.5%.

TESTS

Assay Dissolve about 1.5 g of freshly ignited sample, accurately weighed, and 2.5 g of ammonium chloride in 50.0 mL of 1 *N* sulfuric acid with the aid of gentle heat, if necessary. When solution is complete, add methyl orange TS, and titrate the excess sulfuric acid with 1 *N* sodium hydroxide. Each milliliter of 1 *N* sulfuric acid is equivalent to 40.69 mg of ZnO.
Alkalinity Suspend 2 g of sample in 20 mL of water, boil for 1 min, filter, and add 0.1 mL of phenolphthalein TS to the filtrate. No red color appears.
Cadmium Determine as directed under *Cadmium Limit Test*, Appendix IIIB, using the following as the *Sample Solution*: Transfer 5 g of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in a minimum volume of 2:3 hydrochloric acid, dilute to volume with water, and mix.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Loss on Ignition Ignite about 2 g of sample, accurately weighed, at 800° ± 25° to constant weight.
Substances Not Precipitated by Sulfide Transfer 2 g of sample, accurately weighed, into a 200-mL volumetric flask, dissolve in 20 mL of 1:4 acetic acid, dilute to about 150 mL with water, and mix. Precipitate the zinc completely with ammonium sulfide TS, dilute to volume with water, and mix. Filter through a dry filter, discarding the first portion of filtrate, and collect 100 mL of the subsequent filtrate. Add a few drops of sulfuric acid, and evaporate to dryness on a steam bath in a tared dish. Ignite cautiously until the ammonium salts are volatilized, ignite to constant weight at 800° ± 25°, cool, and weigh. The weight of the residue does not exceed 5 mg.

Packaging and Storage Store in well-closed containers.

Zinc Sulfate

$ZnSO_4 \cdot H_2O$ Formula wt, monohydrate 179.45
$ZnSO_4 \cdot 7H_2O$ Formula wt, heptahydrate 287.54

CAS: monohydrate [7446-19-7]
CAS: heptahydrate [7446-20-0]

DESCRIPTION

Zinc Sulfate occurs as colorless, transparent prisms or small needles, or as a granular, crystalline powder. It contains one or seven molecules of water of hydration. The monohydrate loses water at temperatures above 238°; the heptahydrate effloresces in dry air at room temperature. Its solutions are acid to litmus. The monohydrate is soluble in water and practically insoluble in alcohol. One gram of the heptahydrate dissolves in about 0.6 mL of water and in about 2.5 mL of glycerin; it is insoluble in alcohol.

Function Nutrient.

REQUIREMENTS

Identification A 1:20 aqueous solution gives positive tests for *Zinc* and for *Sulfate*, Appendix IIIA.
Assay *Monohydrate*: Not less than 98.0% and not more than 100.5% of $ZnSO_4 \cdot H_2O$; *Heptahydrate*: Not less than 99.0% and not more than 108.7% of $ZnSO_4 \cdot 7H_2O$.
Acidity Passes test.
Alkalies and Alkaline Earths Not more than 0.5%.
Cadmium Not more than 2 mg/kg.
Lead Not more than 4 mg/kg.
Mercury Not more than 5 mg/kg.
Selenium Not more than 0.003%.

TESTS

Assay Dissolve about 175 mg of the monohydrate, or about 300 mg of the heptahydrate, accurately weighed, in 100 mL of water, add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS, and titrate with 0.05 *M* disodium EDTA until the solution turns deep blue. Each milliliter of 0.05 *M* disodium EDTA is equivalent to 8.973 mg of $ZnSO_4 \cdot H_2O$, or 14.38 mg of $ZnSO_4 \cdot 7H_2O$.
Acidity Add methyl orange TS to a 1:20 aqueous solution. A pink color does not appear.
Alkalies and Alkaline Earths Transfer 2 g of sample into a 200-mL volumetric flask, dissolve in about 150 mL of water, and precipitate the zinc completely with ammonium sulfide TS. Dilute to volume with water, and mix. Filter through a dry filter, rejecting the first portion of the filtrate, and add a few drops of sulfuric acid to 100 mL of the subsequent filtrate. Evaporate to dryness in a tared dish, ignite to constant weight, cool, and weigh. The weight of the residue does not exceed 5 mg.
Cadmium Determine as directed under *Cadmium Limit Test*, Appendix IIIB.
Lead Determine as directed in the *APDC Extraction Method* under *Lead Limit Test*, Appendix IIIB, using a 5-g sample.
Mercury Determine as directed under *Mercury Limit Test*, Appendix IIIB, using the following as the *Sample Preparation*: Dissolve 400 mg of sample in 10 mL of water in a small beaker, add 1 mL of a 1:5 sulfuric acid solution and 1 mL of a 1:25 potassium permanganate solution, cover the beaker, boil for a few seconds, and cool.
Selenium Determine as directed in *Method I* under *Selenium Limit Test*, Appendix IIIB, using 200 mg of sample.

Packaging and Storage Store in tight containers.

3 / *Flavor Chemicals*

Contents

SPECIFICATIONS FOR FLAVOR CHEMICALS

Specifications for flavoring agents other than the essential oils are presented in tabular form rather than as separate monographs. Specifications for all such ingredients from the *Food Chemicals Codex*, Fourth Edition, together with a number of new specifications, are provided on the following pages of this section. Following the tabular specifications are the *Test Methods for Flavor Chemicals* (M-1 through M-17) and the *Gas Chromatographic Assay of Flavor Chemicals* used to determine the assay values and various other physico-chemical properties of the flavors. The infrared spectra, used for identification and comparison purposes, are provided in the section entitled *Infrared Spectra*.

Explanatory Notes to Tabular Specifications

The *Food Chemicals Codex*, Fifth Edition, uses the nomenclature convention (*Z*) and (*E*) to specify the structure of acyclic double bonds in organic chemicals, specifically ''cis'' and ''trans.''

The FCC name of the substance is followed, where available, by the number assigned to the substance by the Flavor and Extract Manufacturers Association (FEMA) and by its synonym(s). The explanatory notes to the specifications in this section apply throughout the tabular series and are as follows:

Note 1 (Odor) Odor terms are general descriptors and do not indicate the source of the material.

Note 2 (Solubility) Approximate solubilities (see *General Provisions*) are indicated by the following abbreviations: *vs = very soluble*; *s = soluble*; *ss = slightly soluble*; *vss = very slightly soluble; m = miscible*; *ins = insoluble or practically insoluble*. Other abbreviations are as follows: alc = alcohol; gly = glycerin; org = organic; prop = propylene (as in propylene glycol); veg = vegetable (as in vegetable oil); vol = volatile.

Note 3 (B.P.) Boiling points (B.P.) are expressed in °C. They are approximate values given for information only and not as requirements.

Note 4 (Solubility in Alcohol) Determine the solubility in alcohol at 25° as directed in the general method, Appendix VI, *Essential Oils and Flavors*.

Note 5 (I.D.) The notation ''IR'' in the identification (I.D.) column indicates that an infrared absorption spectrum is provided for the particular substance in the section entitled *Infrared Spectra*. Where the IR requirement is specified, the infrared absorption spectrum of the sample shall exhibit maxima at the same wavelengths as those shown in the respective spectrum, using the test conditions as specified therein.

Note 6 (Assay) Assay requirements are specified as *minimum* values (unless a range of assay values is given) and are stated in weight percent unless otherwise indicated. References to assay methods are indicated by citations in parentheses, e.g., ''(M-1a),'' to methods provided under *Test Methods for Flavor Chemicals*.

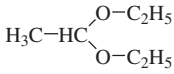
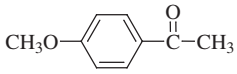
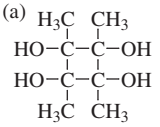
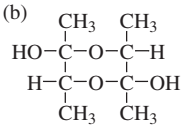
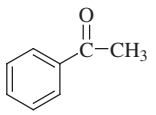
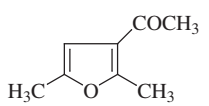
Note 7 (A.V.) Unless otherwise indicated, determine the acid value (A.V.) as directed in M-16, using phenolphthalein TS as the indicator unless another indicator is specified for an individual substance. Where *Method II* is specified, determine the acid value as directed in the general method, Appendix VII, *Fats and Related Substances*.

Note 8 (Ref. Index) Refractive index (Ref. Index) determinations are made at 20° unless another temperature is specified, according to the general method, Appendix II, *Physical Tests and Determinations*.

Note 9 (Sp. Gr.) Specific gravity (Sp. Gr.) determinations are made at 25° unless another temperature is specified by any reliable method (see *General Provisions*).

Note 10 (Other Requirements) Numerical limits for other requirements are specified as *maximum* values unless otherwise indicated (max = maximum; NLT = not lower than or not less than, as appropriate). Test methods are indicated by citations in parentheses, which refer either to methods given in the section that follows this tabular section or to general methods given in Appendix VI, *Essential Oils and Flavors*.

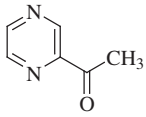
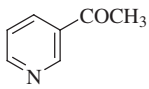
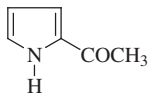
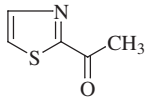
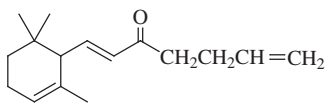
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Acetaldehyde FEMA No. 2003 Acetic Aldehyde; Ethanal	44.05/C_2H_4O/ CH_3CHO	flammable, colorless liq/ pungent, ethereal	*m*—alc, org solvents, water/ 21°	
Acetaldehyde Diethyl Acetal FEMA No. 2002 Acetal	118.17/$C_6H_{14}O_2$/ 	colorless to pale yel liq/ ethereal, fruity	*s*—prop glycol, veg oils; *ss*—water/ 102°	1 mL in 1 mL 95% ethanol
Acetanisole FEMA No. 2005 4-Acetylanisole; *p*-Methoxyacetophenone	150.18/$C_9H_{10}O_2$/ 	colorless to pale yel fused solid/hawthorn	*s*—most fixed oils, prop glycol; *ins*—gly/ 153° (26 mm Hg)	1 g in 5 mL 50% alc
Acetoin FEMA No. 2008 Acetyl Methyl Carbinol; Dimethylketol; 3-Hydroxy-2-butanone	Monomer 88.11/$C_4H_8O_2$/ $CH_3CH(OH)COCH_3$	Monomer colorless to pale yel liq/ buttery	Monomer *m*—alc, prop glycol, water; *ins*—veg oils/ 148°	
	Dimer 176.21/$C_8H_{16}O_4$/ (a)  (b) 	Dimer white to pale yel powder/ odorless	Dimer *s*—hot prop glyc; *ss*—weak alkali; *ins*—most solvents	
Acetophenone FEMA No. 2009 Acetylbenzene; Methyl Phenyl Ketone	120.15/C_8H_8O/ 	practically colorless liq above 20°/ very sweet, pungent	*vs*—most fixed oils, prop glycol; *s*—alc, chloroform, ether; *ss*—water; *ins*—gly/ 202°	1 mL in 5 mL 50% alc
3-Acetyl-2,5-dimethyl Furan FEMA No. 3391 2,5-Dimethyl-3-acetylfuran	138.17/$C_8H_{10}O_2$/ 	yel liq/ powerful, slightly roasted, nutty	*s*—alc, most fixed oils, prop glycol; *ss*—water/ 83° (11 mm Hg)	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	99.0% of C_2H_4O (M-2b)	5.0		0.804–0.811 (0°/20°)	**Residue on Evap.**—0.006% (M-16)
IR	97.0% of $C_6H_{14}O_2$ (M-1b)		1.379–1.384	0.821–0.827	
IR	98.0% of $C_9H_{10}O_2$ (M-1b)				**Chlorinated Cmpds.**—passes test (Appendix VI) **Lead**—10 mg/kg (M-9)
Monomer	Monomer		Monomer	Monomer	
IR	96.0% of $C_4H_8O_2$ (M-1b)		1.417–1.422	0.995–1.019	
Dimer	Dimer				
	96.0% of $C_4H_8O_2$ (M-1b)				
IR	98.0% of C_8H_8O (M-1b)		1.533–1.535	1.025–1.028	**Chlorinated Cmpds.**—passes test (Appendix VI) **Solidification Pt.**—NLT 19° (Appendix IIB)
IR	99.0% of $C_8H_{10}O_2$ (M-1a)		1.484–1.492	1.027–1.048	

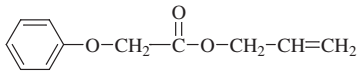
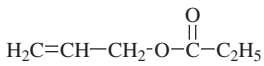
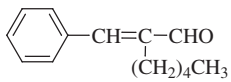
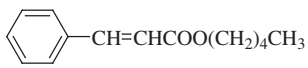
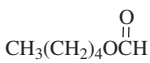
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
2-Acetylpyrazine FEMA No. 3126 Methyl Pyrazinyl Ketone	122.13/$C_6H_6N_2O$/ 	colorless to pale yel cryst/ popcorn		1 g in 20 mL 95% alc
3-Acetylpyridine FEMA No. 3424 Methyl Pyridyl Ketone	121.14/C_7H_7NO/ 	colorless to yel liq/ sweet, nutty, popcorn	*s*—acids, alc, ether, water/ 230°	
2-Acetylpyrrole FEMA No. 3202 Methyl 2-Pyrrolyl Ketone	109.13/C_6H_7NO/ 	white to pale brown fine cryst/ bready	*ins*—prop glycol, veg oils, water/ 220°	1 g in 6 mL ethanol
2-Acetyl Thiazole FEMA No. 3328	127.17/C_5H_5NOS/ 	colorless to pale yel liq/ popcorn	*s*—prop glycol, veg oils; *ins*—water/ 89° at 12 mm 91° (1 mm Hg)	1 mL in 1 mL 95% ethanol
Allyl Cyclohexanepropionate FEMA No. 2026 Allyl-3-cyclohexanepropionate	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/ pineapple	*m*—alc, chloroform, ether; *ins*—gly, water	1 mL in 4 mL 80% alc
Allyl Heptanoate FEMA No. 2031 Allyl Heptoate	170.25/$C_{10}H_{18}O_2$/ $CH_3(CH_2)_5COOC_3H_5$	colorless to pale yel liq/ sweet, pineapple	210°	1 mL in 1 mL 95% alc
Allyl Hexanoate FEMA No. 2032 Allyl Caproate	156.22/$C_9H_{16}O_2$/ $CH_3(CH_2)_4COOCH_2CH{=}CH_2$	colorless to light yel liq/ strong, pineapple	*m*—alc, most fixed oils; *ins*—prop glycol, water/ 185°	1 mL in 6 mL 70% alc
Allyl α-Ionone FEMA No. 2033 Allyl Ionone	232.37/$C_{16}H_{24}O$/ 	colorless to yel liq/ fruity, woody	*s*—alc; *ins*—water/ 265°	1 mL in 1 mL 90% alc gives clear soln

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	99.0% of $C_6H_6N_2O$ (M-1a)				**Melting Range**—between 75° and 78° (Appendix IIB)
IR	98.0% of C_7H_7NO (M-1a)		1.530–1.540	1.100–1.115	**Water**—0.5% (Appendix IIB, KF)
	98.0% of C_6H_7NO (M-1a)				**Melting Range**—between 88° and 92° (Appendix IIB) **Residue on Ignit.**—0.3% (Appendix IIC)
IR	98.0% of C_5H_5NOS (M-1b)		1.542–1.552	1.219–1.226	
IR	98.0% of $C_{12}H_{20}O_2$ (M-1b)	5.0	1.457–1.462	0.945–0.950	**Allyl Alcohol**—NMT 0.1% (M-1b)
IR	97.0% of $C_{10}H_{18}O_2$ (M-1b)	1.0	1.426–1.430	0.880–0.885	**Allyl Alcohol**—NMT 0.1% (M-1b)
IR	98.0% of $C_9H_{16}O_2$ (M-1b)	1.0	1.422–1.426	0.884–0.890	**Allyl Alcohol**—NMT 0.1% (M-1b)
IR	88.0% of $C_{16}H_{24}O$ (M-1b)		1.502–1.507	0.926–0.932	**Allyl Alcohol**—NMT 0.1% (M-1b)

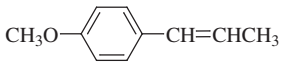
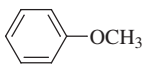
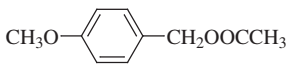
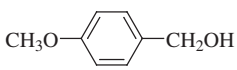
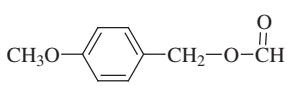
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Allyl Isothiocyanate FEMA No. 2034	99.16/C_4H_5NS/ $CH_2{=}CH{-}CH_2{-}N{=}C{=}S$	colorless to pale yel, strongly refractive liq/ irritating, acrid taste, mustard (caution: lachrymator)	*m*—alc, carbon disulfide, ether/ 150°	
Allyl Isovalerate FEMA No. 2045 Allyl Isopentanoate	142.20/$C_8H_{14}O_2$/ $(CH_3)_2CHCH_2CO_2CH_2CH{=}CH_2$	colorless to pale yel liq/ fruity, apple	155°	1 mL in 1 mL 95% alc
Allyl Phenoxy Acetate FEMA No. 2038	192.21/$C_{11}H_{12}O_3$/ 	colorless to pale yel liq/ honey, pineapple	*ss*—prop glycol; *vss*—water; *ins*—veg oils/ 265°	1 mL in 1 mL 95% ethanol
Allyl Propionate FEMA No. 2040	114.15/$C_6H_{10}O_2$/ 	colorless to pale yel liq/ ethereal, fruity	*s*—prop glycol, veg oils; *ins*—water/ 124°	1 mL in 1 mL 95% ethanol
1-Amyl Alcohol FEMA No. 2056 1-Pentanol	88.15/$C_5H_{12}O$/ $CH_3(CH_2)_4OH$	colorless to pale yel liq/ fusel, winey	*s*—prop glycol, veg oils; water/ 136°	
Amyl Butyrate FEMA No. 2059 1-Pentyl Butyrate	158.23/$C_9H_{18}O_2$/ $CH_3CH_2CH_2COOCH_2(CH_2)_3CH_3$	colorless to pale yel liq/ fruity, banana	*s*—prop glycol, veg oils; water/ 184°–188°	1 mL in 1 mL 95% ethanol
α-Amylcinnamaldehyde FEMA No. 2061 Amylcinnamaldehyde	202.30/$C_{14}H_{18}O$/ 	yel liq/ strong, floral, jasmine on dilution, spicy	*s*—most fixed oils; *ins*—gly, prop glycol/ 285°	1 mL in 5 mL 80% alc
Amyl Cinnamate FEMA No. 2063 Isoamyl Cinnamate; Isoamyl 3-Phenyl Propenate	218.28/$C_{14}H_{18}O_2$/ 	colorless to pale yel liq/ faint, balsamic, cocoa	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly/ 310°	1 mL in 7 mL 80% alc may be opalescent
Amyl Formate FEMA No. 2068 1-Pentyl Formate	116.16/$C_6H_{12}O_2$/ 	colorless to pale yel liq/ fruity	*m*—alc/ 128°–130°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	93.0% of C_4H_5NS (M-1a)		1.527–1.531	1.013–1.020	**Allyl Alcohol**—NMT 0.1% (M-1b) **Distillation Range**—between 148° and 154° (Appendix IIB) **Phenols**—passes test (M-17)
IR	98.0% of $C_8H_{14}O_2$ (one isomer) (M-1b)	1.0	1.413–1.418	0.879–0.884	**Allyl Alcohol**—NMT 0.1% (M-1b)
IR	97.0% of $C_{11}H_{12}O_3$ (M-1b)	1.0	1.513–1.518	1.100–1.105	
IR	97.0% of $C_6H_{10}O_2$ (M-1b)	2.0	1.408–1.413	0.912–0.917	
	98.0% of $C_5H_{12}O$ (M-1b)		1.407–1.412	0.810–0.816	
	98.0% of $C_9H_{18}O_2$ (sum of isomers) (M-1b)	1.0	1.409–1.414	0.863–0.866	
IR	97.0% of $C_{14}H_{18}O$ (sum of two isomers; 90% main isomer) (M-1b)	5.0	1.554–1.559	0.963–0.968	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	96.0% of $C_{14}H_{18}O_2$ (sum of *n*-, 2-methyl butyl, and 3-methyl butyl isomers) (M-1b)	1.0	1.535–1.539	0.992–0.997	
	92.0% of $C_6H_{12}O_2$ (sum of *n*-, 2-methyl butyl, and 3-methyl butyl isomers) (M-1b)	5.0 add ice to soln	1.396–1.402	0.881–0.887	

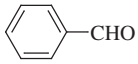
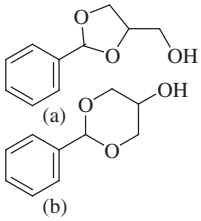
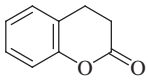
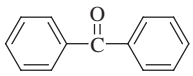
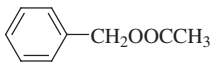
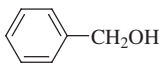
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Amyl Heptanoate FEMA No. 2073 Pentyl Heptanoate	200.32/$C_{12}H_{24}O_2$/ $CH_3(CH_2)_5COO(CH_2)_4CH_3$	colorless to pale yel liq/ fruity	245°	1 mL in 1 mL 95% alc
Amyl Octanoate FEMA No. 2079 Amyl Caprylate; Isoamyl Caprylate; Isoamyl Octanoate	214.35/$C_{13}H_{26}O_2$/ $CH_3(CH_2)_6COOC_5H_{11}$	colorless liq/ fruity	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 260°	1 mL in 7 mL 80% alc remains clear to 10 mL
Amyl Propionate FEMA No. 2082 Isoamyl Propionate	144.21/$C_8H_{16}O_2$/ $CH_3CH_2COOC_5H_{11}$	colorless liq/ fruity, apricot-pineapple	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 160°	1 mL in 3 mL 70% alc
Anethole FEMA No. 2086 *trans*-Anethole; Isoestragole; *p*-Propenylanisole	148.20/$C_{10}H_{12}O$/ 	colorless to faintly yel liq at or above 23°; sweet taste/ anise	*ss*—water; *m*—chloroform, ether/ 234°	1 mL in 2 mL alc
Anisole FEMA No. 2097 Methylphenyl Ether	108.14/C_7H_8O/ 	colorless liq/ phenolic, anise	*s*—alc, ether; *ins*—water/ 154°	
Anisyl Acetate FEMA No. 2098 *p*-Methoxybenzyl Acetate	180.20/$C_{10}H_{12}O_3$/ 	colorless to slightly yel liq/ floral, fruity, balsamic	*s*—alc, most fixed oils; *ins*—gly, prop glycol/ 235°	1 mL in 6 mL 60% alc remains in soln to 10 mL
Anisyl Alcohol FEMA No. 2099 Anisic Alcohol; *p*-Methoxybenzyl Alcohol	138.17/$C_8H_{10}O_2$/ 	colorless to slightly yel liq/ floral	*s*—most fixed oils; *ss*—gly/ 259°	1 mL in 1 mL 50% alc remains in soln to 10 mL
Anisyl Formate FEMA No. 2101 *p*-Methoxybenzyl Formate	166.18/$C_9H_{10}O_3$/ 	colorless to pale yel liq/ sweet, floral, tonka	100°	1 mL in 1 mL 95% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
	93.0% of $C_{12}H_{24}O_2$ (sum of *n*-, 2-methyl butyl, and 3-methyl butyl isomers) (M-1a)	1.0	1.422–1.426	0.859–0.863	
IR	98.0% of $C_{13}H_{26}O_2$ (sum of *n*-, 2-methyl butyl, and 3-methyl butyl isomers) (M-1b)	1.0	1.425–1.429	0.855–0.861	
IR	98.0% of $C_8H_{16}O_2$ (sum of *n*-, 2-methyl butyl, and 3-methyl butyl isomers) (M-1b)	1.0	1.405–1.409	0.866–0.871	
IR	99.0% of $C_{10}H_{12}O$ (M-1b)		1.557–1.562	0.983–0.988	**Angular Rotation**—between −0.15° and +0.15° (Appendix IIB, 100-mm tube) **Distillation Range**—between 231° and 237° (Appendix IIB) **Phenols**—passes test (M-17) **Solidification Pt.**—NLT 20° (Appendix IIB)
IR	97.0% of C_7H_8O (M-1b)		1.515–1.518	0.990–0.993	**Distillation Range**—within a 2° range (Appendix IIB) **Phenols**—passes test (M-17)
IR	97.0% of $C_{10}H_{12}O_3$ (M-1b)	1.0	1.511–1.516	1.104–1.111	
IR	97.0% of $C_8H_{10}O_2$ (M-1b)	1.0	1.542–1.547	1.110–1.115	**Aldehydes**—1.0% as anisaldehyde (M-1b) **Solidification Pt.**—min 23.5° (Appendix IIB)
IR	90.0% of $C_9H_{10}O_3$ (M-1b)	3.0	1.521–1.525	1.138–1.142	

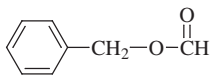
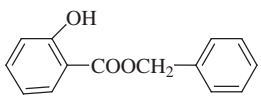
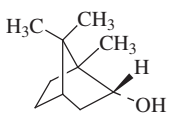
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Benzaldehyde FEMA No. 2127	106.12/C_7H_6O/ 	colorless liq, burning taste/ bitter almond oil	*ss*—water; *m*—alc, ether, most fixed oils, vol oils/ 178°	
Benzaldehyde Glyceryl Acetal FEMA No. 2129 Mixture of 1,2- and 1,3-Benzaldehyde Cyclic Acetals of Glycerin	180.20/$C_{10}H_{12}O_3$/ 	colorless to pale yel liq/ mild almond	185°	1 mL in 1 mL 95% alc
1,2-Benzodihydropyrone FEMA No. 2381 Dihydrocoumarin	148.16/$C_9H_8O_2$/ 	colorless to pale yel liq/ coconut	272°	1 mL in 1 mL 95% alc
Benzophenone FEMA No. 2134 Benzoylbenzene; Diphenyl Ketone	182.22/$C_{13}H_{10}O$/ 	white rhombic cryst or flaky solid/ delicate, persistent, rose	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly/ 305°	1 g in 10 mL 80% alc
Benzyl Acetate FEMA No. 2135	150.18/$C_9H_{10}O_2$/ 	colorless liq/ sweet, floral, fruity	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/ 214°	1 mL in 5 mL 60% alc
Benzyl Alcohol FEMA No. 2137 Phenyl Carbinol	108.14/C_7H_8O/ 	colorless liq with a sharp burning taste/ faint, aromatic	*m*—alc, chloroform, ether, 1 mL in 30 mL water/ 206° (decomp)	
Benzyl Benzoate FEMA No. 2138	212.25/$C_{14}H_{12}O_2$/ 	colorless, oily liq/ slight, aromatic	*m*—alc, chloroform, ether; *ins*—gly, water/ 323°	
Benzyl Butyrate FEMA No. 2140 Benzyl *n*-Butyrate	178.23/$C_{11}H_{14}O_2$/ 	colorless liq/ floral, fruity, plum	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 239°	1 mL in 2 mL 80% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of C_7H_6O (M-1b)		1.544–1.547	1.041–1.046	**Chlorinated Cmpds.**—passes test (Appendix VI) **Hydrocyanic Acid**—passes test (M-8)
IR	95.0% of $C_{10}H_{12}O_3$ (sum of four isomers; each isomer between 5% and 40%) (M-1a)	2.0	1.535–1.541	1.181–1.191	
IR	99.0% of $C_9H_8O_2$ (M-1b)		1.555–1.559	1.186–1.192	**Solidification Pt.**—NLT 22° (Appendix IIB)
IR	98.0% of $C_{13}H_{10}O$ (M-1a)				**Chlorinated Cmpds.**—passes test (Appendix VI) **Lead**—10 mg/kg (M-9) **Solidification Pt.**—NLT 47° (Appendix IIB)
IR	98.0% of $C_9H_{10}O_2$ (M-1b)	1.0 (phenol red TS)	1.501–1.504	1.052–1.056	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	99.0% of C_7H_8O (M-1a)		1.539–1.541	1.042–1.047	**Aldehydes**—0.2% (M-1b) **Chlorinated Cmpds.**—passes test (Appendix VI) **Distillation Range**—NLT 95% between 202.5° and 206.5° (Appendix IIB)
IR	99.0% of $C_{14}H_{12}O_2$ (M-1b)	1.0	1.568–1.570	1.116–1.120	**Chlorinated Cmpds.**—passes test (Appendix VI) **Solidification Pt.**—NLT 18° (Appendix IIB)
IR	98.0% of $C_{11}H_{14}O_2$ (M-1b)	1.0	1.492–1.496	1.006–1.009	

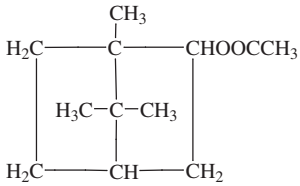
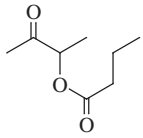
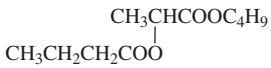
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Benzyl Cinnamate FEMA No. 2142	238.29/$C_{16}H_{14}O_2$/ 	white to pale yel solid/ sweet, balsamic	*s*—most fixed oils; *ins*—gly, prop glycol/ 195° (5 mm Hg)	1 g in 8 mL 90% alc
Benzyl Formate FEMA No. 2145	136.15/$C_8H_8O_2$/ 	colorless to pale yel liq/ sweet, balsamic, floral	203°	1 mL in 1 mL 95% alc
Benzyl Isobutyrate FEMA No. 2141 Benzyl 2-Methyl Propionate	178.23/$C_{11}H_{14}O_2$/ 	colorless liq/ floral, fruity, jasmine	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly/ 229°	1 mL in 6 mL 70% alc
Benzyl Isovalerate FEMA No. 2152 Benzyl 3-Methyl Butyrate	192.26/$C_{12}H_{16}O_2$/ 	colorless liq/ fruity, herbaceous, apple	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 246°	1 mL in 3 mL 80% alc remains in soln on dilution
Benzyl Phenylacetate FEMA No. 2149	226.27/$C_{15}H_{14}O_2$/ 	colorless liq/ sweet, floral, honey undertone	*m*—alc, chloroform, ether/ 317°	1 mL in 3 mL 90% alc gives clear soln
Benzyl Propionate FEMA No. 2150 Benzyl Propanoate	164.20/$C_{10}H_{12}O_2$/ 	colorless liq/ sweet, floral, fruity	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 222°	1 mL in 3 mL 70% alc remains clear to 10 mL
Benzyl Salicylate FEMA No. 2151	228.25/$C_{14}H_{12}O_3$/ 	almost colorless liq/ faint, sweet	*s*—most fixed oils; *ins*—gly, prop glycol/ 300°	1 mL in 5 mL 95% alc
Borneol FEMA No. 2157	154.25/$C_{10}H_{18}O$/ 	white to off-white cryst/ piney, camphoraceous	*ss*—prop glycol; *vss*—water; *ins*—veg oils/ 210°	1 g in 2 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{16}H_{14}O_2$ (M-1b)	1.0			**Chlorinated Cmpds.**—passes test (Appendix VI) **Solidification Pt.**—between 33.0° and 35.0° (Appendix IIB)
IR	95.0% of $C_8H_8O_2$ (M-1b)	3.0	1.508–1.515	1.082–1.092	
IR	97.0% of $C_{11}H_{14}O_2$ (M-1b)	1.0	1.488–1.492	1.000–1.005	
IR	98.0% of $C_{12}H_{16}O_2$ (one isomer) (M-1b)	1.0	1.486–1.490	0.983–0.989	
IR	98.0% of $C_{15}H_{14}O_2$ (M-1b)	1.0	1.553–1.558	1.095–1.099	
IR	98.0% of $C_{10}H_{12}O_2$ (M-1b)	1.0	1.496–1.500	1.028–1.032	
IR	98.0% of $C_{14}H_{12}O_3$ (M-1b)	1.0 (phenol red TS)	1.573–1.582	1.176–1.180	**Solidification Pt.**—NLT 23.5° (Appendix IIB)
IR	97.0% of $C_{10}H_{18}O$ (M-1b)				**Melting Point**—202° min.

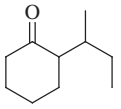
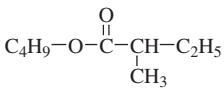
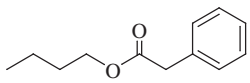
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Bornyl Acetate FEMA No. 2159 L-Bornyl Acetate	196.29/$C_{12}H_{20}O_2$/ 	colorless liq, semicryst mass, or white cryst solid/ sweet, herbaceous, piney	*s*—alc, most fixed oils; *ss*—water; *ins*—gly, prop glycol/ 226°	1 mL in 3 mL 70% alc remains in soln to 10 mL
2-Butanone FEMA No. 2170 Methyl Ethyl Ketone	72.11/C_4H_8O/ $CH_3COCH_2CH_3$	colorless, mobile liq/ ethereal, nauseating	*m*—alc, ether, most fixed oils, 1 mL in 4 mL water/ 78.6°–80°	
Butan-3-one-2-yl Butanoate FEMA No. 3332	158.20/$C_8H_{14}O_3$/ 	white to slightly yel liq/ sweet, red berry character	*s*—alc, prop glycol, most fixed oils; *ins*—water	
Butyl Acetate FEMA No. 2174 *n*-Butyl Acetate	116.16/$C_6H_{12}O_2$/ $CH_3COO(CH_2)_3CH_3$	colorless, mobile liq/ strong, fruity	*m*—alc, ether, prop glycol, 1 mL in 145 mL water/ 126°	
Butyl Alcohol FEMA No. 2178 1-Butanol	74.12/$C_4H_{10}O$/ $CH_3(CH_2)_2CH_2OH$	colorless, mobile liq/ vinous	*m*—alc, ether, 1 mL in 15 mL water/ 117.7°	
Butyl Butyrate FEMA No. 2186 *n*-Butyl *n*-Butyrate	144.21/$C_8H_{16}O_2$/ $CH_3CH_2CH_2COOC_4H_9$	colorless liq/ fruity, pineapple on dilution	*ss*—prop glycol, water, 1 mL in 3 mL 70% alc; *m*—alc, ether, most veg oils/ 165°	
Butyl Butyryllactate FEMA No. 2190 Butyl Ester; Butyrate; Butyryllactic Acid; Lactic Acid	216.28/$C_{11}H_{20}O_4$/ 	colorless liq/ mild, buttery, cream	*s*—prop glycol; *m*—alc, most fixed oils; *ins*—water	1 mL in 3 mL 70% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{12}H_{20}O_2$ (M-1b)	1.0	1.462–1.466	0.981–0.985	**Angular Rotation**—between −39.5° and −45.0° (Appendix IIB, 100-mm tube) **Solidification Pt.**—NLT 25° (Appendix IIB)
IR	99.5% of C_4H_8O (M-1b)	2.0 (M-15)	1.375–1.384	0.801–0.803	**Distillation Range**—within 1.5° (Appendix IIB) **Water**—0.2% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	98.0% of $C_8H_{14}O_3$ (M-1a)		1.408–1.429	0.972–0.992	
IR	98.0% of $C_6H_{12}O_2$ (M-1b)	2.0 (M-15)	1.393–1.396	0.876–0.880	**Distillation Range**—between 120° and 128° (Appendix IIB)
IR	99.5% of $C_4H_{10}O$ (M-1b)	2.0 (M-15)	1.397–1.402	0.807–0.809	**Butyl Ether**—0.15% (M-1b) **Distillation Range**—max. 1.5° between beginning and end (Appendix IIB)
IR	98.0% of $C_8H_{16}O_2$ (M-1b)	1.0	1.405–1.407	0.867–0.871	
IR	95.0% of $C_{11}H_{20}O_4$ (M-1b)	1.0	1.420–1.423	0.970–0.974	

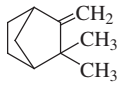
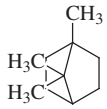
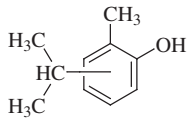
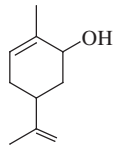
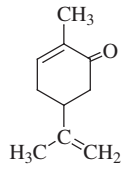
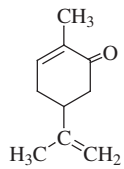
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
2-*sec*-Butyl Cyclohexanone FEMA No. 3261 Freskomenthe	154.25/$C_{10}H_{18}O$/ 	colorless to pale yel liq/ camphoraceous	*s*—prop glycol, veg oils; *ins*—water/ 76° at 8 mm	1 mL in 1 mL 95% ethanol
Butyl Isobutyrate FEMA No. 2188	144.21/$C_8H_{16}O_2$/ $(CH_3)_2CHCOOC_4H_9$	colorless liq/ fresh, fruity, apple-pineapple	*m*—alc, ether, most fixed oils; *ins*—gly, prop glycol, water/ 166°	1 mL in 7 mL 60% alc
Butyl Isovalerate FEMA No. 2218	158.24/$C_9H_{18}O_2$/ $(CH_3)_2CHCH_2COOC_4H_9$	colorless to pale yel liq/ fruity	*s*—alc, most fixed oils; *ins*—prop glycol, water/ 175°	1 mL in 1 mL 95% alc
Butyl 2-Methyl Butyrate FEMA No. 3393	158.24/$C_9H_{18}O_2$/ 	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 173° at 730 mm	1 mL in 1 mL 95% ethanol
Butyl Phenylacetate FEMA No. 2209	196.26/$C_{12}H_{16}O_2$/ 	colorless to pale yel liq/ honey, rose	260°	1 mL in 1 mL 95% alc
Butyl Stearate FEMA No. 2214 Butyl Octadecanoate	340.59/$C_{22}H_{44}O_2$/ $CH_3(CH_2)_{16}COO(CH_2)_3CH_3$	colorless, waxy solid/ odorless to faintly fatty	*s*—alc, most fixed oils; *ins*—prop glycol, water/ 223°	1 mL in 6 mL 95% alc
Butyraldehyde FEMA No. 2219 Butyl Aldehyde	72.11/C_4H_8O/ $CH_3(CH_2)_2CHO$	colorless, mobile liq/ pungent, nutty	*s*—1 mL in 15 mL water; *m*—alc, ether/ 74.8°	
Butyric Acid FEMA No. 2221	88.11/$C_4H_8O_2$/ $CH_3(CH_2)_2COOH$	colorless liq/ strong, rancid, buttery	*m*—alc, most fixed oils, prop glycol water/ 164°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_{10}H_{18}O$ (sum of two isomers) (M-1b)		1.456–1.462	0.910–0.915	
IR	97.0% of $C_8H_{16}O_2$ (one isomer) (M-1b)	1.0	1.401–1.404	0.859–0.864	
IR	97.0% of $C_9H_{18}O_2$ (one isomer) (M-1b)	1.0	1.407–1.411	0.856–0.859	
IR	98.0% of $C_9H_{18}O_2$ (M-1b)	1.0	1.407–1.413	0.858–0.863	
IR	98.0% of $C_{12}H_{16}O_2$ (M-1a)	1.0	1.488–1.492	0.990–0.997	
					Melting Range—between 17° and 21° (Appendix IIB) **Iodine Value**—1 max (Appendix VII) **Saponification Value**—between 165 and 180 (Appendix VI)
IR	98.0% of C_4H_8O (M-2c)	5.0 (methyl red TS)	1.381–1.387	0.797–0.802	**Distillation Range**—between 72° and 80° (first 95%, Appendix IIB) ***para*-Butyraldehyde**—2.5% (M-1b) **Water**—0.5% (Appendix IIB, KF)
IR	99.0% of $C_4H_8O_2$ (M-3a)		1.397–1.399	0.953–0.957	**Reducing Subs.**—passes test (M-14)

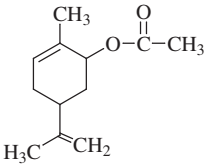
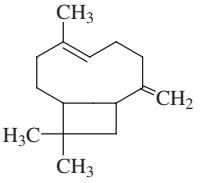
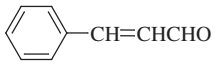
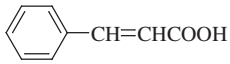
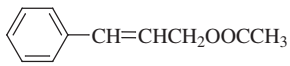
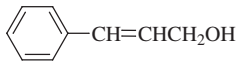
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
γ-Butyrolactone FEMA No. 3291	86.09/$C_4H_6O_2$/ 	colorless to slightly yel liq/ faint, sweet, caramel	*s*—water; *m*—alc/ 204°	
Camphene FEMA No. 2229	136.24/$C_{10}H_{16}$/ 	colorless cryst mass/ camphoraceous-oily	*s*—alc; *m*—most fixed oils; *ins*—water/ 159°	
***d*-Camphor** FEMA No. 2230	152.24/$C_{10}H_{16}O$/ 	white to gray translucent cryst or fused mass/ minty, ethereal	*s*—alc; *ins*—most fixed oils, prop glycol, water/ 204°	1 mL in 1 mL 95% alc
Carvacrol FEMA No. 2245	150.22/$C_{10}H_{14}O$/ 	colorless to pale yel liq/ pungent, spicy, thymol	*s*—alc, ether; *ins*—water/ 238°	1 mL in 4 mL 60% alc gives clear soln
***l*-Carveol** FEMA No. 2247 *p*-Mentha-6,8-dien-2-ol	152.24/$C_{10}H_{16}O$/ 	colorless to pale yel liq/ spearminty	*s*—prop glycol, veg oils; *ins*—water/ 226°–227° (751 mm Hg)	1 mL in 1 mL 95% alc
***d*-Carvone** FEMA No. 2249 *dextro*-Carvone; *d*-1-Methyl-4-isopropenyl-6-cyclohexen-2-one	150.22/$C_{10}H_{14}O$/ 	colorless to light yel liq/ caraway	*s*—prop glycol, most fixed oils; *m*—alc; *ins*—gly/ 230°	1 mL in 5 mL 60% alc
***l*-Carvone** FEMA No. 2249 *levo*-Carvone; l-1-Methyl-4-isopropenyl-6-cyclohexen-2-one	150.22/$C_{10}H_{14}O$/ 	colorless to pale strawberry colored liq/ spearminty	*s*—prop glycol, most fixed oils; *m*—alc; *ins*—gly/ 231°	1 mL in 2 mL 70% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_4H_6O_2$ (M-1a)		1.430–1.440	1.120–1.130	
	80.0% of $C_{10}H_{16}$ (M-1a)				**Solidification Pt**.—40° (Appendix IIB)
IR					**Melting Range**—between 174° and 179° (Appendix IIB) **Angular Rotation**—between +41° and +43° (Appendix IIB)
IR	98.0% of $C_{10}H_{14}O$ (M-1b)		1.521–1.526	0.974–0.980	**Angular Rotation**—between −117° and −130° (Appendix IIB)
IR	96.0% of $C_{10}H_{14}O$ (M-1b)		1.493–1.497	0.947–0.953	**Angular Rotation**—between −117° and −130° (Appendix IIB)
IR	95.0% of $C_{10}H_{16}O$ (M-1b)		1.496–1.499	0.955–0.960	**Angular Rotation**—between +50° and +60° (Appendix IIB, 100-mm tube)
IR	97.0% of $C_{10}H_{14}O$ (M-1b)		1.495–1.502	0.956–0.960	**Angular Rotation**—between −57° and −62° (Appendix IIB, 100-mm tube)

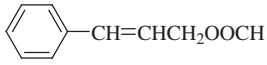
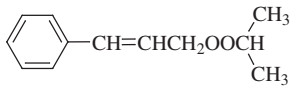
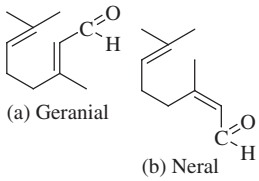
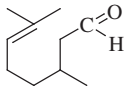
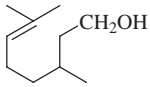
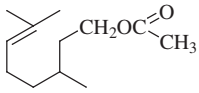
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
***l*-Carvyl Acetate** FEMA No. 2250 *p*-Mentha-6,8-dien-2-yl Acetate	194.27/$C_{12}H_{18}O_2$/ 	colorless to pale yel liq/ spearminty	*s*—alc/ 77°–79° (0.1 mm Hg)	
β-Caryophyllene FEMA No. 2252	204.36/$C_{15}H_{24}$/ 	colorless to slightly yel, oily liq/ woody, spicy	*s*—alc, ether; *ins*—water/ 256°	1 mL in 6 mL 95% alc gives clear soln
Cinnamaldehyde FEMA No. 2286 Cinnamal; Cinnamic Aldehyde	132.16/C_9H_8O/ 	yel, strongly refractive liq/ cinnamon, burning aromatic taste	*m*—alc, chloroform, ether, fixed and vol oils, 1 g in 700 mL water/ 248°	1 mL in 5 mL 60% alc
Cinnamic Acid FEMA No. 2288 3-Phenylpropenoic Acid	148.16/$C_9H_8O_2$/ 	white cryst scales/ honey-floral	*s*—acetic acid, acetone, benzene, most fixed oils, 1 g in 2000 mL water/ 300°	1 g in 7 mL 95% alc
Cinnamyl Acetate FEMA No. 2293	176.22/$C_{11}H_{12}O_2$/ 	colorless to slightly yel liq/ sweet, balsamic, floral	*m*—alc, chloroform, ether, most fixed oils; *ins*—gly, water/ 264°	1 mL in 5 mL 70% alc
Cinnamyl Alcohol FEMA No. 2294 Cinnamic Alcohol	134.18/$C_9H_{10}O$/ 	white to slightly yel cryst solid/ balsamic	*s*—most fixed oils, prop glycol; *ins*—gly/ 258°	1 g in 1 mL 70% alc remains in soln to 10 mL
Cinnamyl Butyrate FEMA No. 2296	204.27/$C_{13}H_{16}O_2$/ 	colorless to pale yel liq/ fruity, balsamic	300°	1 mL in 1 mL 95% alc
Cinnamyl Cinnamate FEMA No. 2298	264.32/$C_{18}H_{16}O_2$/ 	mixture of (*Z*) and (*E*) isomers; low-melting solid	370°	1 mL in 1 mL 95% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{12}H_{18}O_2$ (M-1b)	1.0	1.473–1.479	0.964–0.970	**Angular Rotation**—between −90° and −120° (Appendix IIB)
IR	80.0% of $C_{15}H_{24}$ (M-1a)		1.498–1.504	0.897–0.910	**Angular Rotation**—between −5° and −10° (Appendix IIB, 100-mm tube) **Phenols**—3.0% (M-1b)
IR	98.0% of C_9H_8O (M-1b)	10.0	1.619–1.623	1.046–1.050	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	99.0% of $C_9H_8O_2$ (after drying) (M-3b)				**Melting Range**—NLT 130° (Appendix IIB) **Residue on Ignit.**—0.05% (Appendix IIC)
IR	98.0% of $C_{11}H_{12}O_2$ (M-1b)	1.0	1.539–1.543	1.050–1.054	
IR	98.0% of $C_9H_{10}O$ (M-1b)				**Aldehydes**—1.5% (M-1b) **Chlorinated Cmpds.**—passes test (Appendix VI) **Solidification Pt.**—NLT 31° (Appendix IIB)
IR	96.0% of $C_{13}H_{16}O_2$ (M-1b)	1.0	1.525–1.530	1.010–1.015	
IR	95.0% of $C_{18}H_{16}O_2$ (M-1b)	2.0			

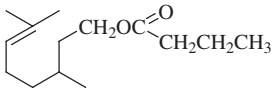
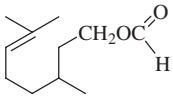
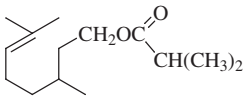
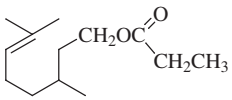
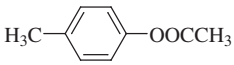
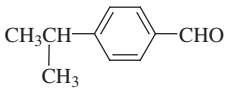
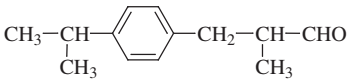
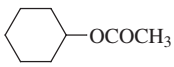
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Cinnamyl Formate FEMA No. 2299	162.19/$C_{10}H_{10}O_2$/ 	colorless to slightly yel liq/ green, herbaceous, balsamic	*m*—alc, chloroform, ether, most fixed oils; *ins*—water/ 250°	1 mL in 2 mL 80% alc gives clear soln
Cinnamyl Isobutyrate FEMA No. 2297	204.27/$C_{13}H_{16}O_2$/ 	colorless to pale yel liq/ sweet, balsamic, fruity	254°	1 mL in 1 mL 95% alc
Cinnamyl Isovalerate FEMA No. 2302	218.30/$C_{14}H_{18}O_2$/ 	colorless to slightly yel liq/ spicy, floral, fruity	*m*—alc, chloroform, most fixed oils, ether; *ins*—gly, prop glycol, water/ 313°	1 mL in 1 mL 90% alc
Cinnamyl Propionate FEMA No. 2301	190.24/$C_{12}H_{14}O_2$/ 	colorless to pale yel liq/ spicy, fruity, balsamic	*m*—alc, chloroform, ether, most fixed oils; *ins*—gly, prop glycol, water/ 289°	
Citral FEMA No. 2303 Mixture of Geranial [(*E*)-3,7-dimethyl-2,6-octadien-1-al] and Neral [the (*Z*) isomer]	152.24/$C_{10}H_{16}O$/  (a) Geranial (b) Neral	pale yel liq/ strong, lemon	*s*—most fixed oils, min oil, prop glycol; *ins*—gly/ 228°	1 mL in 7 mL 70% alc
Citronellal FEMA No. 2307 3,7-Dimethyl-6-octen-1-al	154.25/$C_{10}H_{18}O$/ 	colorless to slightly yel liq/ intense lemon-citronella-rose	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 206°	1 mL in 5 mL 70% alc remains clear on dilution
Citronellol FEMA No. 2309 3,7-Dimethyl-6-octen-1-ol	156.27/$C_{10}H_{20}O$/ 	colorless, oily liq/ rosy	*s*—most fixed oils, prop glycol; *ss*—water; *ins*—gly/ 225°	1 mL in 2 mL 70% alc remains in soln to 10 mL
Citronellyl Acetate FEMA No. 2311 3,7-Dimethyl-6-octen-1-yl Acetate	198.31/$C_{12}H_{22}O_2$/ 	colorless liq/ fruity	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 229°	1 mL in 9 mL 70% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	92.0% of $C_{10}H_{10}O_2$ (M-1a)	3.0	1.550–1.556	1.077–1.082	**Cinnamyl Alcohol**—8.0% (M-1a)
IR	96.0% of $C_{13}H_{16}O_2$ (M-1b)	3.0	1.523–1.528	1.006–1.009	
IR	95.0% of $C_{14}H_{18}O_2$ (one major isomer) (M-1b)	3.0	1.518–1.524	0.991–0.996	
IR	98.0% of $C_{12}H_{14}O_2$ (one isomer) (M-1b)	3.0	1.532–1.537	1.029–1.035	
IR	96.0% of $C_{10}H_{16}O$ (sum of neral and geranial) (M-1b)		1.486–1.490	0.885–0.891	
IR	85.0% of aldehydes as $C_{10}H_{18}O$ (one isomer) (M-1b)	3.0	1.446–1.456	0.850–0.860	**Angular Rotation**—between −1° and +11° (Appendix IIB, 100-mm tube)
IR	90.0% of total alcohols as $C_{10}H_{20}O$ (Appendix VI; 1.2 g/78.13)		1.454–1.462	0.850–0.860	**Aldehydes**—1.0% as citronellal (M-2d; 5 g/66.08) **Esters**—1.0% as citronellyl acetate (Appendix VI; 5 g/99.15)
IR	92.0% of total esters as $C_{12}H_{22}O_2$ (Appendix VI; 1.4 g/99.15)	1.0	1.440–1.450	0.883–0.893	

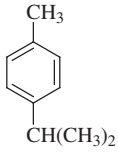
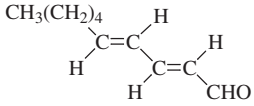
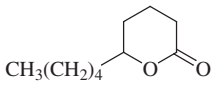
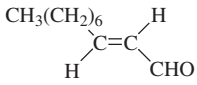
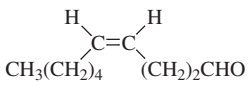
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Citronellyl Butyrate FEMA No. 2312 3,7-Dimethyl-6-octen-1-yl Butyrate	226.36/$C_{14}H_{26}O_2$/ 	colorless liq/ strong, fruity-rosy	*m*—alc, ether, most fixed oils, chloroform; *ins*—water/ 245°	1 mL in 6 mL 80% alc gives clear soln
Citronellyl Formate FEMA No. 2314 3,7-Dimethyl-6-octen-1-yl Formate	184.28/$C_{11}H_{20}O_2$/ 	colorless liq/ strong, fruity, floral	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 235°	1 mL in 3 mL 80% alc remains in soln to 10 mL
Citronellyl Isobutyrate FEMA No. 2313 3,7-Dimethyl-6-octen-1-yl Isobutyrate	226.36/$C_{14}H_{26}O_2$/ 	colorless liq/ fruity-rosy	*m*—alc, chloroform, ether, most fixed oils; *ins*—water/ 249°	1 mL in 6 mL 80% alc gives clear soln
Citronellyl Propionate FEMA No. 2316 Citronellyl Propanoate; 3,7-Dimethyl-6-octen-1-yl Propionate	212.33/$C_{13}H_{24}O_2$/ 	colorless liq/ fruity-rosy	*m*—alc, most fixed oils; *ins*—water/ 242°	1 mL in 4 mL 80% alc gives clear soln
***p*-Cresyl Acetate** FEMA No. 3073 *p*-Methylphenyl Acetate; *p*-Tolyl Acetate	150.18/$C_9H_{10}O_2$/ 	colorless liq/ strong, floral	*s*—most fixed oils, prop glycol; *ins*—gly/ 212°	1 mL in 2 mL 70% alc
Cuminic Aldehyde FEMA No. 2341 Cuminal; Cuminaldehyde; *p*-Cuminic Aldehyde; *p*-Isopropylbenzaldehyde	148.20/$C_{10}H_{12}O$/ 	colorless to pale yel liq/ strong, pungent, cumin oil	*s*—alc, ether; *ins*—water/ 236°	1 mL in 4 mL 70% alc
Cyclamen Aldehyde FEMA No. 2743 2-Methyl-3-(*p*-isopropylphenyl)propionaldehyde	190.29/$C_{13}H_{18}O$/ 	colorless to pale yel liq/ strong, floral	*s*—most fixed oils; *ins*—gly, prop glycol/ 270°	1 mL in 3 mL 80% alc
Cyclohexyl Acetate FEMA No. 2349	142.20/$C_8H_{14}O_2$/ 	colorless to pale yel liq/ green, fruity	*s*—alc/ 174°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	90.0% of total esters as $C_{14}H_{26}O_2$ (Appendix VI; 1.5 g/ 113.2)	1.0	1.444–1.448	0.873–0.883	
IR	86.0% of total esters as $C_{11}H_{20}O_2$ (Appendix VI; 1.0 g/ 92.14)	3.0	1.443–1.452	0.890–0.903	
IR	92.0% of total esters as $C_{14}H_{26}O_2$ (Appendix VI; 1.5 g/ 113.2)	1.0	1.440–1.448	0.870–0.880	
IR	90.0% of total esters as $C_{13}H_{24}O_2$ (Appendix VI; 1.2 g/ 95.12)	1.0	1.443–1.449	0.877–0.886	
IR	98.0% of $C_9H_{10}O_2$ (one isomer) (M-1b)	1.0 (phenol red TS)	1.499–1.502	1.044–1.050	**Free Cresol**—1.0% (M-17)
IR	95.0% of $C_{10}H_{12}O$ (M-2a)	5.0	1.528–1.534	0.975–0.980	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	90.0% of $C_{13}H_{18}O$ (sum of two isomers; major isomer 85.0% min) (M-1b)	5.0	1.503–1.508	0.946–0.952	
	98.0% of $C_8H_{14}O_2$ (M-1b)	1.0	1.436–1.441	0.966–0.970	

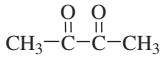
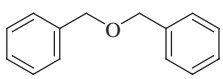
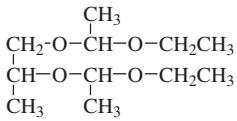
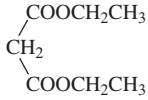
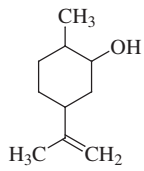
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
***p*-Cymene** FEMA No. 2356	134.22/$C_{10}H_{14}$/ 	colorless to pale yel liq/ kerosene	*s*—veg oils; *ins*—water, prop glycol/ 177°	1 mL in 1 mL 95% alc
(*E*),(*E*)-2,4-Decadienal FEMA No. 3135 *trans*,*trans*-2,4-Decadienal	152.24/$C_{10}H_{16}O$/ 	yel liq/ powerful, oily, chicken fat	*s*—alc, most fixed oils; *ins*—water/ 104° (7 mm Hg)	1 mL in 1 mL 95% ethanol
δ-Decalactone FEMA No. 2361	170.25/$C_{10}H_{18}O_2$/ 	colorless liq/ coconut-fruity, buttery on dilution	*vs*—alc, prop glycol, veg oils; *ins*—water/ 281°	1 mL in 1 mL 95% ethanol
γ-Decalactone FEMA No. 2360 4-Hydroxydecanoic Acid Lactone	170.25/$C_{10}H_{18}O_2$/ 	colorless to pale yel liq/ fruity, peach	*s*—prop glycol; veg oils; *ins*—water/ 281°	1 mL in 1 mL 95% alc
Decanal FEMA No. 2362 Aldehyde C-10; Capraldehyde	156.27/$C_{10}H_{20}O$/ $CH_3(CH_2)_8CHO$	colorless to light yel liq/ fatty, floral-orange on dilution	*m*—alc, most fixed oils, prop glycol (may be turbid); *ins*—gly, water/ 209°	
(*E*)-2-Decenal FEMA No. 2366 *trans*-2-Decenal	154.25/$C_{10}H_{18}O$/ 	slightly yel liq/ orange, wax	*s*—alc, most fixed oils; *ins*—water/ 229°	1 mL in 1 mL 95% ethanol
(*Z*)-4-Decenal FEMA No. 3264 *cis*-4-Decenal	154.25/$C_{10}H_{18}O$/ 	colorless to slightly yel liq/ orange, fatty	*s*—alc, most fixed oils; *ins*—water/ 78°–80° (10 mm Hg)	1 mL in 1 mL 95% ethanol
Decyl Alcohol FEMA No. 2365 Alcohol C-10; 1-Decanol	158.28/$C_{10}H_{22}O$/ $CH_3(CH_2)_8CH_2OH$	colorless liq/ floral, waxy, fruity	*s*—alc, ether, min oil, prop glycol, most fixed oils; *ins*—gly, water/ 233°	1 mL in 3 mL 60% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_{10}H_{14}$ (M-1a)		1.489–1.491	0.853–0.855	
IR	89.0% of $C_{10}H_{16}O$ (sum of two isomers) (M-1a)		1.514–1.519	0.866–0.876	
IR	98.0% of $C_{10}H_{18}O_2$ (M-1b)	5.0 (Appendix VII; A.V. Method II)	1.454–1.459	0.964–0.971	
IR	95.0% of $C_{10}H_{18}O_2$ (M-1a)	1.0	1.447–1.451	0.949–0.954	
IR	92.0% of $C_{10}H_{20}O$ (M-1b)	10.0	1.426–1.430	0.823–0.832	
IR	92.0% of $C_{10}H_{18}O$ (one isomer) (M-1a)		1.452–1.457	0.836–0.846	
IR	90.0% of $C_{10}H_{18}O$ (M-1a)		1.442–1.447	0.843–0.850	
IR	98.0% of $C_{10}H_{22}O$ (M-1b)	1.0	1.435–1.439	0.826–0.831	**Solidification Pt.**—NLT 5° (Appendix IIB)

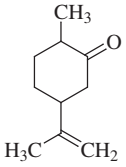
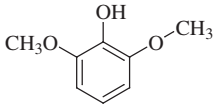
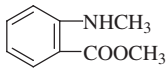
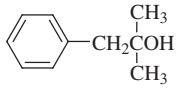
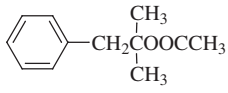
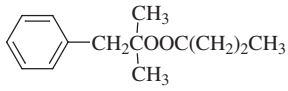
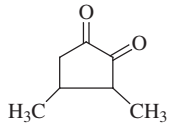
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Diacetyl FEMA No. 2370 2,3-Butanedione; Dimethyldiketone; Dimethylglyoxal	86.09/$C_4H_6O_2$/ 	yel to yel-green liq/ powerful, buttery in very dilute soln	*s*—gly, water; *m*—alc, most fixed oils, prop glycol/ 88°	
Dibenzyl Ether FEMA No. 2371	198.26/$C_{14}H_{14}O$/ 	colorless to pale yel liq/ earthy	298°	1 mL in 1 mL 95% alc
1,2-Di[(1′-ethoxy)ethoxy]propane FEMA No. 3534	220.31/$C_{11}H_{24}O_4$/ 	colorless to pale yel liq/ odorless when pure		
Diethyl Malonate FEMA No. 2375 Ethyl Malonate; Malonic Ester	160.17/$C_7H_{12}O_4$/ 	colorless liq/ slight, fruity	*s*—most fixed oils, prop glycol; *ss*—alc, water; *ins*—gly, min oil/ 200°	1 mL in 1.5 mL 60% alc
Diethyl Sebacate FEMA No. 2376 Ethyl Sebacate	258.36/$C_{14}H_{26}O_4$/ $C_2H_5OOC(CH_2)_8COOC_2H_5$	colorless to slightly yel liq/ faint, winy, fruity	*m*—alc, ether, other org solvents, most fixed oils; *ins*—water/ 302°	
Diethyl Succinate FEMA No. 2377 Ethyl Succinate	174.20/$C_8H_{14}O_4$/ $C_2H_5OOCCH_2CH_2COOC_2H_5$	colorless, mobile liq/ faint, winy, ethereal	*s*—1 mL in 50 mL water; *m*—alc, ether, most fixed oils/ 217°	
Dihydrocarveol FEMA No. 2379	154.25/$C_{10}H_{18}O$/ 	almost colorless, oily liq/ spearmint	*s*—alc, most fixed oils; *ins*—water/ 225°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_4H_6O_2$ (M-1b)		1.393–1.397	0.979–0.985	**Solidification Pt.**—between –2.0° and –4.0° (Appendix IIB)
IR	98.0% of $C_{14}H_{14}O$ (M-1b)		1.557–1.565	1.039–1.044	**Chlorinated Cmpds.**—passes test (Appendix VI)
	97.0% of $C_{11}H_{24}O_4$ (M-1b)	0.1	1.409–1.413	0.915–0.925	
IR	98.0% of $C_7H_{12}O_4$ (M-1b)	1.0	1.413–1.416	1.053–1.056	
IR	98.0% of $C_{14}H_{26}O_4$ (M-1b)	1.0	1.435–1.438	0.960–0.965	
IR	99.0% of $C_8H_{14}O_4$ (M-1a)	2.0	1.036–1.040	1.419–1.423	**Diethyl Maleate**—0.03% (M-1b) **Water**—0.05% (Appendix IIB, KF)
	96.0% of $C_{10}H_{18}O$ (sum of two isomers) (M-1a)		1.477–1.481	0.921–0.926	

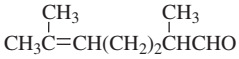
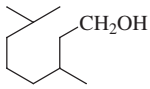
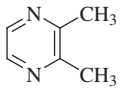
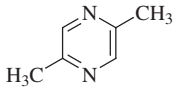
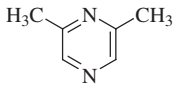
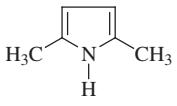
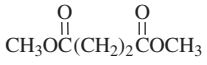
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
***d*-Dihydrocarvone** FEMA No. 3565 *d*-2-Methyl-5-(1-methylethenyl)-cyclohexanone	154.24/$C_{10}H_{16}O$/ 	almost colorless liq/ herbaceous, spearmint	*s*—alc, most fixed oils; *ins*—water/ 222°	1 mL in 1 mL 95% alc
2,6-Dimethoxy Phenol FEMA No. 3137	154.17/$C_8H_{10}O_3$/ 	white to brown cryst/ smoky	*ss*—prop glyc, veg oils; *ins*—water/ 262°	1 g in 2 mL 95% ethanol
Dimethyl Anthranilate FEMA No. 2718 Methyl *N*-Methyl Anthranilate	165.19/$C_9H_{11}NO_2$/ 	pale yel liq with pale blue fluorescence/ grape	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 256°	1 mL in 3 mL 80% alc remains in soln to 10 mL
Dimethyl Benzyl Carbinol FEMA No. 2393 α,α-Dimethylphenethyl Alcohol	150.22/$C_{10}H_{14}O$/ 	white cryst solid; may exist in supercooled form as colorless to pale yel liq/ floral	*s*—min oil, most fixed oils, prop glycol; *ins*—gly	1 mL in 3 mL 50% alc remains in soln to 10 mL
Dimethyl Benzyl Carbinyl Acetate FEMA No. 2392 α,α-Dimethylphenethyl Acetate	192.26/$C_{12}H_{16}O_2$/ 	colorless liq; solidifies at room temp/ floral, fruity	*s*—most fixed oils; *ss*—prop glycol; *ins*—water/ 250°	1 mL in 4 mL 70% alc
Dimethyl Benzyl Carbinyl Butyrate FEMA No. 2394 α,α-Dimethylphenethyl Butyrate	220.31/$C_{14}H_{20}O_2$/ 	almost colorless liq/ prune	*s*—alc, most fixed oils; *ins*—prop glycol, water/ 237°–255°	1 mL in 1 mL 95% ethanol
3,4-Dimethyl 1,2-Cyclopentandione FEMA No. 3268	126.16/$C_7H_{10}O_2$/ 	pale yel to orange cryst/ maple	*ss*—prop glycol; *ins*—veg oils, water/ 142°	1 g in 3 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	92.0% of $C_{10}H_{16}O$ (sum of two isomers) (M-1a)		1.470–1.474	0.923–0.928	**Angular Rotation**—between +14.0° and +22° (Appendix IIB)
IR	98.0% of $C_8H_{10}O_3$ (M-1b)				**Melting Range**—between 53.0° and 56.0° (Appendix IIB)
	98.0–101.3% of total esters as $C_9H_{11}NO_2$ (Appendix VI; 1.1 g/82.60)		1.577–1.583	1.124–1.132	**Solidification Pt.**—NLT 14° (Appendix IIB)
IR	97.0% of $C_{10}H_{14}O$ (M-1b)	1.0	1.514–1.517 (20°, as supercooled liq)	0.972–0.977	**Chlorinated Cmpds.**—passes test (Appendix VI) **Solidification Pt.**—NLT 22° (Appendix IIB)
IR	98.0% of $C_{12}H_{16}O_2$ (M-1b)	1.0	1.490–1.495	0.995–1.002	**Chlorinated Cmpds.**—passes test (Appendix VI) **Solidification Pt.**—NLT 28° (Appendix IIB)
IR	95.0% of $C_{14}H_{20}O_2$ (M-1b)		1.484–1.489	0.960–0.981	
IR	95.0% of $C_7H_{10}O_2$ (M-1b)				**Melting Range**—between 64.0° and 72.0° (Appendix IIB)

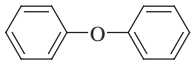
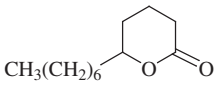
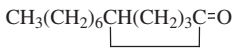
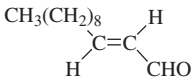
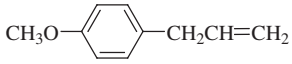
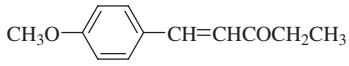
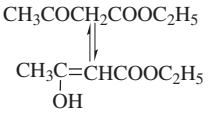
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
2,6-Dimethyl-5-heptenal FEMA No. 2389	140.23/$C_9H_{16}O$/ 	pale yel liq/ melon	*s*—veg oils; *ss*—prop glycol; *ins*—water/ 116°–124° (100 mm Hg)	1 mL in 1 mL 95% alc
3,7-Dimethyl-1-octanol FEMA No. 2391 Dimethyl Octanol; Tetrahydrogeraniol	158.28/$C_{10}H_{22}O$/ 	colorless liq/ sweet, rose	*s*—most fixed oils, prop glycol; *ins*—gly/ 213°	1 mL in 3 mL 70% alc
2,3-Dimethylpyrazine FEMA No. 3271	108.14/$C_6H_8N_2$/ 	colorless to slightly yel liq/ nutty, cocoa	*m*—org solvents, water/ 156°	1 mL in 1 mL 95% ethanol
2,5-Dimethylpyrazine FEMA No. 3272	108.14/$C_6H_8N_2$/ 	colorless to slightly yel liq/ earthy, potato	*m*—water, org solvents/ 155°	
2,6-Dimethylpyrazine FEMA No. 3273	108.14/$C_6H_8N_2$/ 	white to yel, lumpy cryst/ nutty, coffee	*s*—water, org solvents/ 155°	
2,5-Dimethylpyrrole	95.14/C_6H_9N/ 	colorless to yellow, oily liq/ burnt	*vs*—alc, ether; *vss*—water/ 165°	
Dimethyl Succinate FEMA No. 2396	146.14/$C_6H_{10}O_4$/ 	colorless to pale yel liq/ mild, fruity	*s*—prop glycol, veg oils; *ins*—water/ 196°	1 mL in 1 mL 95% alc
Dimethyl Sulfide FEMA No. 2746 Methyl Sulfide; Thiobismethane	62.14/C_2H_6S/ 	colorless to pale yel liq/ disagreeable, intense boiled cabbage	*s*—prop glycol, veg oils; *ins*—water/ 37°	1 mL in 1 mL 95% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	85.0% of $C_9H_{16}O$ (M-2d; 1 g/14.01)	5.0	1.442–1.447	0.848–0.854	
IR	90.0% of total alcohols as $C_{10}H_{22}O$ (Appendix VI; 1.2 g/79.15)	1.0	1.435–1.445	0.826–0.842	
IR	95.0% of $C_6H_8N_2$ (M-1a)		1.506–1.509	1.000–1.022	**Distillation Range**—between 152° and 157° (Appendix IIB) **Solidification Pt.**—11° to 13° (Appendix IIB) **Tri- and Tetrapyrazines**—5% (by GC assay) **Water**—0.5% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	99.0% of $C_6H_8N_2$ (M-1a)		1.497–1.501	0.980–1.000	**Solidification Pt.**—between 12° and 17° (Appendix IIB) **Water**—0.5% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	98.0% of $C_6H_8N_2$ (M-1a)			0.965 (50°)	**Melting Range**—between 35° and 40° (Appendix IIB) **Residue on Ignit.**—0.1% (Appendix IIC) **Water**—0.5% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	98.0% of C_6H_9N (M-1a)		1.503–1.506	0.932–0.942	**Water**—0.5% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
	98.0% of $C_6H_{10}O_4$ (M-1b)	1.0	1.418–1.421	1.114–1.118	
IR	99.0% of C_2H_6S (M-1a)		1.431–1.441	0.842–0.847	

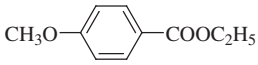
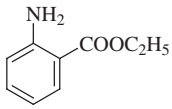
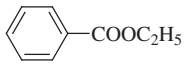
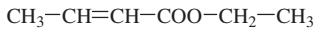
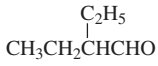
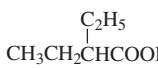
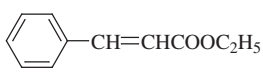
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Diphenyl Ether FEMA No. 3667 Diphenyl Oxide	170.21/$C_{12}H_{10}O$/ 	colorless to white to pale yel liq/ rose	*s*—veg oils; *vss*—water/ 259°	1 g in 2 mL 95% ethanol
δ-Dodecalactone FEMA No. 2401	198.31/$C_{12}H_{22}O_2$/ 	colorless to yel liq/ coconut-fruity, buttery on dilution	*vs*—alc, prop glycol, veg oils; *ins*—water/ 140°–141° (1 mm Hg)	1 mL in 1 mL 95% ethanol
γ-Dodecalactone FEMA No. 2400 4-Hydroxydodecanoic Acid Lactone	198.31/$C_{12}H_{22}O_2$/ 	colorless to pale yel liq/ fruity, peach, pear	*s*—prop glycol, veg oils; *ins*—water/ 131° (1.5 mm Hg)	1 mL in 1 mL 95% alc
(*E*)-2-Dodecen-1-al FEMA No. 2402 *trans*-2-Dodecen-1-al	182.31/$C_{12}H_{22}O$/ 	slightly yel liq/ fatty, citrus	*s*—alc, most fixed oils; *ins*—water/ 272°	1 mL in 1 mL 95% ethanol
Estragole FEMA No. 2411 *p*-Allylanisole; Methyl Chavicol	148.20/$C_{10}H_{12}O$/ 	colorless to light yel liq/ anise	*s*—alc; *ins*—water/ 216°	1 mL in 6 mL 80% alc gives clear soln
Ethone FEMA No. 2673 1-(*p*-Methoxyphenyl)-1-penten-3-one	190.24/$C_{12}H_{14}O_2$/ 	white to pale yel cryst/ nutty, maple		1 g in 7 mL 95% alc
Ethyl Acetate FEMA No. 2414	88.11/$C_4H_8O_2$/ $CH_3COOC_2H_5$	colorless liq; vol at low temp; flammable/ fragrant, acetous, ethereal	*m*—alc, ether, gly, most fixed oils, vol oils, 1 mL in 10 mL water/ 54°	
Ethyl Acetoacetate FEMA No. 2415 Acetoacetic Ester; Ethyl 3-Oxybutanoate	130.14/$C_6H_{10}O_3$/ 	colorless to very light yel, mobile liq/ fruity	*m*—alc, ether, ethyl acetate, 1 mL in 12 mL water/ 181°	
Ethyl Acrylate FEMA No. 2418	100.12/$C_5H_8O_2$/ $CH_2{=}CHCOOC_2H_5$	colorless, mobile liq; lachrymator/ intense, harsh, fruity	*m*—alc, ether, 1 mL in 50 mL water/ 99°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	99.0% of $C_{12}H_{10}O$ (M-1b)		freezes	1.070–1.074	**Melting Range**—between 26.0° and 30.0°
IR	98.0% of $C_{12}H_{22}O_2$ (sum of two isomers; γ isomer 95.0% min) (M-1a)	8.0 (Appendix VII; A.V. Method II)	1.458–1.461	0.942–0.950	**Saponification Value**—between 278 and 286 (Appendix VI, 1-g sample)
	97.0% of $C_{12}H_{22}O_2$ (M-1a)	1.0	1.451–1.456	0.933–0.938	
IR	93.0% of $C_{12}H_{22}O$ (M-1a)		1.454–1.460	0.839–0.849	
IR	95.0% of $C_{10}H_{12}O$ (M-1a)		1.519–1.524	0.960–0.968	
IR	98.0% of $C_{12}H_{14}O_2$ (M-1b)				**Solidification Pt.**—min 59.0° (Appendix IIB)
IR	99.0% of $C_4H_8O_2$ (M-1b)	5.0 (bromocresol purple TS)	1.370–1.375	0.894–0.898	**Distillation Range**—between 76° and 77.5° (Appendix IIB) **Methyl Cmpds.**—passes test (M-10) **Readily Carb. Subs.**—passes test (M-12) **Residue on Evap.**—0.02% (M-16, 10-g sample, 105°)
IR	97.5% of $C_6H_{10}O_3$ (M-1b)	5.0 (bromocresol purple TS)	1.418–1.421	1.022–1.027	
IR	99.5% of $C_5H_8O_2$ (M-1b)	5.0		0.916–0.919	**Antioxidants**—0.022% (M-6) **Water**—0.05% (Appendix IIB, KF)

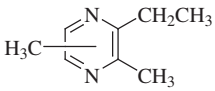
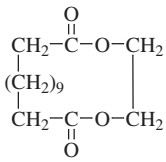
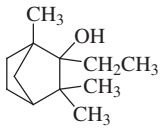
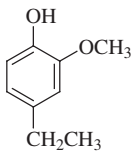
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Ethyl *p*-Anisate FEMA No. 2420 Ethyl *p*-Methoxybenzoate	180.20/$C_{10}H_{12}O_3$/ 	colorless to slightly yel liq/ light, fruity, anise	*s*—alc, chloroform, ether; *ins*—water/ 270°	1 mL in 7 mL 60% alc gives clear soln
Ethyl Anthranilate FEMA No. 2421 Ethyl *o*-Aminobenzoate	165.19/$C_9H_{11}NO_2$/ 	colorless to amber-colored liq/ floral, orange blossom	*s*—alc, most fixed oils, prop glycol/ 267°	1 mL in 2 mL 70% alc
Ethyl Benzoate FEMA No. 2422	150.18/$C_9H_{10}O_2$/ 	colorless liq/ heavy, floral, fruity	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/ 212°	1 mL in 6 mL 60% alc
Ethyl Benzoyl Acetate FEMA No. 2423	192.21/$C_{11}H_{12}O_3$/ 	colorless to light yel liq/ whiskey	265°	
Ethyl-(*E*)-2-butenoate FEMA No. 3486 Ethyl-*trans*-2-butenoate; Ethyl Crotonate	114.14/$C_6H_{10}O_2$/ 	colorless to pale yel liq/ sweet, ethereal	*s*—prop glycol, veg oils; *ins*—water/ 136°	1 mL in 1 mL 95% alc
2-Ethylbutyraldehyde FEMA No. 2426	100.16/$C_6H_{12}O$/ 	colorless, mobile liq/ pungent	*m*—alc, ether, 1 mL in 50 mL water/ 117°	
Ethyl Butyrate FEMA No. 2427	116.16/$C_6H_{12}O_2$/ 	colorless liq/ banana-pineapple	*s*—most fixed oils, prop glycol; *ins*—gly/ 121°	1 mL in 3 mL 60% alc
2-Ethylbutyric Acid FEMA No. 2429	116.16/$C_6H_{12}O_2$/ 	colorless liq/ mildly rancid	*m*—alc, ether, 1 mL in 65 mL water/ 99° (18 mm Hg)	
Ethyl Cinnamate FEMA No. 2430 Ethyl 3-Phenylpropenate	176.22/$C_{11}H_{12}O_2$/ 	colorless, oily liq/ faint, cinnamon	*m*—alc, ether, most fixed oils; *ins*—gly, water/ 272°	1 mL in 5 mL 70% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_{10}H_{12}O_3$ (M-1b)	1.0	1.522–1.526	1.101–1.104	
IR	96.0% of total esters as $C_9H_{11}NO_2$ (Appendix VI; 1.5 g/82.6)	1.0	1.563–1.566	1.115–1.120	**Solidification Pt.**—NLT 13° (Appendix IIB)
IR	98.0% of $C_9H_{10}O_2$ (M-1b)	1.0	1.502–1.506	1.043–1.046	**Chlorinated Cmpds.**—passes test (Appendix VI)
	88.0% of $C_{11}H_{12}O_3$ (M-1b)	2.0	1.528–1.533	1.107–1.120	
IR	97.0% of $C_6H_{10}O_2$ (M-1b)	5.0	1.422–1.427	0.913–0.920	
	95.0% of $C_6H_{12}O$ (M-1b)	2.0	1.398–1.404	0.808–0.814	**Distillation Range**—NLT 95% between 100° and 120° (Appendix IIB)
IR	98.0% of $C_6H_{12}O_2$ (M-1b)	1.0	1.391–1.394	0.870–0.877	
IR	98.0% of $C_6H_{12}O_2$ (M-3b)		1.408–1.418	0.917–0.922	**Distillation Range**—between 190° and 200° (Appendix IIB) **Water**—0.2% (Appendix IIB, KF)
IR	98.0% of $C_{11}H_{12}O_2$ (M-1b)	1.0	1.558–1.561	1.045–1.051	

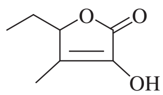
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Ethyl Decanoate FEMA No. 2432 Ethyl Caprate	200.32/$C_{12}H_{24}O_2$/ $CH_3(CH_2)_8COOC_2H_5$	colorless liq/ oily, brandy	*s*—most fixed oils; *ins*—gly, prop glycol/ 243°	1 mL in 4 mL 80% alc
2-Ethyl-3,5(6)-dimethylpyrazine FEMA No. 3149	136.20/$C_8H_{12}N_2$/ 	colorless to slightly yel liq/ roasted cocoa	*s*—prop glycol, veg oils/ 180°–181°	
Ethylene Brassylate FEMA No. 3543	270.37/$C_{15}H_{26}O_4$/ 	colorless to pale yel liq/ sweet, musky	*s*—veg oils; *ins*—prop glycol, water/ 138°–142° (1 mm Hg)	1 mL in 1 mL 95% alc
2-Ethyl Fenchol FEMA No. 3491	182.31/$C_{12}H_{22}O$/ 	pale yel liq/ sharp, camphoraceous, earthy	*s*—alc, prop glycol, most fixed oils; *ins*—water/ 105° (15 mm Hg)	
Ethyl Formate FEMA No. 2434	74.08/$C_3H_6O_2$/ $HCOOC_2H_5$	colorless liq/ sharp, rum	*s*—most fixed oils, prop glycol, water (decomp); *ss*—min oil; *ins*—gly/ 54°	1 mL in 5 mL 50% alc
4-Ethyl Guaiacol FEMA No. 2436 4-Hydroxy-3-methylethylbenzene	152.19/$C_9H_{12}O_2$/ 	colorless to pale yel liq/ warm, spicy, medicinal	*s*—prop glycol, veg oils; *ins*—water/ 235°	1 mL in 1 mL 95% alc
Ethyl Heptanoate FEMA No. 2437 Ethyl Heptoate	158.24/$C_9H_{18}O_2$/ $CH_3(CH_2)_5COOC_2H_5$	colorless liq/ winy-brandy	*ss*—prop glycol; *m*—alc, chloroform, most fixed oils; *ins*—gly/ 189° (72% water azeotrope, 98.5°)	1 mL in 3 mL 70% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{12}H_{24}O_2$ (M-1b)	1.0	1.424–1.427	0.862–0.867	
IR	95.0% of $C_8H_{12}N_2$ (M-1a)		1.500–1.503	0.950–0.970	**Water**—0.1% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	95.0% of $C_{15}H_{26}O_4$ (M-1a)	1.0	1.468–1.473	1.040–1.045	
IR	95.0% of $C_{12}H_{22}O$ (M-1a)		1.470–1.491	0.946–0.967	
IR	95.0% of $C_3H_6O_2$ (M-1b)		1.359–1.363	0.916–0.921	**Acidity**—0.2% (M-5)
IR	98.0% of $C_9H_{12}O_2$ (M-1a)		1.525–1.530	1.061–1.064	
IR	98.0% of $C_9H_{18}O_2$ (M-1b)	1.0	1.411–1.415	0.867–0.872	

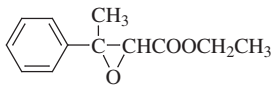
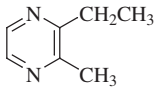
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Ethyl Hexanoate FEMA No. 2439 Ethyl Caproate; Ethyl Capronate	144.21/$C_8H_{16}O_2$/ $CH_3(CH_2)_4COOC_2H_5$	colorless liq/ winy	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly/ 166°	1 mL in 2 mL 70% alc
2-Ethyl Hexanol FEMA No. 3151 2-Ethyl-1-hexanol	130.23/$C_8H_{18}O$/ $CH_3(CH_2)_3CH(C_2H_5)CH_2OH$	colorless to pale yel liq/ green	*s*—prop glycol, veg oils; *ins*—water/ 183°	1 mL in 1 mL 95% ethanol
5-Ethyl 3-Hydroxy 4-Methyl 2(5H)-Furanone FEMA No. 3153 Maple Furanone	142.15/$C_7H_{10}O_3$/ 	pale yel to yel liq/ maple	*s*—prop glycol, veg oils; *ss*—water/ 83° (0.5 mm Hg)	1 mL in 2 mL 95% ethanol
Ethyl Isobutyrate FEMA No. 2428	116.16/$C_6H_{12}O_2$/ $(CH_3)_2CHCOOC_2H_5$	colorless liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 112°–113°	1 mL in 1 mL 95% alc
Ethyl Isovalerate FEMA No. 2463 Ethyl 3-Methylbutyrate	130.19/$C_7H_{14}O_2$/ $(CH_3)_2CHCH_2COOC_2H_5$	colorless liq/ strong, fruity, vinous, apple on dilution	*s*—prop glycol, 1 mL in 350 mL water; *m*—alc, most fixed oils/ 135°	
Ethyl Lactate FEMA No. 2440 Ethyl 2-Hydroxypropionate	118.13/$C_5H_{10}O_3$/ $CH_3CHOHCOOC_2H_5$	colorless liq/ cheesy	*vs*—alc, ether, chloroform, water/ 154°	
Ethyl Laurate FEMA No. 2441 Ethyl Dodecanoate	228.38/$C_{14}H_{28}O_2$/ $CH_3(CH_2)_{10}COOC_2H_5$	colorless, oily liq/ fruity-floral	*m*—alc, chloroform, ether; *ins*—water/ 269°	1 mL in 9 mL 80% alc gives clear soln
Ethyl Levulinate FEMA No. 2442	144.17/$C_7H_{12}O_3$/ $CH_3COCH_2CH_2COOC_2H_5$	colorless to pale yel liq/ fruity, apple, green	*s*—prop glycol, veg oils; *ins*—water/ 93°–94° (18 mm Hg)	1 mL in 1 mL 95% alc
Ethyl 2-Methylbutyrate FEMA No. 2443	130.19/$C_7H_{14}O_2$/ $CH_3CH_2OOCCH(CH_3)CH_2CH_3$	colorless liq/ strong, green-fruity, apple	*s*—alc, prop glycol; *vss*—water; *m*—most fixed oils/ 133°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_8H_{16}O_2$ (M-1b)	1.0	1.406–1.409	0.867–0.871	
	97.0% of $C_8H_{18}O$ (M-1b)		1.429–1.434	0.830–0.834	
IR	95% of $C_7H_{10}O_3$ (M-1b)		1.488–1.493		
IR	98.0% of $C_6H_{12}O_2$ (M-1b)	1.0	1.385–1.391	0.862–0.868	
IR	98.0% of $C_7H_{14}O_2$ (one isomer) (M-1b)	2.0	1.395–1.399	0.862–0.866	
IR	98.0% of $C_5H_{10}O_3$ (M-1b)	1.0	1.410–1.420	1.029–1.032	
IR	98.0% of $C_{14}H_{28}O_2$ (M-1b)	1.0	1.430–1.434	0.858–0.863	
IR	98.0% of $C_7H_{12}O_3$ (M-1b)	2.0	1.420–1.425	1.007–1.013	
	95.0% of $C_7H_{14}O_2$ (one isomer) (M-1b)	2.0	1.393–1.400	0.863–0.870	

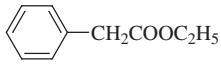
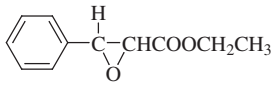
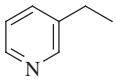
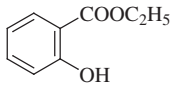
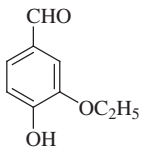
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Ethyl 2-Methylpentanoate FEMA No. 3488	144.21/$C_8H_{16}O_2$/ $CH_3CH_2CH_2CH(CH_3)CO_2CH_2CH_3$	colorless to pale yel liq/ fruity	*s*—veg oils; *ins*—prop glycol, water/ 153°	1 mL in 1 mL 95% ethanol
Ethyl Methylphenylglycidate FEMA No. 2444 Aldehyde C-16; Strawberry Aldehyde	206.24/$C_{12}H_{14}O_3$/ 	colorless to pale yel liq/ strong, fruity, strawberry	*s*—most fixed oils, prop glycol; *ins*—gly/ 272°–275°	1 mL in 3 mL 70% alc
2-Ethyl-3-methylpyrazine FEMA No. 3155	122.17/$C_7H_{10}N_2$/ 	colorless to slightly yel liq/ strong, raw potato	*s*—prop glycol, veg oils, water/ 57° (10 mm Hg)	1 mL in 1 mL 95% ethanol
Ethyl 3-Methylthiopropionate FEMA No. 3343	148.23/$C_6H_{12}O_2S$/ $CH_3SCH_2CH_2COOCH_2CH_3$	colorless to pale yel liq/ onion, fruity, sweet	*s*—prop glycol, veg oils; *ins*—water/ 89°–91° (15 mm Hg)	1 mL in 1 mL 95% ethanol
Ethyl Myristate FEMA No. 2445	256.43/$C_{16}H_{32}O_2$/ $CH_3(CH_2)_{12}COOC_2H_5$	colorless to pale yel liq/ waxy	*s*—veg oils; *ins*—prop glycol, water/ 178°–180° (12 mm Hg)	1 mL in 1 mL 95% alc
Ethyl Nonanoate FEMA No. 2447 Ethyl Pelargonate	186.29/$C_{11}H_{22}O_2$/ $CH_3(CH_2)_7COOC_2H_5$	colorless liq/ fatty, fruity, cognac	*m*—alc, prop glycol; *ins*—water/ 229°	1 mL in 10 mL 70% alc
Ethyl Octanoate FEMA No. 2449 Ethyl Caprylate; Ethyl Octoate	172.27/$C_{10}H_{20}O_2$/ $CH_3(CH_2)_6COOC_2H_5$	colorless liq/ winy-brandy, fruity-floral	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 209°	1 mL in 4 mL 70% alc
Ethyl Oleate FEMA No. 2450 Ethyl 9-Octadecenoate	310.52/$C_{20}H_{38}O_2$/ $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOCH_2CH_3$	colorless to pale yel liq/ floral	*s*—veg oils; *ins*—prop glycol, water/ 205°–208°	1 mL in 1 mL 95% ethanol
Ethyl Oxyhydrate (so-called) FEMA No. 2996 Rum Ether, So-Called		colorless liq/ sharp rum	*m*—alc, gly, prop glycol; *ins*—veg oils, water	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_8H_{16}O_2$ (M-1b)	1.0	1.401–1.404	0.859–0.865	
IR	98.0% of $C_{12}H_{14}O_3$ (sum of two isomers) (M-1b)	2.0	1.504–1.513	1.086–1.096	
IR	98.0% of $C_7H_{10}N_2$ (M-1a)		1.502–1.505	0.978–0.988	**Water**—0.1% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	99.0% of $C_6H_{12}O_2S$ (M-1b)		1.457–1.463	1.030–1.035	
IR	98.0% of $C_{16}H_{32}O_2$ (M-1b)	1.0	1.434–1.438	0.857–0.862	
IR	98.0% of $C_{11}H_{22}O_2$ (M-1b)	3.0	1.420–1.424	0.863–0.867	
IR	98.0% of $C_{10}H_{20}O_2$ (M-1b)	1.0	1.416–1.420	0.863–0.867	
		1.0	1.448–1.453	0.868–0.873	**Saponification Value**—between 175 and 190 (Appendix VI) **Alcohol Content**—min 14.0% by vol, at 15.56° (M-4) **Ester Value**—min 25 (Appendix VI, 1- to 3-g sample)

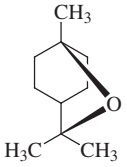
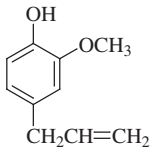
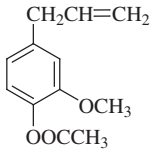
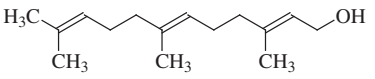
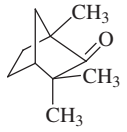
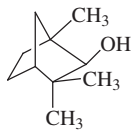
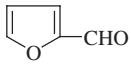
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Ethyl Phenylacetate FEMA No. 2452	164.20/$C_{10}H_{12}O_2$/ 	colorless or nearly colorless liq/ sweet, honey	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 228°	1 mL in 3 mL 70% alc
Ethyl Phenylglycidate FEMA No. 2454	192.21/$C_{11}H_{12}O_3$/ 	colorless to slightly yel liq/ strong, strawberry	*s*—alc, chloroform, ether; *ins*—water/ 96° (0.5 mm Hg)	1 mL in 6 mL 70% alc, and in 1 mL 80% alc gives clear solns
Ethyl Propionate FEMA No. 2456	102.13/$C_5H_{10}O_2$/ $CH_3CH_2COOC_2H_5$	colorless liq/ fruity, rum, ethereal	*s*—most fixed oils, 1 mL in 42 mL water; *m*—alc, prop glycol/ 99°	
3-Ethyl Pyridine FEMA No. 3394	107.16/C_7H_9N/ 	colorless to yel liq/ tobacco	*s*—prop glycol, veg oils; *ins*—water/ 166°	1 mL in 1 mL 95% ethanol
Ethyl Salicylate FEMA No. 2458	166.18/$C_9H_{10}O_3$/ 	colorless liq/ wintergreen	*s*—alc, acetic acid, most fixed oils; *ss*—gly, water/ 234°	1 mL in 4 mL 80% alc gives clear soln
Ethyl 10-Undecenoate FEMA No. 2461	212.33/$C_{13}H_{24}O_2$/ $H_2C{=}CH(CH_2)_8CO_2C_2H_5$	colorless to pale yel liq/ waxy, coconut	*s*—veg oils; *ins*—prop glycol, water/ 258°–259°	1 mL in 1 mL 95% ethanol
Ethyl Valerate FEMA No. 2462 Ethyl *n*-Pentanoate	130.19/$C_7H_{14}O_2$/ $CH_3(CH_2)_3COOCH_2CH_3$	colorless to pale yel liq/ fruity	*s*—veg oils; *ss*—prop glycol; *ins*—water/ 145°	1 mL in 1 mL 95% ethanol
Ethyl Vanillin FEMA No. 2464 3-Ethoxy-4-hydroxybenzaldehyde	166.18/$C_9H_{10}O_3$/ 	fine white or slightly yel cryst; affected by strong light/ strong, vanilla	*s*—alc, chloroform, ether, prop glycol solns of alkali hydroxides, 1 g in 100 mL water at 50°	1 g in 5 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{10}H_{12}O_2$ (M-1b)	1.0	1.496–1.500	1.027–1.032	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	98.0% of $C_{11}H_{12}O_3$ (Appendix VI; Ester Determination; 1.4 g/ 96.11)		1.516–1.521	1.120–1.125	
IR	97.0% of $C_5H_{10}O_2$ (M-1b)	2.0	1.383–1.385	0.886–0.889	
IR	97.0% of C_7H_9N (M-1b)		1.500–1.505	0.951–0.957	
IR	99.0% of $C_9H_{10}O_3$ (M-1b)	1.0 (phenol red TS)	1.520–1.525	1.126–1.130	
	98.0% of $C_{13}H_{24}O_2$ (M-1b)	1.0	1.436–1.440	0.877–0.879	
	98.0% of $C_7H_{14}O_2$ (M-1b)	1.0	1.399–1.404	0.870–0.875	
IR	98.0% of $C_9H_{10}O_3$ (M-1b)				**Melting Range**—76° to 78° (Appendix IIB, dry over P_2O_5/4 h) **Loss on Drying**—0.5% (Appendix IIC, P_2O_5/4 h) **Residue on Ignit.**—0.05% (Appendix IIC, 2-g sample)

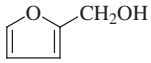
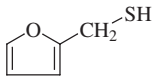
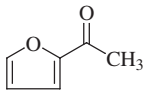
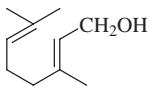
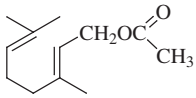
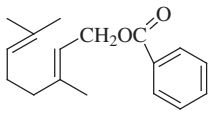
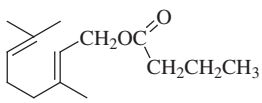
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Eucalyptol FEMA No. 2465 1,8-Cineol; 1,8 Epoxy-*p*-menthane; 1:8 Oxido-*p*-menthane	154.25/$C_{10}H_{18}O$/ 	colorless liq/ camphoraceous; pungent, cooling taste	*s*—alc, most fixed oils, gly, prop glycol/ 176°	1 mL in 5 mL 60% alc
Eugenol FEMA No. 2467 4-Allylguaiacol; 4-Allyl-2-methoxyphenol; Eugenic Acid	164.20/$C_{10}H_{12}O_2$/ 	colorless to pale yel liq/ pungent, spicy taste; darkens and thickens on air exposure/ strong clove	*ss*—water; *m*—alc, chloroform, ether, most fixed oils/ 256°	1 mL in 2 mL 70% alc
Eugenyl Acetate FEMA No. 2469 Aceteugenol; Acetyl Eugenol; 4-Allyl-2-methoxy-phenyl Acetate; Eugenol Acetate	206.24/$C_{12}H_{14}O_3$/ 	fused solid, melts at warm room temp to a pale yel liq/ mild, clove	*s*—alc, ether; *ins*—water/ 282°	1 mL in 5 mL 70% alc
Farnesol FEMA No. 2478 3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	222.37/$C_{15}H_{26}O$/ 	slightly yel liq/ mild, oily	*ins*—water/ 263°	1 mL in 1 mL 95% ethanol
***d*-Fenchone** FEMA No. 2479	152.24/$C_{10}H_{16}O$/ 	colorless to pale yel liq/ camphoraceous	*s*—prop glycol, veg oils; *ins*—water/ 192°	1 mL in 1 mL 95% ethanol
Fenchyl Alcohol FEMA No. 2480	154.25/$C_{10}H_{18}O$/ 	white to pale yel cryst/ camphoraceous	*s*—veg oils; *vss*—water/ 201°	1 g in 1 mL 95% ethanol
Furfural FEMA No. 2489 2-Furaldehyde; Pyromucic Aldehyde	96.09/$C_5H_4O_2$/ 	colorless to yel oily liq, turns red-brown on long storage/ sweet, bready	*s*—veg oils; *ss*—prop glycol, water/ 162°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.5% of $C_{10}H_{18}O$ (M1-a)		1.455–1.460	0.921–0.924	**Angular Rotation**—between 0.5° and +0.5° (Appendix IIB, 100-mm tube)
IR	98.0% of $C_{10}H_{12}O_2$ (M-1b)		1.540–1.542	1.064–1.070	**Hydrocarbons**—passes test (M-7)
IR	98.0% of $C_{12}H_{14}O_3$ (M-1b)	1.0 (phenol red TS)	1.514–1.522 (as supercooled liq)	1.077–1.082 (as melted, supercooled liq)	**Solidification Pt.**—NLT 25° (Appendix IIB)
IR	96.0% of $C_{15}H_{26}O$ (sum of four isomers) (M-1a)		1.487–1.492	0.884–0.891	
IR	97.0% of $C_{10}H_{16}O$ (M-1b)		1.460–1.467	0.940–0.948	**Angular Rotation**—between −68° and −46°
IR	97.0% of $C_{10}H_{18}O$ (M-1b)				**Melting Point**—between 35.0° and 40.0°
IR	96.0% of $C_5H_4O_2$ (M-1b)	1.0	1.522–1.528	1.154–1.158	

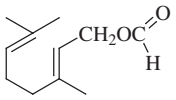
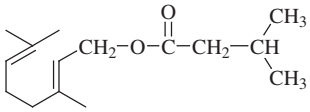
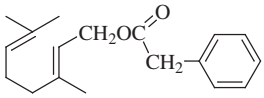
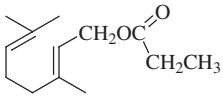
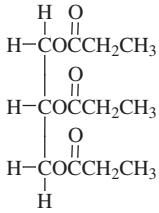
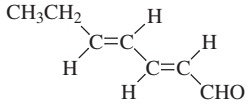
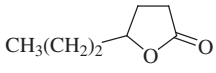
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Furfuryl Alcohol FEMA No. 2491	98.10/$C_5H_6O_2$/ 	pale yel to brown liq/ caramel	*s*—prop glycol, veg oils, water/ 169°	1 mL in 1 mL 95% ethanol
Furfuryl Mercaptan FEMA No. 2493	114.16/C_5H_6OS/ 	yel to brown liq/ coffee	*s*—veg oils; *ss*—prop glycol *ins*—water/ 155°	1 mL in 1 mL 95% ethanol
2-Furyl Methyl Ketone FEMA No. 3163	110.11/$C_6H_6O_2$/ 	yel to brown liq/ coffee	*ss*—prop glycol, veg oils; *vss*—water/ 67° at 10 mm	1 mL in 2 mL 95% ethanol
Fusel Oil, Refined FEMA No. 2497		colorless to pale yel liq/ winy, whiskey	*s*—prop glycol, veg oils; *ins*—water/ 128°–130°	1 mL in 1 mL 95% alc
Geraniol FEMA No. 2507 *trans*-3,7-Dimethyl-2,6-octadien-1-ol; *E*-3,7-Dimethyl-2,6-octadien-1-ol	154.25/$C_{10}H_{18}O$/ 	colorless liq/ rose	*s*—most fixed oils, prop glycol; *ss*—water; *ins*—gly/ 230°	1 mL in 3 mL 70% alc remains in soln to 10 mL
Geranyl Acetate FEMA No. 2509 3,7-Dimethyl-2,6-octadien-1-yl Acetate	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/ floral	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 245°	1 mL in 9 mL 70 % alc
Geranyl Benzoate FEMA No. 2511 3,7-Dimethyl-2,6-octadien-1-yl Benzoate	258.36/$C_{17}H_{22}O_2$/ 	slightly yel liq/ floral, resembling ylang-ylang oil	*m*—alc, chloroform; *ins*—water/ 305°	1 mL in 4 mL 90% alc gives clear soln
Geranyl Butyrate FEMA No. 2512 3,7-Dimethyl-2,6-octadien-1-yl Butyrate	224.34/$C_{14}H_{24}O_2$/ 	colorless to pale yel liq/ fruity, rose	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 253°	1 mL in 6 mL 80% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_5H_6O_2$ (M-1b)		1.481–1.490	1.126–1.136	
IR	95.0% of C_5H_6OS (M1-b)		1.529–1.534	1.124–1.135	
IR	97.0% of $C_{10}H_6O_2$ (M-1b)		1.505–1.510	1.102–1.107	
IR	95.0% of 2- and 3-methyl butanol (M-1a)		1.405–1.410	0.807–0.813	**Angular Rotation**—between −0.5° and −2.0° (Appendix IIB)
IR	88.0% of total alcohols as $C_{10}H_{18}O$ (Appendix VI; 1.2 g/77.13)		1.469–1.478	0.870–0.885	**Aldehydes**—1.0% as citronellal (M-2d; 5 g/77.13) **Esters**—1.0% as geranyl acetate (Appendix VI; 5 g/98.15)
IR	90.0% of total esters as $C_{12}H_{20}O_2$ (Appendix VI; 1.0 g/98.15)		1.458–1.464	0.900–0.914	
IR	95.0% of total esters as $C_{17}H_{22}O_2$ (Appendix VI; Ester Determination; 1.5 g/129.2)	1.0	1.516–1.521	0.983–0.989	
IR	92.0% of total esters as $C_{14}H_{24}O_2$ (Appendix VI; 1.0 g/112.2)	1.0	1.455–1.462	0.889–0.904	

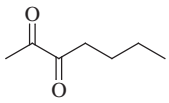
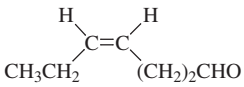
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Geranyl Formate FEMA No. 2514 3,7-Dimethyl-2,6-octadien-1-yl Formate	182.26/$C_{11}H_{18}O_2$/ 	colorless to pale yel liq/ fresh, leafy, rose	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 216°	1 mL in 3 mL 80% alc
Geranyl Isovalerate FEMA No. 2518	238.37/$C_{15}H_{26}O_2$/ 	colorless to pale yel liq/ rose	*s*—prop glycol, veg oils; *ins*—water/ 279°	1 mL in 1 mL 95% ethanol
Geranyl Phenylacetate FEMA No. 2516 3,7-Dimethyl-2,6-octadien-1-yl Phenylacetate	272.39/$C_{18}H_{24}O_2$/ 	yel liq/ honey-rose	*m*—alc, chloroform, ether; *ins*—water/ 278°	1 mL in 4 mL 90% alc gives clear soln
Geranyl Propionate FEMA No. 2517 3,7-Dimethyl-2,6-octadien-1-yl Propionate	210.32/$C_{13}H_{22}O_2$/ 	colorless liq/ rosy, fruity	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 253°	1 mL in 4 mL 80% alc
Glyceryl Tripropanoate FEMA No. 3286 Tripropionin	260.29/$C_{12}H_{20}O_6$/ 	colorless to pale yel liq/ odorless with a bitter taste	175°–176° (20 mm Hg)	
(*E*),(*E*)-2,4-Heptadienal FEMA No. 3164 *trans*,*trans*-2,4-Heptadienal	110.16/$C_7H_{10}O$/ 	slightly yel liq/ fatty, green	*s*—alc, most fixed oils; *ins*—water/ 100° (35 mm Hg)	1 mL in 1 mL 95% ethanol
γ-Heptalactone FEMA No. 2539	128.17/$C_7H_{12}O_2$/ 	colorless, slightly oily liq/ coconut, sweet, malty, caramel	*s*—prop glycol; *m*—alc, most fixed oils; *ins*—water/ 61° (2 mm Hg)	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	85.0% of total esters as $C_{11}H_{18}O_2$ (Appendix VI; 1.0 g/ 91.13)	3.0 (Appendix VI)	1.457–1.466	0.906–0.920	
IR	95.0% of $C_{15}H_{26}O_2$ (sum of neryl and geranyl isomers) (M-1b)	1.0	1.452–1.462	0.881–0.894	
IR	97.0% of total esters as $C_{18}H_{24}O_2$ (Appendix VI; 1.6 g/ 136.2)	2.0	1.506–1.511	0.971–0.978	
IR	92.0% of total esters as $C_{13}H_{22}O_2$ (Appendix VI; 1.6 g/ 105.2)	1.0	1.456–1.464	0.896–0.913	
	97.1% of $C_{12}H_{20}O_6$ (M-1b)	2.0	1.431–1.435	1.078–1.082	
IR	92.0% of $C_7H_{10}O$ (sum of isomers) (M-1a)		1.531–1.537	0.878–0.888	
IR	98.0% of $C_7H_{12}O_2$ (M-1a)		1.439–1.445	0.989–0.998	

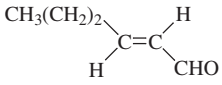
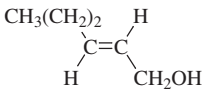
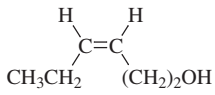
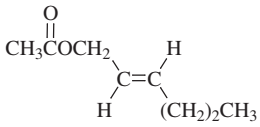
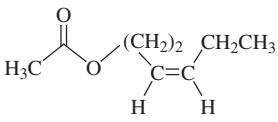
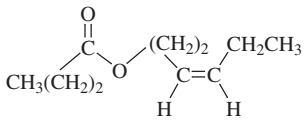
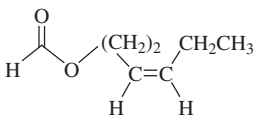
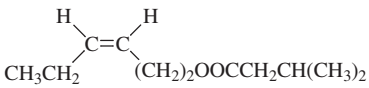
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Heptanal FEMA No. 2540 Aldehyde C-7; Heptaldehyde	114.19/$C_7H_{14}O$/ $CH_3(CH_2)_5CHO$	colorless to slightly yel liq/ penetrating, oily	*ss*—water; *m*—alc, ether, most fixed oils/ 153°	1 mL in 2 mL 70% alc gives clear soln
2,3-Heptandione FEMA No. 2543 Acetyl Valeryl	128.17/$C_7H_{12}O_2$/ 	yel liq/ butter, cheese	*s*—prop glycol, veg oils; *ins*—water/ 64° (18 mm Hg)	1 mL in 1 mL 95% ethanol
2-Heptanone FEMA No. 2544 Methyl Amyl Ketone	114.19/$C_7H_{14}O$/ $CH_3CO(CH_2)_4CH_3$	colorless, mobile liq/ fruity, spicy	*m*—alc, ether, 1 mL in 250 mL water/ 151°	
3-Heptanone FEMA No. 2545 Ethyl Butyl Ketone	114.19/$C_7H_{14}O$/ $CH_3(CH_2)_3COCH_2CH_3$	colorless, mobile liq/ fruity, green, fatty	*m*—alc, ether, 1 mL in 70 mL water/ 149°	
(*Z*)-4-Hepten-1-al FEMA No. 3289 *cis*-4-Hepten-1-al	112.17/$C_7H_{12}O$/ 	slightly yel liq/ fatty, green	*s*—alc, most fixed oils; *ins*—water/ 60° (25 mm Hg)	1 mL in 1 mL 95% ethanol
Heptyl Alcohol FEMA No. 2548 Enanthic Alcohol	116.20/$C_7H_{16}O$/ $CH_3(CH_2)_5CH_2OH$	colorless liq/ fatty, winy	*ss*—water; *m*—alc, ether, most fixed oils/ 175°	1 mL in 2 mL 60% alc gives clear soln
γ-Hexalactone FEMA No. 2556 4-Hydroxyhexanoic Acid Lactone	114.14/$C_6H_{10}O_2$/ 	colorless to pale yel liq/ herbaceous, sweet	*s*—prop glycol, veg oils; *ins*—water/ 220°	1 mL in 1 mL 95% alc
Hexanal FEMA No. 2557 Caproic Aldehyde; Hexaldehyde; Aldehyde C-6	100.16/$C_6H_{12}O$/ $CH_3(CH_2)_4CHO$	almost colorless liq/ fatty-green, grassy	*vss*—water; *m*—alc, prop glycol, most fixed oils/ 131°	
Hexanoic Acid FEMA No. 2559 Caproic Acid	116.16/$C_6H_{12}O_2$/ $CH_3(CH_2)_4COOH$	colorless to very pale yel, oily liq/ cheesy, sweat	*m*—alc, most fixed oils, ether, 1 mL in 250 mL water/ 223°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	92.0% of $C_7H_{14}O$ (M-1b)	10.0	1.412–1.420	0.815–0.820	
IR	97.0% of $C_7H_{12}O_2$ (M-1b)		1.411–1.418	0.916–0.923	
IR	95.0% of $C_7H_{14}O$ (M-1b)	2.0	1.405–1.411	0.811–0.816	**Distillation Range**—between 147° and 154° (Appendix IIB) **Residue on Evap.**—5 mg/100 mL (M-16, 100-mL sample) **Water**—0.3% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	97.0% of $C_7H_{14}O$ (M-1b)	2.0	1.404–1.411	0.813–0.818	**Distillation Range**—between 143° and 151° (Appendix IIB) **Water**—0.3% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	98.0% of $C_7H_{12}O$ [sum of two isomers; (*Z*)-4-isomer 93.0% min] (M-1a)		1.432–1.436	0.843–0.855	
IR	97.0% of $C_7H_{16}O$ (M-1b)	1.0	1.423–1.427	0.820–0.824	**Aldehydes**—1.0% heptanal (M-1b)
IR	98.0% of $C_6H_{10}O_2$ (M-1a)	1.0	1.437–1.442	1.020–1.025	
	97.0% of $C_6H_{12}O$ (M-1a)	10.0	1.402–1.407	0.808–0.817	
IR	98.0% of $C_6H_{12}O_2$ (M-3a)		1.415–1.418	0.923–0.928	**Solidification Pt.**—NLT −4.5° (Appendix IIB)

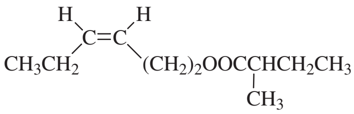
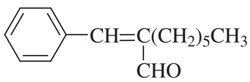
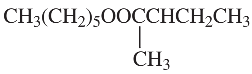
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
(*E*)-2-Hexen-1-al FEMA No. 2560 *trans*-2-Hexen-1-al	98.14/$C_6H_{10}O$/ 	pale yel liq/ strong, fruity-green, vegetable	*s*—alc, prop glycol, most fixed oils; *vss*—water/ 47° (17 mm Hg)	1 mL in 1 mL 95% ethanol
(*E*)-2-Hexen-1-ol FEMA No. 2562 *trans*-2-Hexen-1-ol	100.16/$C_6H_{12}O$/ 	almost colorless liq/ strong, fruity-green	*s*—alc, prop glycol, most fixed oils; *vss*—water/ 158°	
(*Z*)-3-Hexenol FEMA No. 2563 *cis*-3-Hexen-1-ol	100.16/$C_6H_{12}O$/ 	colorless liq/ powerful, grassy-green	*s*—alc, prop glycol, most fixed oils; *vss*—water/ 156°	
(*E*)-2-Hexenyl Acetate FEMA No. 2564 *trans*-2-Hexen-1-yl Acetate	142.20/$C_8H_{14}O_2$/ 	colorless to pale yel liq/ green note	*s*—veg oils; *ss*—prop glycol; *ins*—water/ 166°	1 mL in 1 mL 95% alc
(*Z*)-3-Hexenyl Acetate FEMA No. 3171 *cis*-3-Hexen-1-yl Acetate	142.20/$C_8H_{14}O_2$/ 	colorless to pale yel liq/ powerful green note	*s*—prop glycol, veg oils; *ins*—water/ 198°	1 mL in 1 mL 95% alc
(*Z*)-3-Hexenyl Butyrate FEMA No. 3402	170.25/$C_{10}H_{18}O_2$/ 	colorless to pale yel liq/ green, fruity	*s*—prop glycol, veg oils; *ins*—water/ 96° (20 mm Hg)	1 mL in 1 mL 95% ethanol
(*Z*)-3-Hexenyl Formate FEMA No. 3353	128.17/$C_7H_{12}O_2$/ 	colorless to pale yel liq/ green	*s*—prop glycol, veg oils; *ins*—water/ 72° (40 mm Hg)	1 mL in 1 mL 95% ethanol
(*Z*)-3-Hexenyl Isovalerate FEMA No. 3498 *cis*-3-Hexen-1-yl Isovalerate	184.28/$C_{11}H_{20}O_2$/ 	colorless liq/ sweet, apple	*s*—alc, most fixed oils; prop glycol, water/ 199°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
	92.0% of $C_6H_{10}O$ (M-1a)		1.445–1.449	0.841–0.850	
IR	95.0% of $C_6H_{12}O$ (M-1a)		1.436–1.441	0.839–0.844	
IR	98.0% of $C_6H_{12}O$ [sum of (*Z*)- and (*E*)-isomers; min 92% (*Z*)] (M-1a)		1.436–1.443	0.846–0.850	
IR	98.0% of $C_8H_{14}O_2$ [sum of (*Z*)- and (*E*)-isomers; min 90% (*E*)] (M-1a)		1.425–1.430	0.890–0.897	
IR	98.0% of $C_8H_{14}O_2$ [sum of (*Z*)- and (*E*)-isomers; min 92% (*Z*)] (M-1a)	1.0	1.425–1.429	0.896–0.901	
IR	97.0% of $C_{10}H_{18}O_2$ (one isomer) (M-1b)	1.0	1.427–1.435	0.880–0.887	
IR	95.0% of $C_7H_{12}O_2$ (M-1b)	5.0 (add ice to soln)	1.424–1.430	0.907–0.915	
	95.0% of $C_{11}H_{20}O_2$ [sum of two isomers; (*Z*)-isomer 92.0% min] (M-1a)	2.0	1.429–1.435	0.872–0.877	

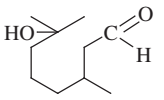
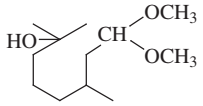
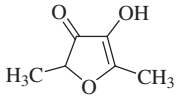
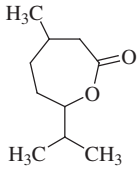
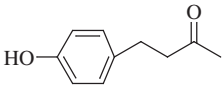
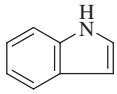
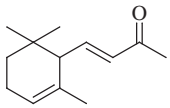
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
(*Z*)-3-Hexenyl 2-Methylbutyrate FEMA No. 3497 *cis*-3-Hexenyl 2-Methylbutyrate	184.28/$C_{11}H_{20}O_2$/ 	almost colorless liq/ powerful, fruity, unripe apples	*s*—alc, most fixed oils; *ins*—water/ 105° (25 mm Hg)	1 mL in 1 mL 95% ethanol
Hexyl Acetate FEMA No. 2565	144.21/$C_8H_{16}O_2$/ $CH_3(CH_2)_5OOCCH_3$	colorless liq/ fruity	*s*—veg oils; *ss*—prop glycol; *ins*—water/ 168°–170°	1 mL in 1 mL 95% alc
Hexyl Alcohol FEMA No. 2567 1-Hexanol; Alcohol C-6	102.18/$C_6H_{14}O$/ $CH_3(CH_2)_4CH_2OH$	colorless, mobile liq/ mild, sweet, green	*m*—alc, ether, 1 mL in 175 mL water/ 157°	
Hexyl-2-butenoate FEMA No. 3354	170.25/$C_{10}H_{18}O_2$/ $CH_3(CH_2)_5OOCCH{=}CHCH_3$	colorless liq/ fruity	*s*—alc, most fixed oils; *ins*—water, prop glycol	
Hexyl Butyrate FEMA No. 2568	172.27/$C_{10}H_{20}O_2$/ $CH_3(CH_2)_5OOC(CH_2)_2CH_3$	colorless to pale yel liq/ fruity	*s*—veg oils; *ss*—prop glycol; *ins*—water/ 205°	1 mL in 1 mL 95% ethanol
α-Hexylcinnamaldehyde FEMA No. 2569	216.32/$C_{15}H_{20}O$/ 	pale yel liq/ jasmine	*s*—most fixed oils; *ins*—gly, prop glycol/ 174° (15 mm Hg)	1 mL in 1 mL 90% alc
Hexyl Hexanoate FEMA No. 2572	200.32/$C_{12}H_{24}O_2$/ $CH_3(CH_2)_5OOC(CH_2)_4CH_3$	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 245°	1 mL in 1 mL 95% ethanol
Hexyl Isovalerate FEMA No. 3500	186.29/$C_{11}H_{22}O_2$/ $(CH_3)_2CHCH_2COOCH_2(CH_2)_4CH_3$	colorless liq/ pungent, fruity	*s*—alc, most fixed oils; *ins*—water/ 215°	1 mL in 1 mL 95% alc
Hexyl 2-Methylbutyrate FEMA No. 3499	186.29/$C_{11}H_{22}O_2$/ 	colorless liq/ strong, fresh-green, fruity	*s*—alc, most fixed oils; *ins*—water/ 217°–219°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
	95.0% of $C_{11}H_{20}O_2$ [sum of two isomers; (*Z*)-isomer 92.0% min] (M-1a)	2.0	1.430–1.434	0.876–0.880	
IR	98.0% of $C_8H_{16}O_2$ (M-1b)	1.0	1.407–1.411	0.868–0.872	
IR	97.0% of $C_6H_{14}O$ (M-1b)	2.0	1.415–1.420	0.816–0.821	
IR	95.0% of $C_{10}H_{18}O_2$ (M-1a)		1.428–1.449	0.880–0.900	
IR	98.0% of $C_{10}H_{20}O_2$ (M-1b)	1.0	1.414–1.420	0.860–0.866	
IR	95.0% of $C_{15}H_{20}O$ (sum of two isomers) (M-1b)	5.0	1.548–1.552	0.953–0.959	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	98.0% of $C_{12}H_{24}O_2$ (M-1b)	1.0	1.421–1.427	0.857–0.863	
	95.0% of $C_{11}H_{22}O_2$ (sum of two isomers; isovalerate isomer 92.0% min) (M-1b)	2.0	1.417–1.421	0.853–0.857	
	95.0% of $C_{11}H_{22}O_2$ (one isomer) (M-1a)	2.0	1.416–1.421	0.854–0.859	

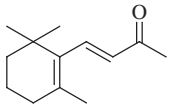
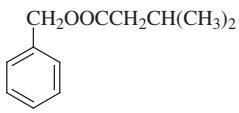
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Hydroxycitronellal FEMA No. 2583 7-Hydroxy-3,7-dimethyl Octanal	172.27/$C_{10}H_{20}O_2$/ 	colorless liq/ sweet, floral, lily	*s*—most fixed oils, prop glycol; *ins*—gly/ 241°	1 mL in 1 mL 50% alc
Hydroxycitronellal Dimethyl Acetal FEMA No. 2585 7-Hydroxy-3,7-dimethyl Octanal: Acetal	218.34/$C_{12}H_{26}O_3$/ 	colorless liq/ floral	*s*—most fixed oils, prop glycol; *ins*—gly/ 252°	1 mL in 2 mL 50% alc
4-Hydroxy-2,5-dimethyl-3(2H)-furanone FEMA No. 3174	128.13/$C_6H_8O_3$/ 	white to pale yel solid/ fruity, caramel, burnt sugar		1 g in 1 mL 95% alc
6-Hydroxy-3,7-dimethyloctanoic Acid Lactone FEMA No. 3355	170.25/$C_{10}H_{18}O_2$/ 	colorless, low-melting solid/ maple syrup or brown sugar	*vs*—-water; *s*—alc	
4-(*p*-Hydroxyphenyl)-2-butanone FEMA No. 2588	164.20/$C_{10}H_{12}O_2$/ 	white solid/ raspberry	*ins*—prop glycol, veg oils, water	1 g in 2 mL 95% alc
Indole FEMA No. 2593	117.15/C_8H_7N/ 	white, lustrous, flaky, cryst solid/ unpleasant odor in high conc., free of fecal quality; floral on dilution	*s*—alc, most fixed oils, prop glycol; *ins*—gly/ 253°–254°	1 g in 3 mL 70% alc
α-Ionone FEMA No. 2594 4-(2,6,6-Trimethyl-2-cyclohexenyl)-3-butene-2-one	192.30/$C_{13}H_{20}O_2$/ 	colorless to pale yel liq/ warm, woody, violet-floral	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/ 237°	1 mL in 10 mL 60% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_{10}H_{20}O_2$ (M-1b)	5.0	1.447–1.450	0.918–0.923	
IR	95.0% of $C_{12}H_{26}O_3$ (M-1b)	1.0	1.441–1.444	0.925–0.930	**Free Hydroxy Citronellal**—3.0% (M-1b)
IR	98.0% of $C_6H_8O_3$ in a suitable solvent (M-1a)				
	90.0% of $C_{10}H_{18}O_2$ (M-1a)		1.457–1.461	0.966–0.973	
IR	98.0% of $C_{10}H_{12}O_2$ (M-1b)				**Melting Range**—between 82° and 84° (Appendix IIB)
IR	99.0% of C_8H_7N (M-1a)				**Solidification Pt.**—NLT 51° (Appendix IIB, dry over H_2SO_4)
IR	85.0% of $C_{13}H_{20}O_2$ (97.0% sum of α-, β-, γ-, and δ-isomers) (M-1b)		1.497–1.502	0.927–0.933	

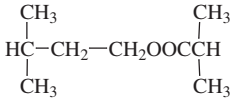
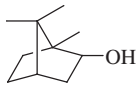
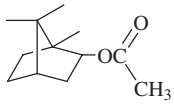
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
β-Ionone FEMA No. 2595 4-(2,6,6-Trimethyl-1-cyclohexenyl)-3-butene-2-one	192.30/$C_{13}H_{20}O$/ 	colorless to pale straw-colored liq/ warm, woody, dry	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/ 239°	1 mL in 1 mL 95% ethanol
Isoamyl Acetate FEMA No. 2055 Amyl Acetate; β-Methyl Butyl Acetate	130.19/$C_7H_{14}O_2$/ $CH_3COOCH_2CH_2CH(CH_3)_2$	colorless liq/ fruity, pear, banana	*ss*—water; *m*—alc, ether, ethyl acetate, most fixed oils; *ins*—gly, prop glycol/ 145°	1 mL in 3 mL 60% alc gives clear soln
Isoamyl Alcohol FEMA No. 2057	88.15/$C_5H_{12}O$/ $(CH_3)_2CHCH_2CH_2OH$	colorless to pale yel liq/ winy	*s*—prop glycol, veg oils; *ins*—water/ 130°	1 mL in 1 mL 95% ethanol
Isoamyl Benzoate FEMA No. 2058	196.26/$C_{12}H_{16}O_2$/ 	colorless to pale yel liq/ pungent fruit	261° (746 mm Hg)	1 mL in 1 mL 95% alc
Isoamyl Butyrate FEMA No. 2060 Amyl Butyrate	158.24/$C_9H_{18}O_2$/ $CH_3(CH_2)_2COOCH_2CH_2CH(CH_3)_2$	colorless liq/ fruity	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 179°	1 mL in 4 mL 70% alc
Isoamyl Formate FEMA No. 2069 Amyl Formate	116.16/$C_6H_{12}O_2$/ $HCOOCH_2CH_2CH(CH_3)_2$	colorless liq/ plum	*s*—alc, most fixed oils, prop glycol; *ss*—water; *ins*—gly/ 124°	1 mL in 4 mL 60% alc remains in soln to 10 mL
Isoamyl Hexanoate FEMA No. 2075 Amyl Hexanoate; Isoamyl Caproate; Pentyl Hexanoate	186.29/$C_{11}H_{22}O_2$/ $CH_3(CH_2)_4COOCH_2CH_2CH(CH_3)_2$	colorless liq/ fruity	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 222°	1 mL in 3 mL 80% alc gives clear soln

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_{13}H_{20}O$ (M-1b)		1.517–1.522	0.940–0.947	
IR	95.0% of $C_7H_{14}O_2$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1b)	1.0	1.400–1.404	0.868–0.878	
	98.0% of $C_5H_{12}O$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1b)		1.405–1.410	0.807–0.813	
IR	98.0% of $C_{12}H_{16}O_2$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1a)	1.0	1.492–1.496	0.986–0.992	
IR	98.0% of $C_9H_{18}O_2$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1b)	1.0	1.409–1.414	0.861–0.866	
IR	92.0% of $C_6H_{12}O_2$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1b)	3.0	1.396–1.400	0.881–0.889	
IR	98.0% of $C_{11}H_{22}O_2$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1b)	1.0	1.418–1.422	0.858–0.863	

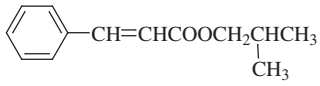
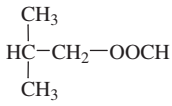
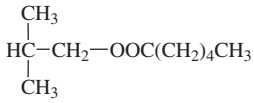
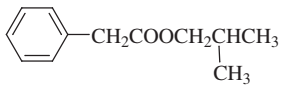
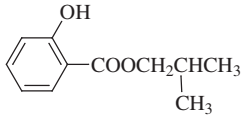
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Isoamyl Isobutyrate FEMA No. 3507	158.24/$C_9H_{18}O_2$/ 	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 169°	1 mL in 1 mL 95% ethanol
Isoamyl Isovalerate FEMA No. 2085 Amyl Valerate; Amyl Isovalerate	172.27/$C_{10}H_{20}O_2$/ $(CH_3)_2CHCH_2COOCH_2CH_2CH(CH_3)_2$	colorless liq/ fruity, apple	*ss*—prop glycol *m*—alc, most fixed oils; *ins*—water/ 192°	1 mL in 6 mL 70% alc
Isoamyl Phenyl Acetate FEMA No. 2081	206.29/$C_{13}H_{18}O_2$/ 	colorless to pale yel liq/ chocolate, honey	268°	1 mL in 1 mL 95% alc
Isoamyl Salicylate FEMA No. 2084 Amyl Salicylate	208.26/$C_{12}H_{16}O_3$/ 	colorless liq/ floral	*m*—alc, chloroform, ether, most fixed oils; *ins*—gly, prop glycol, water/ 277°	1 mL in 3 mL 90% alc remains in soln on dilution
Isoborneol FEMA No. 2158	154.25/$C_{10}H_{18}O$/ 	white cryst solid/ piney, camphoraceous	*ss*—prop glycol; *ins*—veg oils/ 214°	1 g in 1 mL 95% alc
Isobornyl Acetate FEMA No. 2160	196.29/$C_{12}H_{20}O_2$/ 	colorless liq when fresh, yellows upon storage/ camphoraceous, piney, balsamic	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/ 227°	1 mL in 3 mL 70% alc
Isobutyl Acetate FEMA No. 2175	116.16/$C_6H_{12}O_2$/ $CH_3COOCH_2CH(CH_3)_2$	colorless liq/ fruity, banana on dilution	*s*—alc, most fixed oils, prop glycol, 1 mL in 180 mL water/ 116°	1 mL in 1 mL 95% ethanol
Isobutyl Alcohol FEMA No. 2179	74.12/$C_4H_{10}O$/ $(CH_3)_2CHCH_2OH$	colorless, mobile liq/ penetrating, winy	*m*—alc, ether, 1 mL in 140 mL water/ 108°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_9H_{18}O_2$ (M-1b)	1.0	1.404–1.410	0.853–0.859	
	98.0% of $C_{10}H_{20}O_2$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1b)	2.0	1.411–1.414	0.851–0.857	
IR	98.0% of $C_{13}H_{18}O_2$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1b)	1.0	1.485–1.490	0.975–0.981	
IR	98.0% of $C_{12}H_{16}O_3$ (sum of 2-methyl butyl, 3-methyl butyl, and *n*-pentyl isomers) (M-1b)	1.0 (phenol red TS)	1.505–1.509	1.047–1.053	
IR					**Melting Range**—between 212° and 214° (Appendix IIB)
IR	97.0% of $C_{12}H_{20}O_2$ (M-1b)	1.0	1.462–1.465	0.979–0.984	**Angular Rotation**—between −4° and 0° (Appendix IIB, 100-mm tube)
IR	90.0% of $C_6H_{12}O_2$ (M-1b)	1.0	1.389–1.392	0.862–0.871	
IR	98.0% of $C_4H_{10}O$ (M-1a)	2.0	1.392–1.397	0.799–0.801	

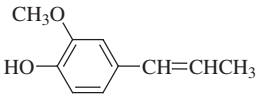
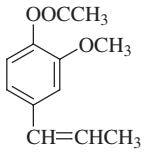
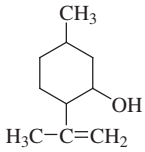
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Isobutyl-2-butenoate FEMA No. 3432	142.20/$C_8H_{14}O_2$/ $(CH_3)_2CHCH_2OOCCH{=}CHCH_3$	colorless liq/ powerful, fruity	*s*—alc, prop glycol, most fixed oils; *ss*—water/ 71°	1 mL in 1 mL 95% ethanol
Isobutyl Butyrate FEMA No. 2187 2-Methyl Propanyl Butyrate	144.21/$C_8H_{16}O_2$/ $C_3H_7COOCH_2CH(CH_3)_2$	colorless liq/ sweet, fruity, apple, pineapple	*s*—alc, most fixed oils; *ss*—water; *ins*—gly/ 157°	1 mL in 8 mL 60% alc
Isobutyl Cinnamate FEMA No. 2193	204.27/$C_{13}H_{16}O_2$/ 	colorless liq/ sweet, fruity, balsamic	*m*—alc, chloroform, ether, most fixed oils; *ins*—water/ 271°	1 mL in 3 mL 80% alc gives clear soln
Isobutyl Formate FEMA No. 2197	102.13/$C_5H_{10}O_2$/ 	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 98°	1 mL in 1 mL 95% ethanol
Isobutyl Hexanoate FEMA No. 2202	172.27/$C_{10}H_{20}O_2$/ 	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 203°	1 mL in 1 mL 95% ethanol
Isobutyl Phenylacetate FEMA No. 2210	192.26/$C_{12}H_{16}O_2$/ 	colorless liq/ rose, honey	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 247°	1 mL in 2 mL 80% alc remains in soln to 10 mL
Isobutyl Salicylate FEMA No. 2213	194.23/$C_{11}H_{14}O_3$/ 	colorless liq/ orchid	*s*—most fixed oils; *ins*—gly, prop glycol/ 260°	1 mL in 9 mL 80% alc remains in soln to 10 mL
Isobutyraldehyde FEMA No. 2220	72.11/C_4H_8O/ $(CH_3)_2CHCHO$	colorless, mobile liq/ sharp, pungent	*m*—alc, ether, 1 mL in 125 mL water/ 64°	
Isobutyric Acid FEMA No. 2222 2-Methyl Propanoic Acid; Isopropylformic Acid	88.11/$C_4H_8O_2$/ $(CH_3)_2CHCOOH$	colorless liq/ strong, penetrating odor of rancid butter	*m*—alc, most fixed oils, gly, prop glycol; *ins*—water/ 155°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_8H_{14}O_2$ (M-1a)		1.426–1.430	0.880–0.900	
IR	98.0% of $C_8H_{16}O_2$ (M-1b)	1.0	1.402–1.405	0.858–0.863	
IR	98.0% of $C_{13}H_{16}O_2$ (sum of two isomers) (M-1b)	1.0	1.539–1.541	1.001–1.004	
IR	94.0% of $C_5H_{10}O_2$ (M-1b)	2.0, add ice to solution			
IR	98.0% of $C_{10}H_{20}O_2$ (M-1b)	1.0	1.411–1.417	0.853–0.859	
IR	98.0% of $C_{12}H_{16}O_2$ (M-1b)	1.0	1.486–1.488	0.984–0.988	
IR	98.0% of $C_{11}H_{14}O_3$ (M-1b)	1.0 (phenol red TS)	1.507–1.510	1.062–1.066	
IR	98.0% of C_4H_8O (M-1b)	5.0 (methyl red TS)		0.783–0.788	
IR	99.0–101.1% of $C_4H_8O_2$ (M-3a)		1.392–1.395	0.944–0.948	**Reducing Subs.**—passes test (M-14)

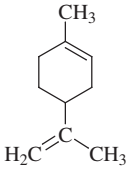
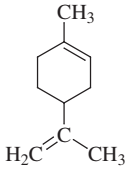
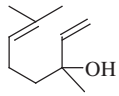
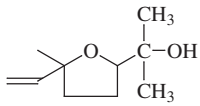
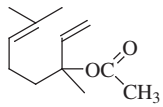
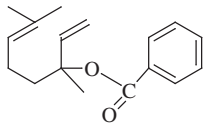
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Isoeugenol FEMA No. 2468 2-Methoxy-4-propenylphenol	164.20/$C_{10}H_{12}O_2$/ 	pale yel, viscous liq/ floral, carnation	*s*—most fixed oils, ether; *ins*—gly/ 266°	1 mL in 5 mL 50% alc
Isoeugenyl Acetate FEMA No. 2470 2-Methoxy-4-propenyl Phenyl Acetate	206.24/$C_{12}H_{14}O_3$/ 	white cryst/ spicy, clove	*s*—alc, most fixed oils, chloroform; *ins*—water	1 g in 27 mL 95% alc gives clear soln
Isopropyl Acetate FEMA No. 2926	102.13/$C_5H_{10}O_2$/ $CH_3COOCH(CH_3)_2$	colorless, mobile liq/ ethereal	*m*—alc, ether, most fixed oils, 1 g in 72 mL water/ 88°	
Isopulegol FEMA No. 2962 *p*-Menth-4-en-3-ol	154.25/$C_{10}H_{18}O$/ 	colorless liq/ harsh, camphoraceous, mint, with rose leaf and geranium background	91° (12 mm Hg)	1 mL in 4 mL 60% alc gives clear soln
Isovaleric Acid FEMA No. 3102 Isopropylacetic Acid	102.13/$C_5H_{10}O_2$/ $(CH_3)_2CHCH_2COOH$	colorless to pale yel liq/ disagreeable, rancid, cheese	*s*—alc, chloroform, ether, water/ 175°	
Lauryl Alcohol FEMA No. 2617 1-Dodecanol; Alcohol C-12	186.34/$C_{12}H_{26}O$/ $CH_3(CH_2)_{10}CH_2OH$	colorless liq above 21°/ fatty	*s*—most fixed oils, prop glycol; *ins*—gly, water/ 259°	1 mL in 3 mL 70% alc remains clear to 10 mL
Lauryl Aldehyde FEMA No. 2615 Aldehyde C-12; Dodecanal	184.32/$C_{12}H_{24}O$/ $CH_3(CH_2)_{10}CHO$	colorless to light yel liq (may solidify at room temp)/ fatty	*s*—alc, most fixed oils, prop glycol (may be turbid); *ins*—gly, water/ 249°	
Levulinic Acid FEMA No. 2627	116.12/$C_5H_8O_3$/ $CH_3COCH_2CH_2COOH$	yel to brown liq; may congeal/ smoky, caramel	245°	1 mL in 1 mL 95% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	99.0% of $C_{10}H_{12}O_2$ (sum of two isomers) (M-1b)		1.572–1.577	1.079–1.085	**Solidification Pt.**—NLT 12° (Appendix IIB)
IR	98.0% of $C_{12}H_{14}O_3$ (sum of two isomers; main isomer 95.0% min) (M-1b)	2.0 (phenol red TS)			**Solidification Pt.**—NLT 76° (Appendix IIB)
IR	99.0% of $C_5H_{10}O_2$ (M-1b)	2.0		0.866–0.869	
IR	95.0% of total alcohols as $C_{10}H_{18}O$ (Appendix VI; 1.2 g/ 77.12 with 2-h reflux)	1.0	1.470–1.475	0.904–0.913	**Aldehydes**—1.0% as citronellal (M-2d; 10 g/ 77.13) **Angular Rotation**—between 0° and −7° (Appendix IIB, 100-mm tube)
IR	99.0% of $C_5H_{10}O_2$ (M-3a)		1.401–1.405	0.923–0.928	
IR	97.0% of $C_{12}H_{26}O$ (M-1b)	1.0	1.440–1.444	0.830–0.836	**Solidification Pt.**—NLT 21° (Appendix IIB)
IR	92.0% of $C_{12}H_{24}O$ (M-1b)	10.0	1.433–1.439	0.826–0.836	
IR	97.0% of $C_5H_8O_3$ (M-3a)		1.440–1.445	1.136–1.142	**Solidification Pt.**—min 27° (Appendix IIB)

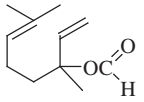
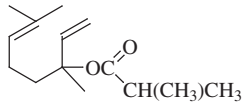
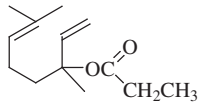
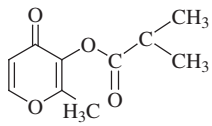
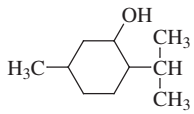
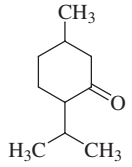
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
***d*-Limonene** FEMA No. 2633 *d-p*-Mentha-1,8-diene; Cinene	136.24/$C_{10}H_{16}$/ 	colorless liq/ mildly citrus, free from camphoraceous and terpene notes	*ss*—gly; *m*—alc, most fixed oils; *ins*—prop glycol, water/ 177°	
***l*-Limonene** *l-p*-Mentha-1,8-diene	136.24/$C_{10}H_{16}$/ 	colorless liq/ refreshing, light, clean	*m*—alc, most fixed oils; *ins*—water/ 177°	
Linalool FEMA No. 2635 3,7-Dimethyl-1,6-octadien-3-ol	154.25/$C_{10}H_{18}O$/ 	colorless liq/ pleasant, floral	*s*—most fixed oils, prop glycol; *ins*—gly/ 198°	1 mL in 4 mL 60% alc
Linalool Oxide FEMA No. 3746	170.25/$C_{10}H_{18}O_2$/ 	colorless to pale yel liq/ floral	*s*—prop glycol, veg oils; *ins*—water/ 188°	1 mL in 1 mL 95% ethanol
Linalyl Acetate FEMA No. 2636 3,7-Dimethyl-1,6-octadien-3-yl Acetate	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/ floral, fruity	*ss*—prop glycol; *m*—alc, most fixed oils; *ins*—gly, water/ 220°	1 mL in 5 mL 70% alc
Linalyl Benzoate FEMA No. 2638 3,7-Dimethyl-1,6-octadien-3-yl Benzoate	258.36/$C_{17}H_{22}O_2$/ 	yel to brown-yel liq/ tuberose	*s*—alc, chloroform, ether; *ins*—water/ 263°	1 mL in 1 mL 90% alc gives clear soln

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	93.0% of $C_{10}H_{16}$ (M-1a)		1.471–1.474	0.838–0.843	**Angular Rotation**—between +96° and +104° (Appendix IIB, 100-mm tube) **Peroxide Value**—5.0 (M-11)
	95.0% of $C_{10}H_{16}$ (M-1a)		1.469–1.473	0.837–0.841	**Angular Rotation**—between −90° and −61° (Appendix IIB, 100-mm tube) **Peroxide Value**—5.0 (M-11)
IR	92.0% of $C_{10}H_{18}O$ (M-1b)		1.461–1.465	0.858–0.867	**Esters**—0.5% as linalylacetate (Appendix VI; 10 g/98.15)
IR	98.0% of $C_{10}H_{18}O_2$ (sum of *cis* and *trans* isomers) (M-1b)		1.449–1.455	0.940–0.947	
IR	90.0% of total esters as $C_{12}H_{20}O_2$ (M-1b)	1.0	1.449–1.457	0.895–0.914	
IR	75.0% of $C_{17}H_{22}O_2$ (M-1b)	5.0	1.505–1.520	0.980–0.999	

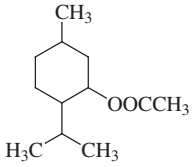
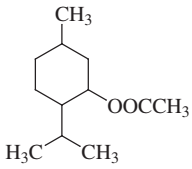
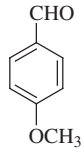
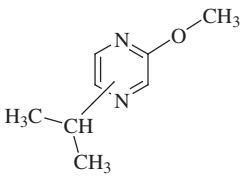
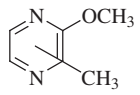
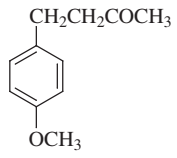
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Linalyl Formate FEMA No. 2642 3,7-Dimethyl-1,6-octadien-3-yl Formate	182.26/$C_{11}H_{18}O_2$/ 	colorless liq/ fresh, citrus, green, herbaceous, bergamot	*s*—alc, most fixed oils; *ss*—prop glycol, water; *ins*—gly/ 202°	1 mL in 6 mL 70% alc
Linalyl Isobutyrate FEMA No. 2640 3,7-Dimethyl-6-octadien-3-yl Isobutyrate	224.34/$C_{14}H_{24}O_2$/ 	colorless to slightly yel liq/ sweet, fresh, rosy	*m*—alc, chloroform, ether; *ins*—water/ 230°	1 mL in 3 mL 80% alc gives clear soln
Linalyl Propionate FEMA No. 2645 3,7-Dimethyl-6-octadien-3-yl Propionate	210.32/$C_{13}H_{22}O_2$/ 	colorless or almost colorless liq/ fresh, floral, sweet, fruity, pear	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly/ 226°	1 mL in 2 mL 80% alc
Maltol Isobutyrate FEMA No. 3462	196.20/$C_{10}H_{12}O_4$/ 	colorless to yel liq/ strawberry	*s*—prop glycol, veg oils; *ins*—water/ 176° (7 mm Hg)	1 mL in 1 mL 95% ethanol
Menthol FEMA No. 2665 3-*p*-Menthanol [NOTE: *l*-Menthol is natural or synthetic, *dl*-Menthol is synthetic]	156.27/$C_{10}H_{20}O$/ 	colorless, hexagonal crysts, usually like needles; fused masses or cryst powder/ peppermint	*vs*—alc, vol oils; *ss*—water/ 212°	1 mL in 1 mL 95% ethanol
***l*-Menthone** FEMA No. 2667 *l*-*p*-Menthan-3-one	154.25/$C_{10}H_{18}O$/ 	almost colorless liq/ mint	*s*—alc, most fixed oils; *vss*—water/ 207°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	90.0% of $C_{11}H_{18}O_2$ (M-1b)	3.0	1.453–1.458	0.910–0.918	
IR	95.0% of $C_{14}H_{24}O_2$ (M-1b)	1.0	1.446–1.451	0.882–0.888	
IR	92.0% of $C_{13}H_{22}O_2$ (M-1b)	1.0	1.449–1.454	0.893–0.902	
IR	96.0% of $C_{10}H_{12}O_4$ (M-1b)	10.0	1.493–1.501	1.140–1.153	
IR					**Melting Range** (*l*-menthol)—41° to 44° (Appendix IIB) **Nonvol. Res.**—0.05% (M-16) **Readily Ox. Subs.** (*dl*-menthol)—pass (M-13) **Specific Rotation** (*l*-menthol)— between −45° and −51° (Appendix IIB) **Specific Rotation** (*dl*-menthol)—between −2° and +2° (Appendix IIB)
	96.0% of $C_{10}H_{18}O$ (sum of two isomers) (M-1b)	1.0	1.448–1.453	0.888–0.895	**Angular Rotation**—min −20° (Appendix IIB, 100-mm tube)

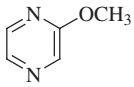
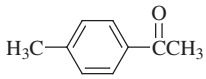
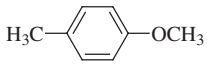
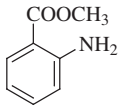
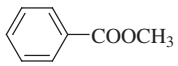
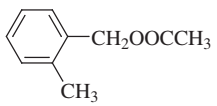
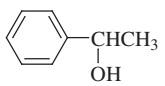
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
***dl*-Menthyl Acetate** FEMA No. 2668 *dl-p*-Menthan-3-yl Acetate	198.31/$C_{12}H_{22}O_2$/ 	colorless liq/ mild, minty	*s*—alc, most fixed oils, prop glycol; *ss*—gly, water/ 228°–229°	1 mL in 1 mL 95% ethanol
***l*-Menthyl Acetate** FEMA No. 2668 *l-p*-Menthan-3-yl Acetate	198.31/$C_{12}H_{22}O_2$/ 	colorless liq/ mild, minty	*s*—alc, prop glycol, most fixed oils; *ss*—water/ 229°–230°	
2-Mercaptopropionic Acid FEMA No. 3180	106.16/$C_3H_6O_2S$/ $CH_3CH(SH)COOH$	colorless to pale yel liq/ roasted, meaty	*m*—water, alc, ether, acetone/ 117°	1 mL in 1 mL 95% alc
***p*-Methoxybenzaldehyde** FEMA No. 2670 Anisic Aldehyde; *p*-Anisaldehyde	136.15/$C_8H_8O_2$/ 	colorless to slightly yel liq/ hawthorn	*s*—prop glycol; *m*—alc, ether, most fixed oils; *ins*—alc, water/ 248°	1 mL in 3 mL 60% alc gives clear soln
2-Methoxy 3- (or 5- or 6-) Isopropyl Pyrazine FEMA No. 3358	152.20/$C_8H_{12}N_2O$/ 	colorless to pale yel liq/ bell pepper, raw potato	*s*—veg oils; water/ 120°–125° (20 mm Hg)	1 mL in 1 mL 95% ethanol
2-Methoxy-3(5)-methylpyrazine FEMA No. 3183	124.14/$C_6H_8N_2O$/ 	colorless liq/ roasted, hazelnut	*s*—org solvents, water	1 mL in 1 mL 95% ethanol
4-*p*-Methoxyphenyl-2-butanone FEMA No. 2672 Anisyl Acetone	178.23/$C_{11}H_{14}O_2$/ 	colorless to pale yel liq/ sweet, floral, fruity	277°	1 mL in 1 mL 95% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
	97.0% of $C_{12}H_{22}O_2$ (sum of two isomers) (M-1b)	2.0	1.443–1.450	0.921–0.926	
	98.0% of $C_{12}H_{22}O_2$ (one major isomer) (M-1b)	2.0	1.443–1.447	0.921–0.926	**Angular Rotation**—between −70° and −69° (Appendix IIB, 100-mm tube)
IR	98.0% of $C_3H_6O_2S$ (M-3a)		1.479–1.484	1.192–1.200	
IR	97.5% of $C_8H_8O_2$ (M-1b)	6.0	1.571–1.574	1.119–1.123	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	97.0% of $C_8H_{12}N_2O$ (sum of three isomers) (M-1b)		1.492–1.499	1.010–1.022	
IR	99.0% of $C_6H_8N_2O$ (sum of two isomers) (M-1b)		1.506–1.510	1.070–1.090	
IR	98.0% of $C_{11}H_{14}O_2$ (M-1a)		1.517–1.521	1.042–1.048	

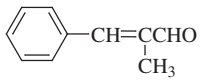
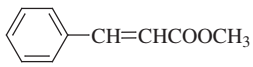
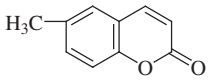
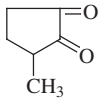
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
2-Methoxypyrazine FEMA No. 3302	110.12/$C_5H_6N_2O$/ 	colorless to yellow liq/ nutty, cocoa	*s*—alc; *ins*—water/ 61° (29 mm Hg)	
Methyl Acetate FEMA No. 2676	74.08/$C_3H_6O_2$/ CH_3COOCH_3	colorless liq/ ethereal, fruity	57.5°	1 mL in 1 mL 95% alc
4-Methyl Acetophenone FEMA No. 2677 Methyl *p*-Tolyl Ketone	134.18/$C_9H_{10}O$/ 	colorless or nearly colorless liq/ fruity-floral, resembling acetophenone	*s*—most fixed oils, prop glycol; *ins*—gly/ 226°	1 mL in 10 mL 50% alc
***p*-Methyl Anisole** FEMA No. 2681 *p*-Cresyl Methyl Ether; Methyl *p*-Cresol	122.17/$C_8H_{10}O$/ 	colorless liq/ ylang-ylang	*s*—most fixed oils; *ins*—gly, prop glycol/ 174°	1 mL in 3 mL 80% alc remains in soln on dilution
Methyl Anthranilate FEMA No. 2682	151.16/$C_8H_9NO_2$/ 	colorless to pale yel liq with blue fluorescence/ grape	*s*—most fixed oils, prop glycol; *ins*—gly/ 256°	1 mL in 5 mL 60% alc remains in soln to 10 mL
Methyl Benzoate FEMA No. 2683	136.15/$C_8H_8O_2$/ 	colorless liq/ deep, pungent, floral	*s*—alc, most fixed oils, prop glycol; *ins*—gly/ 198°	1 mL in 4 mL 60% alc
Methylbenzyl Acetate FEMA No. 3072 *o*-Tolyl Acetate	164.20/$C_{10}H_{12}O_2$/ 	colorless liq/ sweet, nutty	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly	1 mL in 2 mL 70% alc remains clear on dilution
α-Methylbenzyl Alcohol FEMA No. 2685 Methyl Phenylcarbinol; α-Phenethyl Alcohol	122.17/$C_8H_{10}O$/ 	colorless liq above room temp/ mild, hyacinth	*vs*—gly; *s*—most fixed oils, prop glycol/ 204°	1 mL in 3 mL 50% alc
2-Methyl Butanal FEMA No. 2691	86.13/$C_5H_{10}O$/ $CH_3CH_2CH(CH_3)CHO$	colorless to pale yel liq/ chocolate	*s*—prop glycol, veg oils; *ins*—water/ 93°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	99.0% of $C_5H_6N_2O$ (M-1b)		1.508–1.511	1.110–1.140 (20°)	
IR	98.0% of $C_3H_6O_2$ (M-1b)	1.0	1.358–1.363	0.927–0.932	
IR	95.0% of $C_9H_{10}O$ (M-1b)		1.530–1.535	0.996–1.004	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	98.5% of $C_8H_{10}O$ (M-1a)		1.510–1.513	0.966–0.970	**Cresol**—0.5% (M-1b)
IR	98.0% of total esters as $C_8H_9NO_2$ (Appendix VI; 1.0 g/ 75.59)		1.581–1.585 (as supercooled liq)	1.161–1.169	**Solidification Pt.**—NLT 23.8° (Appendix IIB)
IR	98.0% of $C_8H_8O_2$ (M-1b)	1.0	1.514–1.518	1.082–1.088	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	98.0% of $C_{10}H_{12}O_2$ (M-1b)	1.0	1.501–1.504	1.030–1.035	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	99.0% of $C_8H_{10}O$ (M-1b)		1.525–1.529	1.009–1.014	**Ketones**—1.0% as acetophenone (M-2d; 10.0 g/ 60.07) **Solidification Pt.**—NLT 19° (Appendix IIB)
	97.0% of $C_5H_{10}O$ (M-1b)	10.0	1.388–1.393	0.799–0.804	

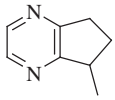
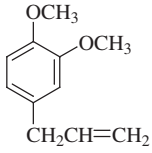
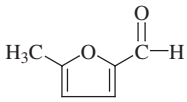
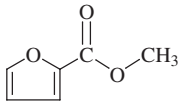
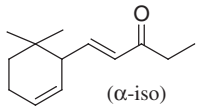
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
3-Methyl Butanal FEMA No. 2692 Isovaleraldehyde	86.13/$C_5H_{10}O$/ $(CH_3)_2CHCH_2CHO$	colorless to pale yel liq/ chocolate	*s*—prop glycol, veg oils; *ins*—water/ 93°	1 mL in 1 mL 95% ethanol
2-Methylbutyl Acetate FEMA No. 3644	130.18/$C_7H_{14}O_2$/ $CH_3CH_2CH(CH_3)CH_2OOCCH_3$	colorless to pale yel liq/ banana	138°	
2-Methylbutyl Isovalerate FEMA No. 3506 2-Methylbutyl-3-methylbutanoate	172.27/$C_{10}H_{20}O_2$/ $CH_3CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}HCH_2OOCCH_2CH(CH_3)_2$	colorless liq/ herbaceous, fruity	*s*—alc, most fixed oils; *ins*—water/ 191°–195°	
Methyl Butyrate FEMA No. 2693	102.13/$C_5H_{10}O_2$/ $CH_3(CH_2)_2COOCH_3$	colorless liq/ fruity	102°	1 mL in 1 mL 95% ethanol
2-Methylbutyric Acid FEMA No. 2695	102.13/$C_5H_{10}O_2$/ $CH_3CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}HCOOH$	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 176°	1 mL in 1 mL 95% alc
α-Methylcinnamaldehyde FEMA No. 2697	146.19/$C_{10}H_{10}O$/ 	yel liq/ cinnamon	*s*—most fixed oils, prop glycol; *ins*—gly/ 148° (27 mm Hg)	1 mL in 3 mL 70% alc remains clear on dilution
Methyl Cinnamate FEMA No. 2698	162.19/$C_{10}H_{10}O_2$/ 	white to slightly yel cryst mass/ fruity, balsamic	*s*—alc, most fixed oils, gly, prop glycol; *ins*—water/ 260°	1 g in 4 mL 80% alc
6-Methylcoumarin FEMA No. 2699	160.17/$C_{10}H_8O_2$/ 	white cryst solid/ coconut	*ins*—prop glycol, veg oils, water/ 303° (725 mm Hg)	1 g in 20 mL 95% alc
Methyl Cyclopentenolone FEMA No. 2700 3-Methylcyclopentane-1,2-dione	112.13/$C_6H_8O_2$/ 	white, cryst powder/ nutty, maple-licorice aroma in dilute soln	*s*—alc, prop glycol; *ss*—most fixed oils, 1 g in 72 mL water	1 g in 5 mL 90% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_5H_{10}O$ (M-1b)	10.0	1.388–1.391	0.795–0.802	
	97.0% of $C_7H_{14}O_2$ (M-1b)	1.0	1.399–1.404	0.872–0.877	
	98.0% of $C_{10}H_{20}O_2$ (M-1a)	2.0	1.413–1.416	0.852–0.857	
	98.0% of $C_5H_{10}O_2$ (M-1b)	1.0	1.386–1.390	0.892–0.897	
IR	98.0% of $C_5H_{10}O_2$ (M-3a)		1.404–1.408	0.932–0.936	
IR	97.0% of $C_{10}H_{10}O$ (one major isomer) (M-1b)	5.0	1.602–1.607	1.035–1.039	
IR	98.0% of $C_{10}H_{10}O_2$ (M-1b)	2.0			**Chlorinated Cmpds**.—passes test (Appendix VI) **Lead**—10 mg/kg (M-9)
IR	99.0% of $C_{10}H_8O_2$ (M-1a)				**Melting Range**—between 73° and 76° (Appendix IIB)
IR					**Melting Range**—between 104° and 108° (Appendix IIB)

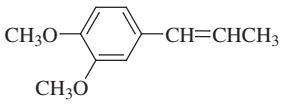
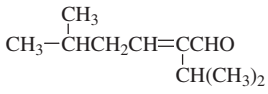
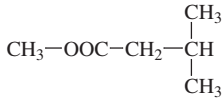
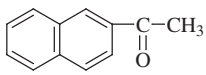
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
5H-5-Methyl-6,7-dihydrocyclopenta[b]pyrazine FEMA No. 3306	134.18/$C_8H_{10}N_2$/ 	yel to brown liq/ peanut	*s*—prop glycol, veg oils; *ss*—water/ 200°	1 mL in 1 mL 95% ethanol
Methyl Eugenol FEMA No. 2475 Eugenyl Methyl Ether; 1,2-Dimethoxy-4-allylbenzene	178.23/$C_{11}H_{14}O_2$/ 	colorless to pale yel liq/ delicate, clove-carnation	*s*—most fixed oils; *ins*—gly, prop glycol/ 249°	1 mL in 2 mL 70% alc remains clear to 10 mL
5-Methyl Furfural FEMA No. 2702	110.11/$C_6H_6O_2$/ 	yel to brown liq/ nutty, caramel	*s*—prop glycol, veg oils; *ins*—water/ 187°	1 mL in 1 mL 95% ethanol
Methyl Furoate FEMA No. 2703	126.11/$C_6H_6O_3$/ 	pale yel to brown liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 181°	1 mL in 1 mL 95% ethanol
6-Methyl-5-hepten-2-one FEMA No. 2707 Methyl Heptenone	126.20/$C_8H_{14}O$/ $CH_3C(CH_3)=CHCH_2CH_2COCH_3$	slightly yel liq/ sharp, citrus-lemongrass	*m*—alc, most fixed oils, ether; *ins*—water/ 73° (18 mm Hg)	1 mL in 2 mL 70% alc gives clear soln
Methyl Hexanoate FEMA No. 2708	130.19/$C_7H_{14}O_2$/ $CH_3—OOC(CH_2)_4CH_3$	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water 151°	1 mL in 1 mL 95% ethanol
Methyl Hexyl Ketone FEMA No. 2802 2-Octanone	128.21/$C_8H_{16}O$/ $CH_3(CH_2)_5COCH_3$	colorless to pale yel liq/ apple	*s*—prop glycol, veg oils; *ins*—water/ 175°	1 mL in 1 mL 95% alc
Methyl Ionones Mixture of α-, β-, γ- or α-iso, and δ-isomers	206.3/$C_{14}H_{22}O$/  (α-iso)	clear to pale yel to yel liq/ woody, orris	232°–270°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
	98.0% of $C_8H_{10}N_2$ (M-1b)		1.525–1.535	1.048–1.059	
IR	98.0% of $C_{11}H_{14}O_2$ (one major isomer) (M-1b)		1.532–1.536	1.032–1.036	**Eugenol**—1.0% (M-1b)
IR	97.0% of $C_6H_6O_2$ (M-1b)	5.0	1.525–1.535	1.095–1.110	
IR	98.0% of $C_6H_6O_3$ (M-1b)	5.0	1.483–1.500	1.174–1.180	
IR	98.0% of $C_8H_{14}O$ (M-1b)		1.438–1.442	0.846–0.851	
IR	98.0% of $C_7H_{14}O_2$ (M-1b)	2.0	1.402–1.408	0.880–0.886	
IR	95.0% of $C_8H_{16}O$ (M-1a)	1.0	1.414–1.418	0.813–0.818	
	88.0% of $C_{14}H_{22}O$ (sum of four isomers) (M-1b)	5.0	1.497–1.507	0.925–0.934	

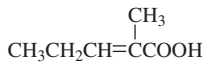
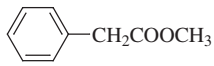
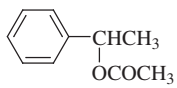
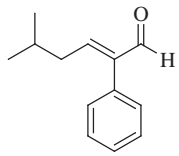
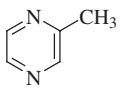
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Methyl Isobutyrate FEMA No. 2694	102.13/$C_5H_{10}O_2$/ $CH_3COOCH(CH_3)_2$	colorless liq/ fruity	90°	1 mL in 1 mL 95% alc
Methyl Isoeugenol FEMA No. 2476 4-Allyl-1,2-dimethoxy Benzene; Isoeugenyl Methyl Ether; 4-Propenyl Veratrole	178.23/$C_{11}H_{14}O_2$/ 	colorless to pale yel liq/ delicate, clove-carnation	*s*—most fixed oils; *ins*—gly, prop glycol/ 270°	1 mL in 2 mL 70% alc remains in soln to 10 mL
5-Methyl-2-isopropyl-2-hexenal FEMA No. 3406 Isodihydrolavandulal	154.25/$C_{10}H_{18}O$/ 	slightly yel liq/ herbaceous, woody, fruity, chocolate	*s*—alc, most fixed oils; *ins*—water, prop glycol/ 73° (10 mm Hg)	
Methyl Isovalerate FEMA No. 2753	116.16/$C_6H_{12}O_2$/ 	colorless to pale yel liq/ apple	*s*—prop glycol, veg oils; *ins*—water/ 114°	1 mL in 1 mL 95% ethanol
Methyl 2-Methylbutyrate FEMA No. 2719 Methyl 2-Methylbutanoate	116.16/$C_6H_{12}O_2$/ $CH_3OOCCHCH_2CH_3$ | CH_3	almost colorless liq/ sweet, fruity, apple	*s*—alc, most fixed oils; *ins*—water/ 115°	
Methyl-3-methylthiopropionate FEMA No. 2720	134.19/$C_5H_{10}O_2S$/ $CH_3SCH_2CH_2CO_2CH_3$	colorless to pale yel liq/ onion	*s*—prop glycol, veg oils; *ins*—water/ 74°–75° (18 mm Hg)	1 mL in 1 mL 95% ethanol
Methyl β-Naphthyl Ketone FEMA No. 2723 2-Acetonaphthone	170.21/$C_{12}H_{10}O$/ 	white or nearly white cryst solid/ orange blossom	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly/ 300°	1 g in 5 mL 95% alc
Methyl 2-Octynoate FEMA No. 2729 Methyl Heptine Carbonate	154.21/$C_9H_{14}O_2$/ $CH_3(CH_2)_5{\equiv}CCOOCH_3$	colorless to slightly yel liq/ powerful, unpleasant, violet when diluted	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly/ 217°	1 mL in 5 mL 70% alc
2-Methylpentanoic Acid FEMA No. 2754	116.16/$C_6H_{12}O_2$/ $CH_3CH_2CH_2CHCOOH$ | CH_3	colorless to pale yel liq/ caramel, pungent	196°–197°	1 mL in 1 mL 95% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_5H_{10}O_2$ (M-1b)	1.0	1.382–1.386	0.884–0.888	
IR	85.0% of $C_{11}H_{14}O_2$ (one isomer) (M-1a)		1.566–1.569	1.047–1.053	**Isoeugenol**—1.0% (M-1b)
IR	90.0% of $C_{10}H_{18}O$ (sum of isomers) (M-1a)		1.448–1.454	0.842–0.850	
IR	95.0% of $C_6H_{12}O_2$ (M-1b)	1.0	1.390–1.396	0.878–0.884	
	92.0% of $C_6H_{12}O_2$ (M-1b)	2.0	1.393–1.397	0.879–0.883	
IR	97.0% of $C_5H_{10}O_2S$ (M-1a)	1.0	1.462–1.468	1.069–1.078	
IR	99.0% of $C_{12}H_{10}O$ (M-1b)				**Solidification Pt.**—NLT 53° (Appendix IIB)
IR	96.0% of $C_9H_{14}O_2$ (M-1b)	1.0	1.446–1.449	0.919–0.924	**Chlorinated Cmpds**.—passes test (Appendix VI)
IR	98.0% of $C_6H_{12}O_2$ (M-3a)		1.411–1.416	0.916–0.923	

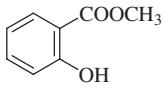
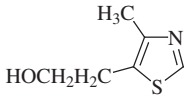
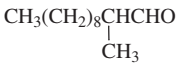
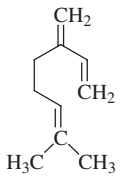
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
4-Methylpentanoic Acid FEMA No. 3463	116.16/$C_6H_{12}O_2$/ 	colorless to pale yel liq/ sour, penetrating	199°–201°	1 mL in 1 mL 95% alc
4-Methyl-2-pentanone FEMA No. 2731 Methyl Isobutyl Ketone	100.16/$C_6H_{12}O$/ $CH_3COCH_2CH(CH_3)_2$	colorless, mobile liq/ fruity, ethereal	*m*—alc, ether, 1 mL in 50 mL water/ 117°	
2-Methyl-2-pentenoic Acid FEMA No. 3195	114.14/$C_6H_{10}O_2$/ 	colorless to pale yel liq (high-purity material may solidify at room temp, with a melting point range of 24°–26°)	123° (30 mm Hg)	1 mL in 1 mL 95% alc
Methyl Phenylacetate FEMA No. 2733	150.18/$C_9H_{10}O_2$/ 	colorless or nearly colorless liq/ honey, jasmine	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 215°	1 mL in 6 mL 60% alc
Methyl Phenylcarbinyl Acetate FEMA No. 2684 α-Phenyl Ethyl Acetate	164.20/$C_{10}H_{12}O_2$/ 	colorless liq/ gardenia	*s*—most fixed oils, gly; *ins*—water/ 214°	1 mL in 7 mL 60% alc
5-Methyl 2-Phenyl 2-Hexenal FEMA No. 3199	188.27/$C_{13}H_{16}O$/ 	colorless to pale yel liq/ cocoa	*s*—prop glycol, veg oils; *ins*—water/ 89° (26 mm Hg)	1 mL in 1 mL 95% ethanol
2-Methyl Propyl 3-Methyl Butyrate FEMA No. 3369 Isobutyl Isovalerate	158.24/$C_9H_{18}O_2$/ $(CH_3)_2CHCH_2COOCH_2CH(CH_3)_2$	colorless to pale yel liq/ fruity	*m*—alc/ 170°	
2-Methylpyrazine FEMA No. 3309	94.12/$C_5H_6N_2$/ 	colorless to slightly yel liq/ nutty, cocoa	*m*—water, alc, acetone, most fixed oils/ 137°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_6H_{12}O_2$ (M-3a)		1.412–1.417	0.919–0.926	
	99.0% of $C_6H_{12}O$ (M-2d)	2.0 (M-15)	1.392–1.397	0.796–0.799	**Distillation Range**—between 114° and 117° (Appendix IIB) **Water**—0.1% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	98.0% of $C_6H_{10}O_2$ (M-3a)		1.455–1.465	0.978–0.985	
IR	98.0% of $C_9H_{10}O_2$ (M-1b)	1.0	1.503–1.509	1.061–1.067	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	97.0% of $C_{10}H_{12}O_2$ (M-1b)	2.0	1.493–1.497	1.023–1.026	**Chlorinated Cmpds.**—passes test (Appendix VI)
	92.0% of $C_{13}H_{16}O$ [sum of (*E*)- and (*Z*)-isomers] (M-1b)	4.0	1.529–1.536	0.963–0.979	
	98.0% of $C_9H_{18}O_2$ (M-1b)	1.0	1.404–1.408	0.850–0.854	
IR	99.0% of $C_5H_6N_2$ (M-1a)		1.504–1.506	1.010–1.030	**Water**—0.5% (Appendix IIB, KF; use freshly dist. pyridine as solvent)

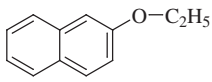
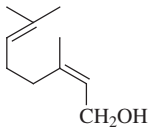
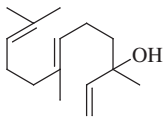
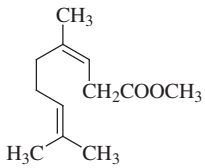
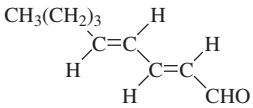
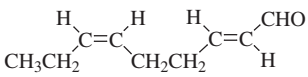
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Methyl Salicylate FEMA No. 2745	152.15/$C_8H_8O_3$/ 	colorless, to yel liq/ wintergreen	*s*—alc, glacial acetic acid; *ss*—water/ 222° (decomp)	1 mL in 7 mL 70% alc may be slightly cloudy
4-Methyl-5-thiazole Ethanol FEMA No. 3204 Sulfurol	143.20/C_9H_9NOS/ 	colorless to pale yel liq; may darken upon aging/ meaty	135° (7 mm Hg)	
Methyl Thiobutyrate FEMA No. 3310	118.20/$C_5H_{10}OS$/ $CH_3—SOC(CH_2)_2CH_3$	colorless to pale yel liq/ pungent	*s*—prop glycol, veg oils; *ins*—water/ 143°	1 mL in 1 mL 95% ethanol
3-Methylthiopropionaldehyde FEMA No. 2747 Methional	104.17/C_4H_8OS/ 	colorless to pale yel liq/ meaty potato	165°–166°	1 mL in 1 mL 95% alc
2-Methylundecanal FEMA No. 2749 Aldehyde C-12 MNA; Methyl *n*-Nonyl Acetaldehyde	184.32/$C_{12}H_{24}O$/ 	colorless to slightly yel liq/ fatty	*s*—most fixed oils, alc, prop glycol (may be turbid); *ins*—gly/ 171°	
Methyl Valerate FEMA No. 2752	116.16/$C_6H_{12}O_2$/ $CH_3—OOC(CH_2)_3CH_3$	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 128°	1 mL in 1 mL 95% ethanol
Myrcene FEMA No. 2762 7-Methyl-3-methylene-1,6-octadiene	136.24/$C_{10}H_{16}$/ 	colorless to pale yel liq/ sweet, balsamic	*s*—alc, most fixed oils; *ins*—water/ 167°	
Myristaldehyde FEMA No. 2763 Tetradecanal	212.38/$C_{14}H_{28}O$/ $CH_3(CH_2)_{12}CHO$	colorless to pale yel liq/ fatty, orris	*ins*—ethanol, prop glycol, veg oils, water/ 260°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_8H_8O_3$ (M-1b)	1.0 (phenol red TS)	1.535–1.538	1.180–1.185	
IR	98.0% of C_9H_9NOS (M-1b)		1.548–1.552	1.196–1.210	
IR	97.0% of $C_5H_{10}OS$ (M-1b)	3.0	1.461–1.467	0.964–0.970	
IR	98.0% of C_4H_8OS (M-1a)		1.484–1.493	1.038–1.048	
IR	94.0% of $C_{12}H_{24}O$ (M-1b)	10.0	1.431–1.436	0.822–0.830	
IR	98.0% of $C_6H_{12}O_2$ (M-1b)	1.0	1.395–1.401	0.885–0.891	
	90.0% of $C_{10}H_{16}$ (M-1a)		1.466–1.471	0.789–0.793	**Peroxide Value**—50.0 (M-11)
	85.0% of $C_{14}H_{28}O$ (M-2a)	5.0	1.438–1.445	0.825–0.830	

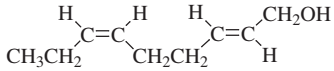
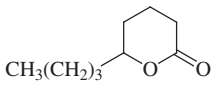
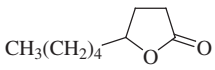
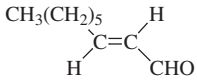
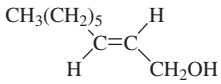
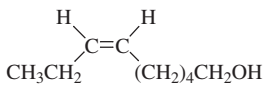
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Myristyl Alcohol 1-Tetradecanol; Tetradecyl Alcohol	214.38/$C_{14}H_{30}O$/ $CH_3(CH_2)_{12}CH_2OH$	colorless to white, waxy, solid flakes/ waxy	*s*—ether; *ss*—alc; *ins*—water/ 289°	
β-Naphthyl Ethyl Ether FEMA No. 2768 Nerolin II; Nerolin Bromelia	172.23/$C_{12}H_{12}O$/ 	white to pale yel cryst/ floral	*ss*—prop glycol, veg oils; *ins*—water/ 282°	1 mL in 5 mL 95% ethanol
Nerol FEMA No. 2770 *cis*-3,7-Dimethyl-2,6-octadien-1-ol	154.25/$C_{10}H_{18}O$/ 	colorless liq/ fresh, sweet, rose	*m*—alc, chloroform, ether; *ins*—water/ 227°	1 mL in 9 mL 50% alc gives clear soln
Nerolidol FEMA No. 2772 3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	222.37/$C_{15}H_{26}O$/ 	colorless to straw-colored liq/ faint, floral, rose, apple	*s*—most fixed oils, prop glycol; *ins*—gly/ 276°	1 mL in 4 mL 70% alc
Neryl Acetate FEMA No. 2773 *cis*-3,7-Dimethyl-2,6-octadien-1-yl Acetate	196.29/$C_{12}H_{20}O_2$/ 	colorless to pale yel liq/ sweet, floral	*s*—veg oils; *ss*—prop glycol; *ins*—water/ 134° (25 mm Hg)	1 mL in 1 mL 95% alc
(*E*),(*E*)-2,4-Nonadienal FEMA No. 3212 *trans*,*trans*-2,4-Nonadienal	138.21/$C_9H_{14}O$/ 	slightly yel liq/ strong, fatty, floral	*s*—alc, most fixed oils; *ins*—water/ 97° (10 mm Hg)	1 mL in 1 mL 95% ethanol
(*E*),(*Z*)-2,6-Nonadienal FEMA No. 3377 *trans*,*cis*-2,6-Nonadienal	138.21/$C_9H_{14}O$/ 	slightly yel liq/ powerful, violet, cucumber	*s*—alc, most fixed oils; *ins*—water/ 94° (18 mm Hg)	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
	98.0% of $C_{14}H_{30}O$ (M-1b)	1.0			**Melting Range**—between 38° and 41° (Appendix IIB) **Iodine Value**—3.0 max. (Appendix VII) **Saponification Value**—1.0 max. (Appendix VI)
IR	97.0% of $C_{12}H_{12}O$ (M-1b)				**Melting Point**—NLT 30.0° (Appendix IIB)
IR	95.0% of total alcohols as $C_{10}H_{18}O$ (Appendix VI; 1.2 g/77.13)		1.467–1.478	0.875–0.880	
IR	97.0% of $C_{15}H_{26}O$ (sum of two isomers) (M-1b)		1.478–1.483	0.870–0.880	**Angular Rotation** (Natural)—between +11° and +14° (Appendix IIB, 100-mm tube) **Esters**—0.5% as nerolidyl acetate (Appendix VI; 10 g/132.7)
IR	96.0% of $C_{12}H_{20}O_2$; [predominantly (*Z*)-isomer by M-1a] (M-1b)	1.0	1.458–1.464	0.905–0.914	
IR	89.0% of $C_9H_{14}O$ (one major isomer) (M-1a)		1.517–1.523	0.865–0.880	
IR	96.0% of $C_9H_{14}O$ (sum of two isomers; 90.0% major isomer) (M-1a)		1.470–1.475	0.850–0.870	

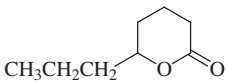
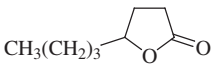
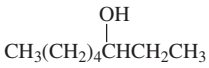
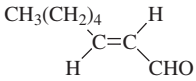
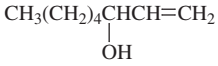
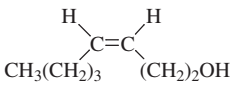
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
(*E*),(*Z*)-2,6-Nonadienol FEMA No. 2780 *trans*,*cis*-2,6-Nonadienol	140.22/$C_9H_{16}O$/ 	white to yel liq/ powerful, green, vegetable	*ins*—water/ 196°	1 mL in 1 mL 95% ethanol
δ-Nonalactone FEMA No. 3356 5-Hydroxynonanoic Acid, Lactone	156.22/$C_9H_{16}O_2$/ 	colorless to pale yel liq/ coconut	*s*—prop glycol; veg oils; *ins*—water/ 250°	1 mL in 1 mL 95% alc
γ-Nonalactone FEMA No. 2781 Aldehyde C-18, So-Called	156.22/$C_9H_{16}O_2$/ 	colorless to slightly yel liq/ coconut	*s*—alc, most fixed oils, prop glycol; *ins*—water/ 121°–122° (6 mm Hg)	1 mL in 5 mL 60% alc
Nonanal FEMA No. 2782 Aldehyde C-9; Pelargonic Aldehyde	142.24/$C_9H_{18}O$/ $CH_3(CH_2)_7CHO$	colorless to light yel liq/ fatty; citrus-rose on dilution	*s*—alc, most fixed oils, prop glycol; *ins*—gly/ 93° (23 mm Hg)	
Nonanoic Acid FEMA No. 2784	158.24/$C_9H_{18}O_2$/ $CH_3(CH_2)_7COOH$	colorless to pale yel liq/ fatty	*s*—prop glycol, veg oils; *ins*—water/ 254°	1 mL in 1 mL ethanol
2-Nonanone FEMA No. 2785 Methyl Heptyl Ketone	142.24/$C_9H_{18}O$/ $CH_3CO(CH_2)_6CH_3$	colorless to pale yel liq/ fruity, floral, fatty, herbaceous	*s*—prop glycol, veg oils; *ins*—water/ 195°	1 mL in 1 mL 95% alc
(*E*)-2-Nonenal FEMA No. 3213 *trans*-2-Nonenal	140.22/$C_9H_{16}O$/ 	white to slightly yel liq/ fatty, violet	*s*—alc, most fixed oils; *ins*—water/ 88° (12 mm Hg)	1 mL in 1 mL 95% ethanol
(*E*)-2-Nonen-1-ol FEMA No. 3379 *trans*-2-Nonenol	142.24/$C_9H_{18}O$/ 	white liq/ fatty, violet	*ins*—water/ 105° (12 mm Hg)	1 mL in 1 mL 95% ethanol
(*Z*)-6-Nonen-1-ol FEMA No. 3465 *cis*-6-Nonen-1-ol	142.24/$C_9H_{18}O$/ 	white to slightly yel liq/ powerful, melon	*ins*—water/ 115° (20 mm Hg)	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	92.0% of $C_9H_{16}O$ (one major isomer) (M-1a)		1.464–1.471	0.860–0.880	
IR	98.0% of $C_9H_{16}O_2$ (M-1a)		1.454–1.459	0.980–0.986	
IR	98.0% of $C_9H_{16}O_2$ (M-1b)	2.0	1.446–1.450	0.958–0.966	
IR	92.0% of $C_9H_{18}O$ (M-1b)	10.0	1.422–1.429	0.820–0.830	
	98.0% of $C_9H_{18}O_2$ (M-3a)		1.431–1.435	0.901–0.906	
IR	97.0% of $C_9H_{18}O$ (M-1a)		1.418–1.423	0.817–0.823	
IR	92.0% of $C_9H_{16}O$ (one major isomer) (M-1a)		1.450–1.460	0.840–0.850	
IR	96.0% of $C_9H_{18}O$ (one major isomer) (M-1a)		1.444–1.452	0.830–0.850	
IR	95.0% of $C_9H_{18}O$ (one major isomer) (M-1a)		1.446–1.452	0.850–0.870	

General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Nonyl Acetate FEMA No. 2788	186.29/$C_{11}H_{22}O_2$/ $CH_3COO(CH_2)_8CH_3$	colorless liq/ floral, fruity	*s*—alc, ether; *ins*—water/ 212°	1 mL in 6 mL 70% alc gives clear soln
Nonyl Alcohol FEMA No. 2789 1-Nonanol; Alcohol C-9	144.26/$C_9H_{20}O$/ $CH_3(CH_2)_7CH_2OH$	colorless liq/ rose-citrus	*m*—alc, chloroform, ether; *ins*—water/ 213°	1 mL in 3 mL 60% alc gives clear soln
δ-Octalactone FEMA No. 3214 5-Hydroxyoctanoic Acid Lactone	142.20/$C_8H_{14}O_2$/ 	colorless to pale yel liq/ coconut	*s*—prop glycol, veg oils; *ins*—water/ 234°	1 mL in 1 mL 95% ethanol
γ-Octalactone FEMA No. 2796	142.20/$C_8H_{14}O_2$/ 	colorless to slightly yel liq/ sweet, coconut, fruity	*s*—alc; *ss*—water/ 234°	
Octanal FEMA No. 2797 Aldehyde C-8; Caprylic Aldehyde	128.21/$C_8H_{16}O$/ $CH_3(CH_2)_6CHO$	colorless to light yel liq/ fatty-orange	*s*—alc, most fixed oils, prop glycol; *ins*—gly/ 171°	
3-Octanol FEMA No. 3581	130.23/$C_8H_{18}O$/ 	colorless liq/ strong, oily-nutty, herbaceous	*s*—alc, most fixed oils; *ins*—water/ 174°	1 mL in 1 mL 95% ethanol
(*E*)-2-Octen-1-al FEMA No. 3215 *trans*-2-Octen-1-al	126.20/$C_8H_{14}O$/ 	slightly yel liq/ fatty, green	*s*—alc, most fixed oils; *ss*—water/ 84° (19 mm Hg)	
1-Octen-3-ol FEMA No. 2805 Amyl Vinyl Carbinol	128.21/$C_8H_{16}O$/ 	colorless to pale yel liq/ mushroom, herbaceous	*s*—prop glycol, veg oils; *ins*—water/ 175°	1 mL in 1 mL 95% alc
(*Z*)-3-Octen-1-ol FEMA No. 3467 *cis*-3-Octen-1-ol	128.21/$C_8H_{16}O$/ 	white to slightly yel liq/ musty, mushroom	*ins*—water/ 174°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_{11}H_{22}O_2$ (M-1b)	1.0	1.422–1.426	0.864–0.868	
IR	97.0% of $C_9H_{20}O$ (M-1b)	1.0	1.431–1.435	0.824–0.830	
IR	98.0% of $C_8H_{14}O_2$ (M-1a)		1.452–1.458	0.995–1.000	
IR	95.0% of $C_8H_{14}O_2$ (M-1a)	8.0	1.443–1.447	0.970–0.980	
IR	92.0% of $C_8H_{16}O$ (M-1b)	10.0	1.417–1.425	0.810–0.830	
	97.0% of $C_8H_{18}O$ (M-1a)		1.425–1.429	0.817–0.824	
IR	92.0% of $C_8H_{14}O$ [as (*E*)-isomer] (M-1a)		1.450–1.455	0.830–0.850	
IR	97.0% of $C_8H_{16}O$ (M-1a)		1.434–1.442	0.831–0.839	
IR	95.0% of $C_8H_{16}O$ [as (*Z*)-isomer] (M-1a)		1.440–1.446	0.830–0.850	

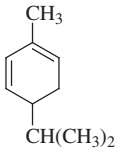
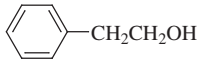
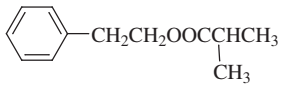
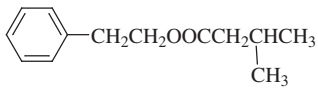
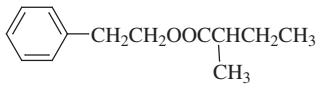
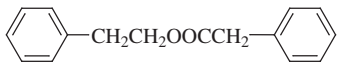
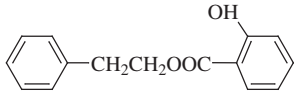
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
1-Octen-3-yl Acetate FEMA No. 3582	170.25/$C_{10}H_{18}O_2$/ $CH_3(CH_2)_3CH_2CHOOCCH_3$ | $CH{=}CH_2$	almost colorless liq/ metallic, mushroom	*s*—alc, most fixed oils; *ins*—water, prop glycol/ 80° (15 mm Hg)	1 mL in 1 mL 95% ethanol
1-Octen-3-yl Butyrate FEMA No. 3612	198.31/$C_{12}H_{22}O_2$/ $CH_3(CH_2)_3CH_2CHOOC(CH_2)_2CH_3$ | $CH{=}CH_2$	almost colorless liq/ metallic, mushroom	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—water/ 225°–229°	1 mL in 1 mL 95% ethanol
Octyl Acetate FEMA No. 2806	172.27/$C_{10}H_{20}O_2$/ $CH_3COO(CH_2)_7CH_3$	colorless liq/ fruity, orange, jasmine	*m*—alc, most fixed oils, org solvents; *ins*—water/ 208°	1 mL in 4 mL 70% alc gives clear soln
3-Octyl Acetate FEMA No. 3583	172.27/$C_{10}H_{20}O_2$/ $CH_3(CH_2)_3CH_2CHOOCCH_3$ | CH_2CH_3	colorless liq/ rosy-minty	*s*—alc, prop glycol, most fixed oils; *ss*—water/ 187°	
Octyl Alcohol FEMA No. 2800 Alcohol C-8; 1-Octanol; Capryl Alcohol	130.23/$C_8H_{14}O$/ $CH_3(CH_2)_6CH_2OH$	colorless liq/ sharp fatty-citrus	*s*—most fixed oils, prop glycol; *ins*—gly/ 195°	1 mL in 5 mL 50% alc
Octyl Formate FEMA No. 2809	158.24/$C_9H_{18}O_2$/ $HCOO(CH_2)_7CH_3$	colorless liq/ fruity	*s*—most fixed oils, min oil, prop glycol; *ins*—gly/ 200°	1 mL in 5 mL 70% alc remains in soln to 10 mL
Octyl Isobutyrate FEMA No. 2808 Octyl 2-Methylpropanoate	200.32/$C_{12}H_{24}O_2$/ $CH_3(CH_2)_7OOCCH(CH_3)_2$	colorless to pale yel liq/ refreshing, herbaceous	245°	1 mL in 1 mL 95% alc
ω-Pentadecalactone FEMA No. 2840 Cyclopentadecanolide; Exaltolide; Thibetolide	240.38/$C_{15}H_{28}O_2$/ $(CH_2)_{14}$ $C{=}O$ | O	white to tan or blue-gray cryst/ musky	*s*—veg oils; *ins*—prop glycol/ 137° (2 mm Hg)	1 g in 1 mL 95% alc
2,3-Pentanedione FEMA No. 2841 Acetyl Propionyl	100.12/$C_5H_8O_2$/ $CH_3CH_2C(=O)C(=O)CH_3$	yel to yel-green liq/ penetrating, buttery on dilution	*m*—alc, prop glycol, most fixed oils; *ins*—gly, water/ 108°	1 mL in 3 mL 50% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_{10}H_{18}O_2$ (M-1b)		1.418–1.428	0.865–0.886	
IR	95.0% of $C_{12}H_{22}O_2$ (M-1b)		1.423–1.433	0.859–0.880	
IR	98.0% of $C_{10}H_{20}O_2$ (M-1b)	1.0	1.418–1.421	0.865–0.868	
	98.0% of $C_{10}H_{20}O_2$ (M-1b)	2.0	1.414–1.419	0.856–0.860	
IR	98.0% of $C_8H_{18}O$ (M-1b)	1.0	1.428–1.431	0.822–0.830	
IR	96.0% of $C_9H_{18}O_2$ (M-1b)	1.0	1.418–1.420	0.869–0.874	
	98.0% of $C_{12}H_{24}O_2$ (M-1a)	1.0	1.420–1.425	0.853–0.858	
IR	99.0% of $C_{15}H_{28}O_2$ (M-1b)				**Solidification Pt.**—NLT 35° (Appendix IIB)
IR	93.0% of $C_5H_8O_2$ (M-1b)		1.402–1.406	0.952–0.962	

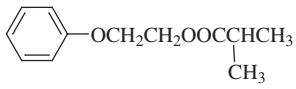
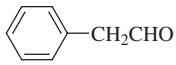
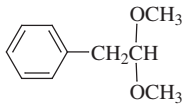
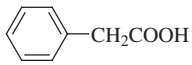
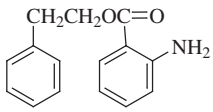
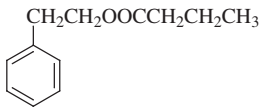
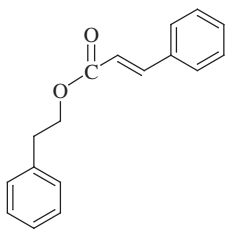
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
2-Pentanone FEMA No. 2842 Methyl Propyl Ketone	86.13/$C_5H_{10}O$/ $CH_3COCH_2CH_2CH_3$	colorless, mobile liq/ fruity, ethereal	*m*—alc, ether, 1 mL in 25 mL water/ 102°	
α-Phellandrene FEMA No. 2856 *p*-Mentha-1,5-diene	136.24/$C_{10}H_{16}$/ 	colorless to slightly yel liq/ herbaceous; minty background	*s*—alc; *ins*—water	1 mL in 1 mL 95% alc gives clear soln
Phenethyl Acetate FEMA No. 2857 2-Phenethyl Acetate	164.20/$C_{10}H_{12}O_2$/ 	colorless liq/ sweet, rosy, honey	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/ 232°	1 mL in 2 mL 70% alc remains clear to 10 mL
Phenethyl Alcohol FEMA No. 2858 2-Phenylethyl Alcohol	122.17/$C_8H_{10}O$/ 	colorless liq/ rose	*s*—most fixed oils, water, prop glycol/ 219°	1 mL in 2 mL 50% alc remains clear to 10 mL
Phenethyl Isobutyrate FEMA No. 2862	192.26/$C_{12}H_{16}O_2$/ 	colorless to slightly yel liq/ fruity, rosy	*s*—alc, most fixed oils; *ins*—water/ 230°	1 mL in 3 mL 80% alc gives clear soln
Phenethyl Isovalerate FEMA No. 2871	206.28/$C_{13}H_{18}O_2$/ 	colorless to slightly yel liq/ fruity, rosy	*s*—alc, most fixed oils; *ins*—water/ 263°	1 mL in 3 mL 80% alc gives clear soln
2-Phenethyl 2-Methylbutyrate FEMA No. 3632	206.28/$C_{13}H_{18}O_2$/ 	colorless liq/ floral-fruity	*s*—alc, most fixed oils; *ins*—water	
Phenethyl Phenylacetate FEMA No. 2866	240.30/$C_{16}H_{16}O_2$/ 	colorless to slightly yel liq above 26°/ rosy, hyacinth	*s*—alc; *ins*—water/ 325°	1 mL in 4 mL 90% alc gives clear soln
Phenethyl Salicylate FEMA No. 2868	242.27/$C_{15}H_{14}O_3$/ 	white cryst/ balsamic	*s*—alc; *ins*—water/ 370°	1 g in 20 mL 95% alc gives clear soln

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_5H_{10}O$ (M-1b)	2.0	1.387–1.392	0.801–0.806	
IR			1.471–1.477	0.835–0.865	**Angular Rotation**—between −80° and −120° (Appendix IIB, 100-mm tube)
IR	98.0% of $C_{10}H_{12}O_2$ (M-1b)	1.0	1.497–1.501	1.030–1.034	
IR	99.0% of $C_8H_{10}O$ (one isomer) (M-1a)		1.531–1.534	1.017–1.020	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	98.0% of $C_{12}H_{16}O_2$ (M-1b)	1.0	1.486–1.490	0.987–0.990	
IR	98.0% of $C_{13}H_{18}O_2$ (one major isomer) (M-1b)	1.0	1.484–1.486	0.973–0.976	
	95.0% of $C_{13}H_{18}O_2$ (M-1b)	2.0	1.484–1.488	0.973–0.977	
IR	98.0% of $C_{16}H_{16}O_2$ (M-1b)	1.0	1.548–1.552 (may solidify)	1.079–1.082	**Solidification Pt.**—NLT 26° (Appendix IIB)
IR	98.0% of $C_{15}H_{14}O_3$ (M-1b)	1.0 (phenol red TS)			**Solidification Pt.**—NLT 41° (Appendix IIB)

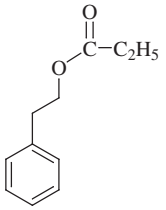
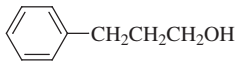
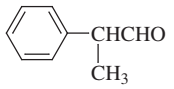
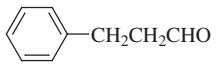
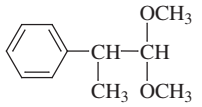
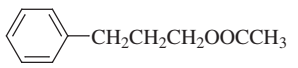
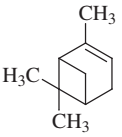
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Phenoxyethyl Isobutyrate FEMA No. 2873	208.26/$C_{12}H_{16}O_3$/ 	colorless liq/ honey, rose	*m*—alc, chloroform, ether; *ins*—water/ 125°–127° (4 mm Hg)	1 mL in 3 mL 70% alc gives clear soln
Phenylacetaldehyde FEMA No. 2874 α-Toluic Aldehyde	120.15/C_8H_8O/ 	colorless to slightly yel, oily liq; becomes more viscous on aging/ harsh; hyacinth on dilution	*s*—most fixed oils, prop glycol; *ins*—gly/ 195°	1 mL in 2 mL 80% alc
Phenylacetaldehyde Dimethyl Acetal FEMA No. 2876	166.22/$C_{10}H_{14}O_2$/ 	colorless liq/ green, spicy, floral	*s*—most fixed oils, prop glycol; *ins*—gly/ 219°	1 mL in 2 mL 70% alc remains clear to 10 mL
Phenylacetic Acid FEMA No. 2878 α-Toluic Acid	136.15/$C_8H_8O_2$/ 	glistening white cryst solid/ persistent, disagreeable, suggestive of geranium leaf and rose when diluted	*s*—most fixed oils, gly; *ss*—water/ 265°	
Phenylethyl Anthranilate FEMA No. 2859	241.29/$C_{15}H_{15}NO_2$/ 	colorless to pale yel cryst mass/ neroli, grape undertone	*s*—alc/ 324°	
Phenylethyl Butyrate FEMA No. 2861	192.26/$C_{12}H_{16}O_2$/ 	colorless to pale yel liq/ green, hay	238°	1 mL in 1 mL 95% alc
Phenyl Ethyl Cinnamate FEMA No. 2863	252.31/$C_{17}H_{16}O_2$/ 	white to pale yel cryst/ floral	*s*—prop glycol, veg oils; *ins*—water	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_{12}H_{16}O_3$ (M-1b)	1.0	1.492–1.495	1.044–1.048	
IR	90.0% of C_8H_8O (M-1b)	5.0	1.525–1.545	1.025–1.045	
IR	95.0% of $C_{10}H_{14}O_2$ (M-1b)	1.0	1.493–1.496	1.000–1.006	**Chlorinated Cmpds.**—passes test (Appendix VI) **Free Phenyl Acetaldehyde**—1.0% (M-1b)
IR	99.0% of $C_8H_8O_2$ (after drying) (M-3b)				**Melting Range**—between 76° and 78° (Appendix IIB, Class Ia) **Lead**—10 mg/kg (M-9)
IR	98.0% of $C_{15}H_{15}NO_2$ (M-1b)	1.0			**Solidification Pt.**—NLT 40° (Appendix IIB)
IR	98.0% of $C_{12}H_{16}O_2$ (M-1b)	1.0	1.487–1.492	0.991–0.995	
IR	99.0% of $C_{17}H_{16}O_2$ (M-1b)	1.0			**Melting Point**—NLT 54.0° (Appendix IIB)

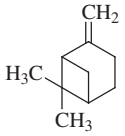
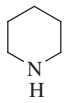
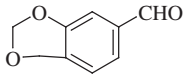
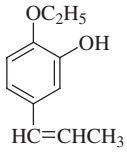
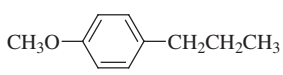
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Phenyl Ethyl Propionate FEMA No. 2867	178.23/$C_{11}H_{14}O_2$/ 	colorless to pale yel liq/ rose	*s*—prop glycol, veg oils; *ins*—water/ 245°	1 mL in 1 mL 95% ethanol
3-Phenyl-1-propanol FEMA No. 2885 Phenylpropyl Alcohol; Hydrocinnamyl Alcohol	136.19/$C_9H_{12}O$/ 	colorless, slightly viscous liq/ sweet, hyacinth-mignonette	*s*—most fixed oils, prop glycol; *ins*—gly/ 236°	1 mL in 1 mL 70% alc
2-Phenylpropionaldehyde FEMA No. 2886 Hydratropic Aldehyde; α-Methyl Phenylacetaldehyde	134.18/$C_9H_{10}O$/ 	water-white to pale yel liq/ floral	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly/ 222°	
3-Phenylpropionaldehyde FEMA No. 2887 Hydrocinnamaldehyde; Phenylpropyl Aldehyde	134.18/$C_9H_{10}O$/ 	colorless to slightly yel liq/ strong, pungent, floral, hyacinth	*m*—alc, ether; *ins*—water/ 97°–98° (12 mm Hg)	1 mL in 7 mL 60% alc remains clear on dilution
2-Phenylpropionaldehyde Dimethyl Acetal FEMA No. 2888 Hydratropic Aldehyde Dimethyl Acetal	180.25/$C_{11}H_{16}O_2$/ 	colorless to slightly yel liq/ mushroom	*s*—alc, ether; *ins*—water/ 241°	1 mL in 7 mL 60% alc, and in 3 mL 70% alc gives clear solns
3-Phenylpropyl Acetate FEMA No. 2890	178.23/$C_{11}H_{14}O_2$/ 	colorless liq/ spicy, floral	*s*—alc; *ins*—water/ 244°	1 mL in 3 mL 70% alc gives clear soln
α-Pinene FEMA No. 2902 2,6,6-Trimethylbicyclo(3.1.1)hept-2-ene; 2-Pinene; l-α-Pinene	136.24/$C_{10}H_{16}$/ 	colorless liq/ fresh, piney	*s*—alc, most fixed oils; *ins*—water/ 155°	1 mL in 3 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{11}H_{14}O_2$ (M-1b)	1.0	1.491–1.497	1.009–1.015	
IR	98.0% of $C_9H_{12}O$ (M-1b)		1.524–1.528	0.998–1.002	**Free 3-Phenyl Propionaldehyde**—0.5% (M-1b)
IR	95.0% of $C_9H_{10}O$ (M-1b)	5.0	1.515–1.520	0.998–1.006	
IR	90.0% of aldehydes (M-1b)	10.0	1.520–1.532	1.010–1.020	**Chlorinated Cmpds.**—passes test (Appendix VI)
IR	95.0% of $C_{11}H_{16}O_2$ (M-1b)		1.492–1.497	0.989–0.994	**Free 2-Phenylpropionaldehyde**—3.0% (M-1b)
IR	98.0% of $C_{11}H_{14}O_2$ (M-1b)	1.0	1.494–1.497	1.012–1.015	
	97.0% of $C_{10}H_{16}$ (M-1a)		1.464–1.468	0.855–0.860	**Angular Rotation**—between −20° and −50° (Appendix IIB)

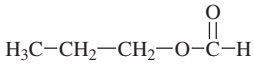
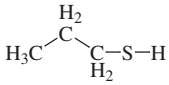
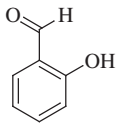
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
β-Pinene FEMA No. 2903 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane	136.24/$C_{10}H_{16}$/ 	colorless liq/ resinous-piney	*s*—most fixed oils; *ins*—water, prop glycol/ 165°	1 mL in 3 mL 95% ethanol
Piperidine FEMA No. 2908 Hexahydropyridine	85.15/$C_5H_{11}N$/ 	colorless to pale yel liq/ ammoniacal, fishy, nauseating	106°	
Piperonal FEMA No. 2911 3,4-(Methylenedioxy)-benzaldehyde; Heliotropine; Piperonyl Aldehyde	150.13/$C_8H_6O_3$/ 	white cryst substance/ floral, heliotrope, free from safrole by-odor	*vs*—alc; *s*—most fixed oils, prop glycol; *ins*—gly, water/ 264°	1 g in 4 mL 70% alc
Propenylguaethol FEMA No. 2922 1-Ethoxy-2-hydroxy-4-propenylbenzene	178.23/$C_{11}H_{14}O_2$/ 	white cryst powder/ vanilla	*s*—veg oils; *ins*—water, 1 g in 20 mL 95% alc	1 g in 15 mL 95% ethanol
Propionaldehyde FEMA No. 2923	58.08/C_3H_6O/ CH_3CH_2CHO	colorless, mobile liq/ sharp, pungent	*m*—alc, ether, water/ 49°	
Propyl Acetate FEMA No. 2925 *n*-Propyl Acetate	102.13/$C_5H_{10}O_2$/ $CH_3CH_2CH_2OOCCH_3$	colorless liq/ ethereal	102°	1 mL in 1 mL 95% alc
Propyl Alcohol FEMA No. 2928 *n*-Propanol	60.09/C_3H_8O/ $CH_3CH_2CH_2OH$	colorless liq/ ethereal	*s*—prop glycol, veg oils; *m*—water/ 97°	1 mL in 1 mL 95% alc
***p*-Propyl Anisole** FEMA No. 2930 Dihydroanethole	150.22/$C_{10}H_{14}O$/ 	colorless to pale yel liq/ anise, with sassafras background	*s*—most fixed oils; *ins*—gly, prop glycol/ 215°	1 mL in 5 mL 80% alc remains in soln on dilution

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
	97.0% of $C_{10}H_{16}$ (M-1a)		1.477–1.481	0.867–0.871	**Angular Rotation**—between −20° and −50° (Appendix IIB)
IR	98.0% of $C_5H_{11}N$ (M-1a)		1.450–1.454	0.858–0.862	
IR	99.0% of $C_8H_6O_3$ (M-1b)				**Lead**—10 mg/kg (M-9) **Solidification Pt.**—NLT 35° (Appendix IIB)
IR	99.0% of $C_{11}H_{14}O_2$ (M-1a)				**Melting Range**—between 85° and 88° (Appendix IIB) **Residue on Ignit.**—0.1% (Appendix IIC, 2-g sample)
IR	97.0% of C_3H_6O (M-2c)	5.0 (M-15)		0.800–0.805	**Distillation Range**—between 46° and 50° (first 97%, Appendix IIB) **Water**—2.5% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	97.0% of $C_5H_{10}O_2$ (M-1b)	1.0	1.382–1.387	0.880–0.886	
IR	99.0% of C_3H_8O (M-1b)		1.383–1.388	0.800–0.805	
IR	99.0% of $C_{10}H_{14}O$ (M-1a)		1.502–1.506	0.940–0.943	

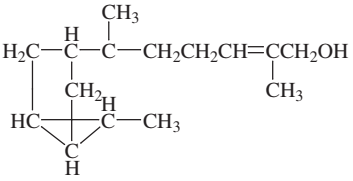
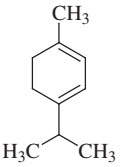
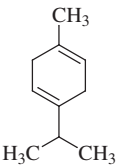
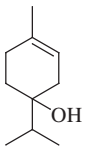
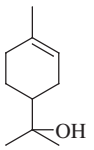
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Propyl Formate FEMA No. 2943	88.11/$C_4H_8O_2$/ 	colorless to pale yel liq/ ethereal	*s*—prop glycol, veg oils; *ins*—water/ 80°	1 mL in 1 mL 95% ethanol
Propyl Mercaptan FEMA No. 3521	76.16/C_3H_8S/ 	colorless to pale yel liq/ onion	*s*—prop glycol, veg oils; *ins*—water/ 67°	1 mL in 1 mL 95% ethanol
Propyl Propionate FEMA No. 2958 *n*-Propyl Propionate	116.16/$C_6H_{12}O_2$/ $CH_3CH_2CH_2OOCCH_2CH_3$	colorless to pale yel liq/ fruity	123°	1 mL in 1 mL 95% alc
Pyrrole FEMA No. 3386	67.09/C_4H_5N/ 	colorless to yel liq, darkens on aging/ nutty, sweet, warm, ethereal	*s*—alc, most fixed oils; *ss*—water/ 130° (decomp)	
Rhodinol FEMA No. 2980	[see *Citronellol*, *Geraniol*, and *Nerol*]	colorless liq/ pronounced rose	*s*—most fixed oils, prop glycol; *ins*—gly/ 68°–70° (1.8 mm Hg)	1 mL in 1.2 mL 70% alc
Rhodinyl Acetate FEMA No. 2981	[see *Citronellyl Acetate* and *Geranyl Acetate*]	colorless to slightly yel liq/ light, fresh, rose	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 237°	1 mL in 2 mL 80% alc remains in soln to 10 mL
Rhodinyl Formate FEMA No. 2984	[see *Citronellyl Formate*]	colorless to slightly yel liq/ leafy, rose	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/ 220°	1 mL in 2 mL 80% alc gives clear soln
Salicylaldehyde FEMA No. 3004	122.12/$C_7H_6O_2$/ 	colorless to yel liq/ phenolic	*s*—prop glycol, veg oils; *ins*—water/ 197°	1 mL in 1 mL 95% ethanol

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_4H_8O_2$ (M-1b)	5.0 (add ice to soln)	1.374–1.380	0.898–0.904	
IR	97.0% of C_3H_8S (M-1b)		1.436–1.442	0.838–0.844	
IR	98.0% of $C_6H_{12}O_2$ (M-1b)	1.0	1.391–1.396	0.873–0.879	
IR	98.0% of C_4H_5N (M-1a)		1.507–1.510	0.950–0.980 (20°)	**Distillation Range**—between 125° and 130° (Appendix IIB) **Water**—0.5% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	82.0% of total alcohols as $C_{10}H_{20}O$ (Appendix VI; 1.2 g/ 78.14)		1.463–1.473	0.860–0.880	**Angular Rotation**—between −4° and −9° (Appendix IIB, 100-mm tube) **Esters**—1.0% as citronellyl acetate (Appendix VI; 5 g/99.15)
IR	87.0% of total esters as $C_{12}H_{22}O_2$ (Appendix VI; 1.3 g/ 99.15)	1.0	1.450–1.458	0.895–0.908	**Angular Rotation**—between −2° and −6° (Appendix IIB, 100-mm tube)
IR	85.0% of total esters as $C_{11}H_{20}O_2$ (Appendix VI; 1.3 g/ 92.14)	2.0	1.453–1.458	0.901–0.908	
IR	97.0% of $C_7H_6O_2$ (M-1b)	10.0 (use phenol red indicator)	1.570–1.576	1.159–1.170	

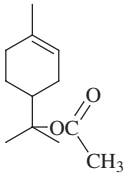
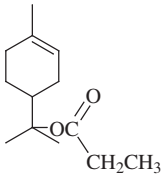
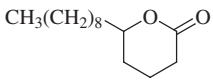
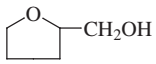
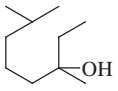
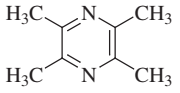
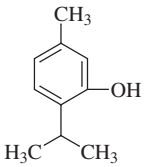
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Santalol FEMA No. 3006 [Mixture of α- and β-isomers]	220.35/$C_{15}H_{24}O$/ 	colorless to slightly yel, viscous liq/ sandalwood	*vs*—alc, most fixed oils, prop glycol; *ins*—gly, water/ 302°	1 mL in 4 mL 70% alc gives clear soln
Santalyl Acetate FEMA No. 3007	[mixture of α- and β- isomers from acetylation of *Santalol*]	colorless to slightly yel liq/ sandalwood	*s*—alc; *ins*—water/ 315°	1 mL in 9 mL 80% alc gives clear soln
α-Terpinene FEMA No. 3558 1-Methyl-4-(1-methylethyl)-1,3-cyclohexadiene	136.24/$C_{10}H_{16}$/ 	colorless liq/ lemon	*s*—alc, most fixed oils; *ins*—water/ 173°	1 mL in 2 mL 95% ethanol
γ-Terpinene FEMA No. 3559 1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	136.24/$C_{10}H_{16}$/ 	colorless liq/ herbaceous, citrus	*s*—alc, most fixed oils; *ins*—water/ 182°	1 mL in 3 mL 95% ethanol
Terpinen-4-ol FEMA No. 2248 4-Carvomenthenol	154.25/$C_{10}H_{18}O$/ 	colorless to pale yel liq/ piney	*s*—alc/ 88° (6 mm Hg)	1 mL in 1 mL 95% ethanol
α-Terpineol FEMA No. 3045 *p*-Menth-1-en-8-ol	154.25/$C_{10}H_{18}O$/ 	colorless, viscous liq (high-purity material may solidify)/ lilac	*s*—prop glycol, veg oils; *ss*—gly, water/ 217°	1 mL in 2 mL 70% alc, 4 mL 60% alc, 8 mL 50% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of total alcohols as $C_{15}H_{24}O$ (Appendix VI; 1.6 g/110.18)		1.505–1.509	0.965–0.975	**Angular Rotation**—between −11° and −19° (Appendix IIB, 100-mm tube)
IR	95.0% of total esters as $C_{17}H_{26}O_2$ (Appendix VI; 1.6 g/131.2)	1.0	1.488–1.491	0.980–0.986	
	89.0% of $C_{10}H_{16}$ (M-1a)		1.475–1.480	0.833–0.838	
	95.0% of $C_{10}H_{16}$ (M-1a)		1.473–1.477	0.841–0.845	
	92.0% of $C_{10}H_{18}O$ (M-1b)		1.476–1.480	0.928–0.934	
IR	96.0% of $C_{10}H_{18}O$ [sum of α-, (*E*)-β-, (*Z*)-β-, γ-, terpinen-4-ol, and terpinen-1-ol isomers] (M-1a)		1.482–1.485	0.930–0.936	

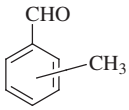
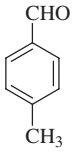
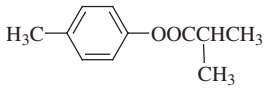
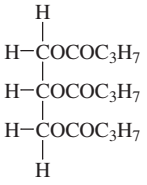
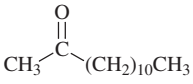
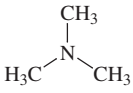
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Terpinyl Acetate FEMA No. 3047 Menthen-1-yl-8 Acetate	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/ sweet, refreshing, herbaceous	*s*—alc, most fixed oils, min oil, prop glycol; *ss*—gly; *ins*—water/ 220°	1 mL in 5 mL 70% alc remains in soln to 10 mL
Terpinyl Propionate FEMA No. 3053 Menthen-1-yl-8 Propionate	210.32/$C_{13}H_{22}O_2$/ 	colorless to slightly yel liq/ sweet, floral, herbaceous, lavender	*s*—gly; *ss*—prop glycol; *m*—alc, chloroform, ether, most fixed oils; *ins*—water/ 240°	1 mL in 2 mL 80% alc gives clear soln
δ-Tetradecalactone FEMA No. 3590	226.36/$C_{14}H_{26}O_2$/ 	colorless to pale yel liq/ fruity	*s*—prop glycol, veg oils; *ins*—water/ 130° (5 mm Hg)	1 mL in 1 mL 95% ethanol
Tetrahydrofurfuryl Alcohol FEMA No. 3056	102.13/$C_5H_{10}O_2$/ 	colorless liq/ mild, warm, oily, caramel	178°	
Tetrahydrolinalool FEMA No. 3060 3,7-Dimethyl-3-octanol	158.28/$C_{10}H_{22}O$/ 	colorless liq/ distinct floral	*s*—alc, most fixed oils; *ins*—water/ 71° (6 mm Hg)	
2,3,5,6-Tetramethylpyrazine FEMA No. 3237	136.20/$C_8H_{12}N_2$/ 	white cryst or powder/ fermented soybeans	*s*—alc, prop glycol, most fixed oils; *ss*—water/ 190°	
Thymol FEMA No. 3066	150.22/$C_{10}H_{14}O$/ 	white cryst/ phenol	*s*—water, prop glycol, veg oils/ 232°	1 g in 1 mL 95% alc

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_{12}H_{20}O_2$ (sum of α-, (*E*)-, β-, (*Z*)-β-, γ-, terpinen-4-ol, and terpinen-1-ol isomers) (M-1b)		1.464–1.467	0.953–0.962	
IR	95.0% of $C_{13}H_{22}O_2$ (sum of α-, (*E*)-, β-, (*Z*)-β-, γ-, terpinen-4-ol, and terpinen-1-ol isomers) (M-1b)	1.0	1.462–1.468	0.947–0.952	
IR	98.0% of $C_{14}H_{26}O_2$ (M-1b)	5.0	1.459–1.465	0.931–0.937	
IR	99.0% of $C_5H_{10}O_2$ (M-1a)		1.452–1.453	1.050–1.052	
	95.0% of $C_{10}H_{22}O$ (M-1a)		1.431–1.435	0.823–0.829	
IR	95.0% of $C_8H_{12}N_2$ (M-1a)				**Melting Range**—between 85° and 90° (Appendix IIB) **Water**—0.2% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	99.0% of $C_{10}H_{14}O$ (M-1a)				**Melting Range**—between 49° and 51° (Appendix IIB)

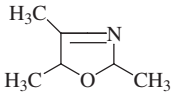
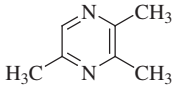
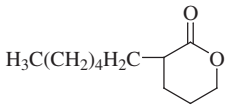
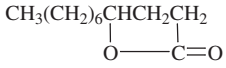
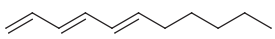
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Tolualdehyde, Mixed Isomers FEMA No. 3068 Tolyl Aldehyde, mixed isomers; Methylbenzaldehyde	120.15/C_8H_8O/ 	colorless liq/ cherry	198°	1 mL in 1 mL 95% alc
***p*-Tolualdehyde** FEMA No. 3068 *p*-Tolyl Aldehyde; *p*-Methylbenzaldehyde	120.15/C_8H_8O/ 	colorless liq/ cherry	83°–85° (11 mm Hg)	1 mL in 1 mL 95% alc
***p*-Tolyl Isobutyrate** FEMA No. 3075 *p*-Cresyl Isobutyrate	178.23/$C_{11}H_{14}O_2$/ 	colorless liq/ fruity	*s*—alc; *ins*—water/ 237°	1 mL in 7 mL 70% alc gives clear soln
Tributyrin FEMA No. 2223 Glyceryl Tributyrate; Butyrin	302.37/$C_{15}H_{26}O_6$/ 	colorless, somewhat oily liq/ almost odorless, slightly fatty	*s*—alc, chloroform, ether; *ins*—water/ 308°	
2-Tridecanone FEMA No. 3388	198.35/$C_{13}H_{26}O$/ 	white to pale yel solid/ herbal	*s*—prop glycol, veg oils; *ins*—water/ 134° (10 mm Hg)	1 g in 1 mL 95% ethanol
2-Tridecenal FEMA No. 3082	196.33/$C_{13}H_{24}O$/ $CH_3(CH_2)_9CH{=}CHCHO$	white or slightly yellow liq/ oily, citrus	*s*—alc, most fixed oils; *ins*—water	1 mL in 1 mL 95% ethanol
Trimethylamine FEMA No. 3241	59.11/C_3H_9N/ 	gas/ pungent, fishy, ammoniacal	2.9°	
3,5,5-Trimethyl Hexanal FEMA No. 3524	142.24/$C_9H_{18}O$/ $(CH_3)_3CCH_2CH(CH_3)CH_2CHO$	colorless to pale yel liq/ melon, green	67° (2.5 mm Hg)	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	94.0% of C_8H_8O (sum of three isomers) (M-1b)	5.0	1.540–1.548	1.019–1.029	
	97.0% of C_8H_8O (M-1b)	5.0	1.542–1.548	1.012–1.018	
IR	95.0% of $C_{11}H_{14}O_2$ (M-1b)	1.0	1.485–1.489	0.990–0.996	
IR	99.0% of $C_{15}H_{26}O_6$ (M-1b)	5.0	1.431–1.441	1.034–1.037	
IR	95.0% of $C_{13}H_{26}O$ (M-1b)				**Melting Point**—NLT 27.0° (Appendix IIB)
IR	92.0% of $C_{13}H_{24}O$ (M-1a)		1.455–1.460	0.842–0.862	
	98.0% of C_3H_9N in a suitable solvent (M-1a)				
	97.0% (M-1b)	5.0	1.419–1.424	0.817–0.823	

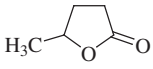
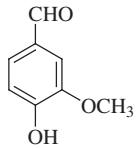
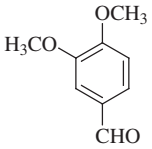
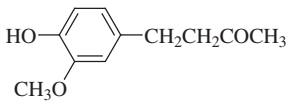
General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
2,4,5-Trimethyl δ-3-Oxazoline FEMA No. 3525	113.16/$C_6H_{11}NO$/ 	yel orange liq/ powerful, musty, slight green, wood, nut	*s*—alc, prop glycol, water; *ins*—most fixed oils	
2,3,5-Trimethylpyrazine FEMA No. 3244	122.17/$C_7H_{10}N_2$/ 	colorless to slightly yel liq/ baked potato, peanut	*s*—org solvents, water/ 171°	1 mL in 1 mL 95% ethanol
δ-Undecalactone FEMA No. 3294 5-Hydroxyundecanoic Acid Lactone	184.28/$C_{11}H_{20}O_2$/ 	colorless to pale yel liq/ creamy, peach	152°–155° (10.5 mm Hg)	1 mL in 1 mL 95% alc
γ-Undecalactone FEMA No. 3091 Aldehyde C-14 Pure, So-Called; Peach Aldehyde	184.28/$C_{11}H_{20}O_2$/ 	colorless to slightly yel liq/ fruity, peach	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/ 297°	1 mL in 5 mL 60% alc
Undecanal FEMA No. 3092 Aldehyde C-11 Undecyclic; *n*-Undecyl Aldehyde	170.30/$C_{11}H_{22}O$/ $CH_3(CH_2)_9CHO$	colorless to slightly yel liq/ sweet, fatty, floral	*s*—most fixed oils, prop glycol; *ins*—gly, water/ 223°	
2-Undecanone FEMA No. 3093 Methyl Nonyl Ketone	170.30/$C_{11}H_{22}O$/ $CH_3CO(CH_2)_9CH_3$	colorless to pale yel liq/ citrus, fatty, rue	231°–232°	1 mL in 1 mL 95% alc
1,3,5-Undecatriene FEMA No. 3795	150.26/$C_{11}H_{18}$/ 	clear, colorless to pale yel liq/ oily, waxy, peppery	88° 1 Torr	1 mL in 25 mL 95% alc
10-Undecenal FEMA No. 3095 Aldehyde C-11 Undecylenic; Undecen-10-al	168.28/$C_{11}H_{20}O$/ $H_2C{=}CH(CH_2)_8CHO$	colorless to light yel liq/ fatty; rose on dilution	*s*—most fixed oils, prop glycol; *ins*—gly, water/ 235°	
(*E*)-2-Undecenol	170.30/$C_{11}H_{22}O$/ $CH_3(CH_2)_7CH{=}CHCH_2OH$	white to slightly yel liq/ oily, sweet, floral	*ins*—water	1 mL in 1 mL 95% ethanol
Undecyl Alcohol FEMA No. 3097 Alcohol C-11	172.31/$C_{11}H_{24}O$/ $CH_3(CH_2)_9CH_2OH$	colorless liq/ fatty-floral	*s*—most fixed oils; *ins*—water/ 146° (30 mm Hg)	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	94.0% of $C_6H_{11}NO$ (M-1a)		1.414–1.435	0.911–0.932	
IR	98.0% of $C_7H_{10}N_2$ (M-1a)		1.503–1.507	0.970–0.980	**Water**—0.2% (Appendix IIB, KF; use freshly dist. pyridine as solvent)
IR	98.0% of $C_{11}H_{20}O_2$ (sum of two isomers; δ isomer 96.0% min) (M-1a)		1.457–1.461	0.956–0.961	
IR	98.0% of $C_{11}H_{20}O_2$ (sum of two isomers; γ isomer 96.0% min) (M-1b)	5.0	1.448–1.453	0.942–0.945	
IR	92.0% of $C_{11}H_{22}O$ (M-1b)	10.0	1.430–1.435	0.825–0.832	
IR	96.0% of $C_{11}H_{22}O$ (M-1a)	5.0	1.428–1.432	0.822–0.826	
IR	90% of $C_{11}H_{18}$ (sum of isomers 90% min) (M-1b)		1.508–1.517	0.787–0.793	
IR	90.0% of $C_{11}H_{20}O$ (M-1b)	6.0	1.441–1.447	0.840–0.850	
IR	92.0% of $C_{11}H_{22}O$ (M-1a)		1.448–1.453	0.840–0.846	
IR	97.0% of $C_{11}H_{24}O$ (M-1a)		1.437–1.443	0.820–0.840	

General Information and Description

Name of Substance/ Synonyms	Formula Wt/Formula/ Structure	Physical Form/ Odor[1]	Solubility[2]/ B.P.[3]	Solubility in Alcohol[4]
Valeraldehyde FEMA No. 3098	86.13/$C_5H_{10}O$/ $CH_3CH_2CH_2CH_2CHO$	colorless to pale yel liq/ chocolate	103°	1 mL in 1 mL 95% alc
Valeric Acid FEMA No. 3101 Pentanoic Acid	102.13/$C_5H_{10}O_2$/ $CH_3(CH_2)_3COOH$	colorless to pale yel, mobile liq/ unpleasant, penetrating, rancid	*m*—alc, ether, 1 mL in 40 mL water/ 186°	
γ-Valerolactone FEMA No. 3103	100.12/$C_5H_8O_2$/ 	colorless to slightly yel liq/ warm, sweet, herbaceous	*m*—alc, most fixed oils, water/ 207°	
Vanillin FEMA No. 3107 4-Hydroxy-3-methoxybenzaldehyde	152.15/$C_8H_8O_3$/ 	fine, white to slightly yel cryst, usually needles/ odor and taste of vanilla	*s*—alc, chloroform, ether, 1 g in 100 mL water at 25°, in 20 mL gly, in 20 mL water at 80°	
Veratraldehyde FEMA No. 3109 Methyl Vanillin; Veratryl Aldehyde; 3,4-Dimethoxybenzaldehyde	166.18/$C_9H_{10}O_3$/ 	white to tan or blue-gray flakes or solid/ sweet, vanilla	281°	1 g in 1 mL 95% alc
Zingerone FEMA No. 3124	194.23/$C_{11}H_{14}O_3$/ 	yel to yel-brown liq (may solidify at room temp)/ spicy	290°	

Requirements

I.D. Test[5]	Assay Min. %[6]	A.V. Max.[7]	Ref. Index[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_5H_{10}O$ (M-2a)	5.0	1.390–1.395	0.805–0.809	
IR	99.0% of $C_5H_{10}O_2$ (M-3b)		1.405–1.412	0.935–0.940	
IR	95.0% of $C_5H_8O_2$ (M-1b)		1.431–1.434	1.047–1.054	
IR	97.0% of $C_8H_8O_3$ (on dried basis) (M-1b)				**Loss on Drying**—0.5% (Appendix IIC, silica gel/4 h) **Melting Range**—between 81° and 83° (Appendix IIB) **Residue on Ignit.**—0.05% (Appendix IIC, 2-g sample)
	95.0% of $C_9H_{10}O_3$ (M1-b)				**Solidification Pt.**—NLT 40° (Appendix IIB)
IR	95.0% of $C_{11}H_{14}O_3$ (M-1b)		1.538–1.545	1.136–1.140	

TEST METHODS FOR FLAVOR CHEMICALS

This section provides the test methods by which certain flavor chemicals listed in the preceding tabular section are to be analyzed.

M-1 ASSAY BY GAS CHROMATOGRAPHY

M-1a General Method, Polar Column

Proceed as directed under *GC Assay of Flavor Chemicals*. The composition of the polar column and the conditions of analysis may be varied at the discretion of the analyst, provided that such changes would result in equal or improved separations and/or quantification as would be obtained by use of the particular column material and test conditions specified therein.

M-1b General Method, Nonpolar Column

Proceed as directed under *GC Assay of Flavor Chemicals*. The composition of the nonpolar column and the conditions of analysis may be varied at the discretion of the analyst, provided that such changes would result in equal or improved separations and/or quantification as would be obtained by use of the particular column material and test conditions specified therein.

M-2 ASSAYS FOR CERTAIN ALDEHYDES AND KETONES

M-2a Aldehydes—Hydroxylamine *tert*-Butyl Alcohol Method

Hydroxylamine Solution Dissolve 45 g of reagent-grade hydroxylamine hydrochloride in 130 mL of water, add 850 mL of *tert*-butyl alcohol, mix, and using a pH meter, neutralize to a pH of 3.0 to 3.5 with sodium hydroxide.

Caution: Do not heat the solution.

Procedure Transfer an accurately weighed quantity of sample, as specified below, into a 250-mL glass-stoppered flask. Add 50 mL of the *Hydroxylamine Solution*, mix thoroughly, and allow to stand at room temperature for the time specified. Titrate with 0.5 *N* sodium hydroxide to the same pH as that of the *Hydroxylamine Solution* used. Calculate the percent aldehyde or ketone by the equation

$$AK = (S)(100e)/W,$$

in which AK is the percent aldehyde or ketone; S is the number of milliliters of 0.5 *N* sodium hydroxide consumed in the titration of the sample; e is the equivalence factor given below; and W is the weight, in milligrams, of the sample taken.

Substance	Sample Weight (g)	Reaction Time (min)	1 mL of 0.5 *N* NaOH Equivalent to
Cuminic Aldehyde	1	60	74.11 mg of $C_{10}H_{12}O$
Myristaldehyde	1.5	60	106.18 mg of $C_{14}H_{28}O$
Valeraldehyde	1	60	43.07 mg of $C_5H_{10}O$

M-2b Procedure Requiring the Use of Sealed Glass Vials or Ampules

Transfer 65 mL of 0.5 *N* hydroxylamine hydrochloride and 50.0 mL of 0.5 *N* triethanolamine into a suitable heat-resistant pressure bottle provided with a tight closure that can be fastened securely. Replace the air in the bottle by passing a gentle stream of nitrogen for 2 min through a glass tube positioned so that the end is just above the surface of the liquid. Add the quantity of sample specified below, contained in a sealed glass ampule, to the mixture in the pressure bottle. Introduce several pieces of 8-mm glass rod, cap the bottle, and shake vigorously to break the ampule. Allow the bottle to stand at room temperature for the time specified, swirling occasionally. Cool, if necessary, and uncap the bottle cautiously to prevent any loss of the contents. Titrate with 0.5 *N* sulfuric acid to pH 3.4, using a suitable pH meter. Perform a residual blank titration (see *General Provisions*). Each milliliter of 0.5 *N* sulfuric acid is equivalent to the amount specified below.

Substance	Sample Weight (mg)	Reaction Time (min)	1 mL of 0.5 *N* H_2SO_4 Equivalent to
Acetaldehyde	600	30	22.03 mg of C_2H_4O

M-2c Aldehydes—Hydroxylamine Method

Hydroxylamine Hydrochloride Solution Dissolve 50 g of hydroxylamine hydrochloride (preferably reagent grade or freshly recrystallized before using) in 90 mL of water, and dilute to 1000 mL with aldehyde-free alcohol. Adjust the solution to a pH of 3.4 with 0.5 *N* alcoholic potassium hydroxide.

Procedure Transfer an accurately weighed quantity of sample, as specified in the table below, into a 125-mL Erlenmeyer flask. Add 30 mL of *Hydroxylamine Hydrochloride Solution*, mix thoroughly, and allow to stand at room temperature for the time specified below. Titrate with 0.5 *N* alcoholic potassium

hydroxide to a green-yellow endpoint that matches the color of 30 mL of *Hydroxylamine Hydrochloride Solution* in a 125-mL flask when the same volume of bromophenol blue TS has been added to each flask, or preferably, using a suitable pH meter, titrate to a pH of 3.4. Calculate the percent aldehyde (A) by the equation

$$A = (S - b)(100e)/W,$$

in which S is the number of milliliters of 0.5 N alcoholic potassium hydroxide consumed in the titration of the sample; b is the number of milliliters of 0.5 N alcoholic potassium hydroxide consumed in the titration of the blank; e is the equivalence factor given below; and W is the weight, in milligrams, of the sample taken.

Substance	Sample Weight (mg)	Reaction Time (min)	1 mL of 0.5 N KOH Equivalent to
Butyraldehyde	900	60	36.06 mg of C_4H_8O
Isobutyraldehyde	900	60	36.06 mg of C_4H_8O
Propionaldehyde	750	30	29.04 mg of C_3H_6O

M-2d Ketones—Hydroxylamine Method

Hydroxylamine Solution Dissolve 20 g of hydroxylamine hydrochloride (reagent grade or, preferably, freshly crystallized) in 40 mL of water, and dilute to 400 mL with alcohol. While stirring, add 300 mL of 0.5 N alcoholic potassium hydroxide, and filter. Use this solution within 2 days.

Procedure Transfer an accurately weighed quantity of sample, as specified below, into a 250-mL glass-stoppered flask. Add 75.0 mL of *Hydroxylamine Solution* to this flask and to a similar flask for a residual blank titration (see *General Provisions*). Attach the flask to a suitable condenser, reflux the mixture for the time specified, and then cool to room temperature. Titrate both flasks with 0.5 N hydrochloric acid to the same green-yellow endpoint using bromophenol blue TS as the indicator or, preferably, using a pH meter, to a pH of 3.4. (If the indicator is used, the endpoint color must be the same as that produced when the blank is titrated to a pH of 3.4.) Calculate the percent ketone by the equation

$$K = (b - S)(100e)/W,$$

in which K is the percent ketone; b is the number of milliliters of 0.5 N hydrochloric acid consumed in the residual blank titration; S is the number of milliliters of 0.5 N hydrochloric acid consumed in the titration of the sample; e is the equivalence factor given below; and W is the weight, in milligrams, of the sample taken.

Substance	Sample Weight (mg)	Reaction Time (min)	1 mL of 0.5 N HCl Equivalent to
4-Methyl-2-pentanone	1200	60	50.08 mg of $C_6H_{12}O$

M-3 ASSAY BY TITRIMETRIC PROCEDURES

M-3a Direct Aqueous Acid Base Titrations

Transfer an accurately weighed amount of sample, as specified below, into a 250-mL Erlenmeyer flask containing 75 to 100 mL of water, add phenolphthalein TS, and titrate with 0.5 N sodium hydroxide to the first pink color that persists for 15 s. Each milliliter of 0.5 N sodium hydroxide is equivalent to the amount of substance as specified below.

Substance	Sample Weight (g)	1 mL of 0.5 N NaOH Equivalent to
Butyric Acid	1.5	44.06 mg of $C_4H_8O_2$
Hexanoic Acid	2.0	58.08 mg of $C_6H_{12}O_2$
Isobutyric Acid	1.5	44.06 mg of $C_4H_8O_2$
Isovaleric Acid	1.5	51.07 mg of $C_5H_{10}O_2$
Levulinic Acid	1.0	58.08 mg of $C_5H_8O_3$
2-Mercaptopropionic Acid	1.0	53.08 mg of $C_3H_6O_2S$
2-Methyl-2-pentenoic Acid	2.0	57.02 mg of $C_6H_{10}O_2$
2-Methylbutyric Acid	1.0	51.06 mg of $C_5H_{10}O_2$
4-Methylpentanoic Acid	2.0	58.05 mg of $C_6H_{12}O_2$
2-Methylpentanoic Acid	2.0	58.05 mg of $C_6H_{12}O_2$
Nonanoic Acid	1.0	79.12 mg of $C_9H_{18}O_2$

M-3b Direct Aqueous Alcoholic Acid Base Titrations

Dissolve 1 g of sample, accurately weighed, in 50% ethanol/water that previously has been neutralized to phenolphthalein TS with 0.1 N sodium hydroxide. Titrate with 0.5 N sodium hydroxide to a pink color. Each milliliter of titrant is equivalent to the amount of substance specified below.

Conditions for Direct Aqueous Alcoholic Acid Base Titrations

Substance	1 mL of 0.5 N NaOH Equivalent to
Cinnamic Acid (dried in desiccator 3 h over silica gel)	74.08 mg of $C_9H_8O_2$
2-Ethylbutyric Acid	58.08 mg of $C_6H_{12}O_2$
Phenylacetic Acid (dried 3 h over H_2SO_4)	68.08 mg of $C_8H_8O_2$
Valeric Acid	51.07 mg of $C_5H_{10}O_2$

M-4 ALCOHOL CONTENT OF ETHYL OXYHYDRATE

Mix 25.0 mL of sample with an equal volume of water in a separator, saturate with sodium chloride, and extract with three 25-mL portions of solvent hexane. Extract the combined solvent hexane extracts with three 10-mL portions of a saturated solution of sodium chloride, and then discard the solvent

hexane solutions. Combine the saline solutions in a suitable distillation flask, and distill, collecting 25 mL of distillate. The specific gravity of the distillate is not greater than 0.9814, indicating an alcohol content of not less than 14.0% by volume.

M-5 ACIDITY DETERMINATION BY IODOMETRIC METHOD

Ethyl Formate (Acidity as Formic Acid) Transfer about 5 g of sample, accurately weighed, into a glass-stoppered flask containing a solution of 500 mg of potassium iodate and 2 g of potassium iodide in 50 mL of water. Titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 4.603 mg of CH_2O_2.

M-6 LIMIT TEST FOR ANTIOXIDANTS IN ETHYL ACRYLATE

Preliminary Examination of the Sample Wash a 25-mL portion of the sample with 25 mL of a 1:10 solution of sodium hydroxide. Any yellow or brown coloration in the extract indicates the presence of hydroquinone, in which case both of the procedures below (*A* and *B*) must be followed to determine the antioxidant content. If the sodium hydroxide extract remains colorless, the first procedure (*A*) need not be run, and the antioxidant content is determined by the second procedure (*B*) alone.

A. Determination of Hydroquinone

Carbonyl-Free Methanol Add 5 g of 2,4-dinitrophenylhydrazine to 500 mL of anhydrous methanol, heat the mixture under a reflux condenser for 2 h, and then recover the methanol by distillation. Store the carbonyl-free methanol in tight containers.

2,4-Dinitrophenylhydrazine Solution Dissolve 100 mg of 2,4-dinitrophenylhydrazine in 50 mL of *Carbonyl-Free Methanol*, add 4 mL of hydrochloric acid, and dilute to 100 mL with water.

Sodium Carbonate Solution Dissolve 530 mg of sodium carbonate in sufficient water to make 100 mL.

Pyridine–Diethanolamine Solution Mix 5 mL of diethanolamine with 500 mL of freshly distilled pyridine.

Calibration Curve Transfer 25 mg of hydroquinone, accurately weighed, into a 100-mL volumetric flask, add sufficient butyl acetate to volume, and mix thoroughly (250 μg/mL). Prepare a series of standards by transferring 1.0-, 2.0-, 3.0-, 4.0-, and 6.0-mL portions of this solution into separate 50-mL volumetric flasks, and diluting each aliquot to 50.0 mL with butyl acetate. One milliliter of each of these standards contains 5, 10, 15, 20, and 30 μg, respectively, of hydroquinone. Transfer 1.0 mL of each solution into separate 25-mL glass-stoppered graduates, and continue as directed in the *Procedure*, beginning with ''. . . add 2.0 mL of water. . . .'' Plot a calibration curve of absorbance versus micrograms of hydroquinone. Fifteen micrograms of hydroquinone should be equivalent to approximately 0.30 units of absorbance, and the curve should intersect the origin.

Procedure Using a hypodermic syringe, transfer 0.2 mL of sample, accurately weighed, into a 25-mL glass-stoppered graduate, add 2.0 mL of water, stopper the graduate, and mix the contents well without allowing contact between the liquid and the stopper. Add 0.5 mL of *Sodium Carbonate Solution* to the mixture, and immediately shake gently for 5 s, avoiding contact between the solution and the stopper. Immediately add 1.0 mL of a 15% (v/v) solution of sulfuric acid, shake as previously directed, and add 1 mL of *2,4-Dinitrophenylhydrazine Solution*. Stopper the graduate and place it in a water bath, maintained at a temperature between 70° and 72°, for 1 h. Shake samples three times during the heating period. Cool the graduate to room temperature, dilute the contents to 15 mL with water, add 5.8 mL of benzene, stopper, shake vigorously, and then allow the phases to separate. Using a suitable pipet, transfer 2.0 mL of the benzene layer into a test tube, add 10.0 mL of *Pyridine–Diethanolamine Solution*, and mix. Transfer a portion of this solution into a 2-cm cell, and determine the absorbance at 620 nm with a suitable spectrophotometer, using as a blank 1.0 mL of butyl acetate treated in the same manner as the sample except that 5.0 mL of benzene is used for the extraction instead of 5.8 mL. From the previously prepared *Calibration Curve*, read the micrograms of hydroquinone and/or benzoquinone corresponding to the absorbance of the solution from the sample, and record this value as *w*. Calculate the milligrams per kilogram of hydroquinone (mg/kg HQ) in the sample by the formula

$$1000w/W,$$

in which *W* is the weight, in milligrams, of the sample taken.

B. Determination of Hydroquinone Monomethyl Ether

Antioxidant-Free Ethyl Acrylate Wash a suitable volume of the sample with three separate, similar-sized volumes of a 1:10 sodium hydroxide solution. After the last washing, add a small amount of sodium chloride, if necessary, to remove any turbidity that may be present.

Calibration Curve Transfer 25.0 mg of hydroquinone monomethyl ether, accurately weighed, into a 100-mL volumetric flask, add *Antioxidant-Free Ethyl Acrylate* to volume, and shake to effect complete solution (250 μg/mL). Prepare a series of standards by transferring 1.0-, 5.0-, 10.0-, and 20.0-mL portions of this solution into separate 25-mL volumetric flasks, diluting each to volume with *Antioxidant-Free Ethyl Acrylate*, and mixing. One milliliter of each of the standards contains 10, 50, 100, and 200 μg, respectively, of hydroquinone monomethyl ether. Transfer 5.0 mL of each solution into separate 50-mL volumetric flasks, dilute each to volume with isooctane, and mix. Determine the absorbance of each

solution in a 1-cm silica cell at 292 nm with a suitable spectrophotometer, using a 1:10 dilution of *Antioxidant-Free Ethyl Acrylate* as the blank. Plot a calibration curve of absorbance versus micrograms of hydroquinone monomethyl ether. The curve should be linear and should intersect the origin.

Procedure Transfer 5.0 mL of sample, accurately weighed, into a 50-mL volumetric flask, dilute to volume with isooctane, and mix. Determine the absorbance of this solution in a 1-cm silica cell at 292 nm with a suitable spectrophotometer, using a 1:10 dilution of *Antioxidant-Free Ethyl Acrylate* in isooctane as the blank. From the previously prepared *Calibration Curve* read the micrograms of hydroquinone monomethyl ether corresponding to the absorbance of the sample solution, and record this value as *w*. Calculate the milligrams per kilogram of hydroquinone monomethyl ether (mg/kg HMME) in the sample by the formula

$$w/W,$$

in which *W* is the weight, in grams, of the sample taken.

Note: If the first sodium hydroxide extract obtained under *Preliminary Examination of the Sample* (or under *Antioxidant-Free Ethyl Acrylate*) showed a yellow coloration, the true mg/kg HMME is obtained by subtracting the mg/kg HQ, obtained under section *A*, from the apparent mg/kg HMME.

M-7 LIMIT TEST FOR HYDROCARBONS IN EUGENOL

Dissolve 1 mL of sample in 20 mL of 0.5 *N* sodium hydroxide contained in a stoppered 50-mL tube, add 18 mL of water, and mix. A clear mixture results immediately, but it may become turbid when exposed to air.

M-8 LIMIT TEST FOR HYDROCYANIC ACID IN BENZALDEHYDE

Shake 0.5 mL of sample with 5 mL of water, add 0.5 mL of 1 *N* sodium hydroxide and 0.1 mL of ferrous sulfate TS, and warm the mixture gently. Upon the addition of a slight excess of hydrochloric acid, no green-blue color or blue precipitate evolves within 15 min.

M-9 LIMIT TEST FOR LEAD

A *Sample Solution* containing a 1-g sample and prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 10 μg of lead (Pb) ion in the control.

M-10 LIMIT TEST FOR METHYL COMPOUNDS IN ETHYL ACETATE

Transfer 20 mL of sample into a 500-mL separator, add a solution of 20 g of sodium hydroxide in 50 mL of water, stopper the separator, and wrap it securely in a towel for protection against the heat of the reaction. Shake the mixture vigorously for about 5 min, cautiously opening the stopcock from time to time to permit the escape of air. Continue shaking the mixture vigorously until a homogeneous liquid results, then distill, and collect about 25 mL of the distillate. Add 1 drop of dilute phosphoric acid (1:20) and 1 drop of a 1:20 solution of potassium permanganate to 1 drop of the distillate. Mix, allow to stand for 1 min, and add, dropwise, a 1:20 solution of sodium bisulfite until the color disappears. If a brown color remains, add 1 drop of the dilute phosphoric acid. Add to the colorless solution 5 mL of a freshly prepared 1:2000 solution of chromotropic acid in 75% sulfuric acid, and heat on a steam bath for 10 min at 60°. No violet color appears.

M-11 LIMIT TEST FOR PEROXIDE VALUE

Add 10 mL of sample to 50 mL of a 3:2 (v/v) mixture of glacial acetic acid and chloroform. Add 1 mL of a saturated solution of potassium iodide to this solution, allow to stand for exactly 1 min with gentle shaking, and then introduce 100 mL of water and a few drops of starch TS. Titrate immediately with 0.1 *N* sodium thiosulfate. Each milliliter of 0.1 *N* sodium thiosulfate, multiplied by 5, equals the peroxide value, expressed in millimoles of peroxide per liter of the sample.

M-12 LIMIT TEST FOR READILY CARBONIZABLE SUBSTANCES IN ETHYL ACETATE

Carefully pour 2 mL of sample onto 10 mL of 95% sulfuric acid to form separate layers. No discoloration appears within 15 min.

M-13 LIMIT TEST FOR READILY OXIDIZABLE SUBSTANCES IN *dl*-MENTHOL

Transfer 500 mg of *dl*-menthol into a clean, dry test tube, and add 10 mL of potassium permanganate solution (prepared by diluting 3 mL of 0.1 *N* potassium permanganate to 100

mL with water). Place the test tube in a beaker of water maintained between 45° and 50°. At 30-s intervals, quickly remove the test tube from the bath and shake. The color of potassium permanganate is still apparent after 5 min.

M-14 LIMIT TEST FOR REDUCING SUBSTANCES

Dilute 2 mL of sample in a glass-stoppered flask with 50 mL of water and 5 mL of sulfuric acid, shaking the flask during the addition. While the solution is still warm, titrate with 0.1 *N* potassium permanganate. Not more than 1 mL is required to produce a pink color that persists for 30 min.

M-15 ACID VALUE

Dissolve about 10 g of sample, accurately weighed, in 50 mL of alcohol, previously neutralized to phenolphthalein with 0.1 *N* sodium hydroxide. (Add 50 g of ice when testing cinnamyl formate, citronellyl formate, geranyl formate, isoamyl formate, or linalyl formate.) Add 1 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 10 s, unless otherwise directed in the individual monograph. Calculate the acid value (*AV*) by the equation

$$AV = (5.61 \times S)/W,$$

in which *S* is the number of milliliters of 0.1 *N* sodium hydroxide consumed in the titration of the sample, and *W* is the weight, in grams, of the sample.

When phenol red TS is specified as the indicator in the individual monograph, proceed as directed above, and titrate with 0.1 *N* sodium hydroxide to the appearance of the first endpoint, a yellow-orange color.

M-16 RESIDUE ON EVAPORATION

Transfer the quantity of sample specified in the monograph, accurately weighed, into a suitable evaporating dish that has previously been heated on a steam bath, cooled to room temperature in a desiccator, and accurately weighed. Weigh the sample in the dish. Heat the evaporating dish containing the sample on the steam bath for 1 h. Cool the dish and its contents to room temperature in a desiccator, and accurately weigh. Calculate the residue as percent of the sample used.

M-17 QUALITATIVE TEST FOR PHENOLS USING FERRIC CHLORIDE

Allyl Isothiocyanate Dilute 1 mL of the sample with 5 mL of alcohol, and add 1 drop of ferric chloride TS. A blue color does not immediately appear.

Anethole Shake 1 mL of sample with 20 mL of water, and allow the liquids to separate. Filter the water layer through a filter paper previously moistened with water, and add 3 drops of ferric chloride TS to 10 mL of the filtrate. No purple color appears.

Anisole Shake 1 mL of sample with about 20 mL of water, allow the layers to separate, collect the water layer in a test tube, and add a few drops of ferric chloride TS. No green, blue, or purple color appears.

Cresyl Acetate (Test for Free Cresol)

Ferric Chloride Solution Add 1.5 g of anhydrous ferric chloride to 850 mL of chloroform contained in a 2-L beaker. Add 100 mL of ethylene glycol monobutyl ether. When the ferric chloride has dissolved, add 50 mL of pyridine, mix, and filter through a Büchner funnel.

Procedure Transfer 5 mL of sample into a 15-mm test tube, and add 10 mL of the *Ferric Chloride Solution*. The color of the solution is not a darker green than is a solution of 5 mL of a 1% solution of cresol in cresol-free methyl *p*-cresol mixed with 10 mL of the *Ferric Chloride Solution*.

GAS CHROMATOGRAPHIC (GC) ASSAY OF FLAVOR CHEMICALS

This procedure applies both to the assay of flavor chemicals and to the quantitation of minor components in flavor chemicals. Analysts following this procedure and performing the test should obtain sufficient resolution of major and even trace components of a mixture to calculate accurately the concentration of the desired component; should be familiar with the general principles, usual techniques, and instrumental variables normally met in gas chromatographic analysis; and should pay particular attention to the following:

1. Stability of baseline, return to baseline before and after each peak of interest, and minimum use of recorder attenuation.
2. Any incompatibility between a sensitive sample component and column support, liquid substrate, or construction material.
3. The response to different components of the same or different detectors. Because sizable errors may be encountered in correlating area percent directly to weight percent, analysts must know the methods for calculating response factors.
4. Where limits for minor components are specified in the column entitled *Other Requirements* in the above tabular specifications for flavor chemicals, analysts should use authentic materials to confirm the retention times of minor components. Determine the quantity of components following the instructions below under *Calculations and Methods*.

I GC CONDITIONS FOR ANALYSIS

Column: Open tubular capillary column of fused silica 30 m × 0.25 to 0.53 mm (id), or equivalent.

Stationary phase:

1. For a **nonpolar column** (or equivalent): methyl silicone gum, or equivalent (preferably a bonded and cross-linked dimethyl polysiloxane);
2. For a **polar column** (or equivalent): polyethylene glycol, or equivalent (preferably a bonded and cross-linked polyethylene glycol);
3. The stationary phase coating should have a thickness of 0.25 to 3 μm.

Carrier gas: Helium flowing at a linear velocity of 20 to 40 cm/s.

Sample size: 0.1 to 1.0 μL.

Split ratio: [for 0.25-mm to 0.35-mm (id) columns only] 50:1 to 200:1, typically, making sure that no one component exceeds the capacity of the column. Peak fronting is indicative of an overloaded column.

Inlet temperature: 225° to 275°.

Detector temperature: 250° to 300°.

Detectors: Use a thermal conductivity detector or a flame ionization detector or a mass spectrometer, operating all as recommended by the manufacturer.

Oven program: 50° to 240°, increasing the temperature by 5°/min; and holding at 240° for 5 min.

Analysts may also use any GC conditions providing separations equal to (or better than) those obtained with the above method, but in the case of a dispute, the above method must stand.

II CALCULATIONS AND METHODS

A. Peak area integration with total area detected normalized to 100%, using electronic integrators: Use an electronic peak integrator in accordance with the manufacturer's recommendations, ensuring that the integration parameters permit proper integration of the peaks of a variety of shapes and magnitudes and do not interpret baseline shifts and noise spikes as area contributed by the sample. Use internal or external standards as needed to confirm that the total GC peak area corresponds to 100% of the components present in the sample.

B. Results obtained as described above are based on the assumption that the entire sample has eluted and the peaks of all of the components have been included in the calculation. They will be incorrect if any part of the sample does not elute or if not all of the peaks are measured. In such cases, and in all methods described above, the internal standard method may be used to determine percentages based on the total sample. For this method, measurements are required of the peaks of the component(s) being assayed and of the internal standard.

An accurately weighed or pipetted mixture of the internal standard and the sample is prepared and chromatographed, the area ratio(s) of the component(s) to the standard is computed, and the percentage(s) of the component(s) is calculated.

If this calculation is to be applied, the substance used as the standard should be one that meets the following criteria:

a. Its detector response is similar to that of the component(s) to be determined. In general, the more nearly the chemical structure of the component resembles that of the standard, the closer the response will be.

b. Its retention time is close to, but not well resolved from, that of the component(s).

c. The internal standard is never a natural component found in the sample.

The weight ratio of the internal standard to the sample should be such that the internal standard and the component sought produce approximately equal peaks. This is, of course, not possible if several components of interest are at different levels of concentration.

If the internal standard method is applied properly, it may be assumed that the ratio of the weight of component to the weight of internal standard is exactly proportional to the peak area ratio, and under these conditions no correction factor is needed. The sample is first run by itself to determine whether the internal standard would mask any component by peak superposition. If there is no interference, a mixture is prepared of the sample and of the internal standard in the specified weight ratio, and the percentages of the internal standard and of the sample in the mixture are calculated. The mixture is chromatographed, and the areas of the component peak and the internal standard peak are calculated by one of the methods described above.

The calculations are as follows:

1a. % Component in Mixture / % Internal Standard in Mixture = Component Area / Internal Standard Area

or

1b. % Component in Mixture = % Internal Standard in Mixture × (Component Area / Internal Standard Area)

2. % Component in Sample = (% Component in Mixture × 100) / % Sample in Mixture

If calibration is necessary, mixtures should be prepared of the internal standard and component, either of 100% or of known purity. The number of mixtures and the weight ratios to be used depend on the component being analyzed. Usually, a minimum of three mixtures will be required. The weight ratio of one is chosen so that the heights of component and standard are equal. The ratios of the other two may be two-thirds and four-thirds of this value. Each mixture should be chromatographed at least three times, and the areas calculated. The factor for each chromatograph should be calculated as specified below, and the averages taken for each mixture. An overall average factor is calculated from them. The calibration should be performed periodically.

1. Factor = [(Weight Component × % Purity) / (Weight of Internal Standard × % Purity)] × [Internal Standard Area / Component Area]

2. % Component in Sample Mixture = (Component Area × Factor × % Internal Standard in Sample Mixture) / Internal Standard Area

3. % Component in Sample = (% Component in Sample Mixture × 100) / % Sample in Sample Mixture

III GC SYSTEM SUITABILITY TEST SAMPLE

The GC system suitability test sample consists of an equal-weight mixture of FCC-quality acetophenone, benzyl alcohol, benzyl acetate, linalool, and hydroxycitronellal.

Using the test sample described below, periodically test the performance of and resolution provided by the gas chromatograph employed. The test sample must display results comparable in quantitative composition, peak shape, and elution order to those specified herein. The quantitative composition should not deviate from the results listed below by more than ±10%. Analyze the GC test sample using the *GC Conditions for Analysis* given above.

	Order of Elution		Normalized % Area (FID)	
Component in Test Sample	Nonpolar	Polar	Nonpolar	Polar
Benzyl Alcohol	1	4	22.0	21.3
Acetophenone	2	2	21.1	21.4
Linalool	3	1	20.8	21.0
Benzyl Acetate	4	3	18.6	19.1
Hydroxycitronellal	5	5	16.7	16.7

4 / *Infrared Spectra*

INTRODUCTION

The infrared absorption spectra contained in this section are provided in conjunction with the requirements for *Identification* as specified for a number of substances in this edition

ORGANIZATION

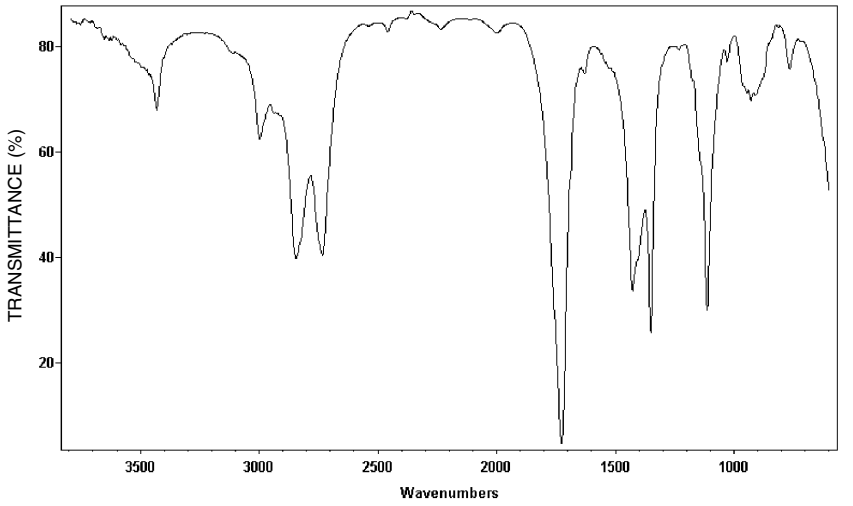
The spectra are arranged in alphabetical order.

SAMPLE PREPARATION

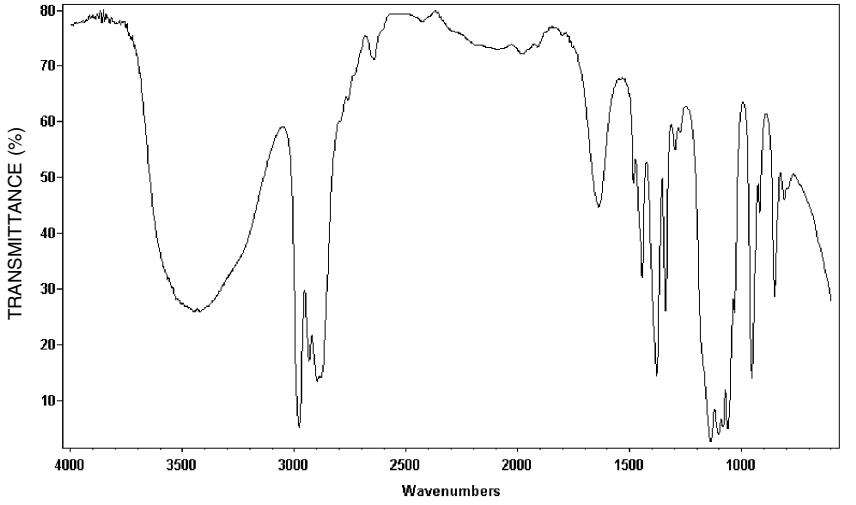
Most of the substances for which spectra are provided are liquids at or near room temperature. Unless otherwise noted in the caption for an individual spectrum, the spectra for essential oils and flavor chemicals were obtained on the neat liquids contained in fixed-volume sodium chloride cells or between salt plates. For solids, the sample was prepared as a potassium bromide pellet or a mineral oil (Nujol or equivalent) dispersion, as indicated in the individual spectrum captions. The remaining substances were prepared as directed under *Identification* in the individual monographs.

Table of Contents for Infrared Spectra

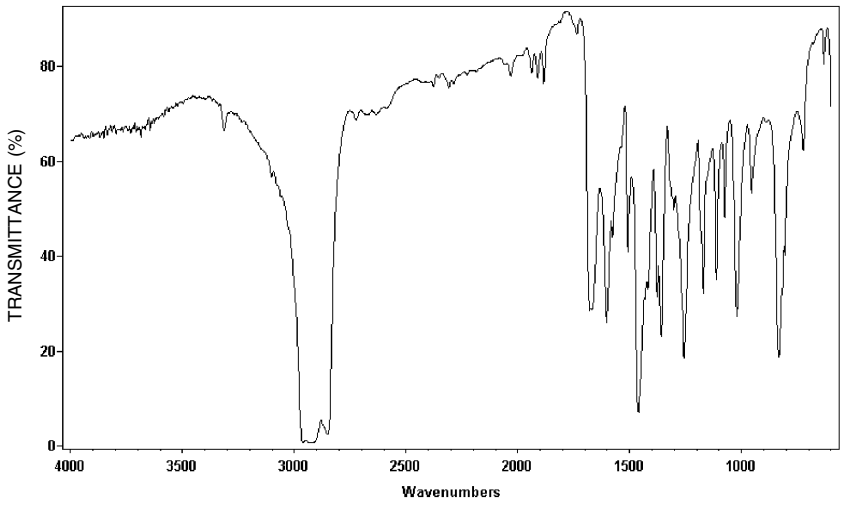
Acetaldehyde View Flavor Chemical



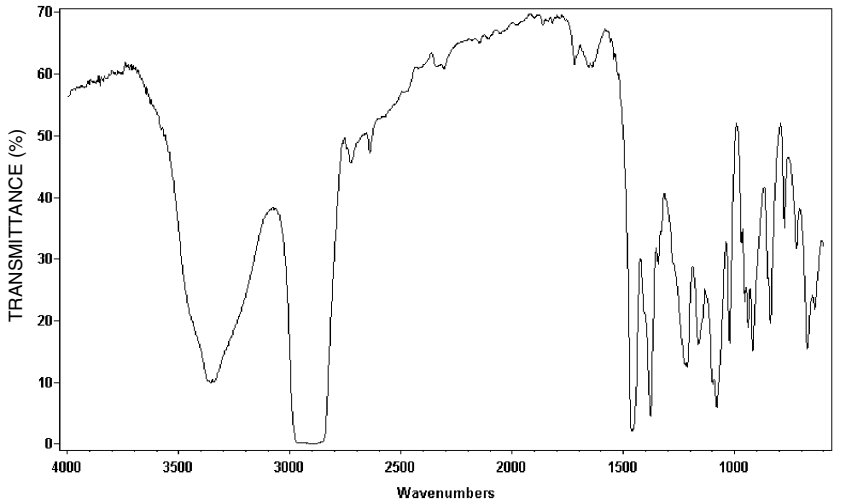
Acetaldehyde Diethyl Acetal View Flavor Chemical



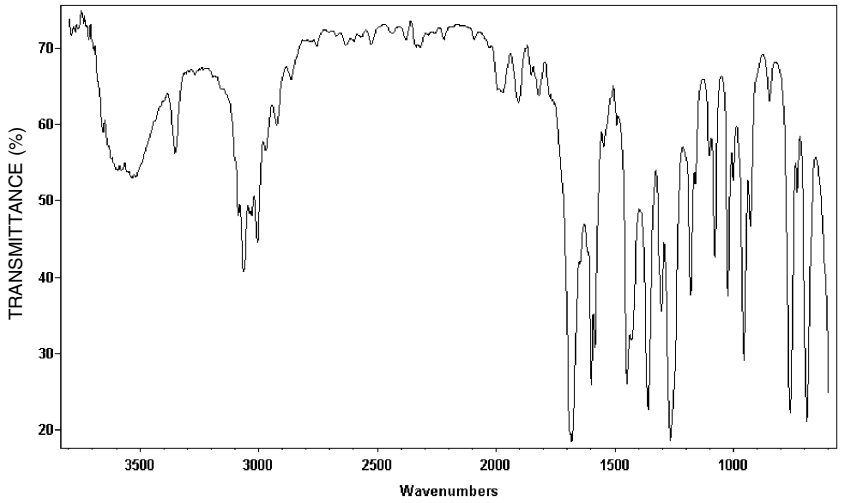
Acetanisole View Flavor Chemical



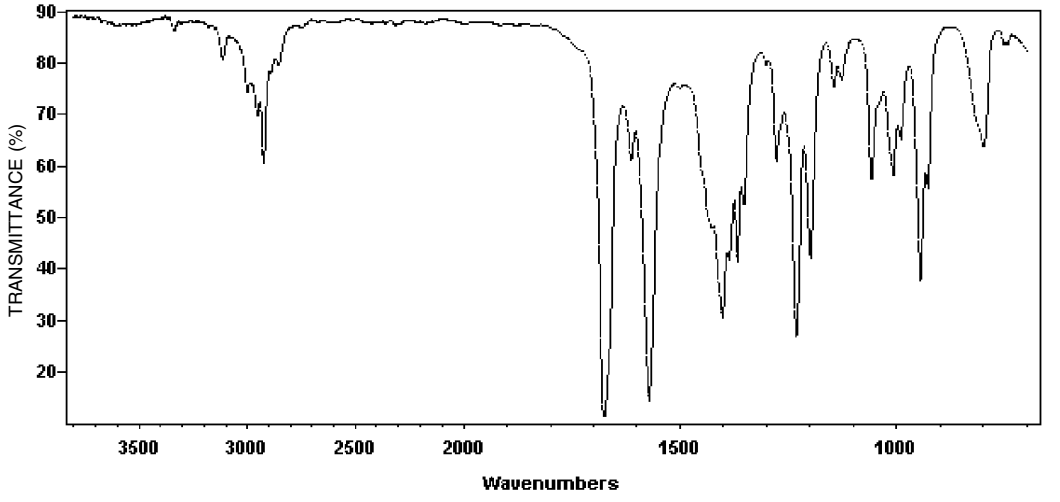
Acetoin View Flavor Chemical



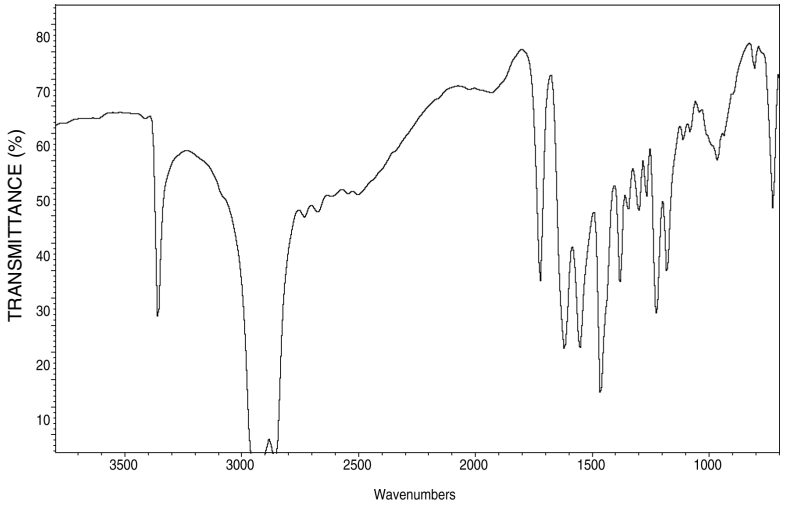
Acetophenone View Flavor Chemical



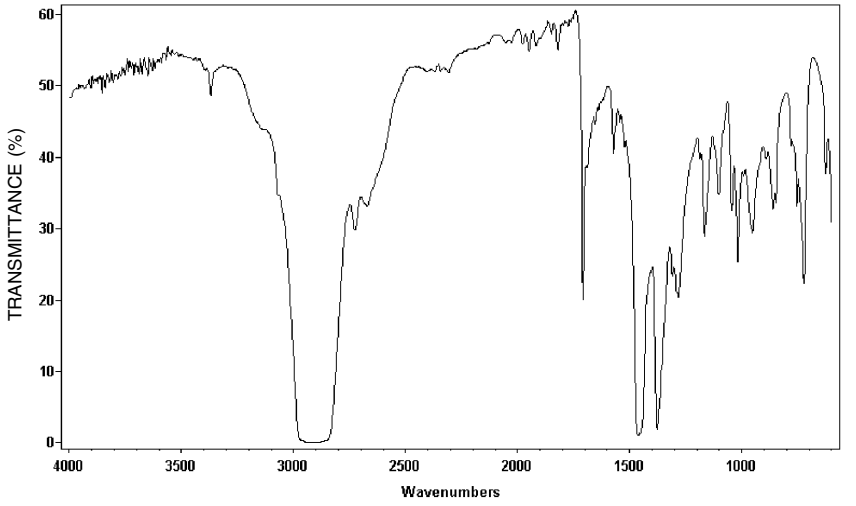
3-Acetyl-2,5-dimethyl Furan View Flavor Chemical



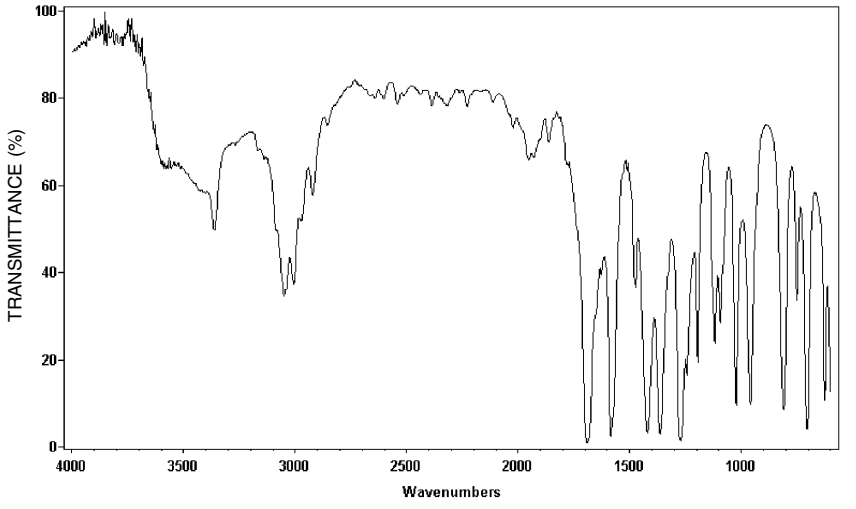
_N_-Acetyl-L-methionine (Mineral Oil Mull) View Monograph



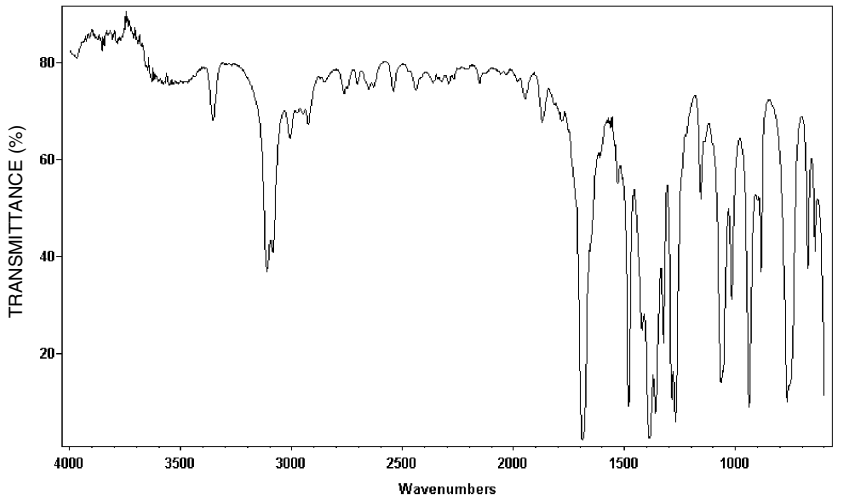
2-Acetylpyrazine (Mineral Oil Mull) View Flavor Chemical



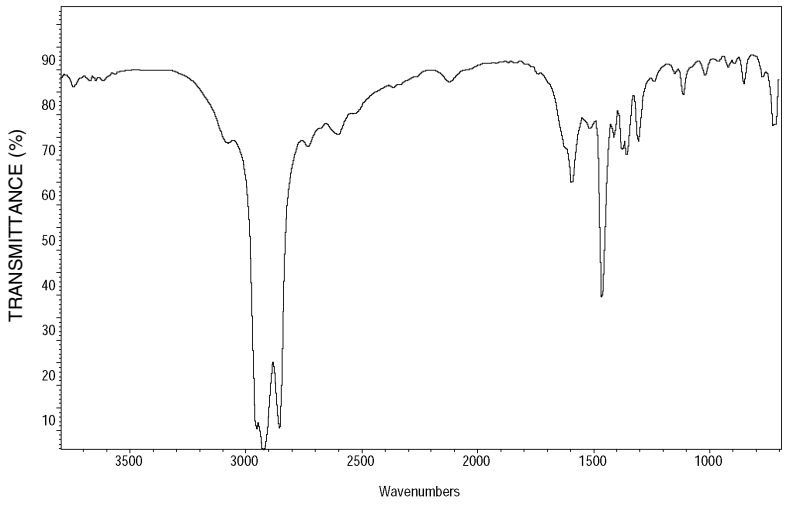
3-Acetylpyridine View Flavor Chemical



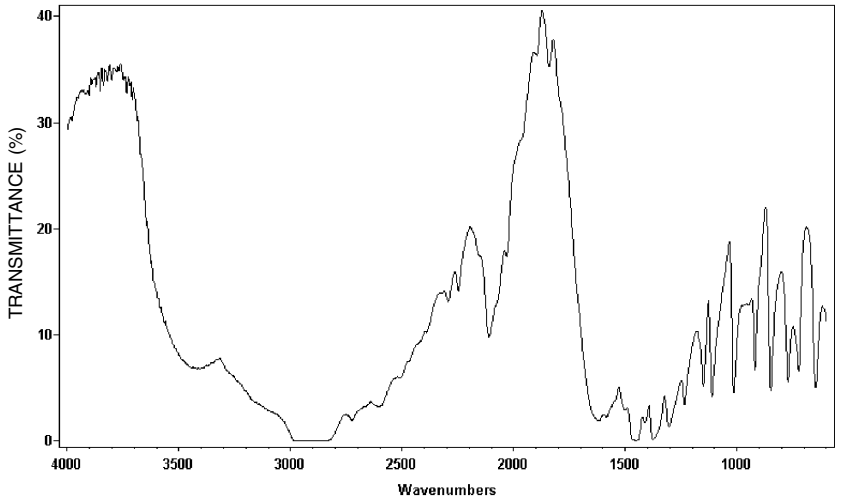
2-Acetyl Thiazole **View Flavor Chemical**



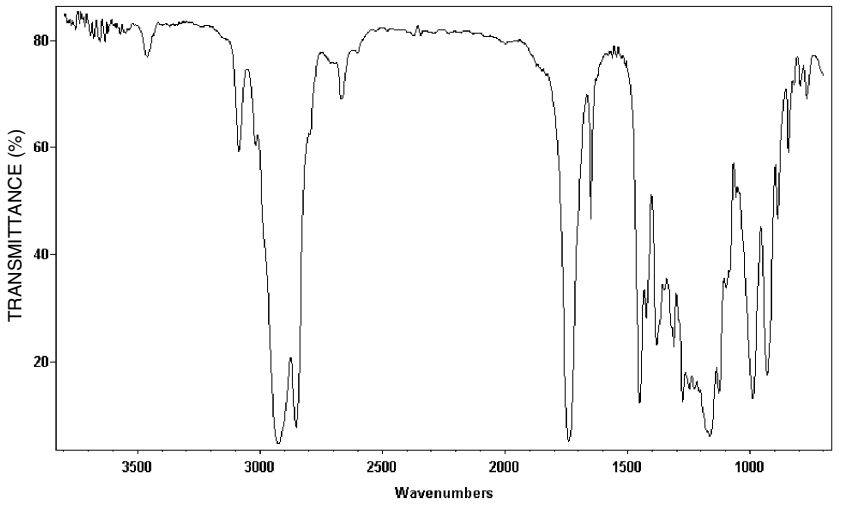
DL-Alanine (Mineral Oil Mull) **View Monograph**



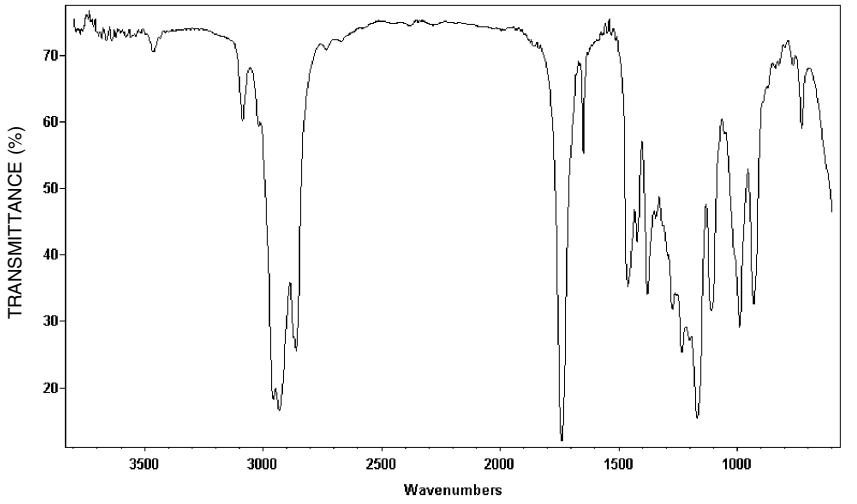
L-Alanine (Mineral Oil Mull) **View Monograph**



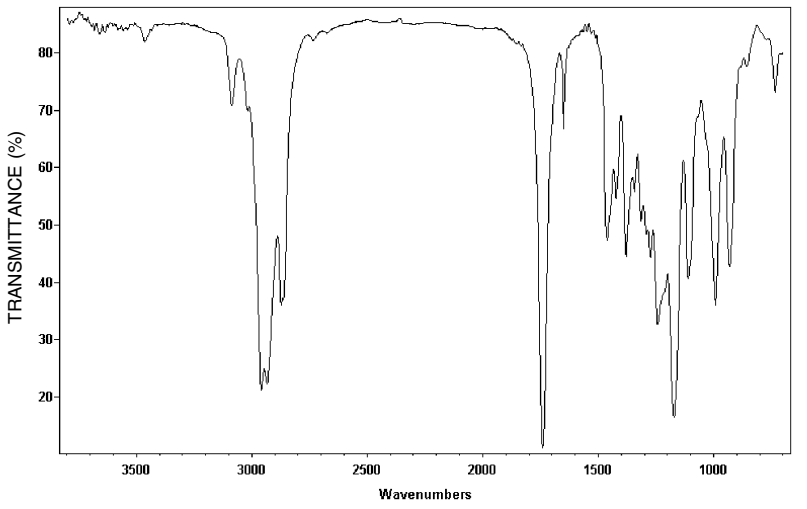
Allyl Cyclohexanepropionate View Flavor Chemical



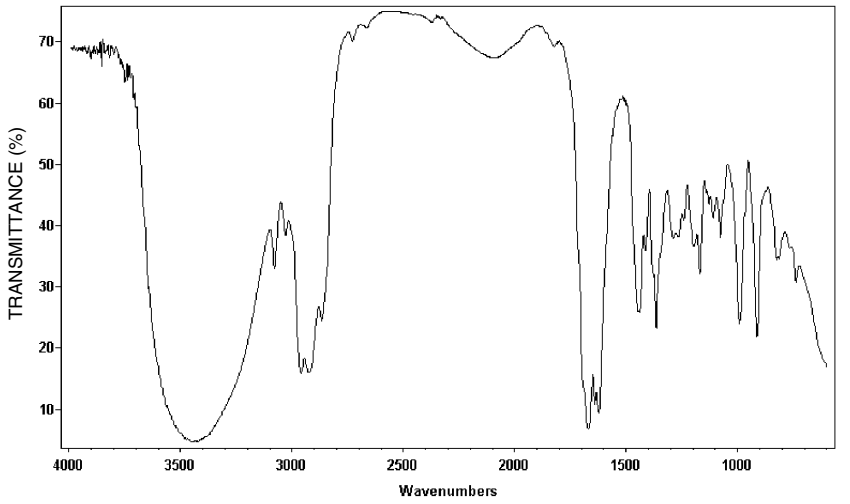
Allyl Heptanoate View Flavor Chemical



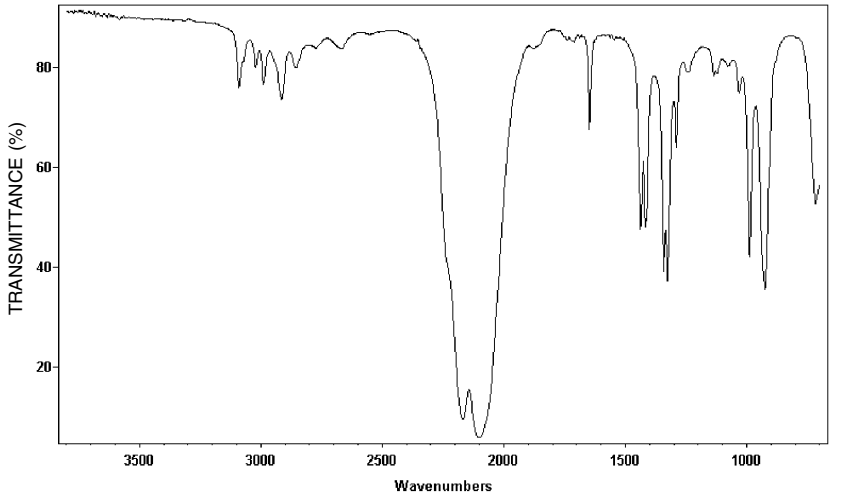
Allyl Hexanoate View Flavor Chemical



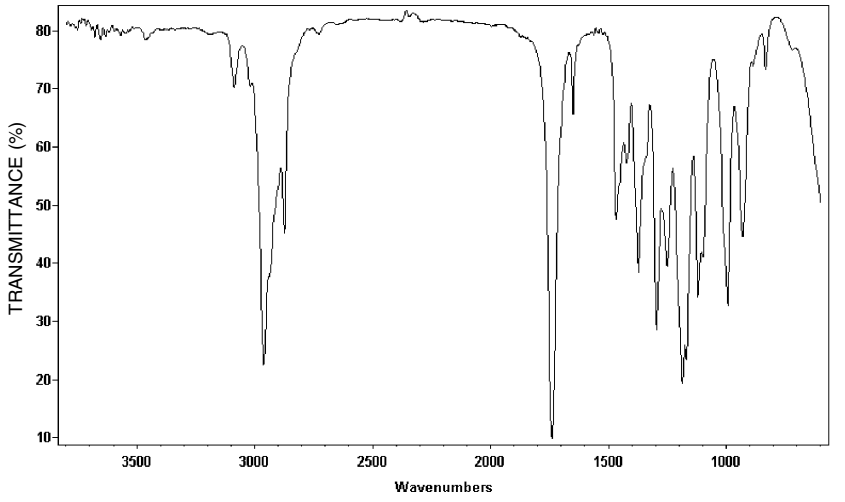
Allyl α-Ionone View Flavor Chemical



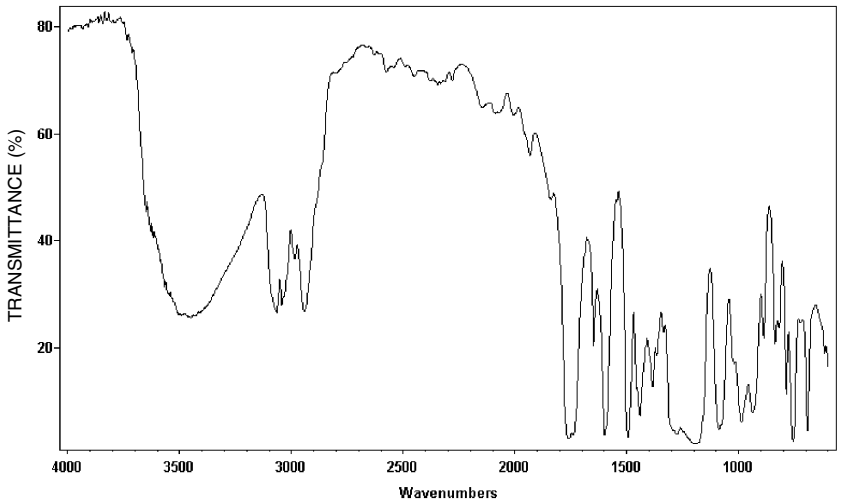
Allyl Isothiocyanate View Flavor Chemical



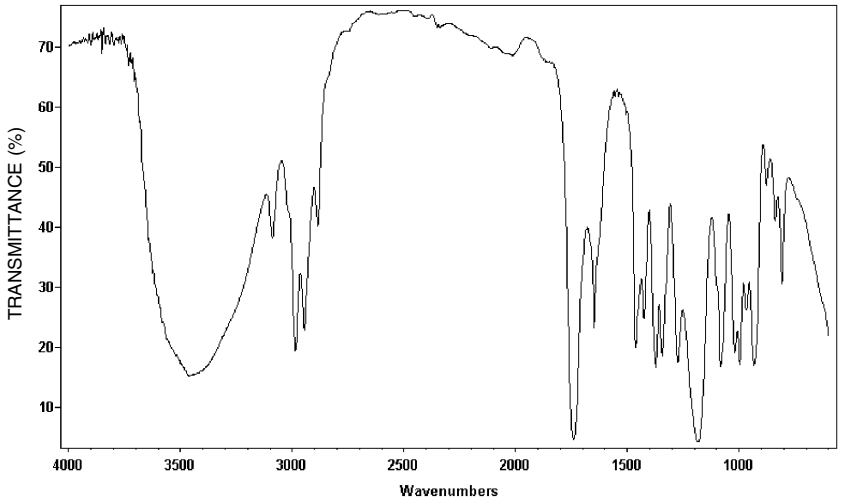
Allyl Isovalerate View Flavor Chemical



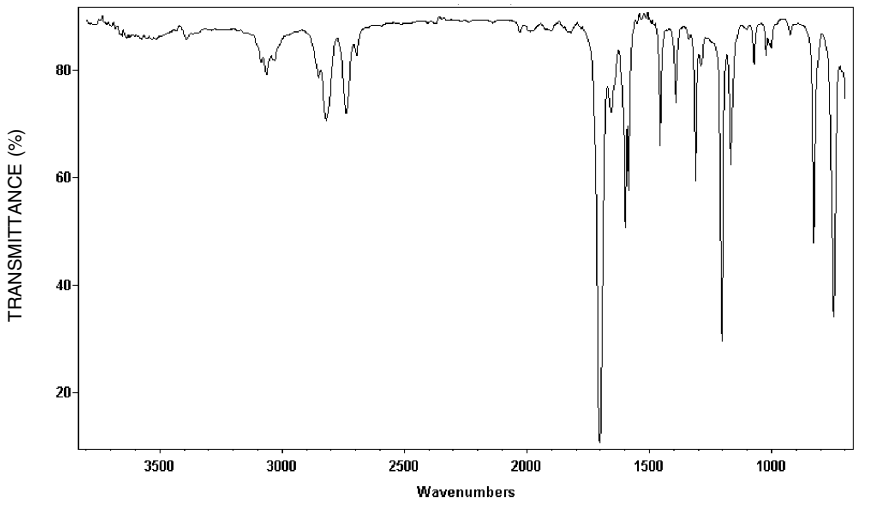
Allyl Phenoxy Acetate View Flavor Chemical



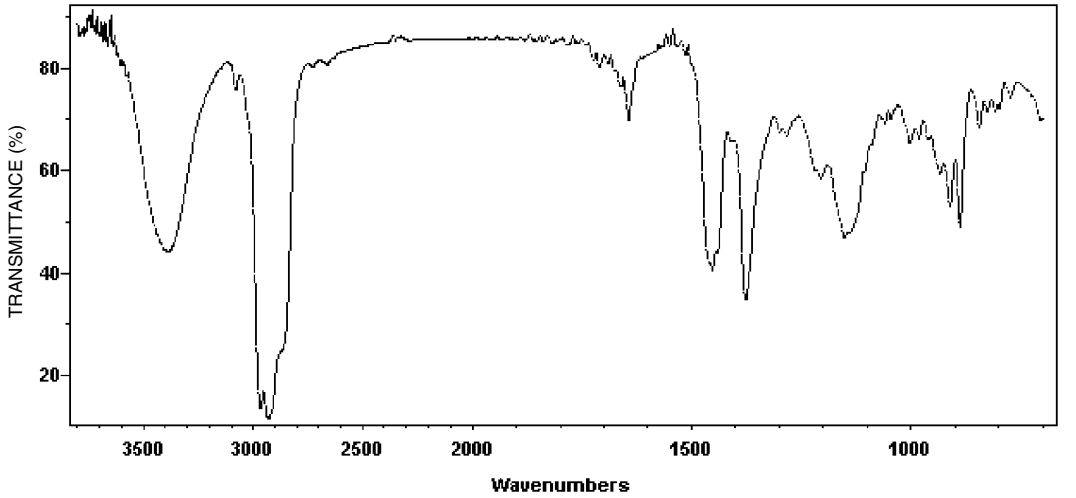
Allyl Propionate View Flavor Chemical



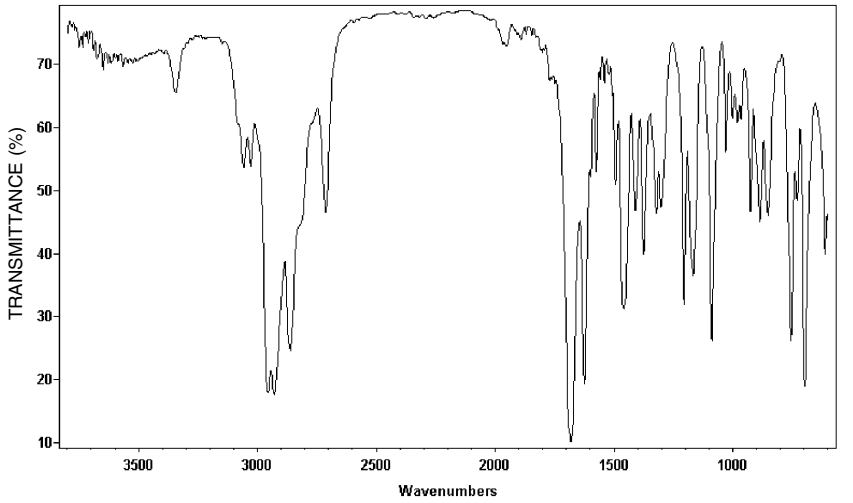
Almond Oil, Bitter, FFPA View Monograph



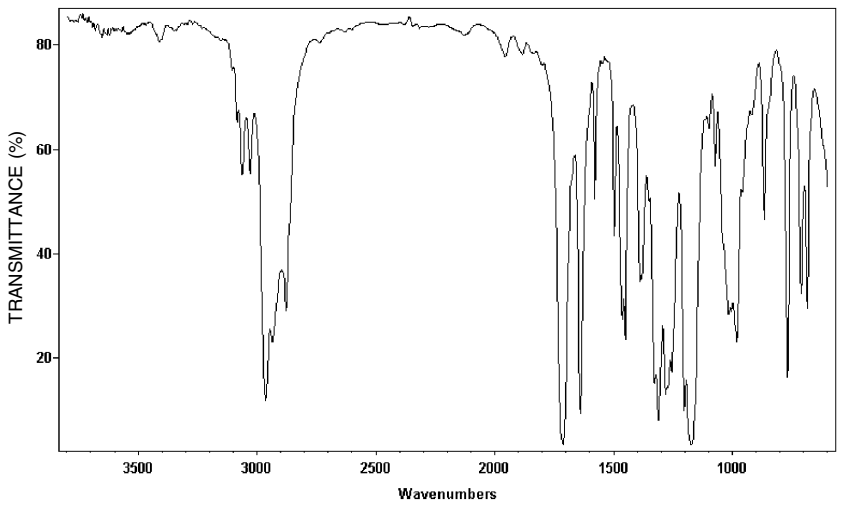
Ambrette Seed Oil View Monograph



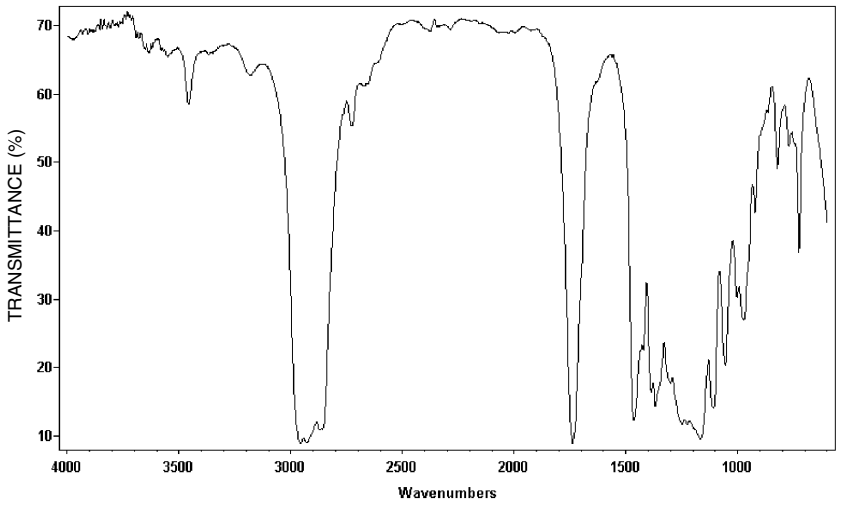
α-Amylcinnamaldehyde View Flavor Chemical



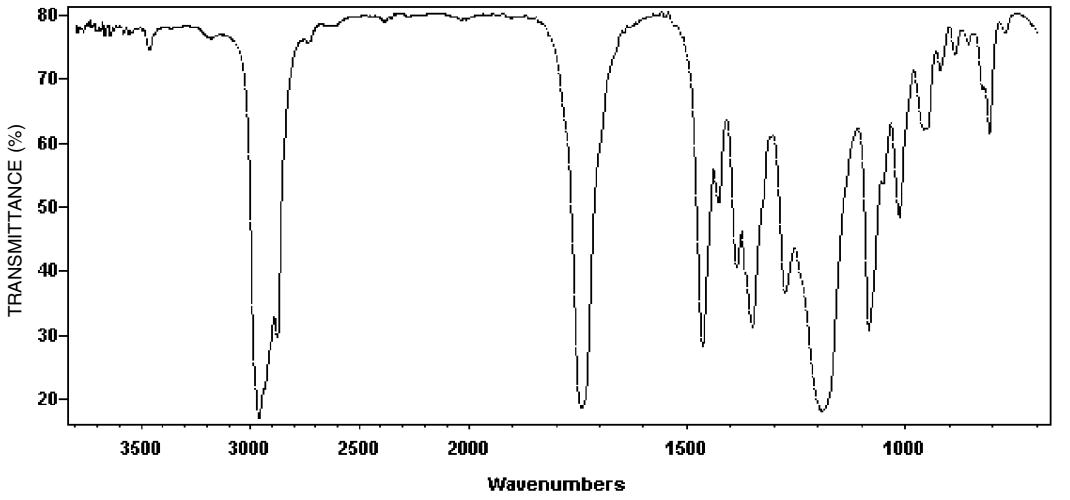
Amyl Cinnamate View Flavor Chemical



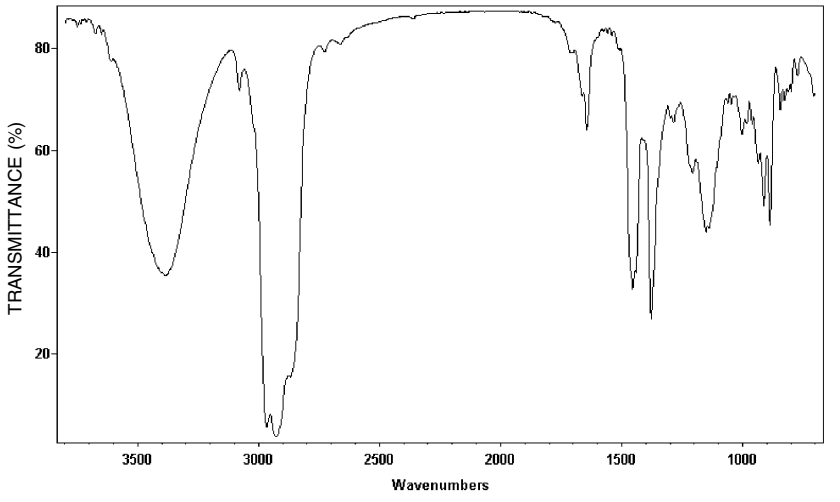
Amyl Octanoate View Flavor Chemical



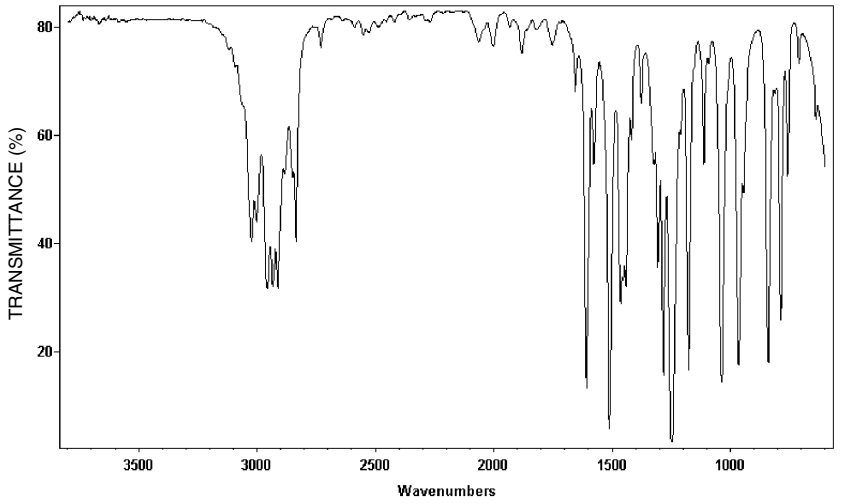
Amyl Propionate View Flavor Chemical



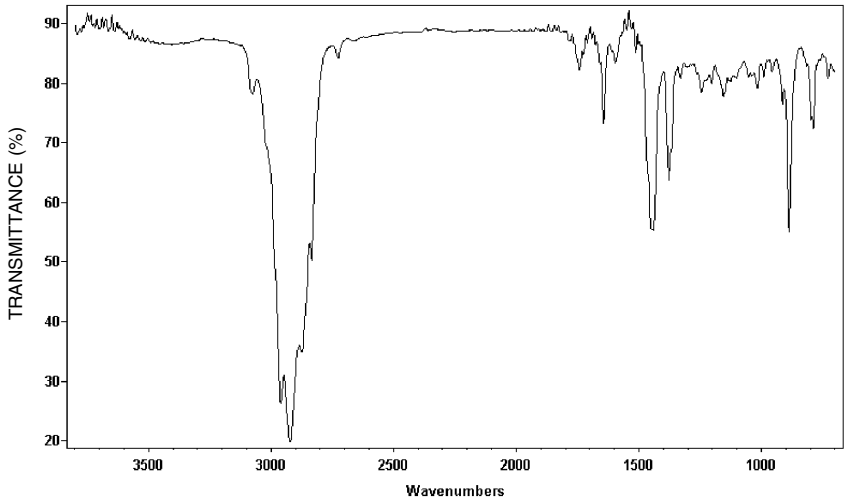
Amyris Oil, West Indian Type View Monograph



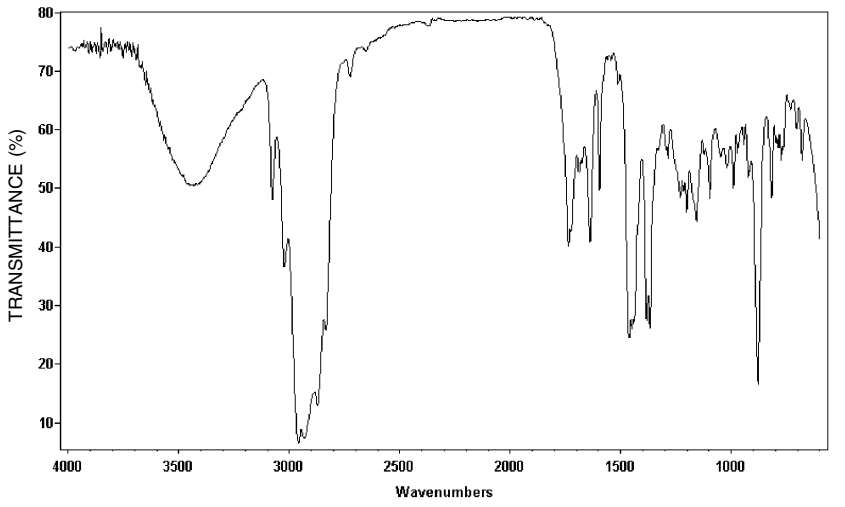
Anethole View Flavor Chemical



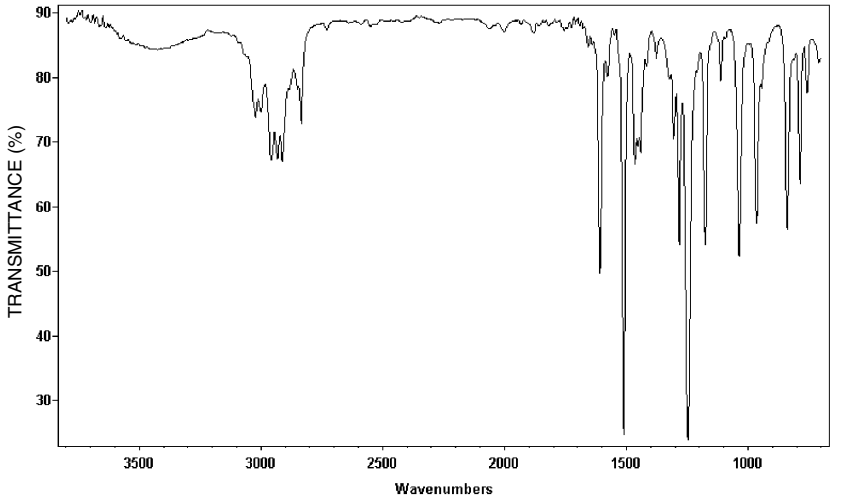
Angelica Root Oil View Monograph



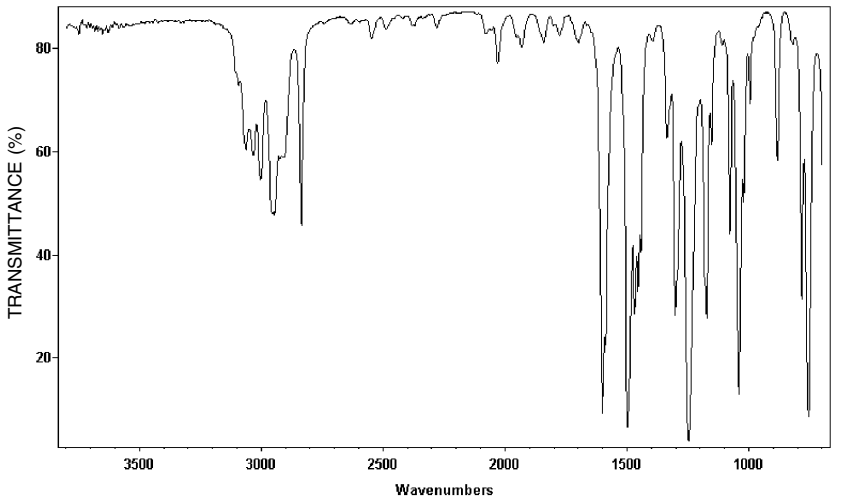
Angelica Seed Oil View Monograph



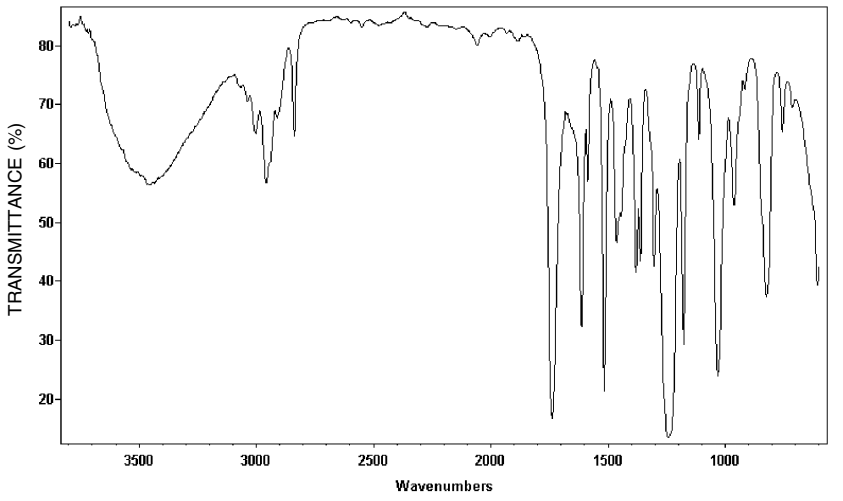
Anise Oil View Monograph



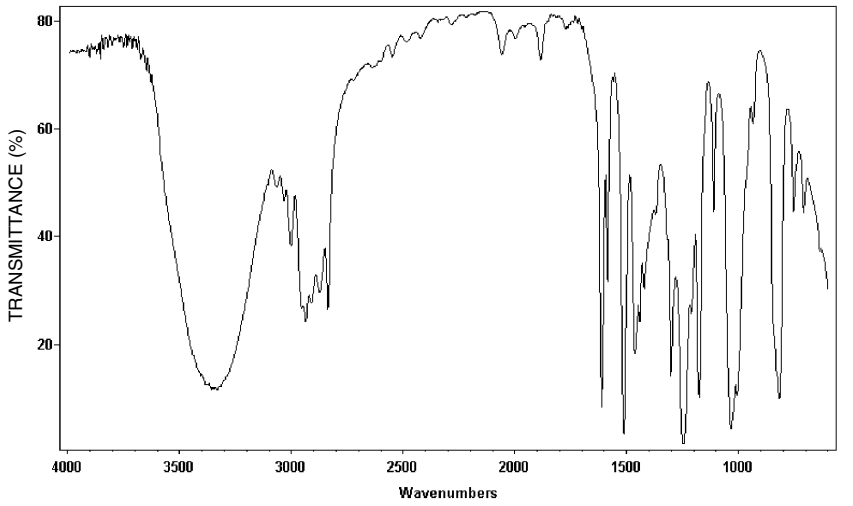
Anisole View Flavor Chemical



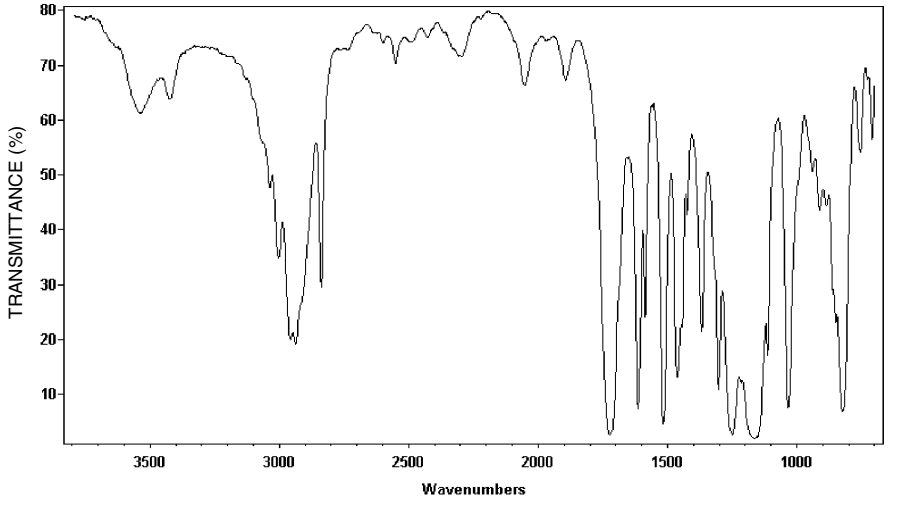
Anisyl Acetate View Flavor Chemical



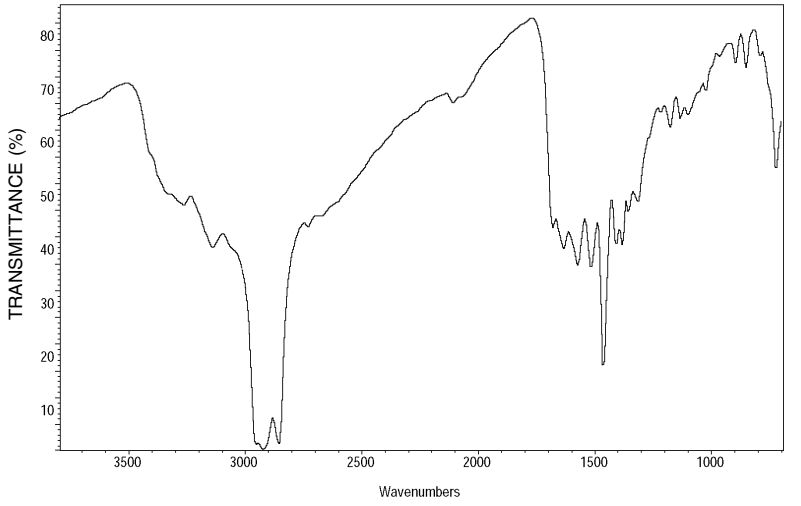
Anisyl Alcohol View Flavor Chemical



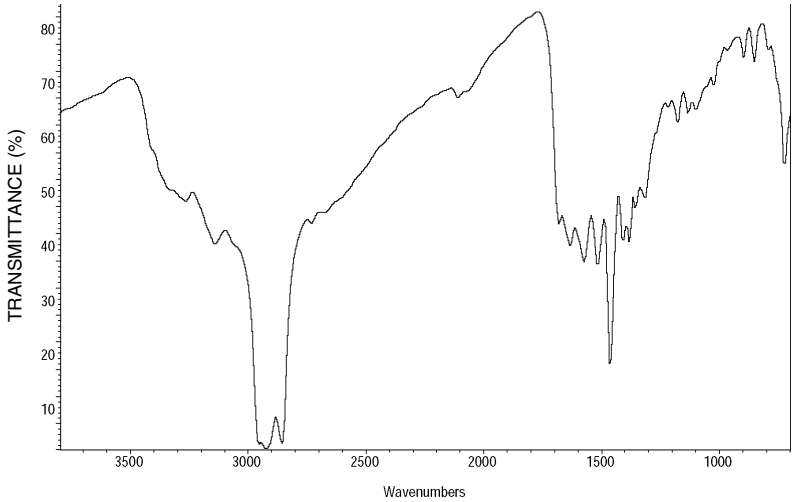
Anisyl Formate View Flavor Chemical



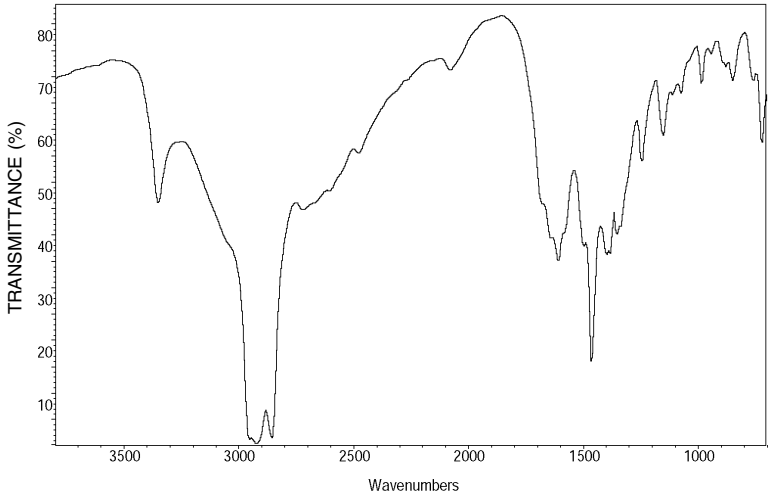
L-Arginine (Mineral Oil Mull) View Monograph



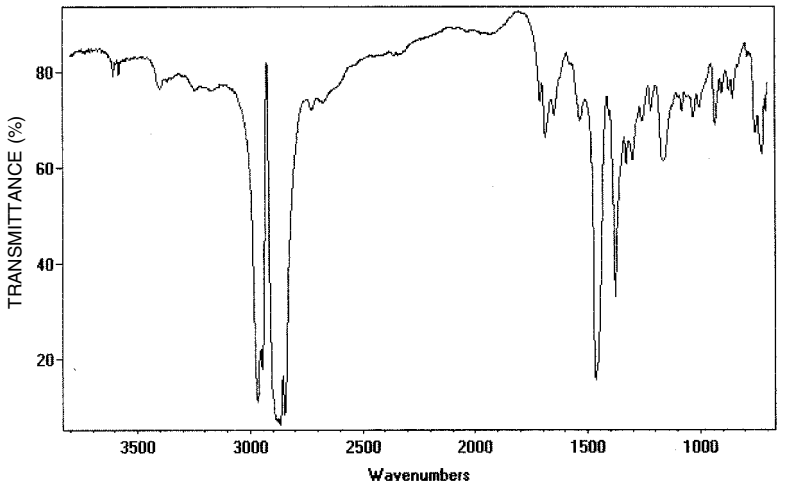
L-Arginine Monohydrochloride (Mineral Oil Mull) View Monograph



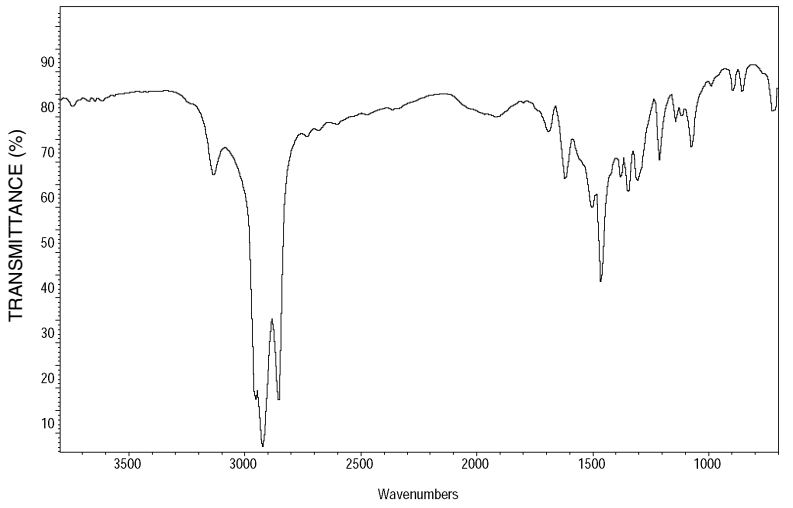
L-Asparagine (Mineral Oil Mull) View Monograph



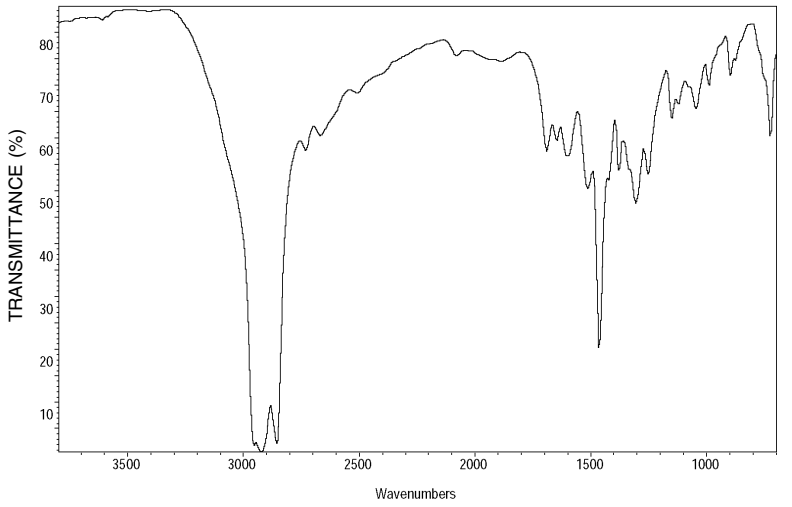
Aspartame-Acesulfame Salt (Mineral Oil Mull) View Monograph



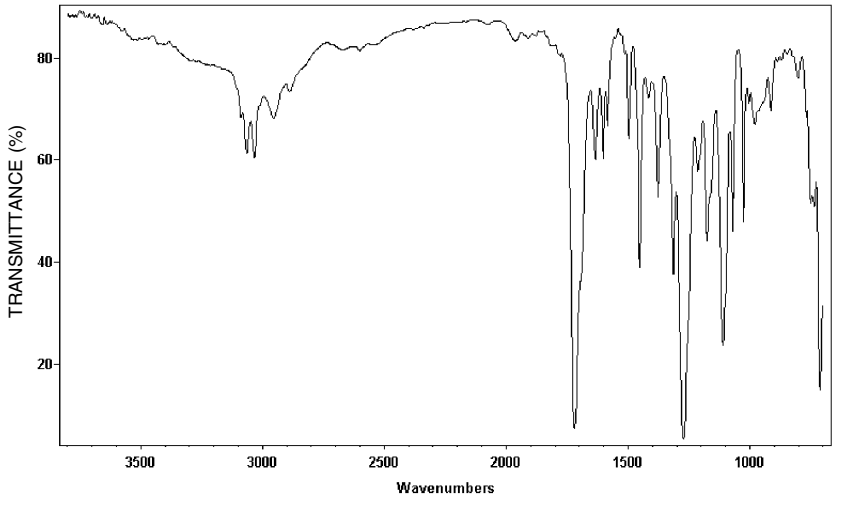
DL-Aspartic Acid (Mineral Oil Mull) **View Monograph**



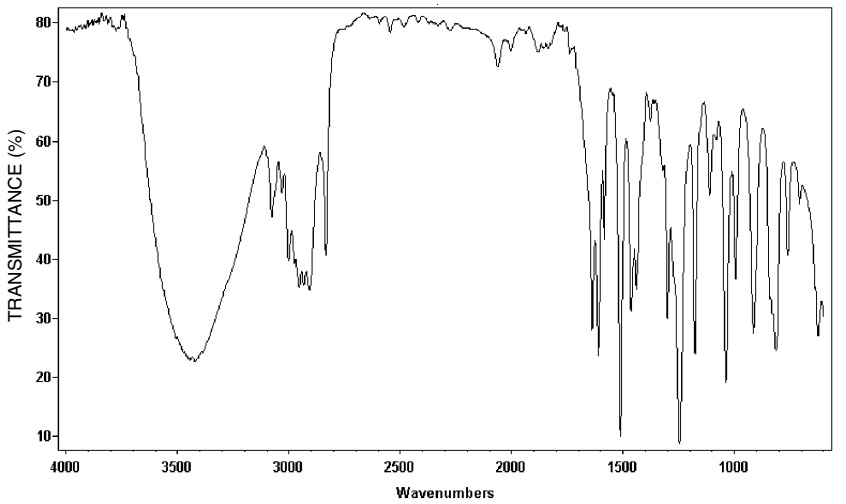
L-Aspartic Acid (Mineral Oil Mull) **View Monograph**



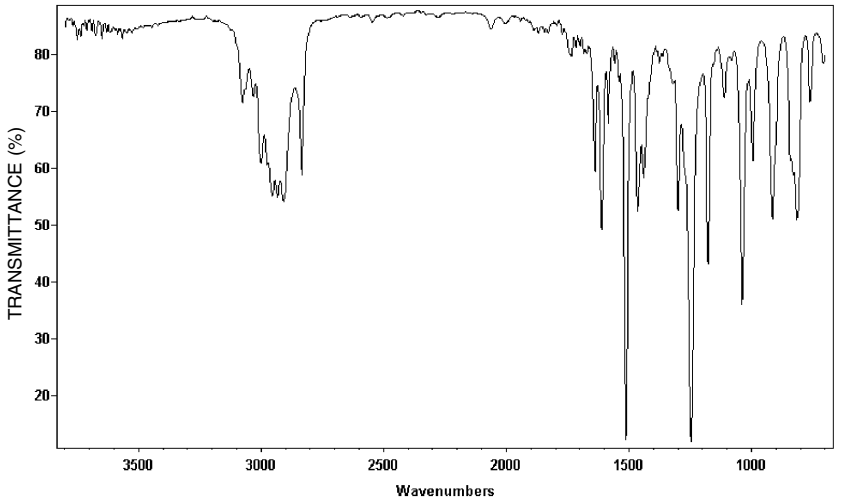
Balsam Peru Oil **View Monograph**



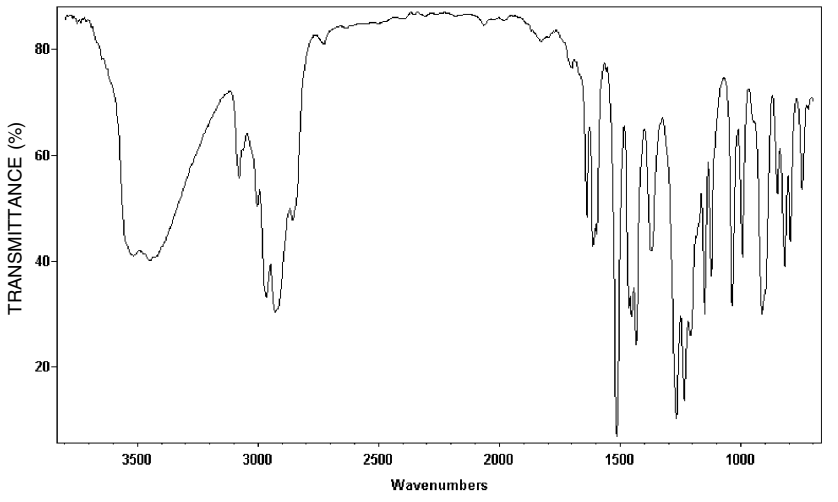
Basil Oil, Comoros Type View Monograph



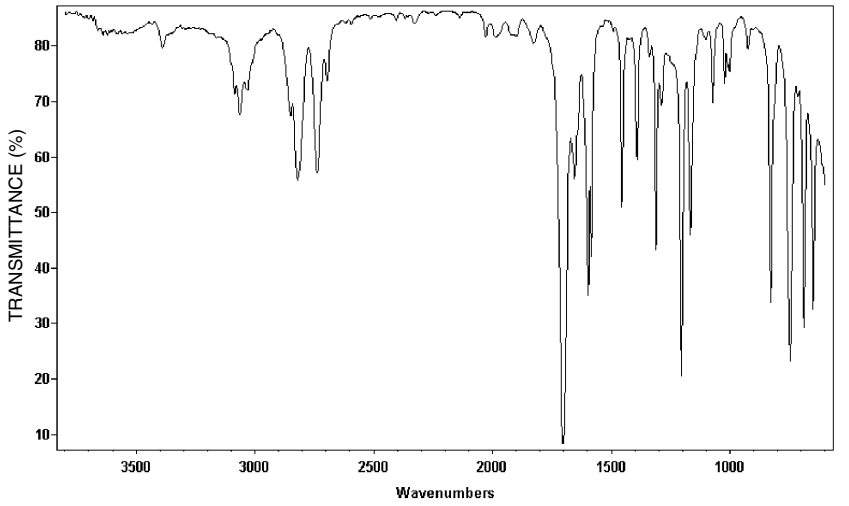
Basil Oil, European Type View Monograph



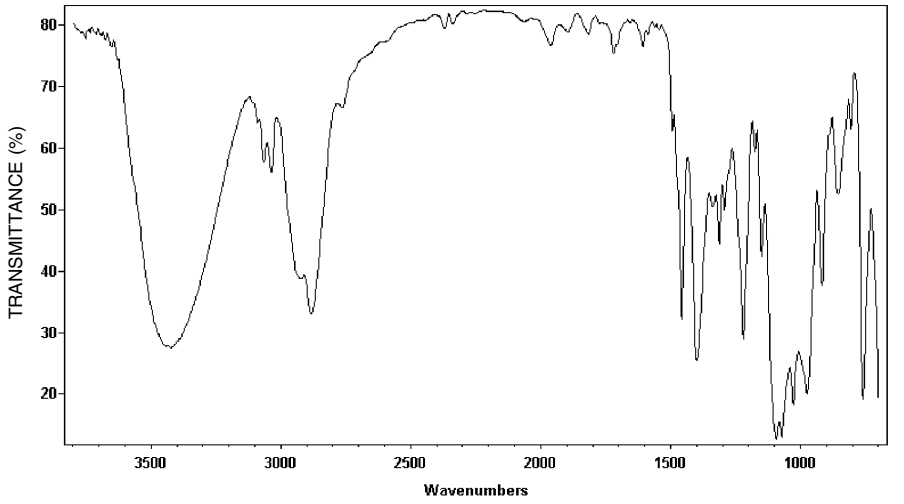
Bay Oil View Monograph



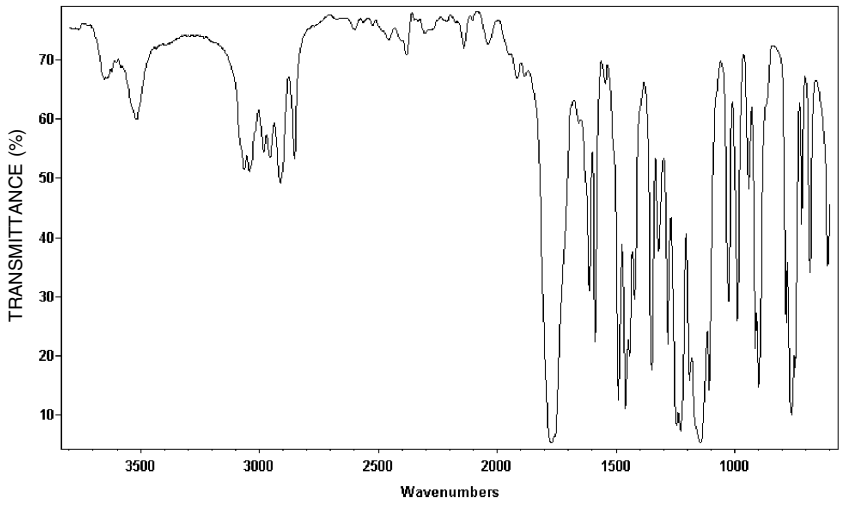
Benzaldehyde **View Flavor Chemical**



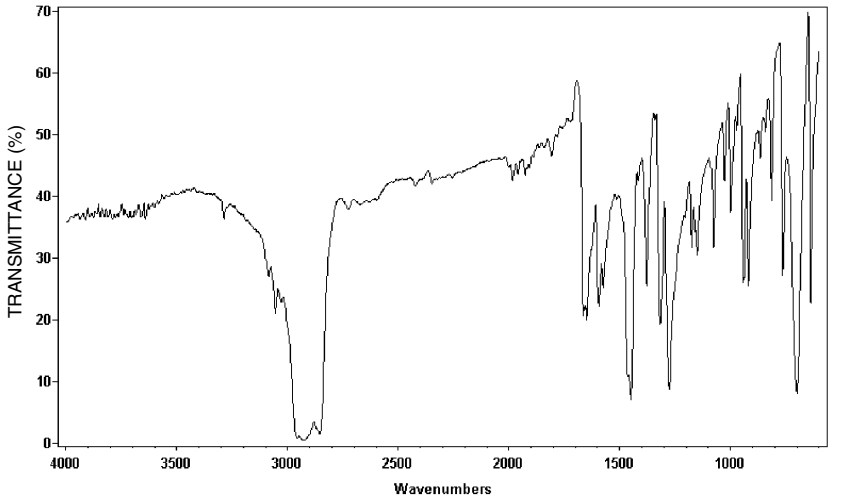
Benzaldehyde Glyceryl Acetal **View Flavor Chemical**



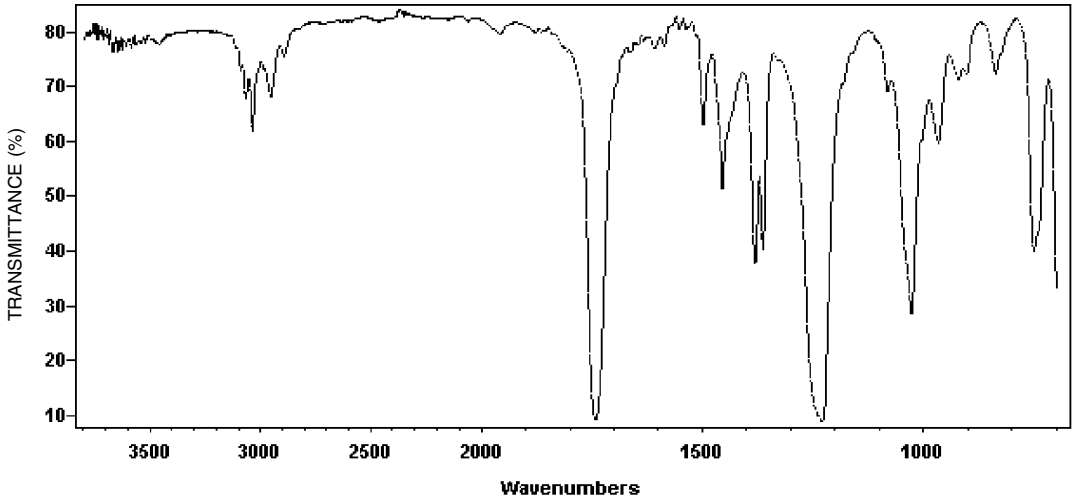
1,2-Benzodihydropyrone **View Flavor Chemical**



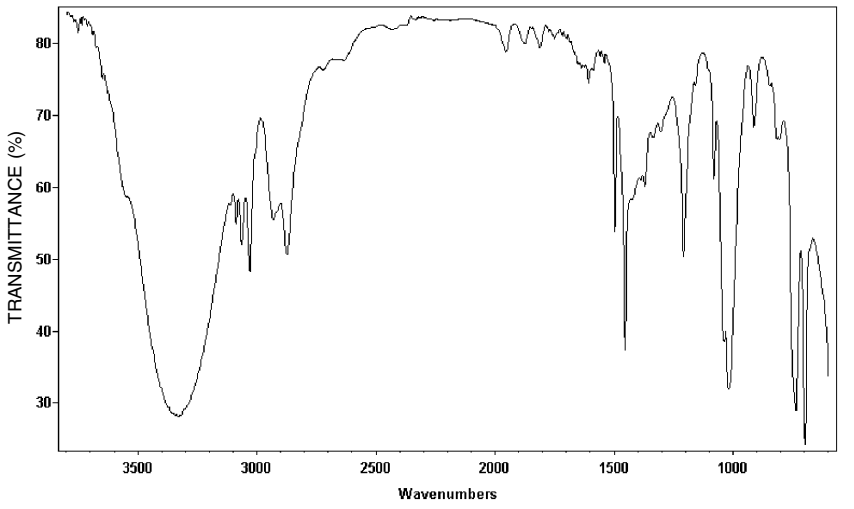
Benzophenone View Flavor Chemical



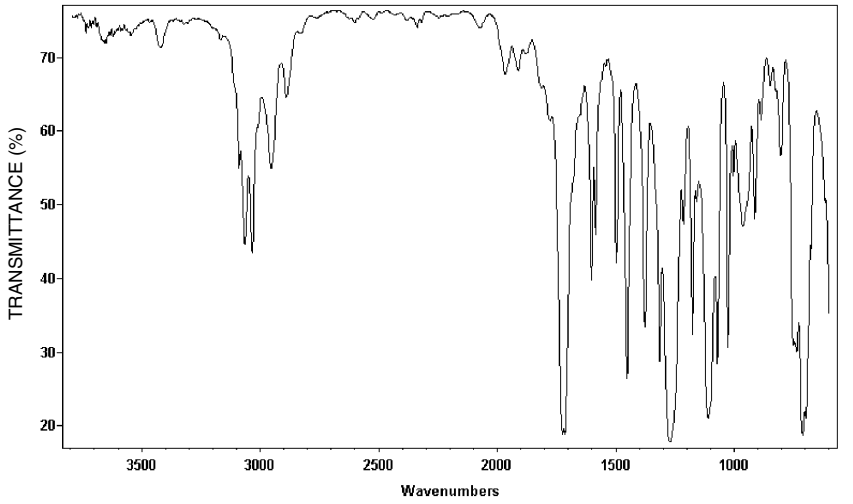
Benzyl Acetate View Flavor Chemical



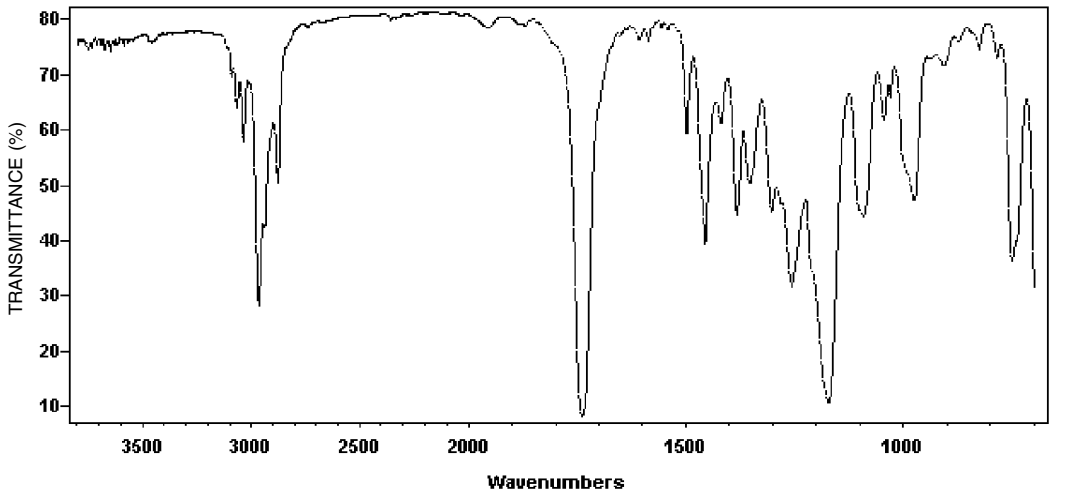
Benzyl Alcohol View Flavor Chemical



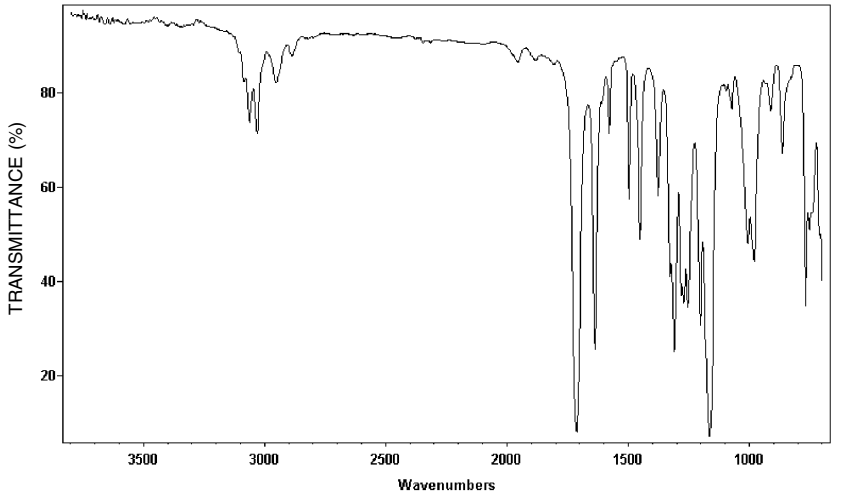
Benzyl Benzoate View Flavor Chemical



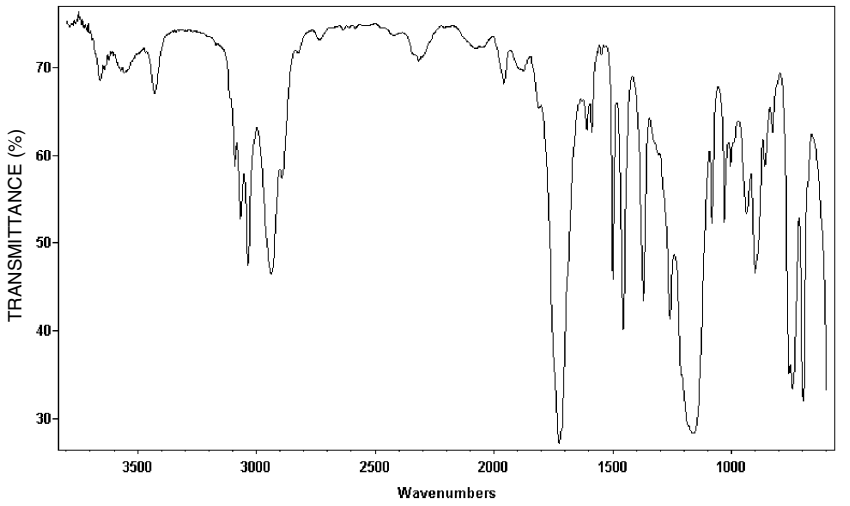
Benzyl Butyrate View Flavor Chemical



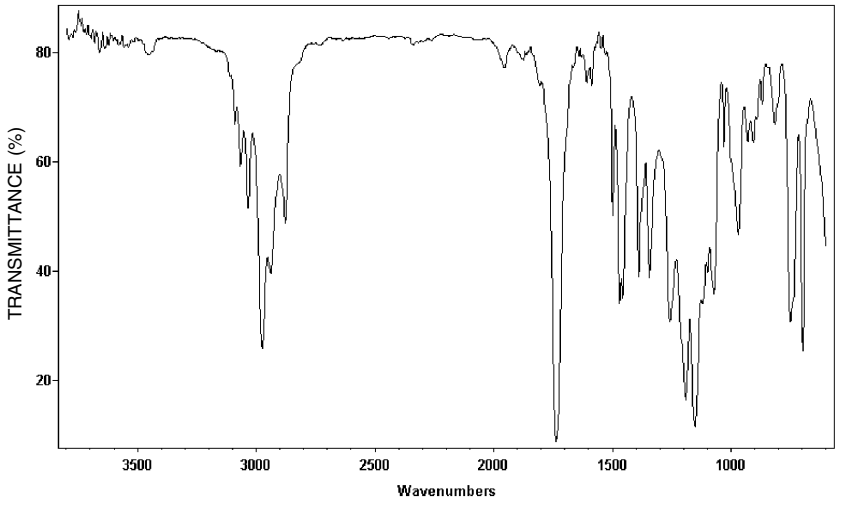
Benzyl Cinnamate View Flavor Chemical



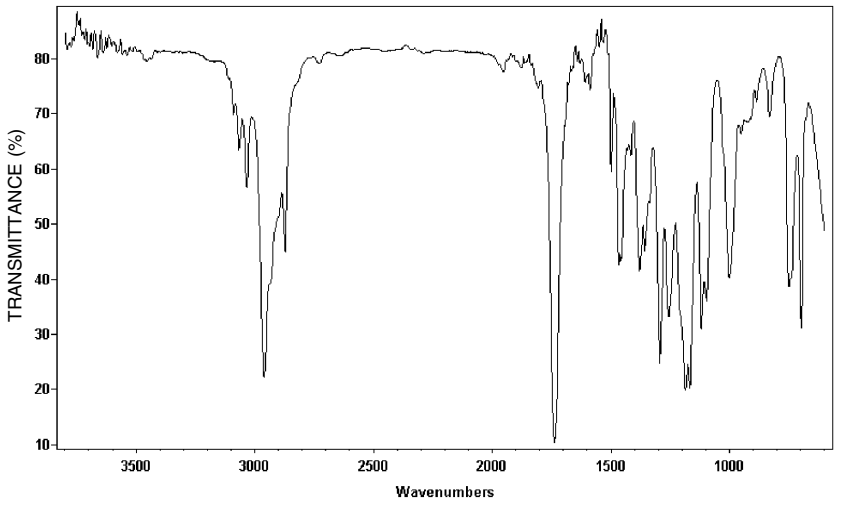
Benzyl Formate View Flavor Chemical



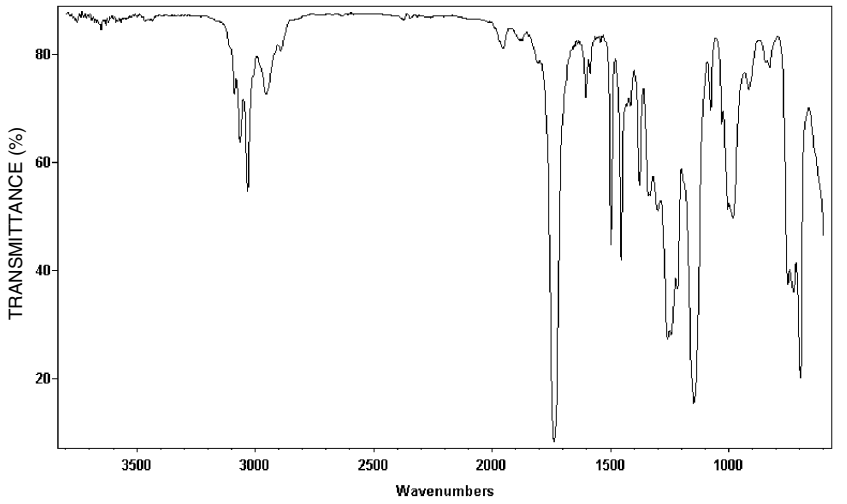
Benzyl Isobutyrate View Flavor Chemical



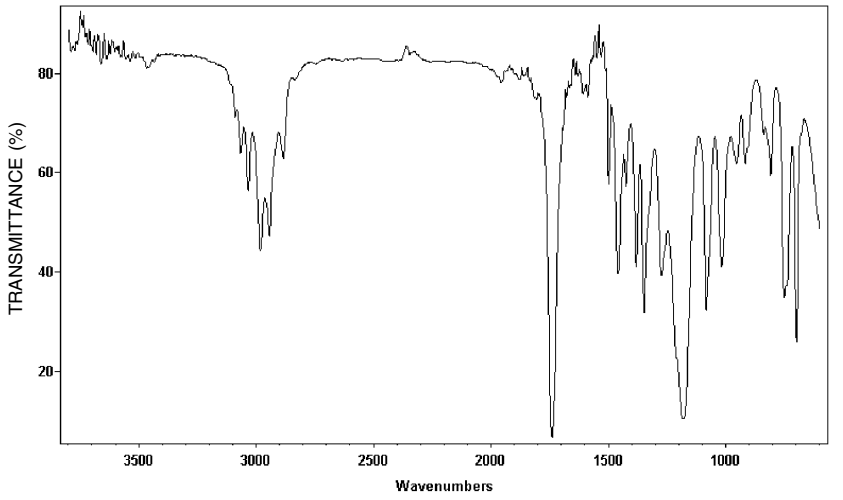
Benzyl Isovalerate View Flavor Chemical



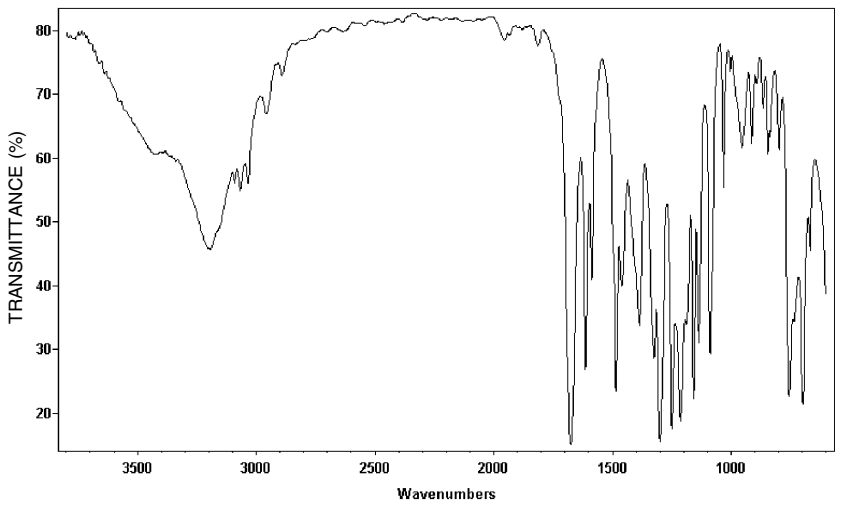
Benzyl Phenylacetate View Flavor Chemical



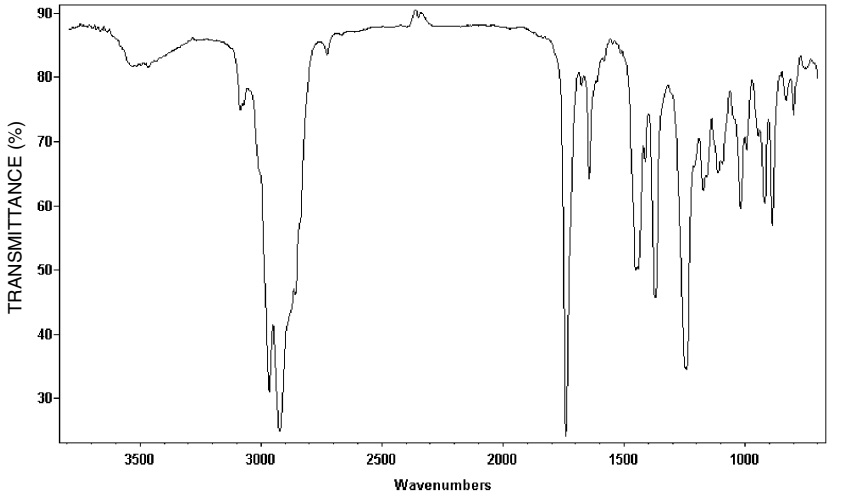
Benzyl Propionate View Flavor Chemical



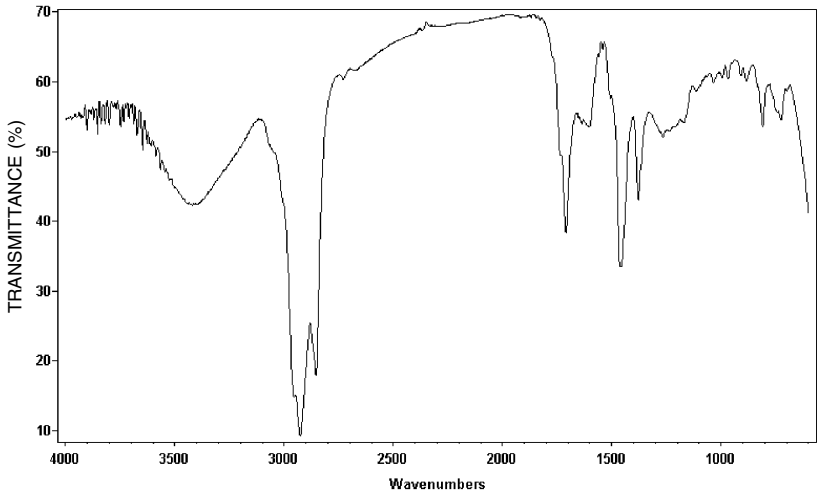
Benzyl Salicylate View Flavor Chemical



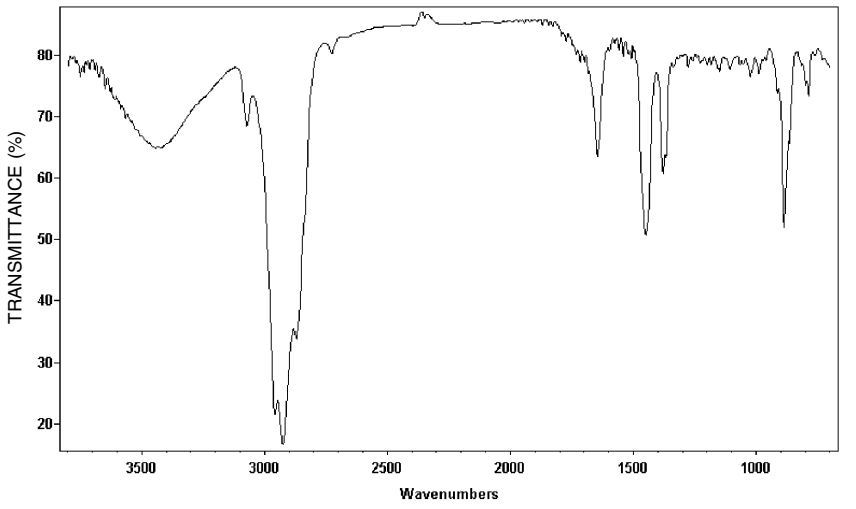
Bergamot Oil, Coldpressed View Monograph



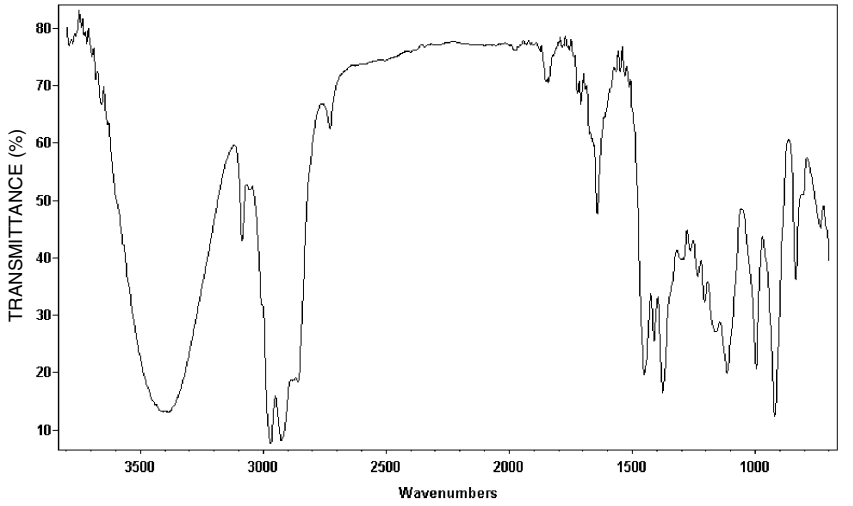
Birch Tar Oil, Rectified View Monograph



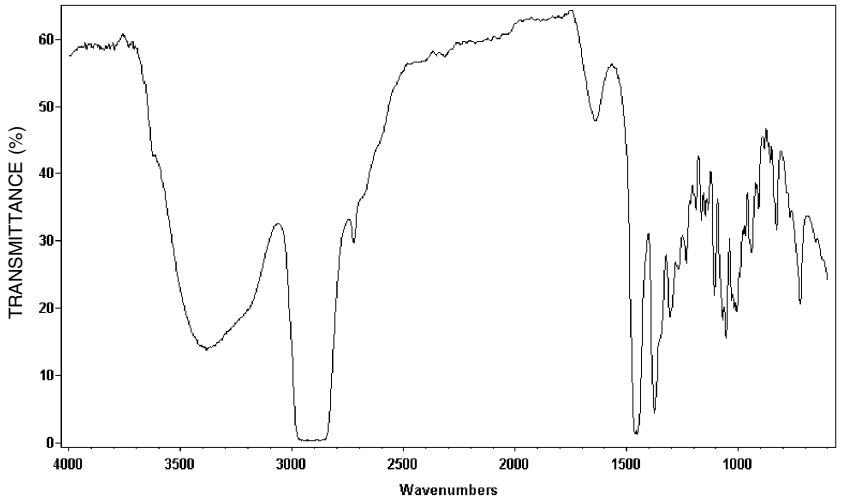
Black Pepper Oil View Monograph



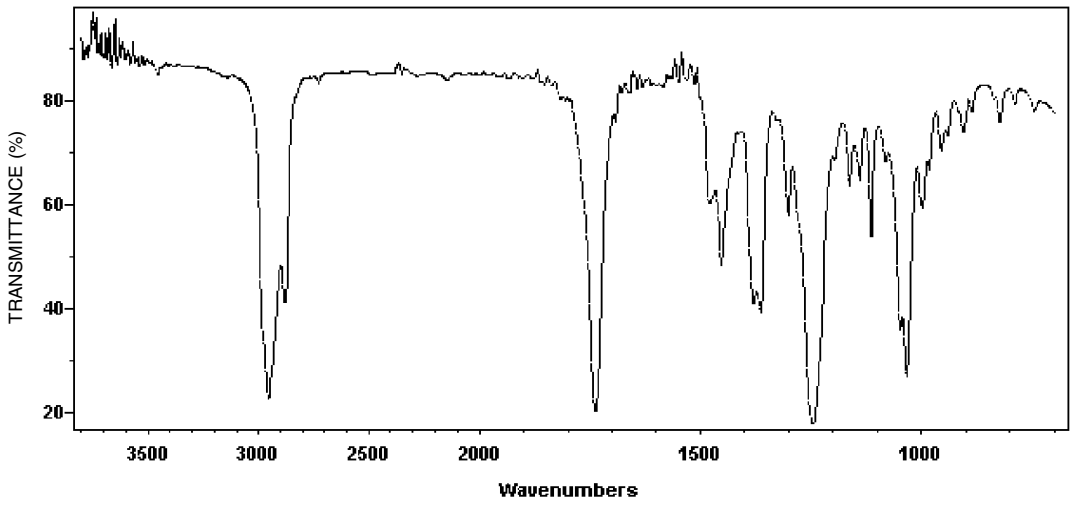
Bois de Rose Oil View Monograph



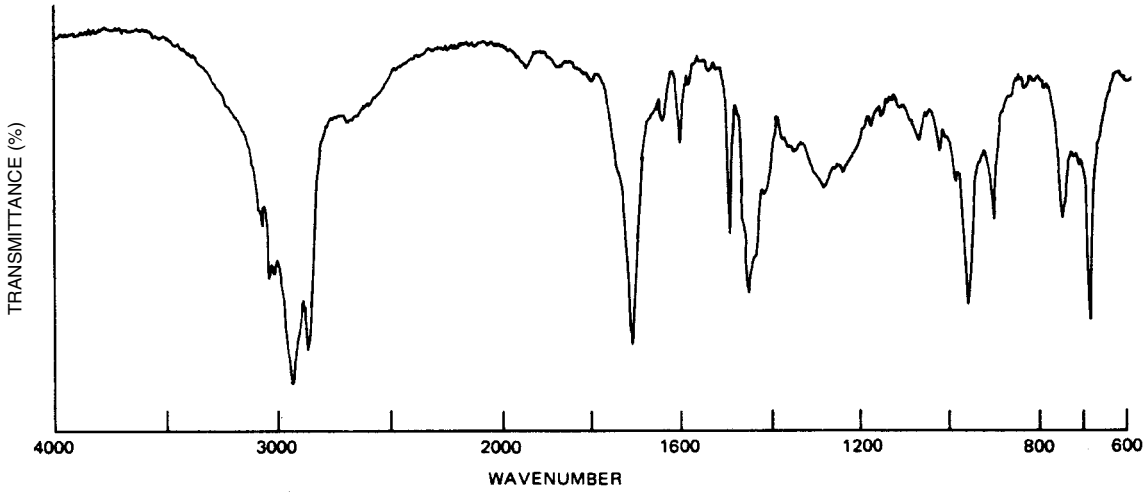
Borneol View Flavor Chemical



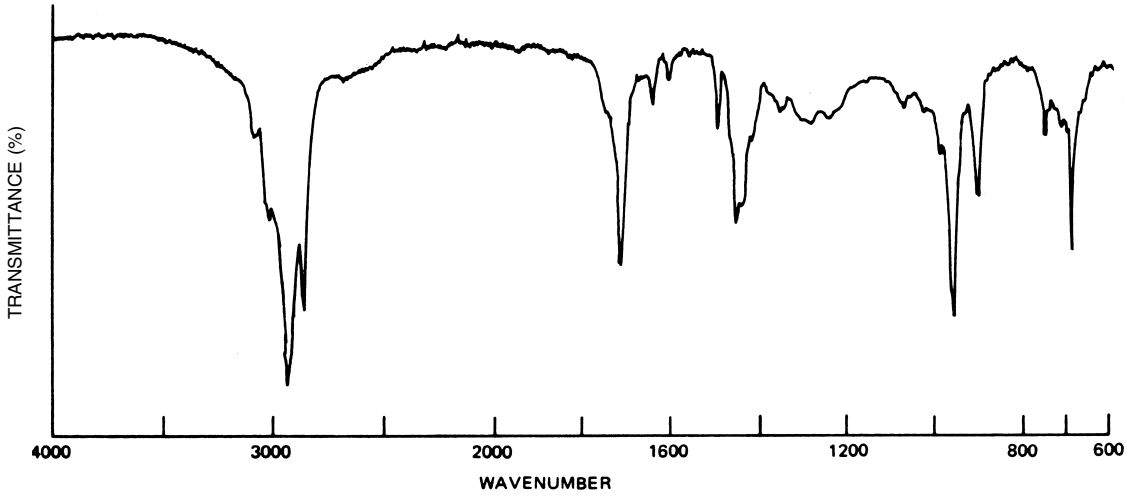
Bornyl Acetate View Flavor Chemical



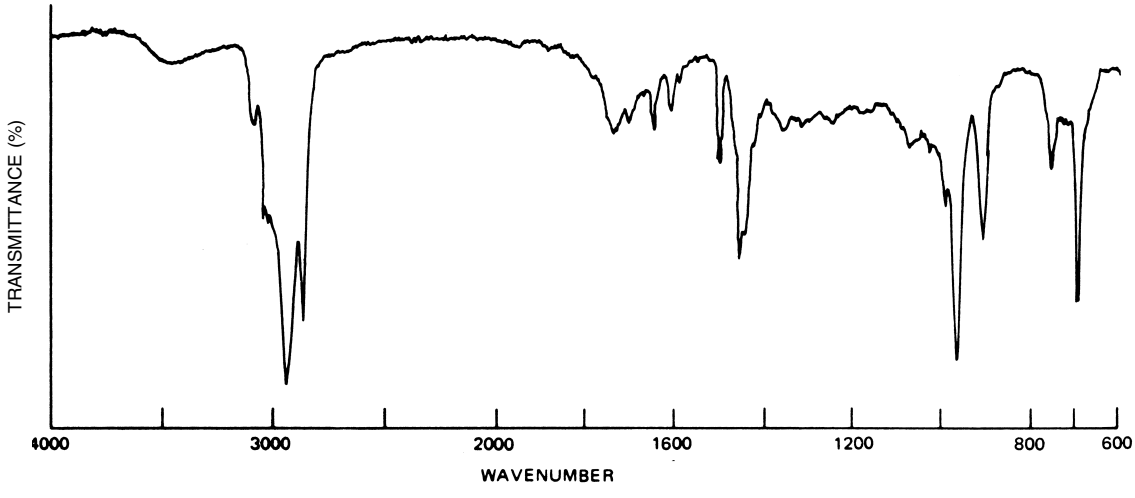
Butadiene-Styrene 50/50 Rubber (Solid)



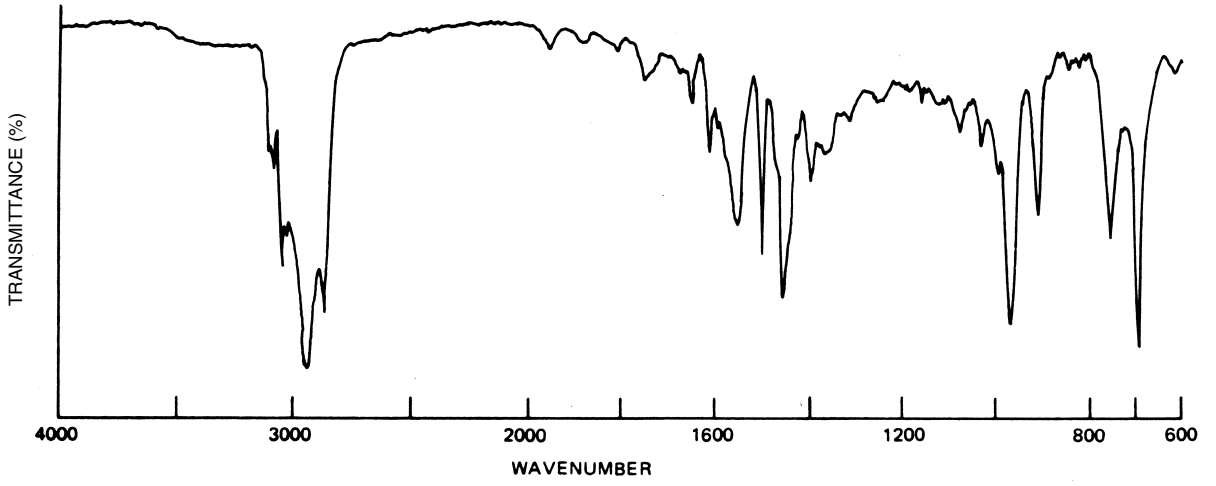
Butadiene-Styrene 75/25 Rubber (Emulsion-Polymerized Latex)



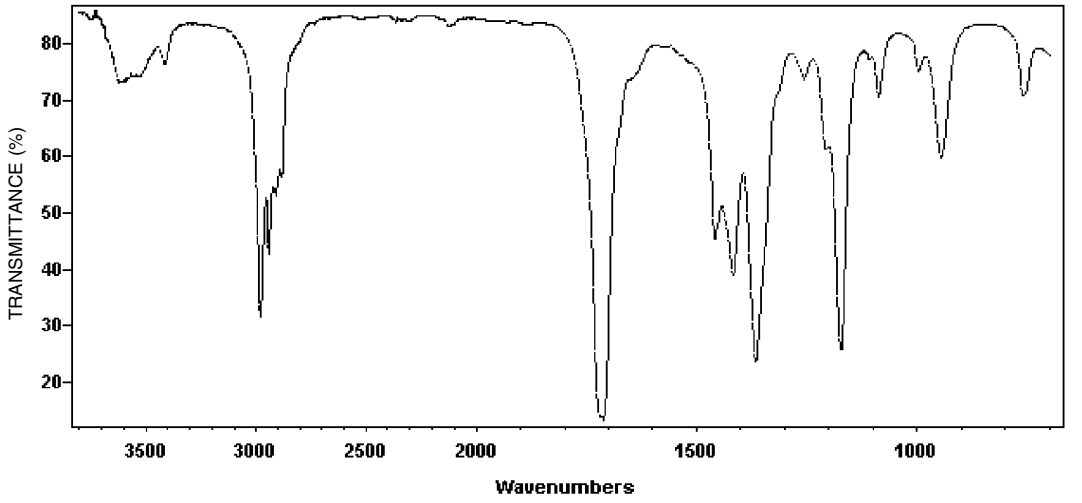
Butadiene-Styrene 75/25 Rubber (Emulsion-Polymerized Solid)



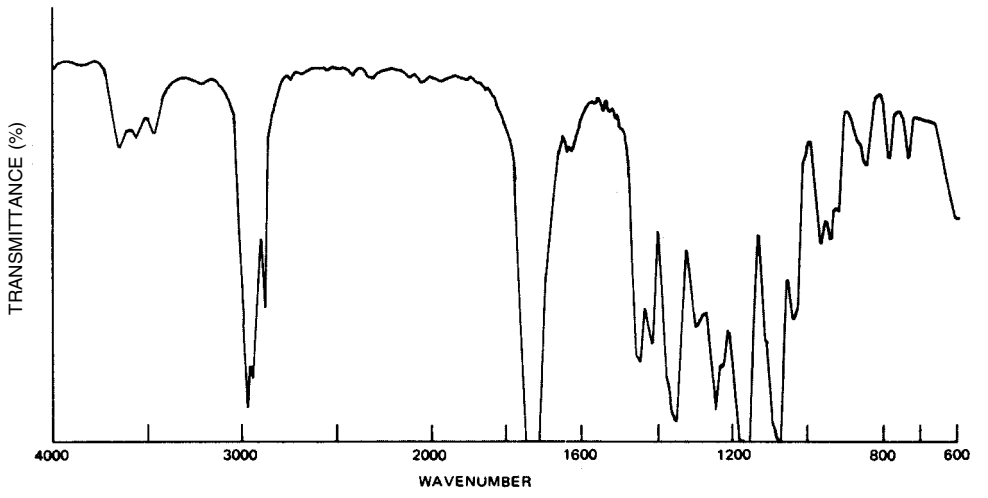
Butadiene-Styrene 50/50 Rubber (Latex)



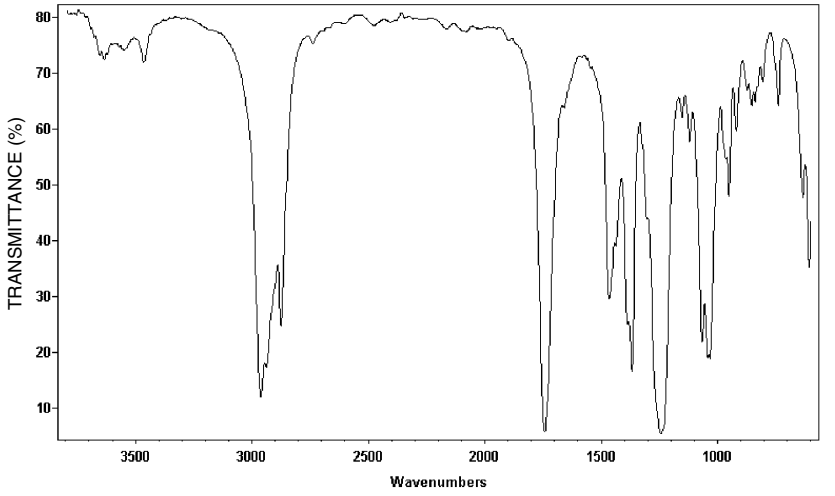
2-Butanone View Flavor Chemical



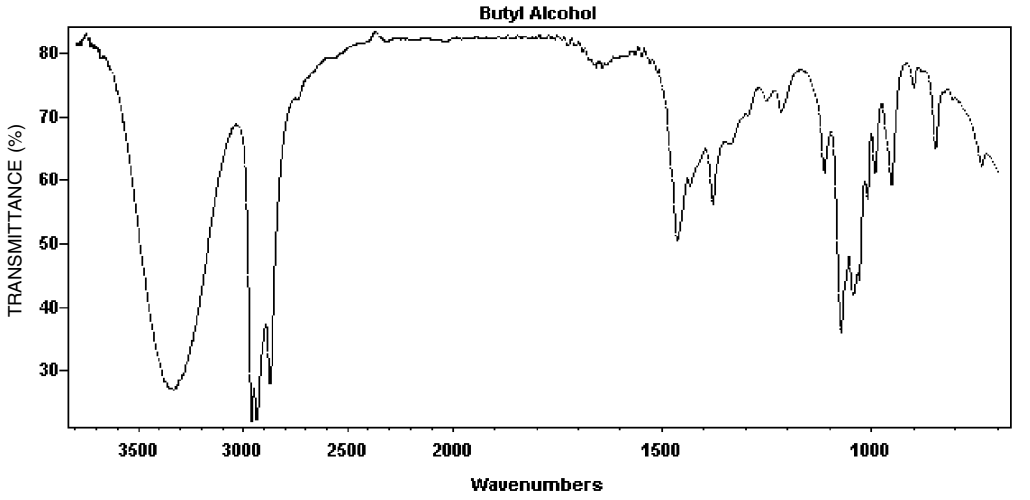
Butan-3-one-2-yl Butanoate View Flavor Chemical



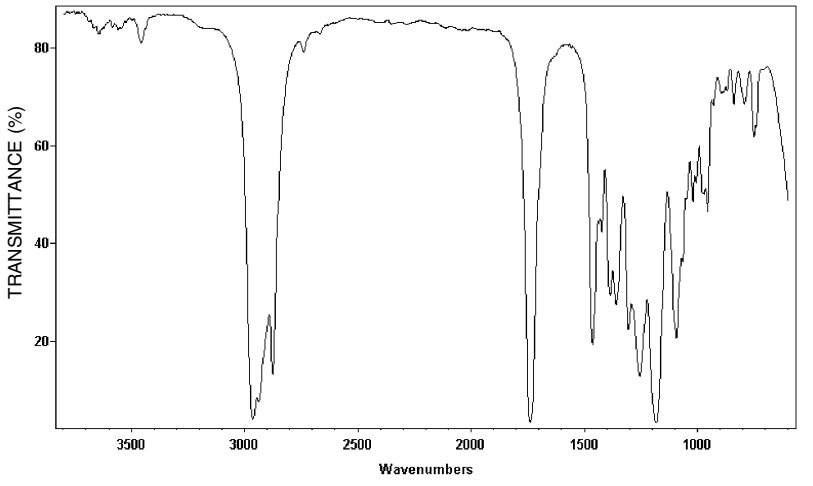
Butyl Acetate View Flavor Chemical



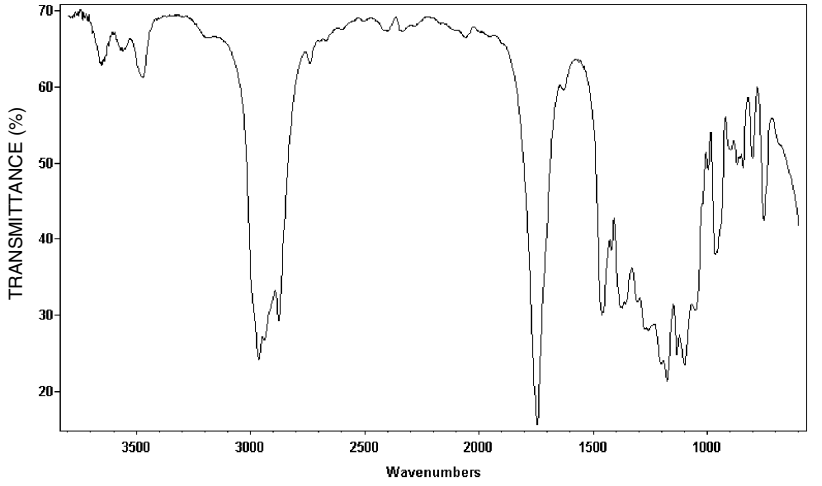
Butyl Alcohol View Flavor Chemical



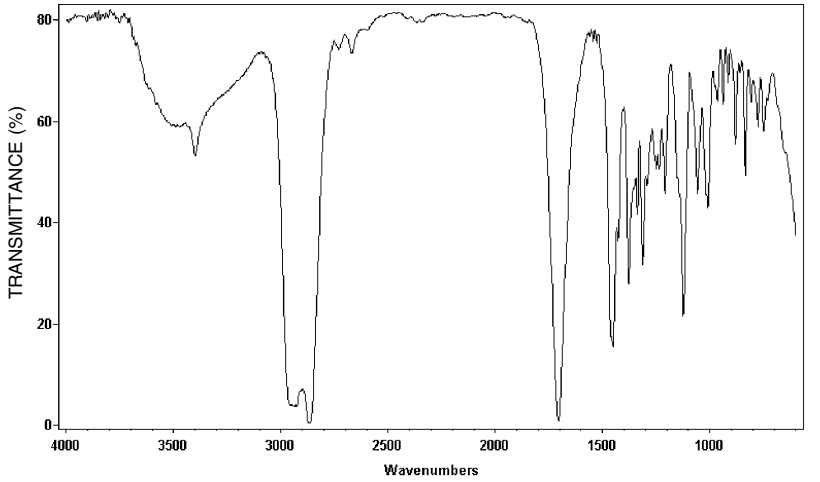
Butyl Butyrate View Flavor Chemical



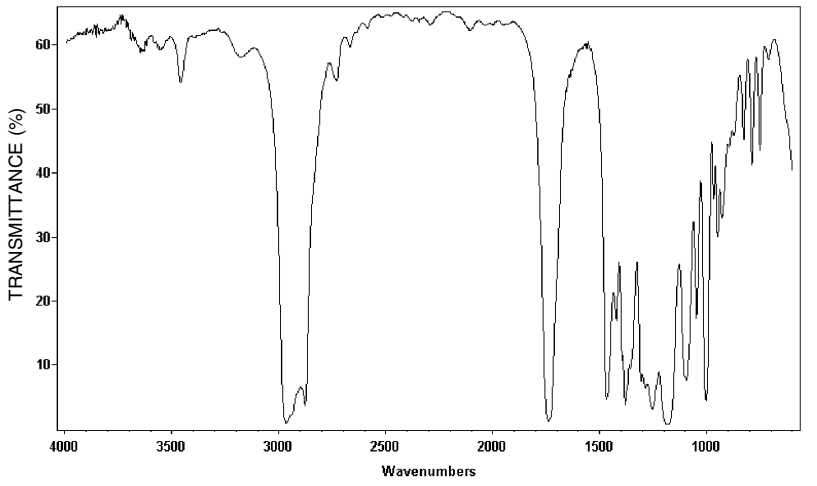
Butyl Butyryllactate View Flavor Chemical



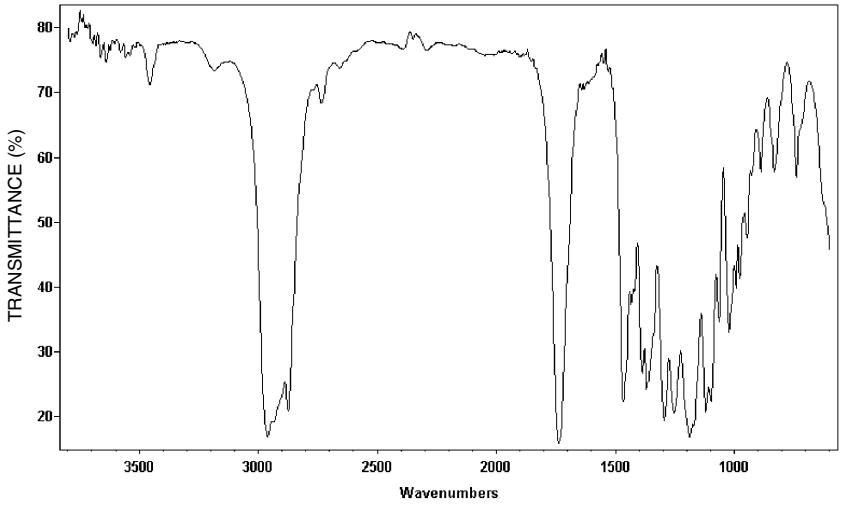
2-*sec*-Butyl Cyclohexanone View Flavor Chemical



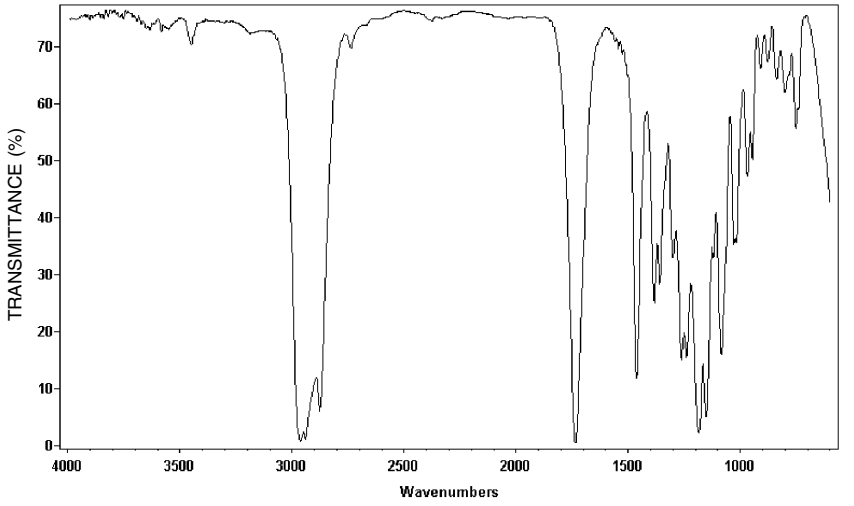
Butyl Isobutyrate View Flavor Chemical



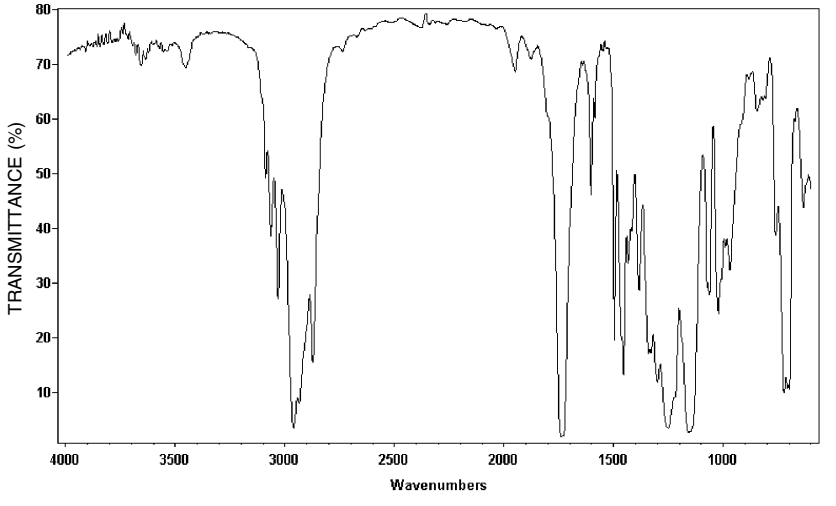
Butyl Isovalerate View Flavor Chemical



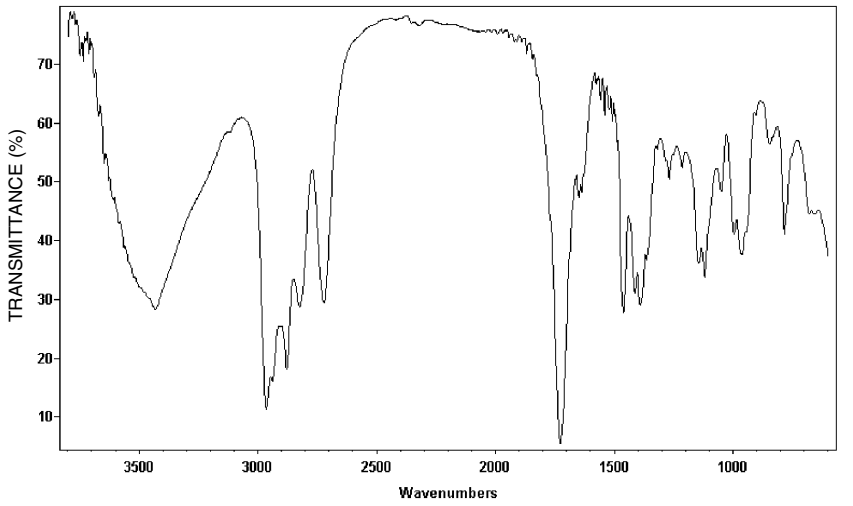
Butyl 2-Methyl Butyrate View Flavor Chemical



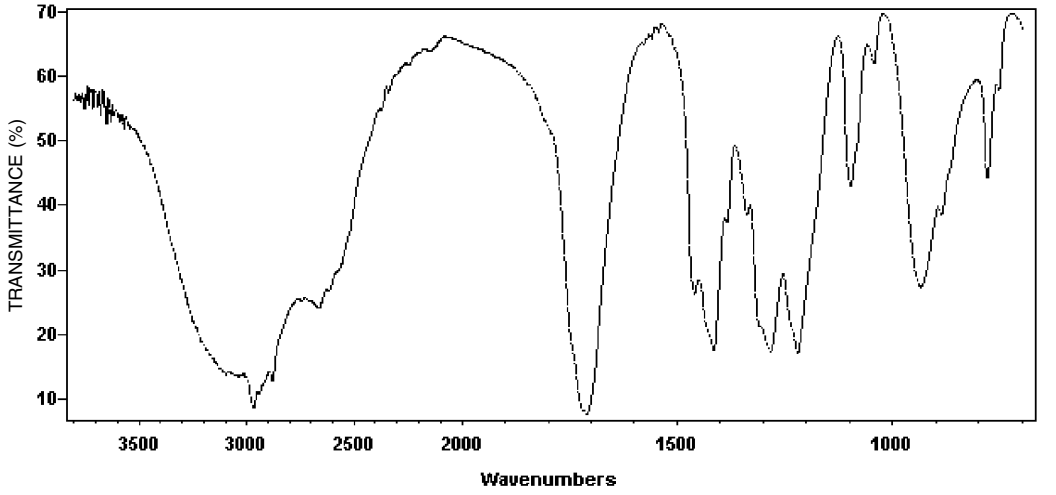
Butyl Phenylacetate View Flavor Chemical



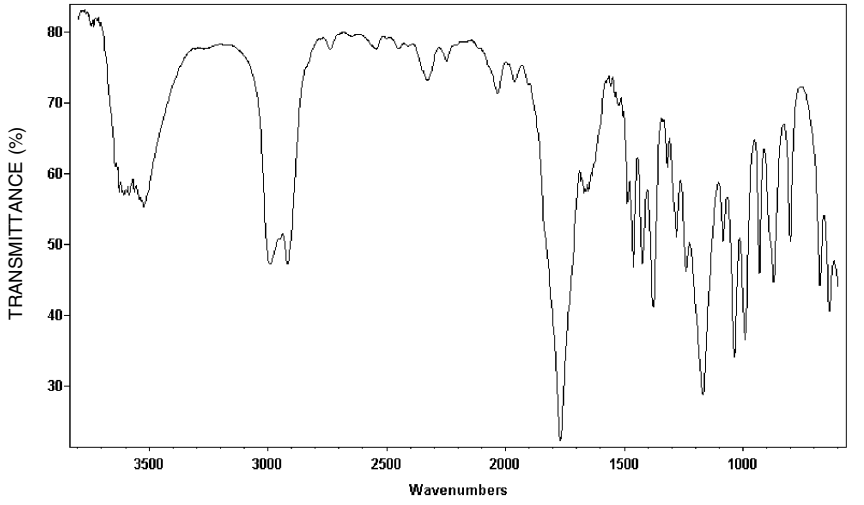
Butyraldehyde View Flavor Chemical



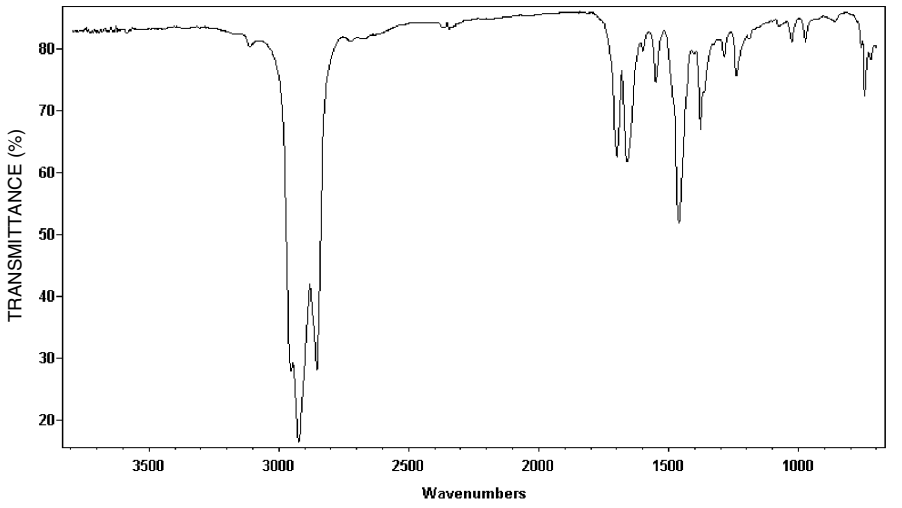
Butyric Acid View Flavor Chemical



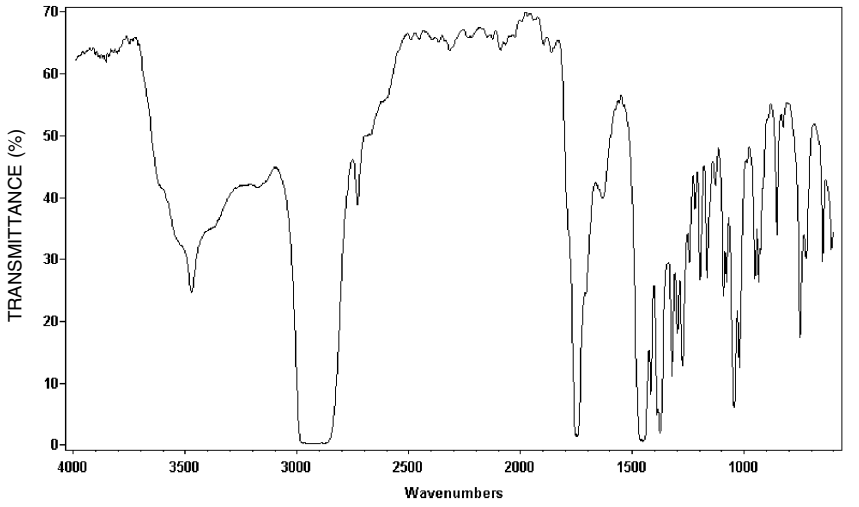
γ-Butyrolactone View Flavor Chemical



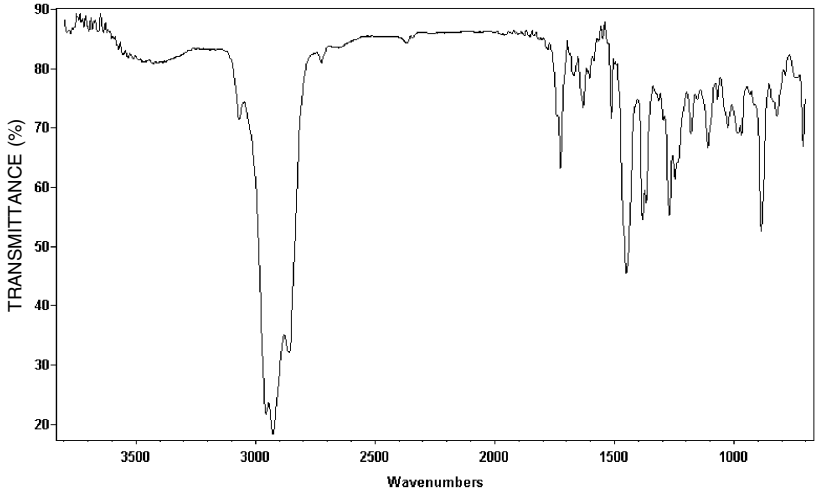
Caffeine (Mineral Oil Mull) View Monograph



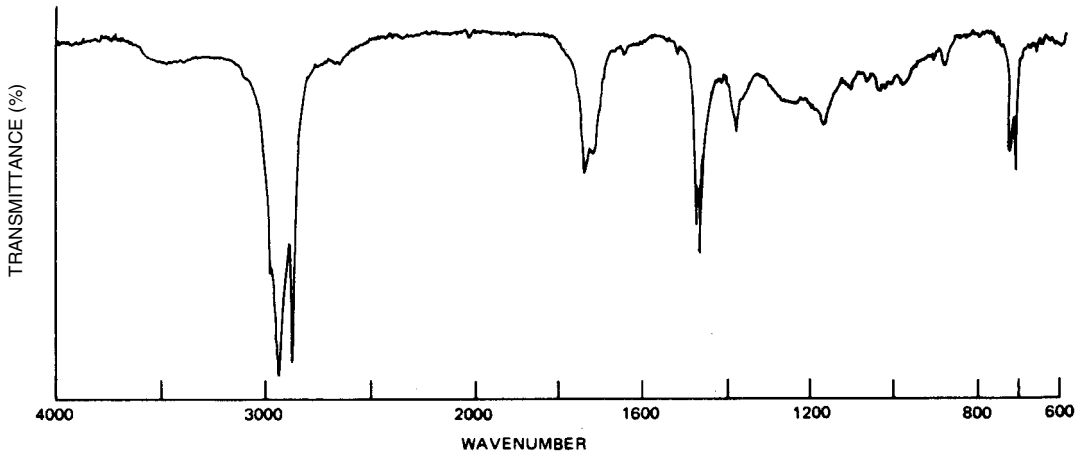
***d*-Camphor** View Flavor Chemical



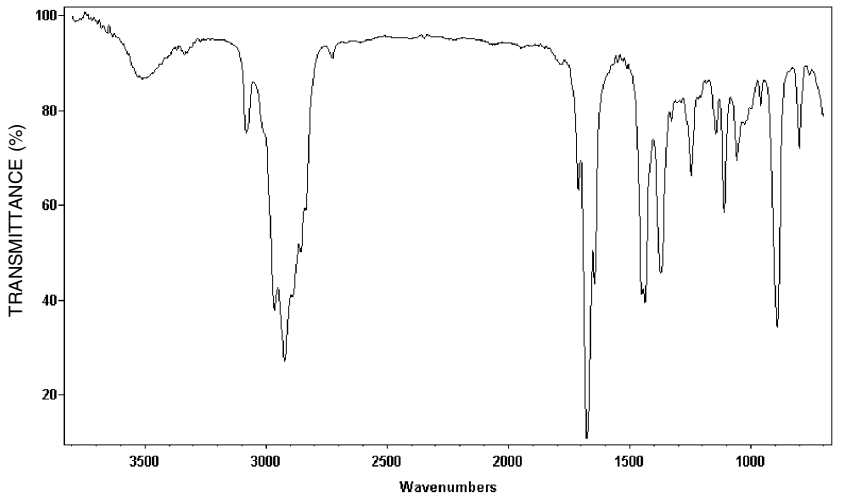
Cananga Oil View Monograph



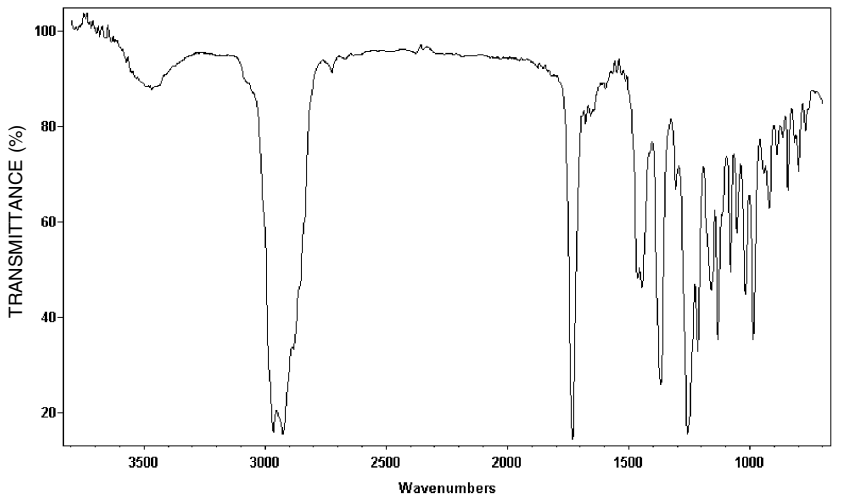
Candelilla Wax View Monograph



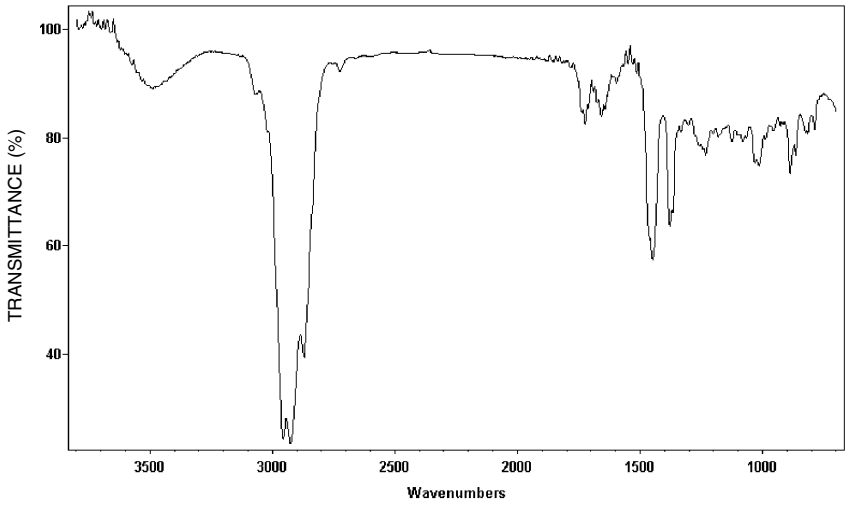
Caraway Oil View Monograph



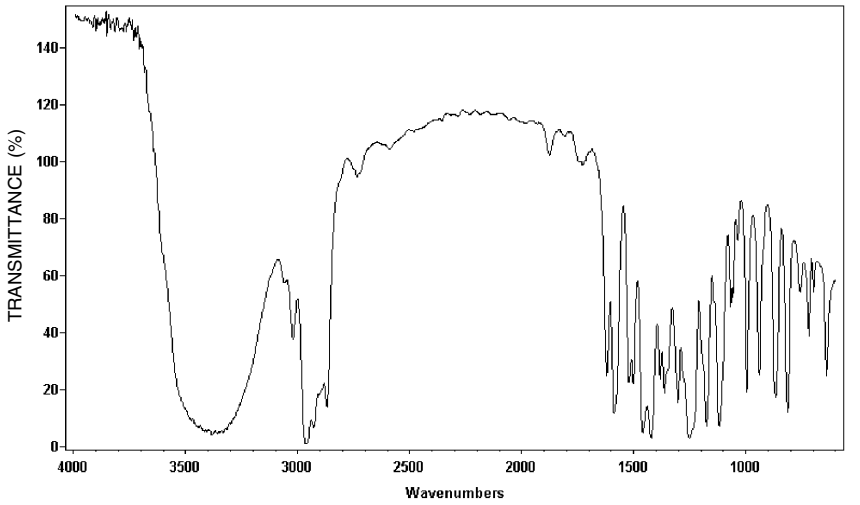
Cardamom Oil View Monograph



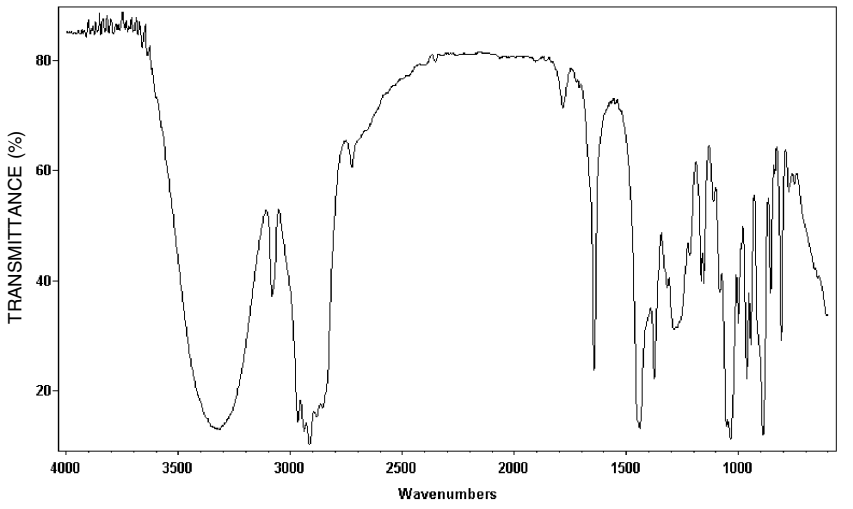
Carrot Seed Oil View Monograph



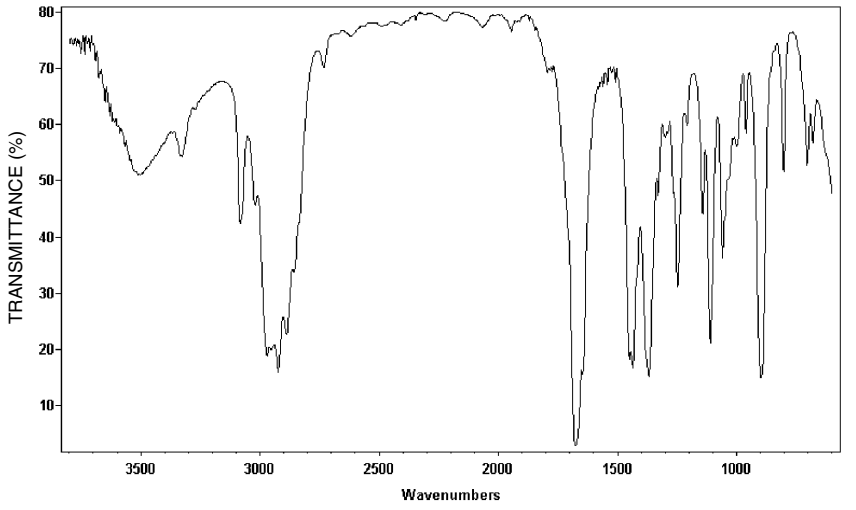
Carvacrol View Flavor Chemical



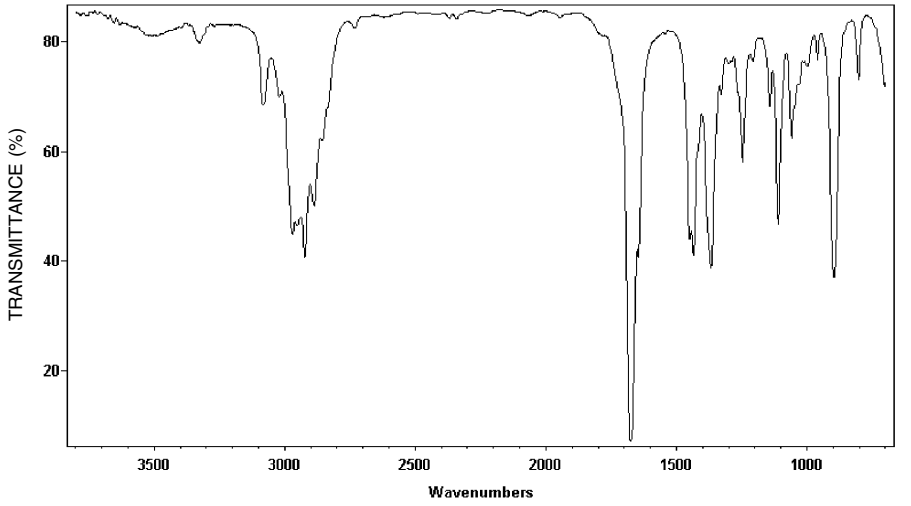
***l*-Carveol** View Flavor Chemical



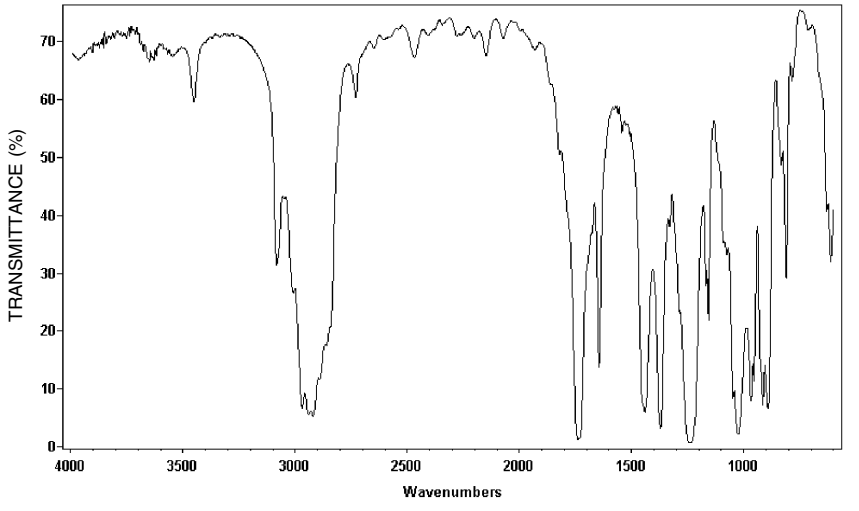
***d*-Carvone** **View Flavor Chemical**



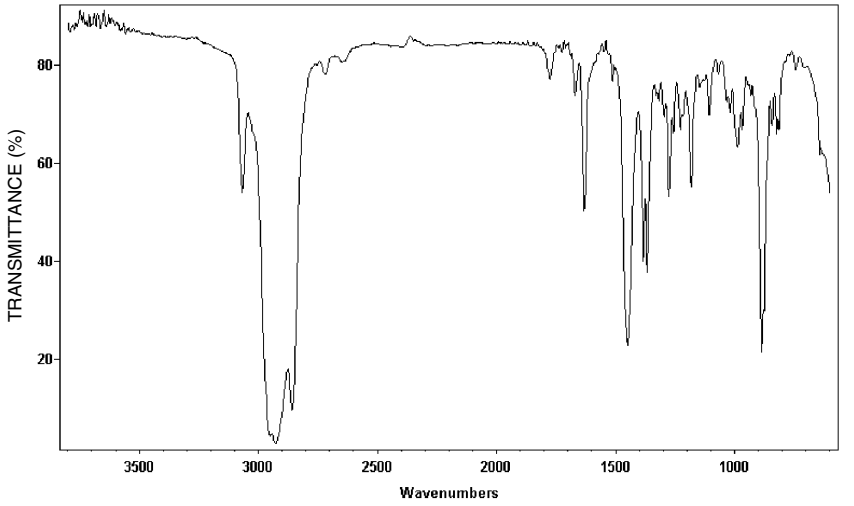
***l*-Carvone** **View Flavor Chemical**



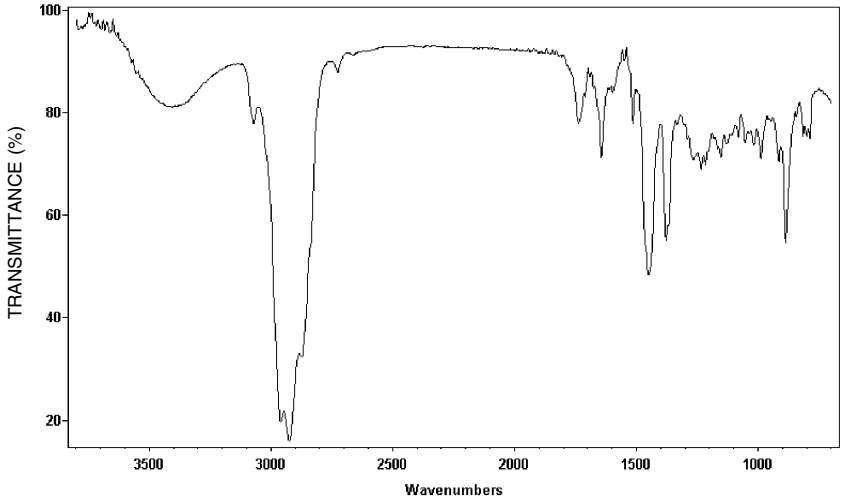
***l*-Carvyl Acetate** **View Flavor Chemical**



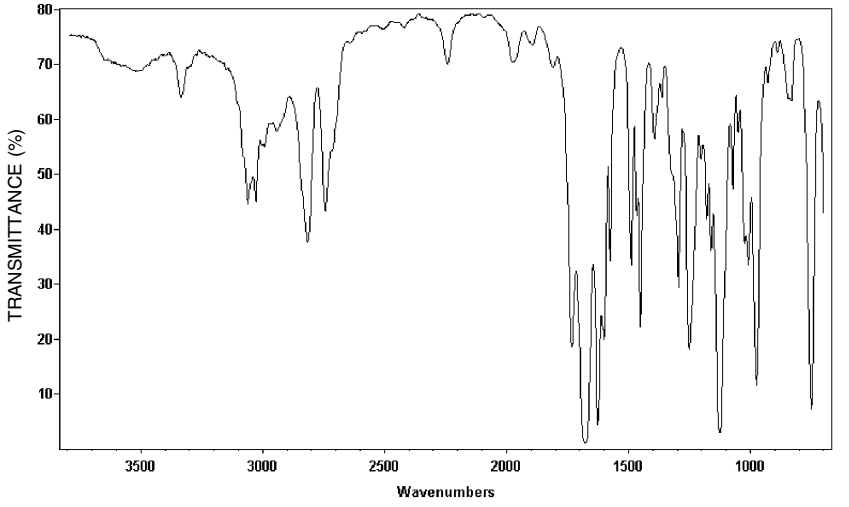
β-Caryophyllene View Flavor Chemical



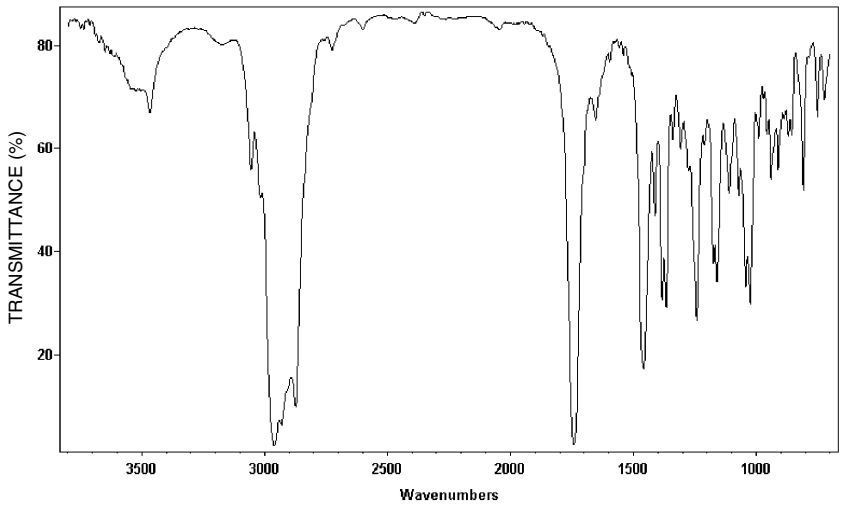
Cascarilla Oil View Monograph



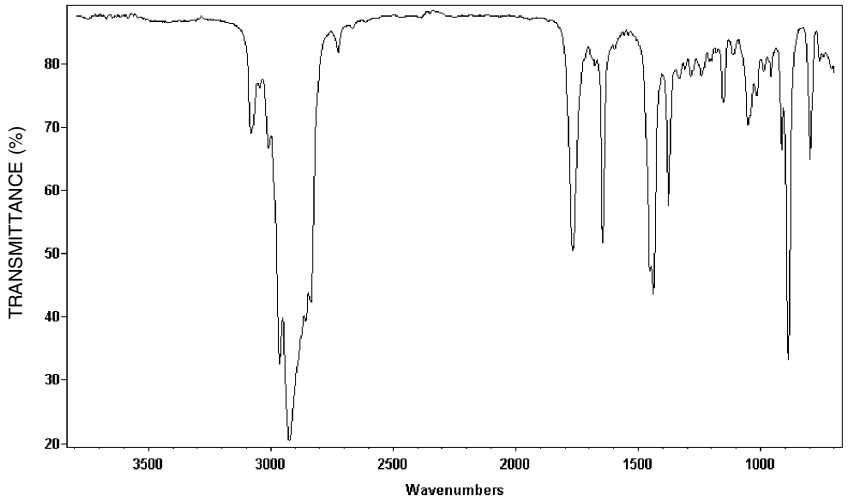
Cassia Oil View Monograph



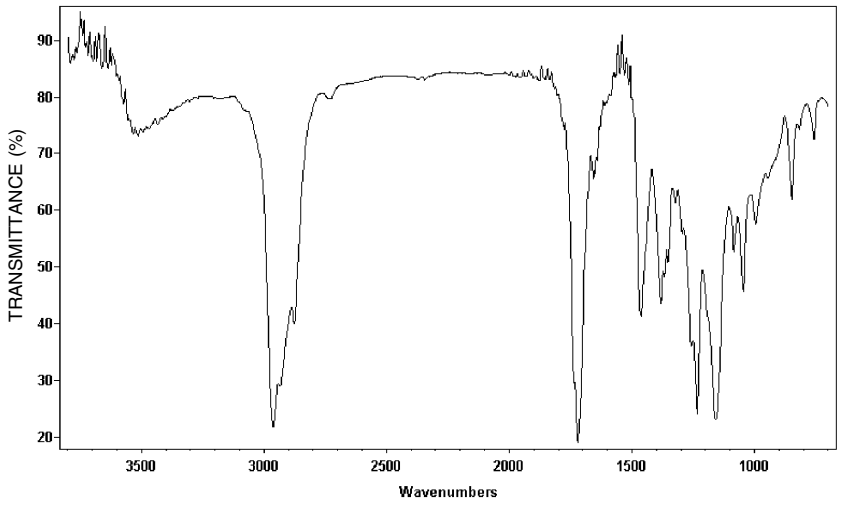
Cedar Leaf Oil View Monograph



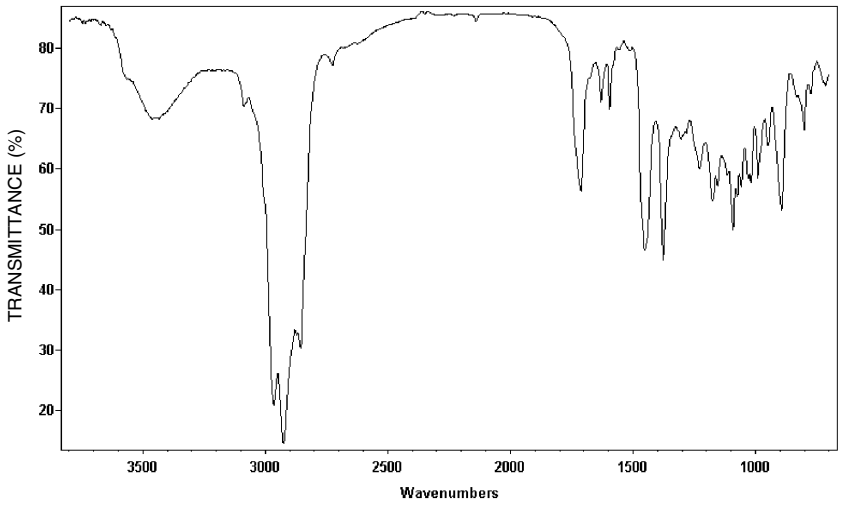
Celery Seed Oil View Monograph



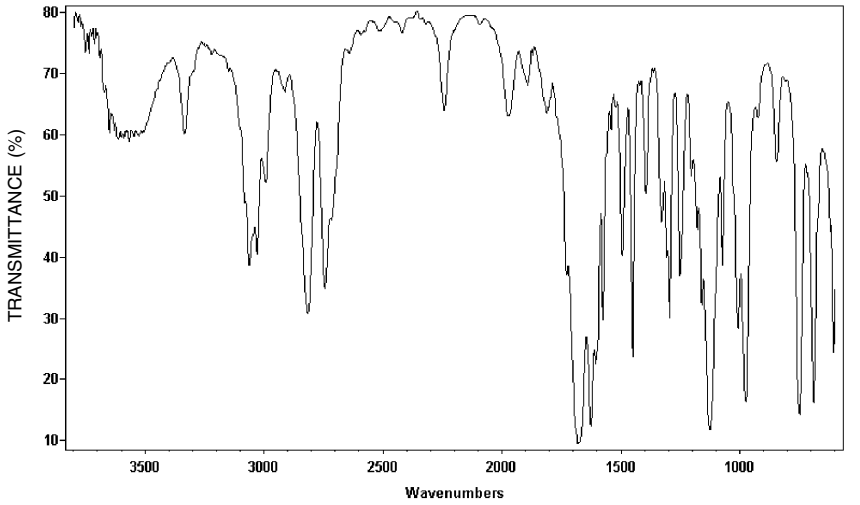
Chamomile Oil, English Type View Monograph



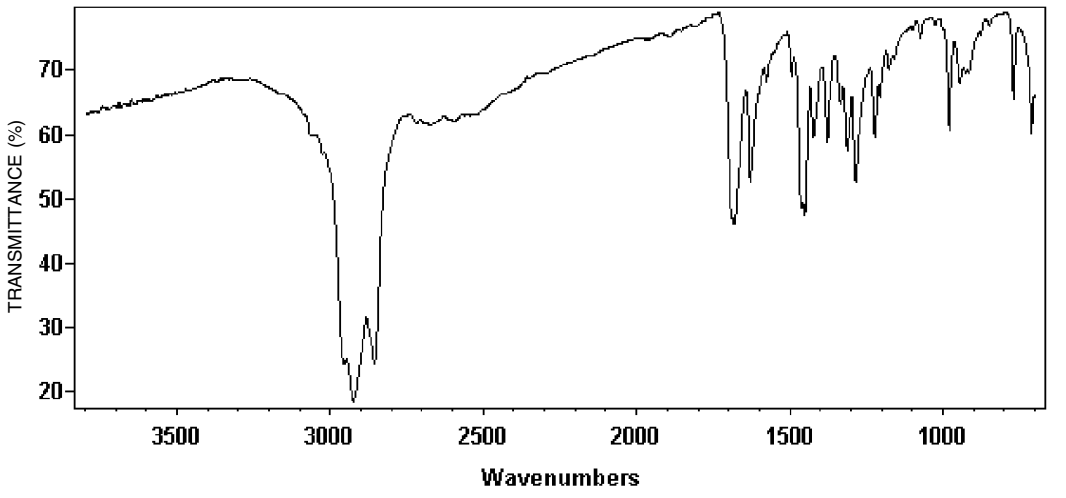
Chamomile Oil, German Type View Monograph



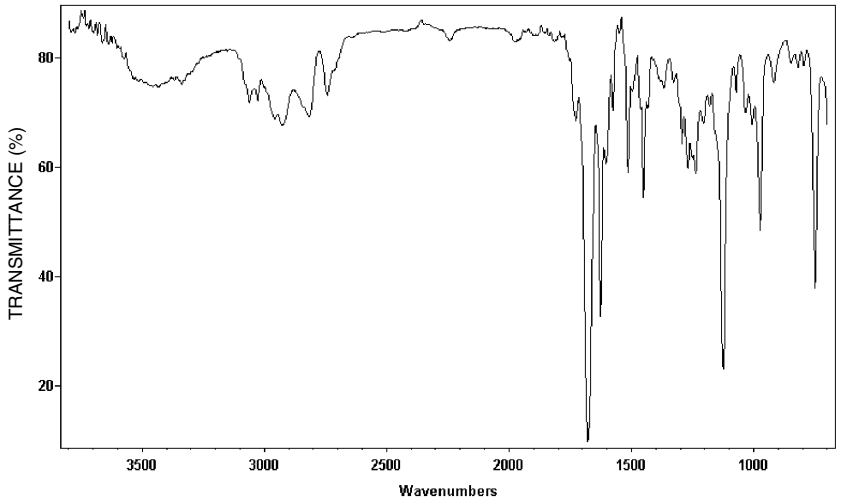
Cinnamaldehyde View Flavor Chemical



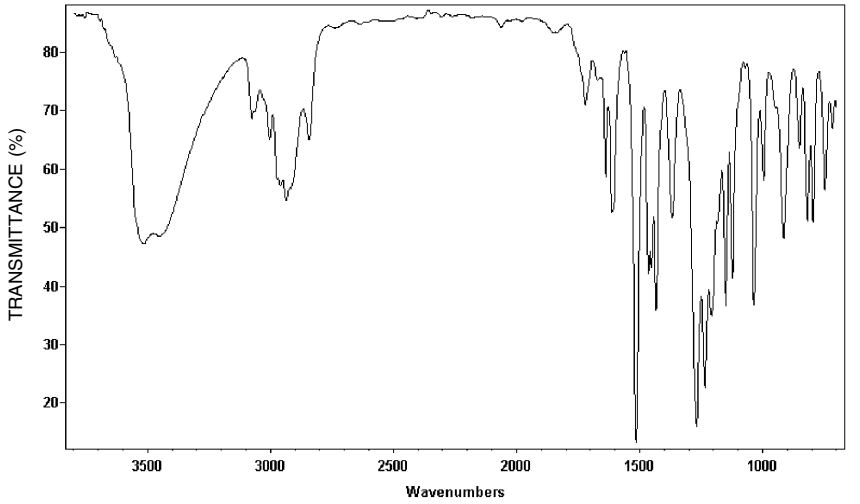
Cinnamic Acid View Flavor Chemical



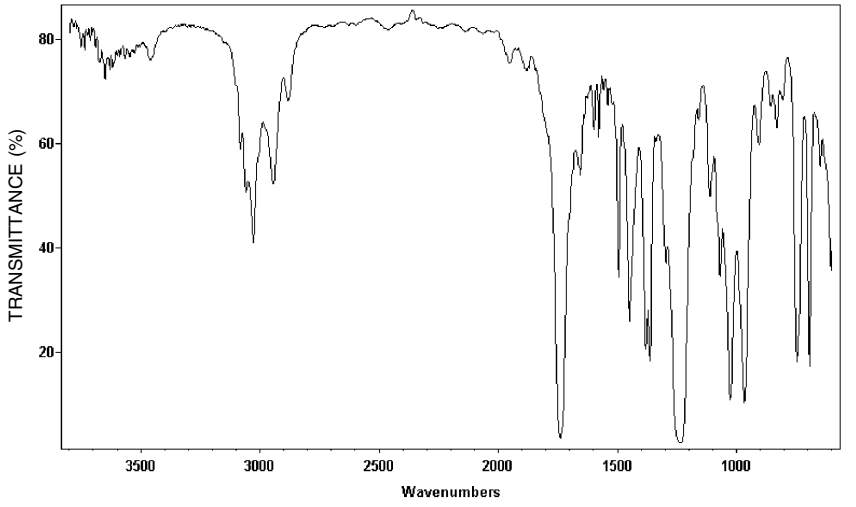
Cinnamon Bark Oil, Ceylon Type View Monograph



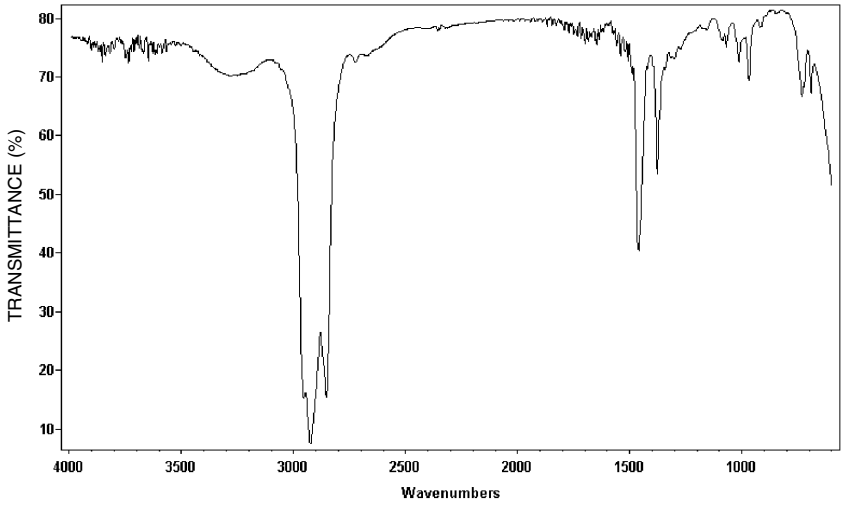
Cinnamon Leaf Oil View Monograph



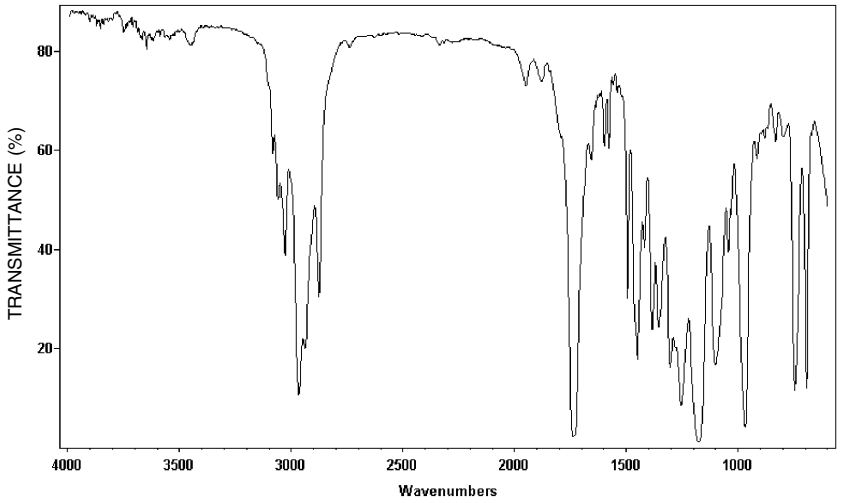
Cinnamyl Acetate View Flavor Chemical



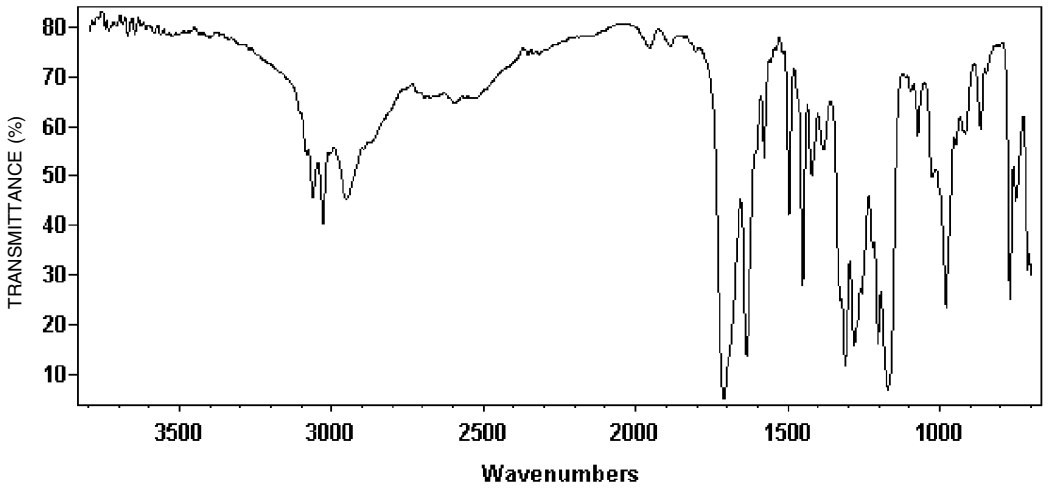
Cinnamyl Alcohol View Flavor Chemical



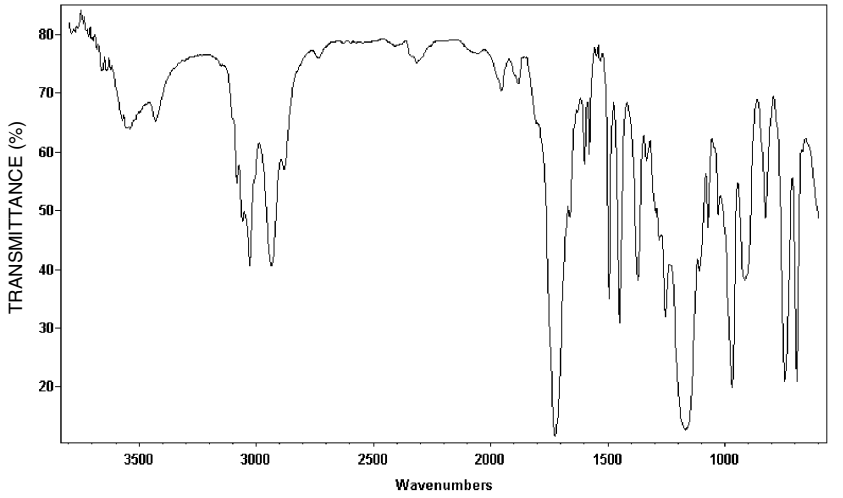
Cinnamyl Butyrate View Flavor Chemical



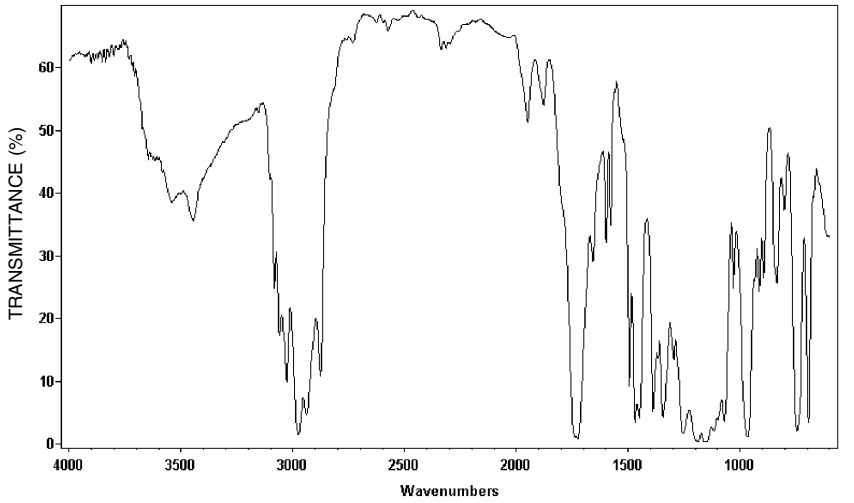
Cinnamyl Cinnamate View Flavor Chemical



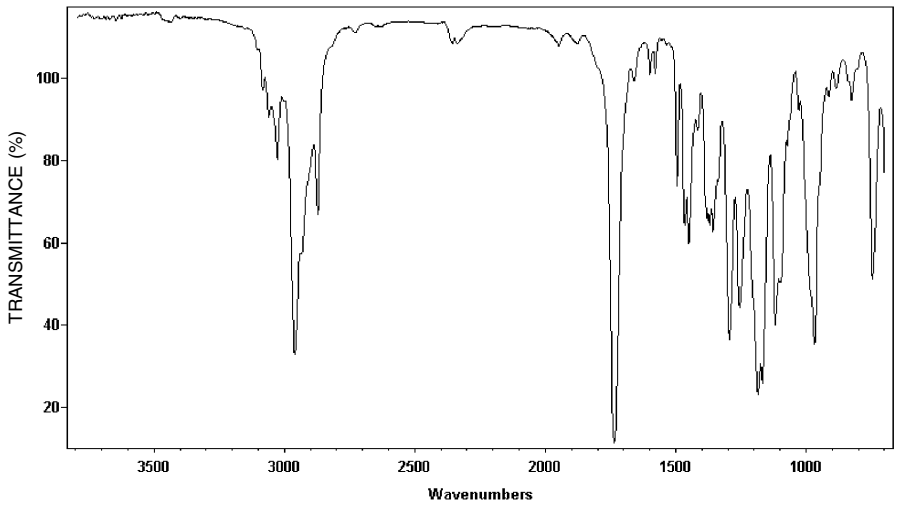
Cinnamyl Formate View Flavor Chemical



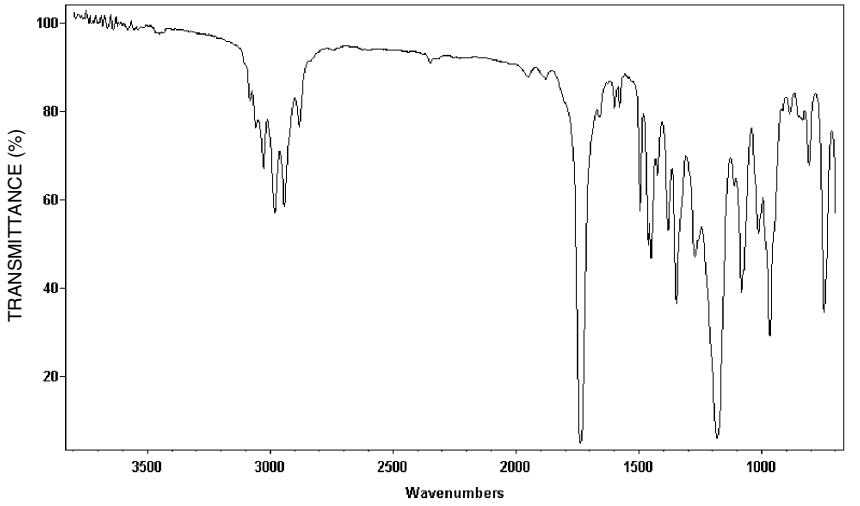
Cinnamyl Isobutyrate View Flavor Chemical



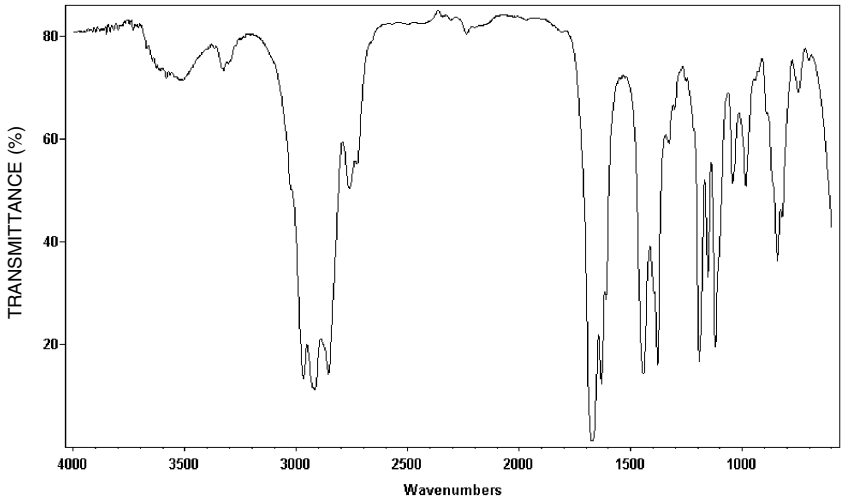
Cinnamyl Isovalerate View Flavor Chemical



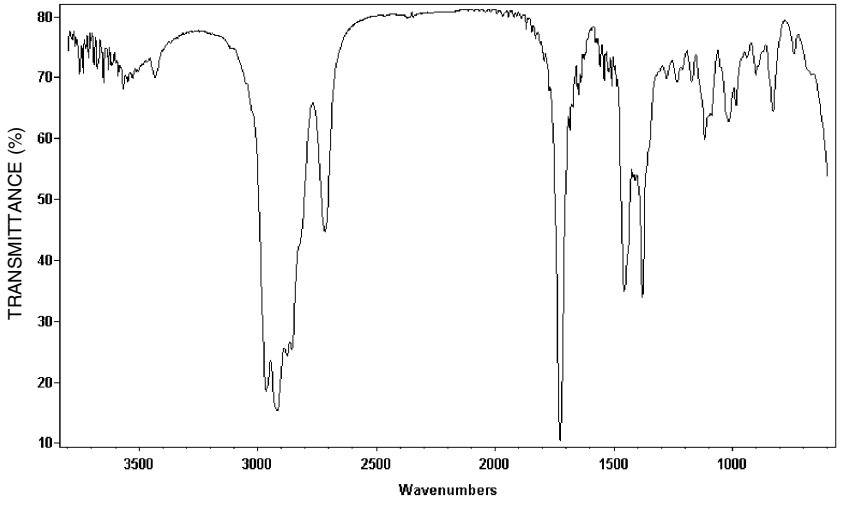
Cinnamyl Propionate View Flavor Chemical



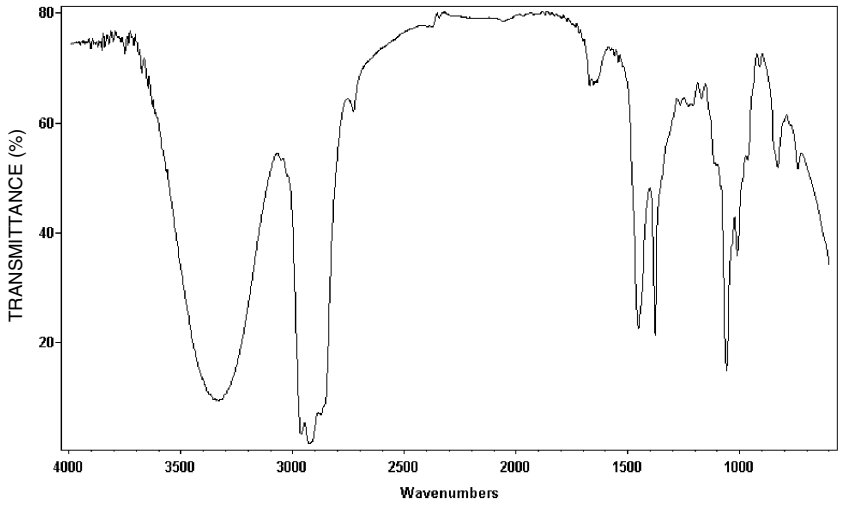
Citral View Flavor Chemical



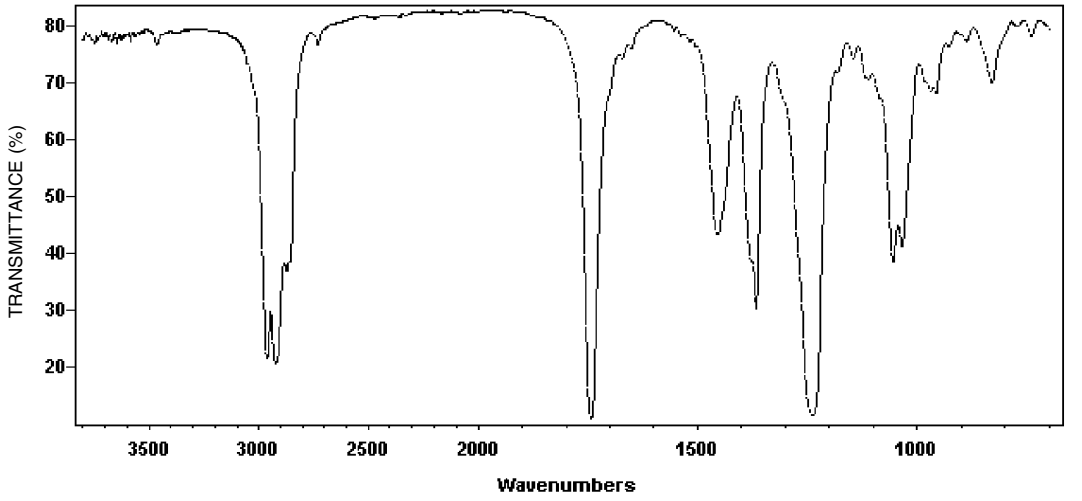
Citronellal View Flavor Chemical



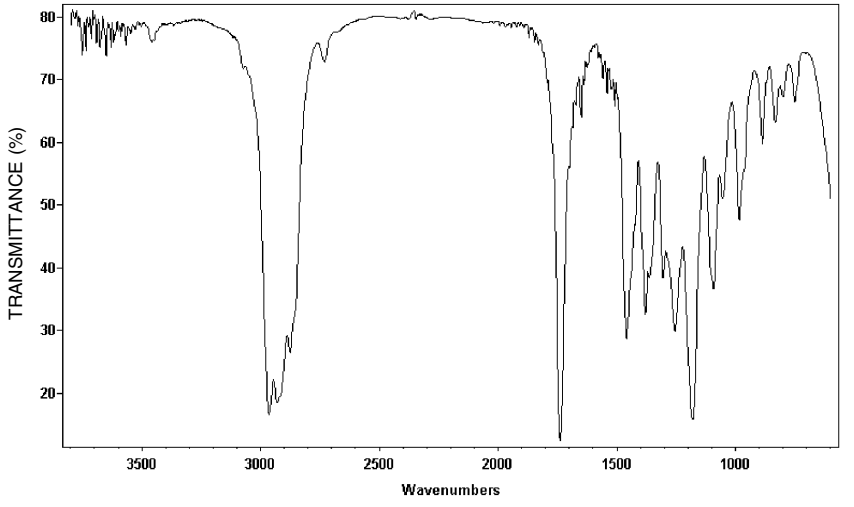
Citronellol View Flavor Chemical



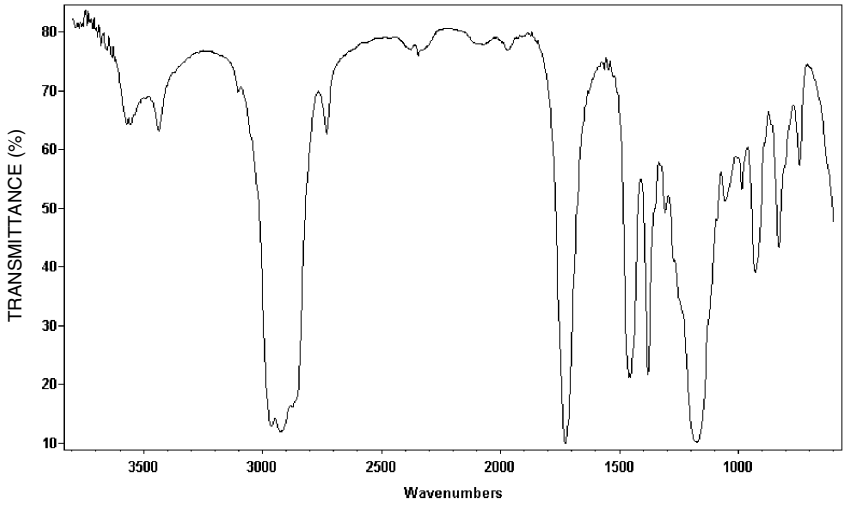
Citronellyl Acetate View Flavor Chemical



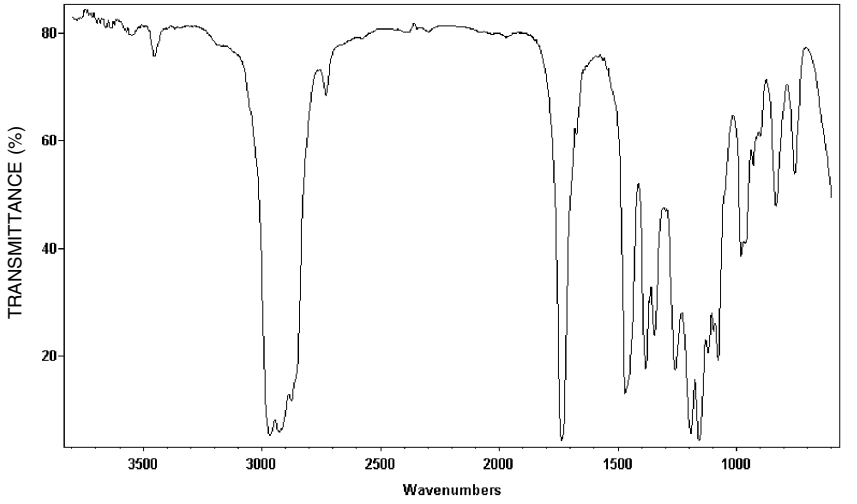
Citronellyl Butyrate View Flavor Chemical



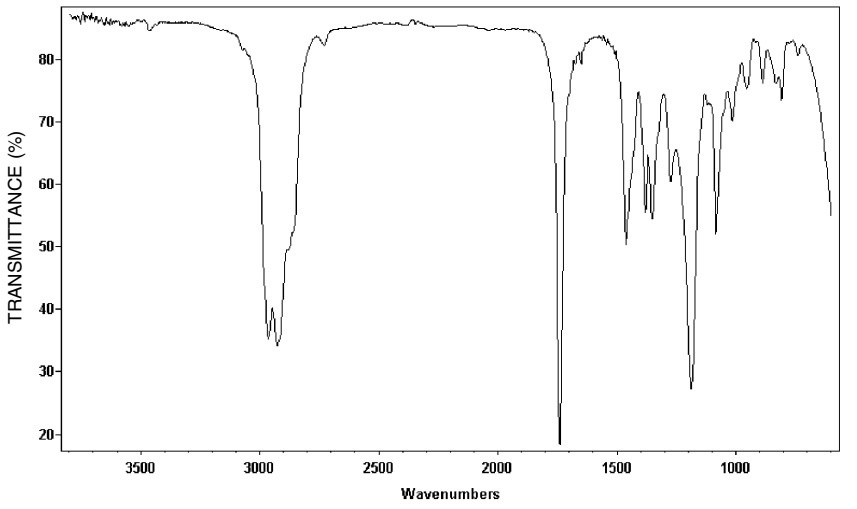
Citronellyl Formate View Flavor Chemical



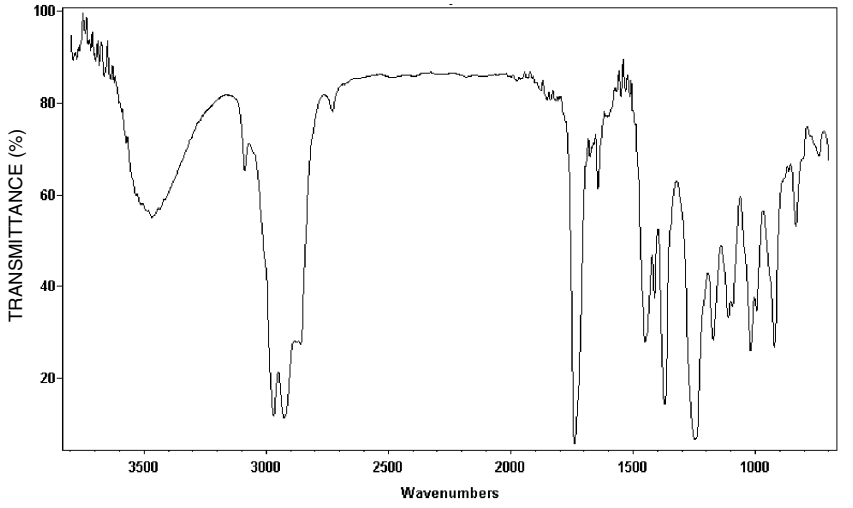
Citronellyl Isobutyrate View Flavor Chemical



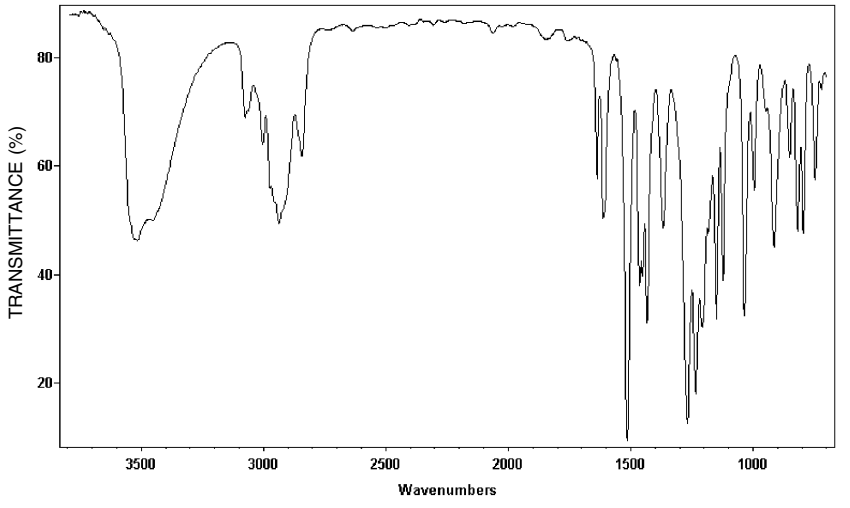
Citronellyl Propionate View Flavor Chemical



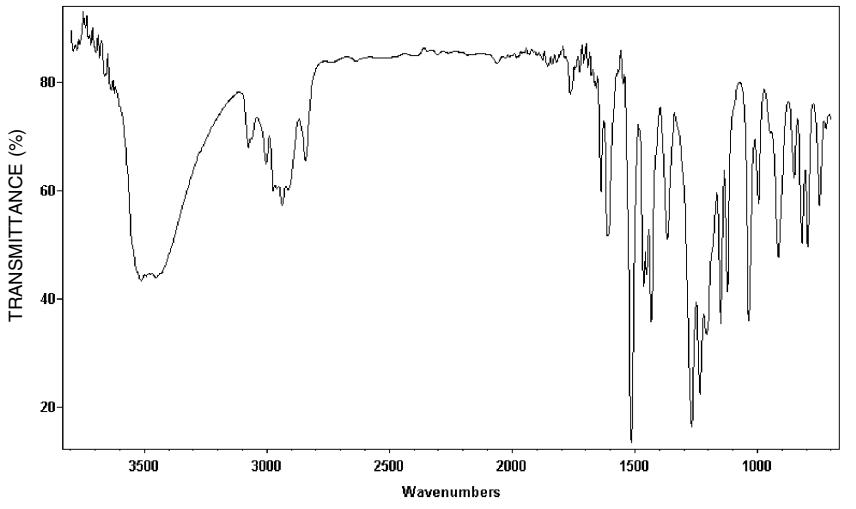
Clary Oil View Monograph



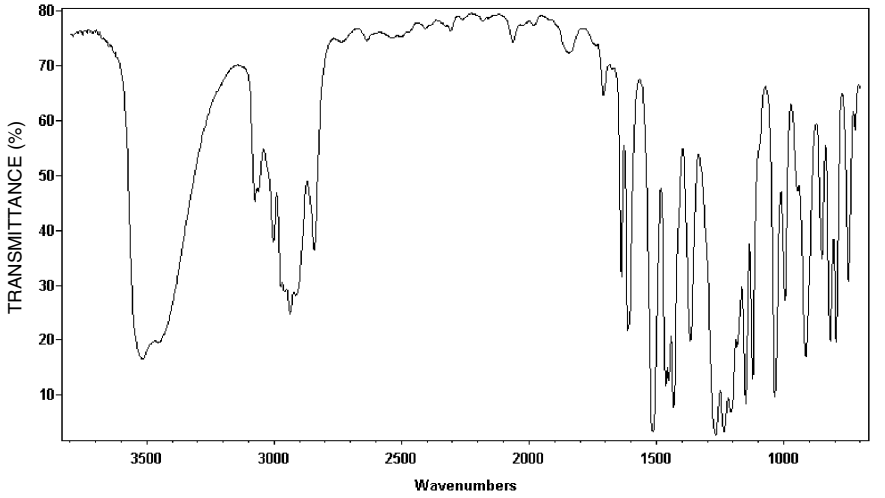
Clove Leaf Oil View Monograph



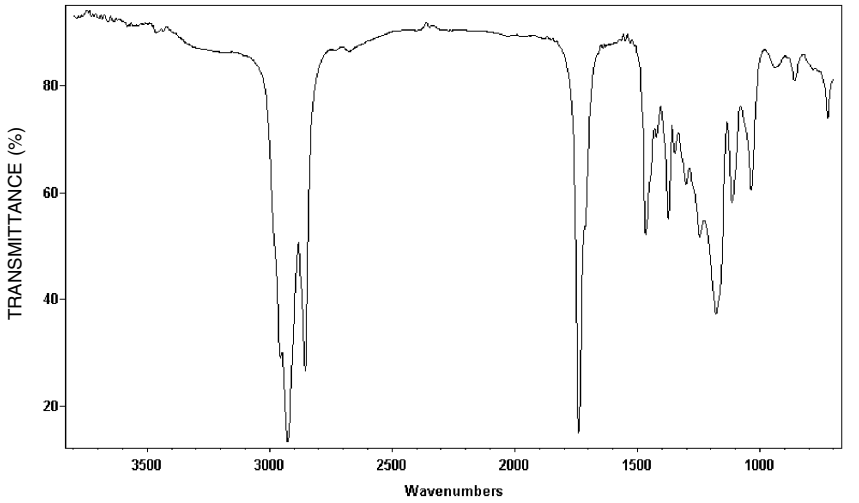
Clove Oil View Monograph



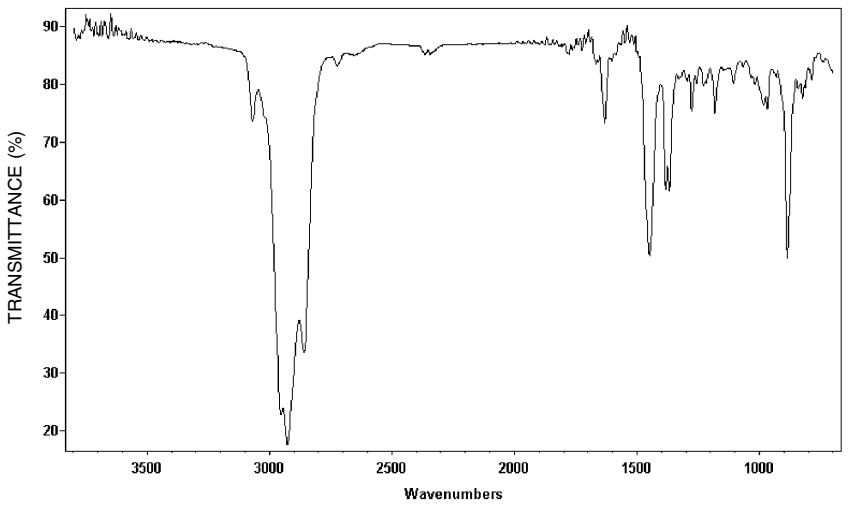
Clove Stem Oil View Monograph



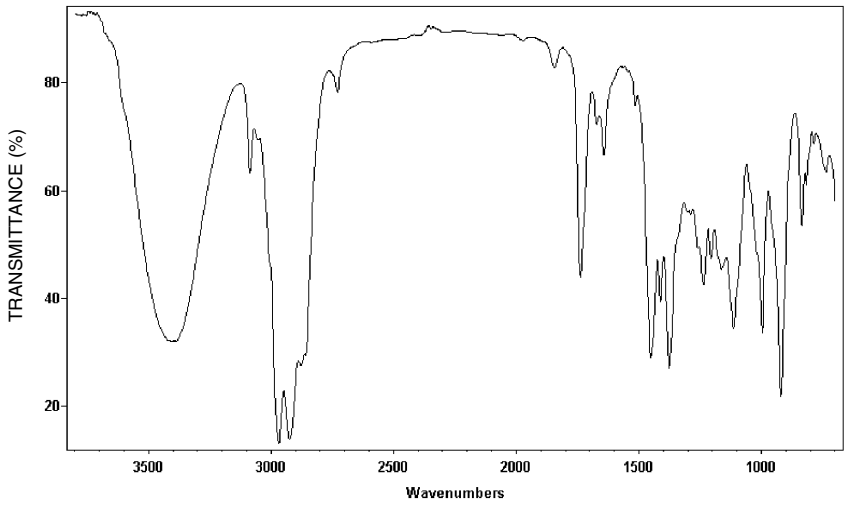
Cognac Oil, Green View Monograph



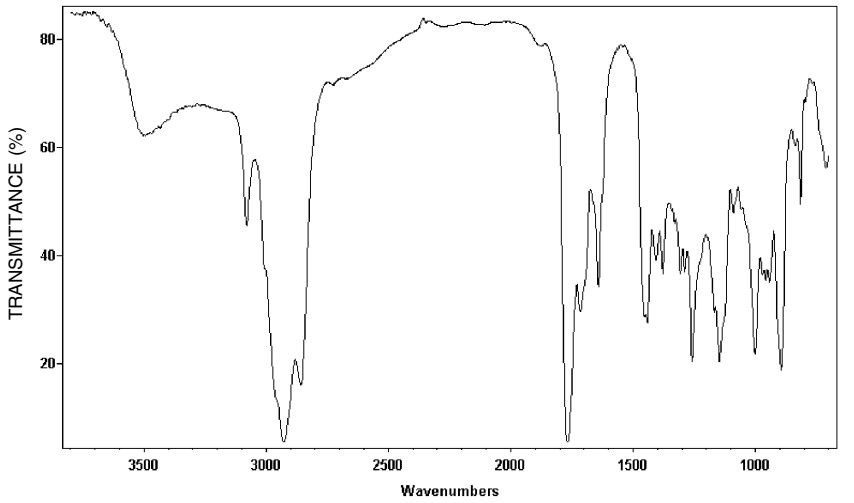
Copaiba Oil View Monograph



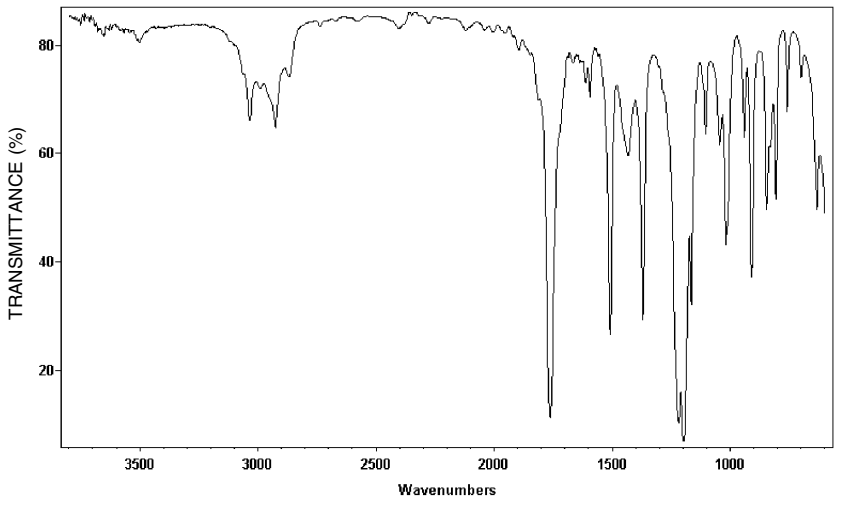
Coriander Oil View Monograph



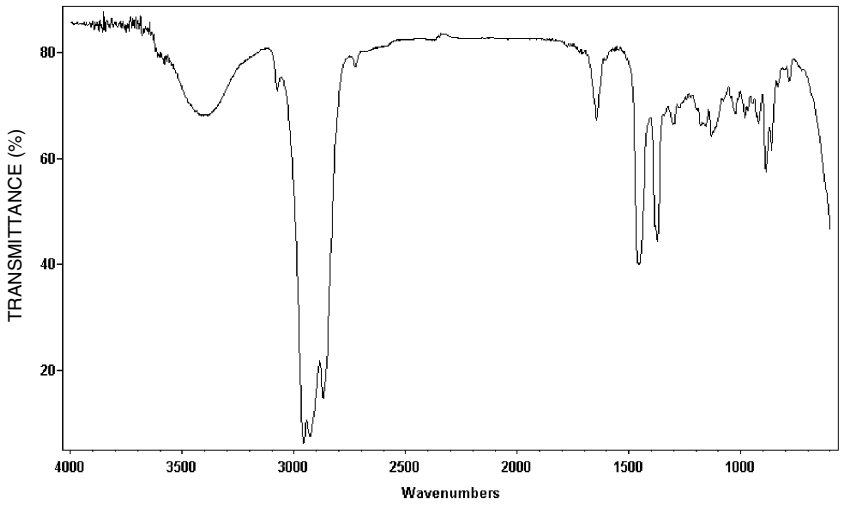
Costus Root Oil View Monograph



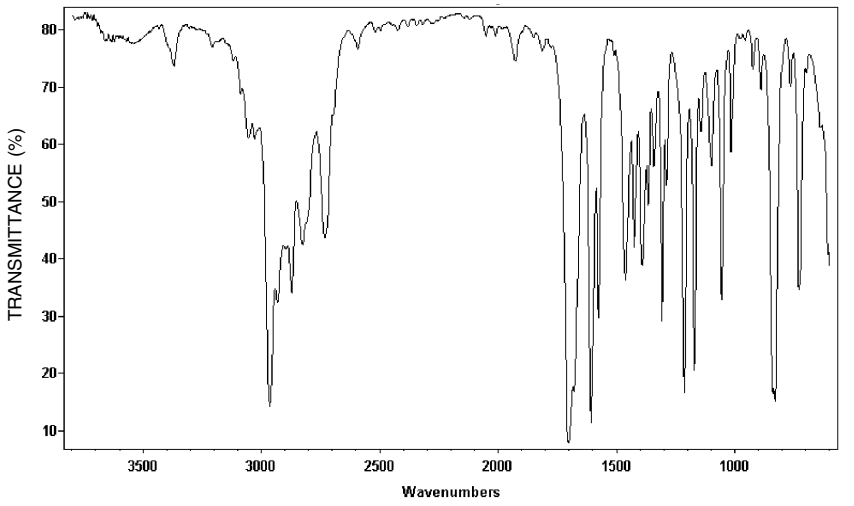
***p*-Cresyl Acetate** View Flavor Chemical



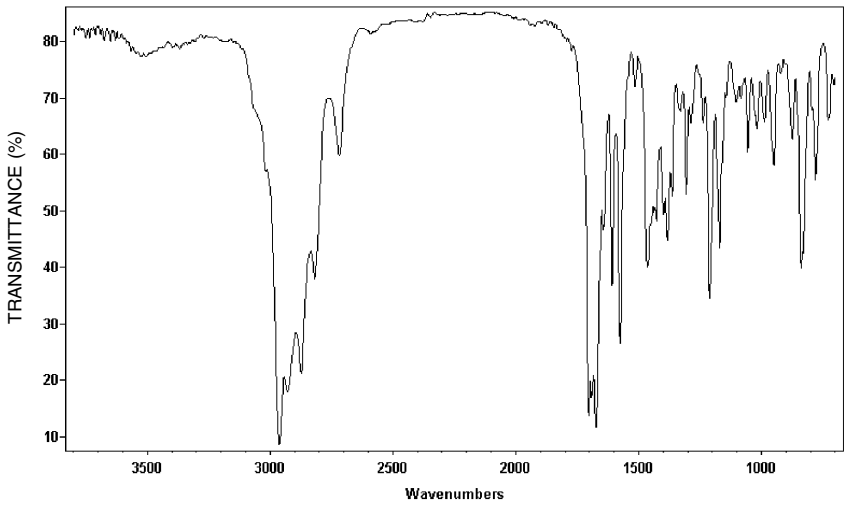
Cubeb Oil View Monograph



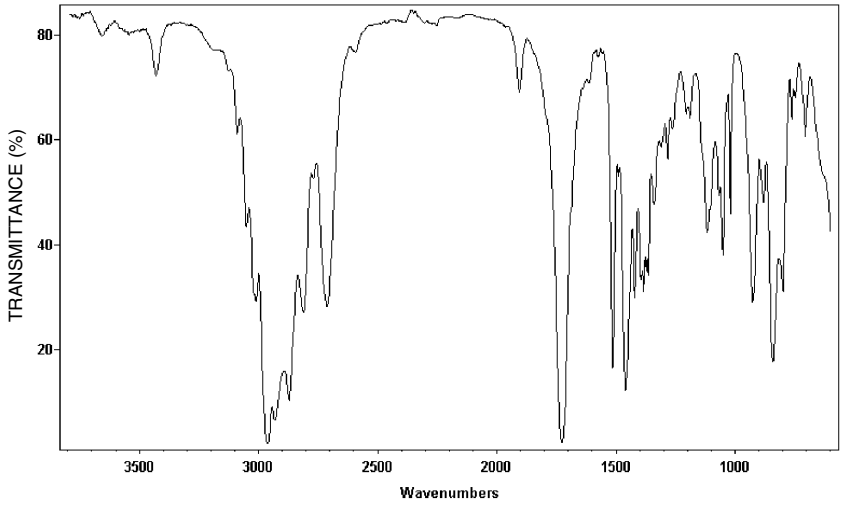
Cuminic Aldehyde View Flavor Chemical



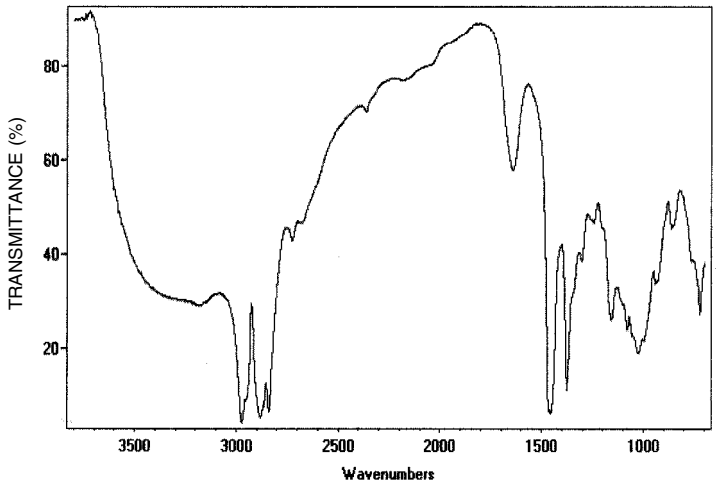
Cumin Oil View Monograph



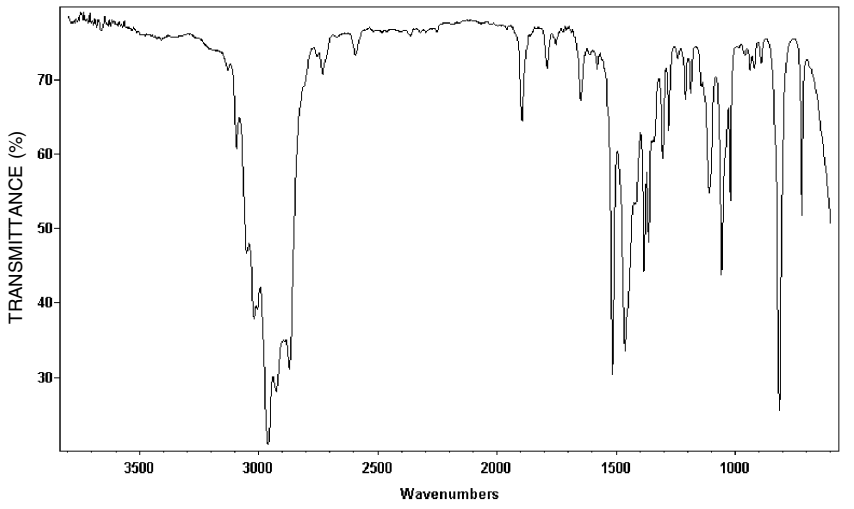
Cyclamen Aldehyde View Flavor Chemical



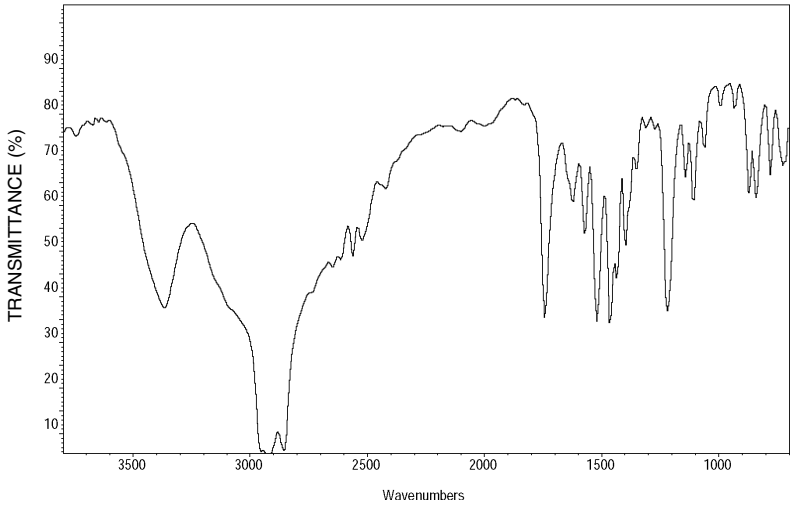
γ-Cyclodextrin (Mineral Oil Mull) View Monograph



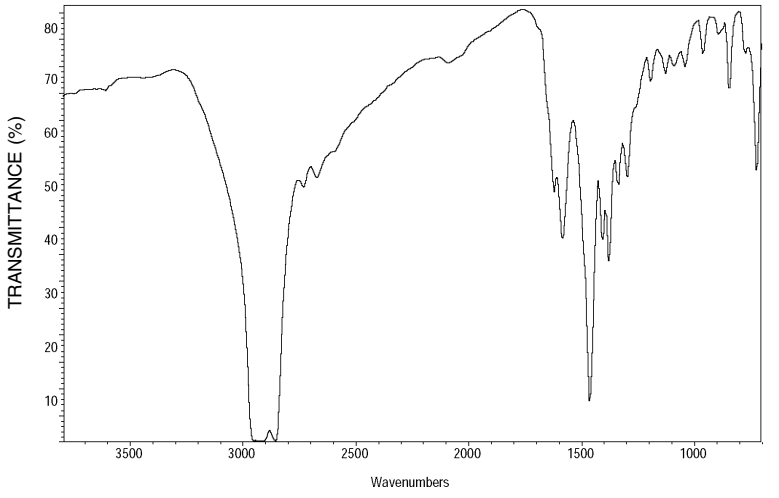
***p*-Cymene** View Flavor Chemical



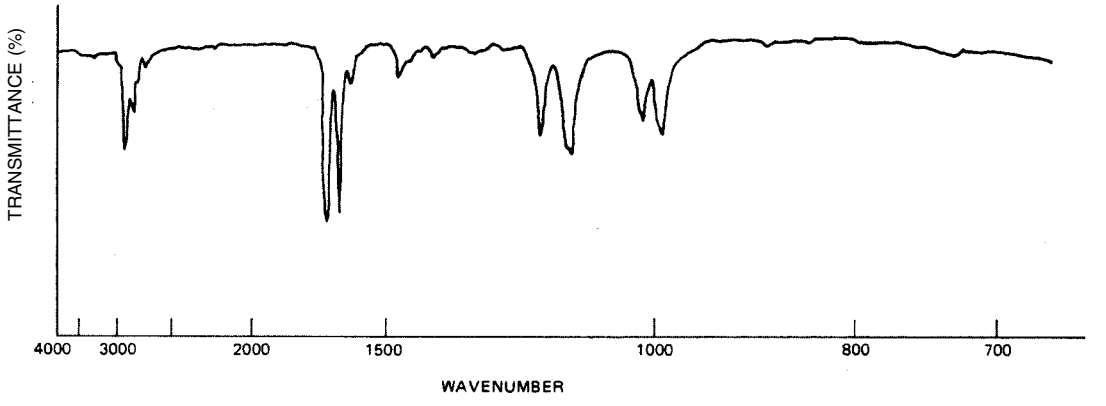
L-Cysteine Monohydrochloride View Monograph



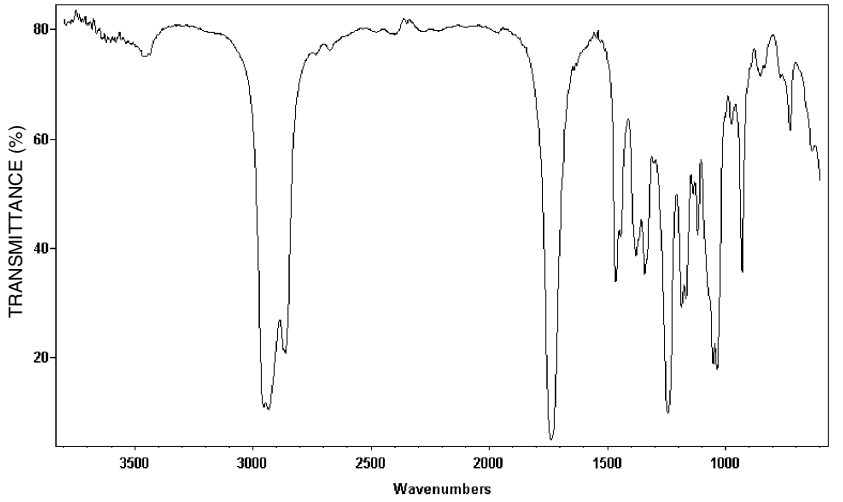
L-Cystine (Mineral Oil Mull) View Monograph



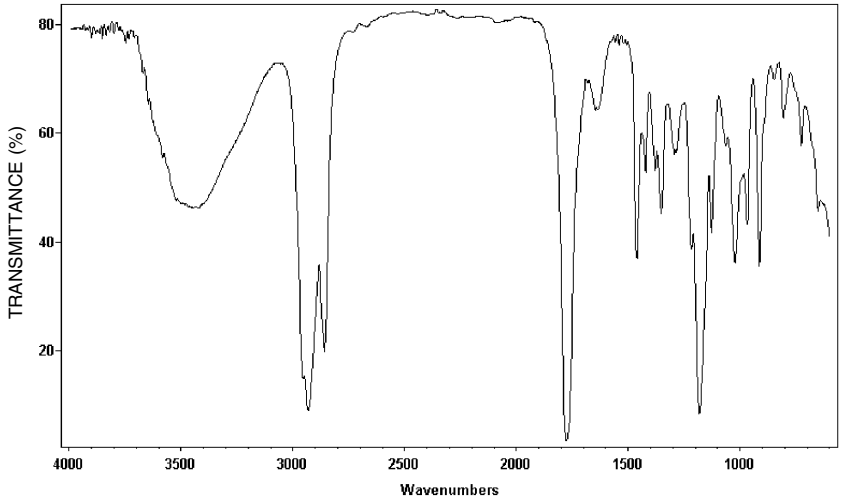
(*E*),(*E*)-2,4-Decadienal View Flavor Chemical



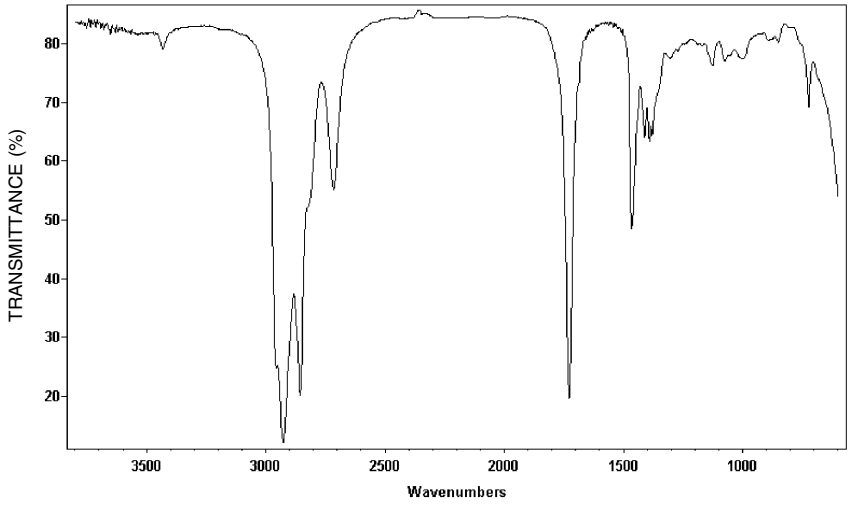
δ-Decalactone **View Flavor Chemical**



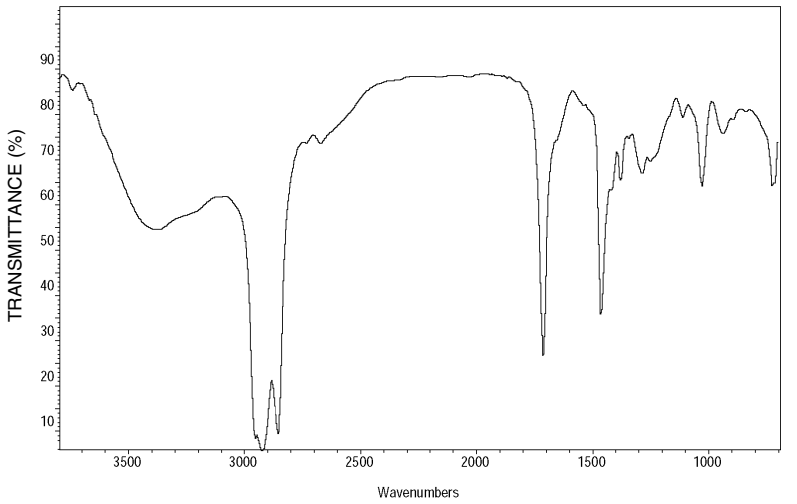
γ-Decalactone **View Flavor Chemical**



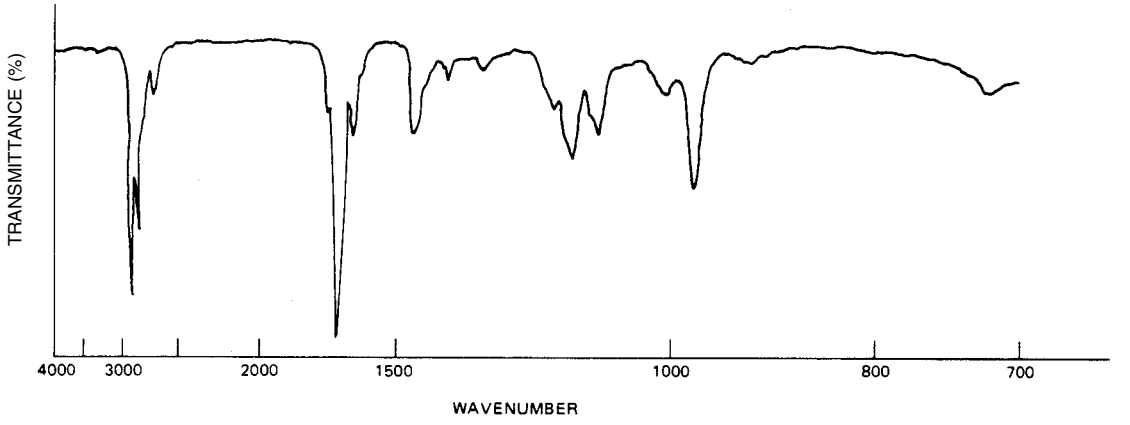
Decanal **View Flavor Chemical**



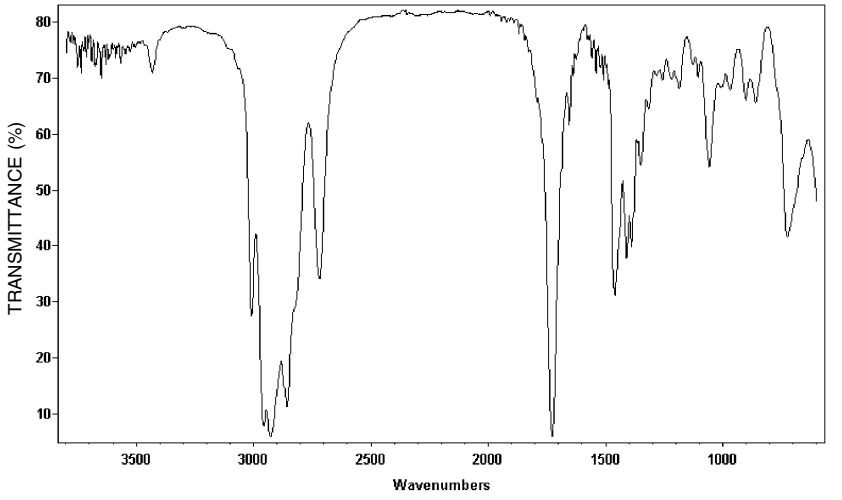
Decanoic Acid (Nujol Mull) View Monograph



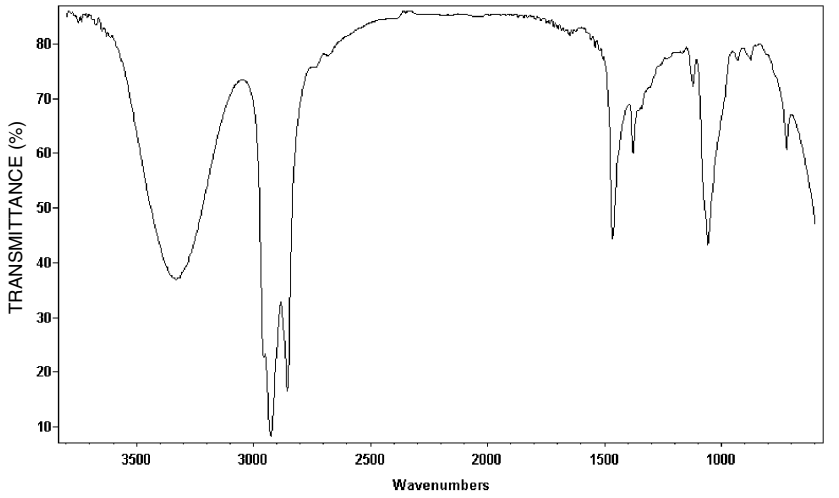
(*E*)-2-Decenal View Flavor Chemical



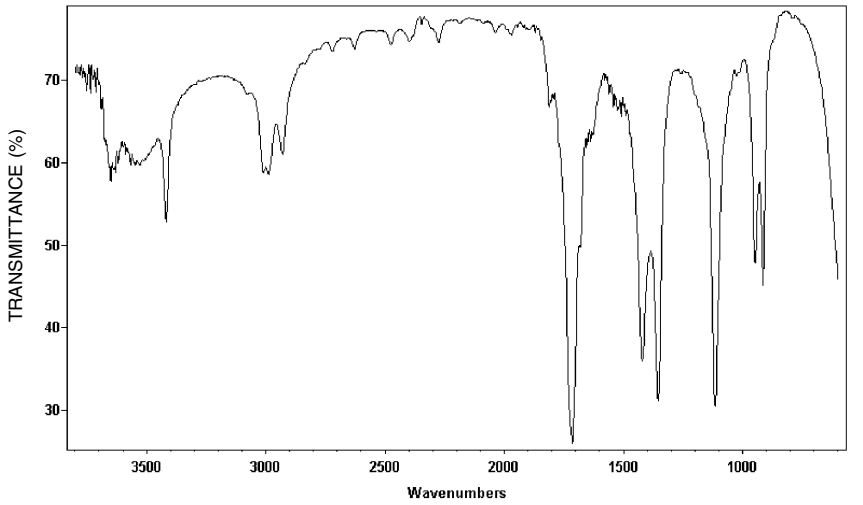
(*Z*)-4-Decenal View Flavor Chemical



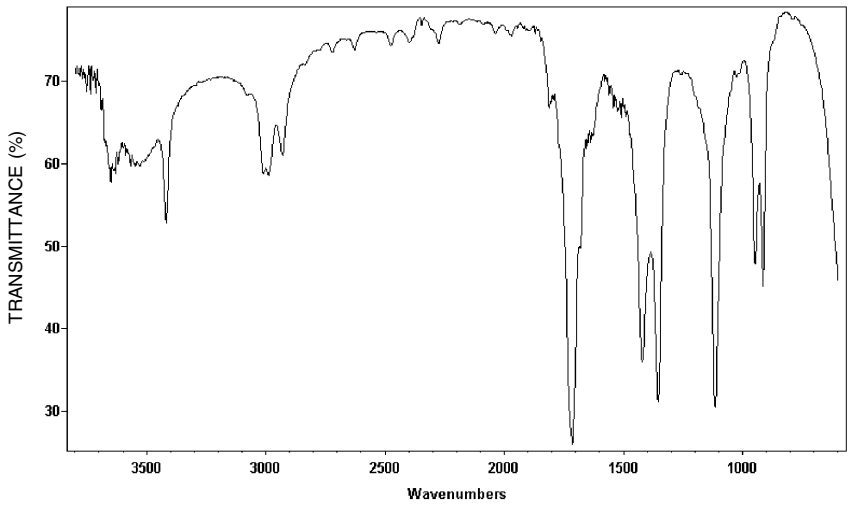
Decyl Alcohol View Flavor Chemical



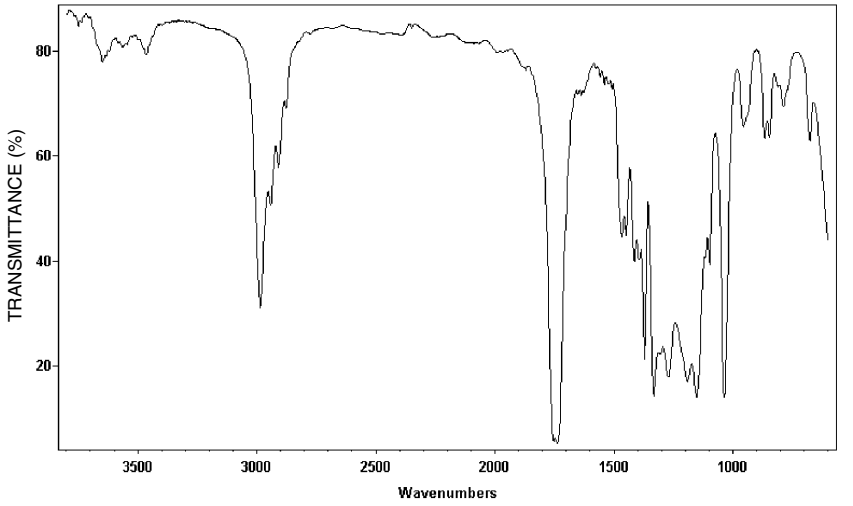
Diacetyl View Flavor Chemical



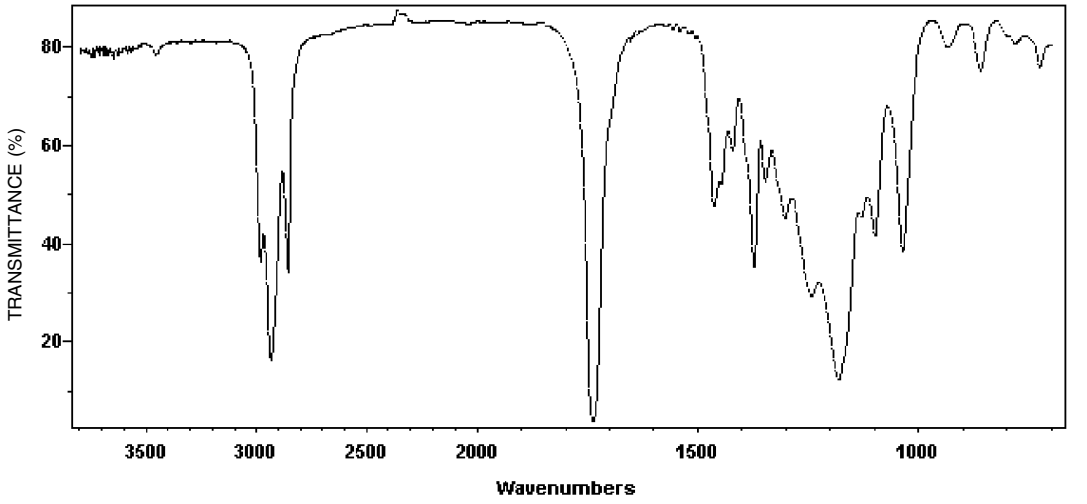
Dibenzyl Ether View Flavor Chemical



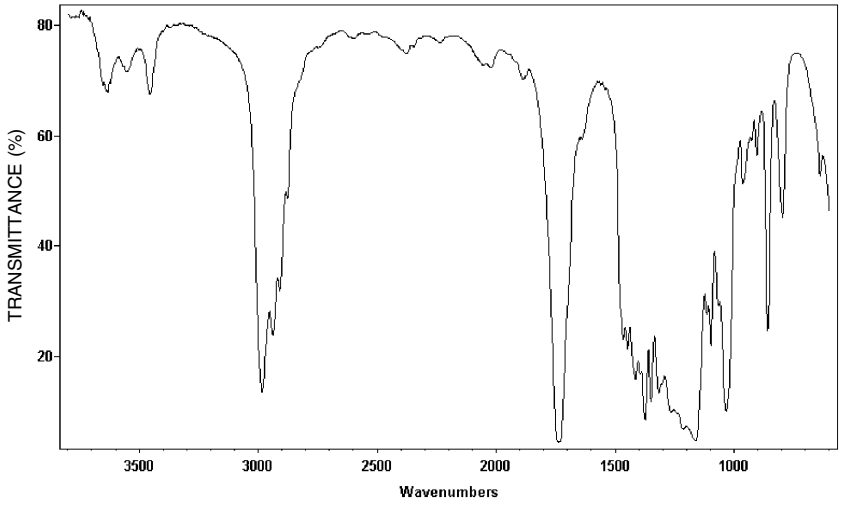
Diethyl Malonate View Flavor Chemical



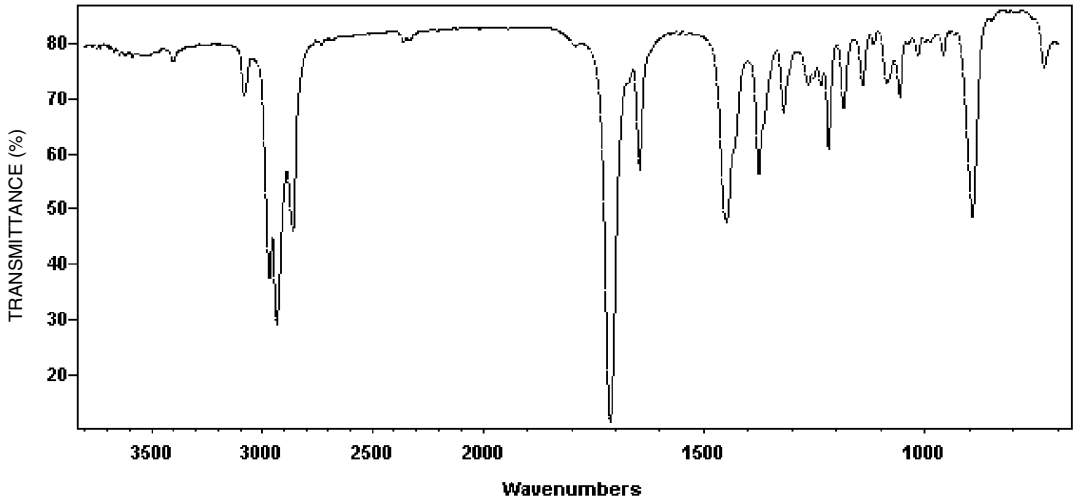
Diethyl Sebacate View Flavor Chemical



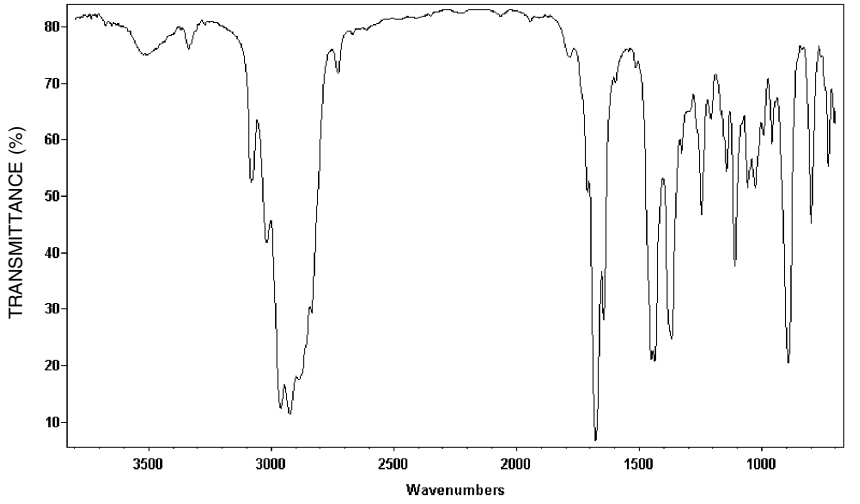
Diethyl Succinate View Flavor Chemical



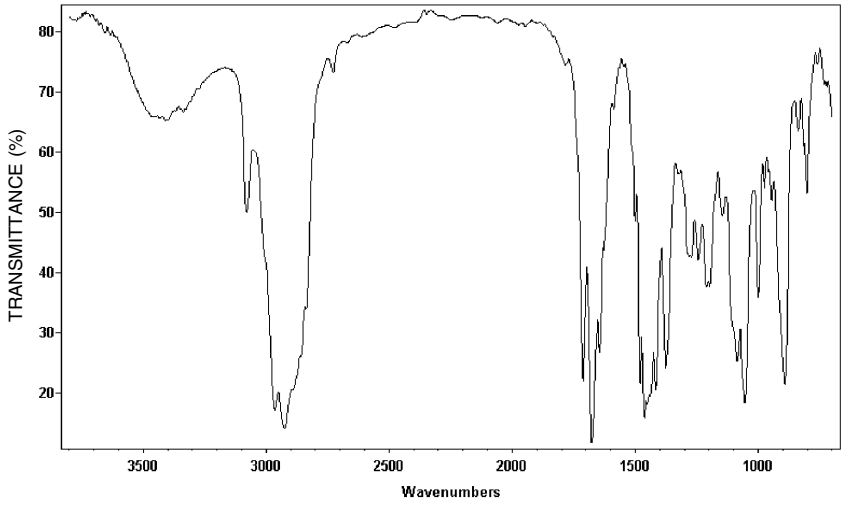
***d*-Dihydrocarvone** View Flavor Chemical



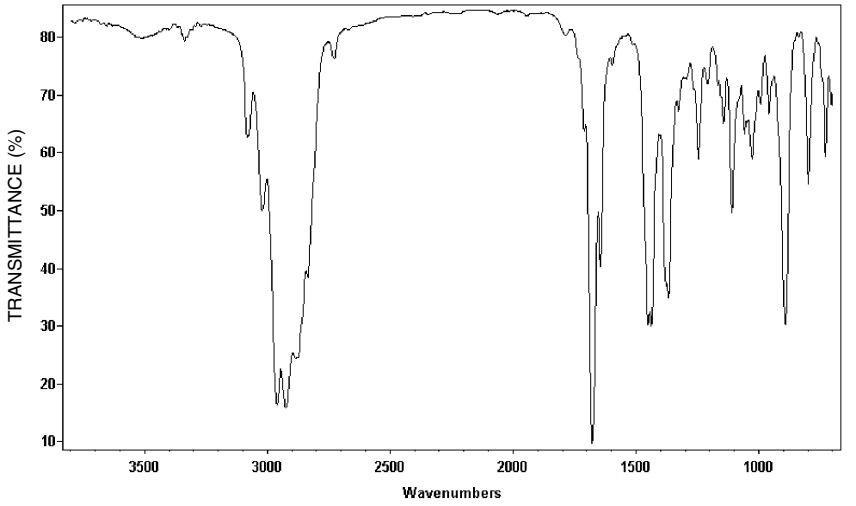
Dill Seed Oil, European Type View Monograph



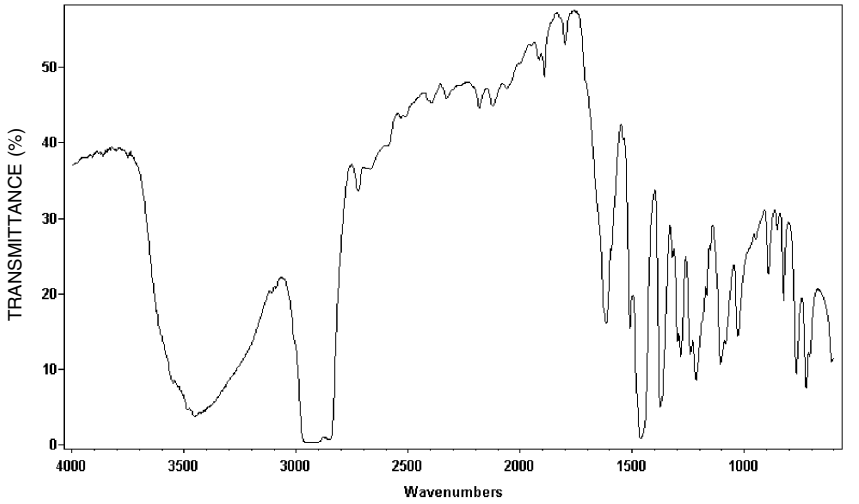
Dill Seed Oil, Indian Type View Monograph



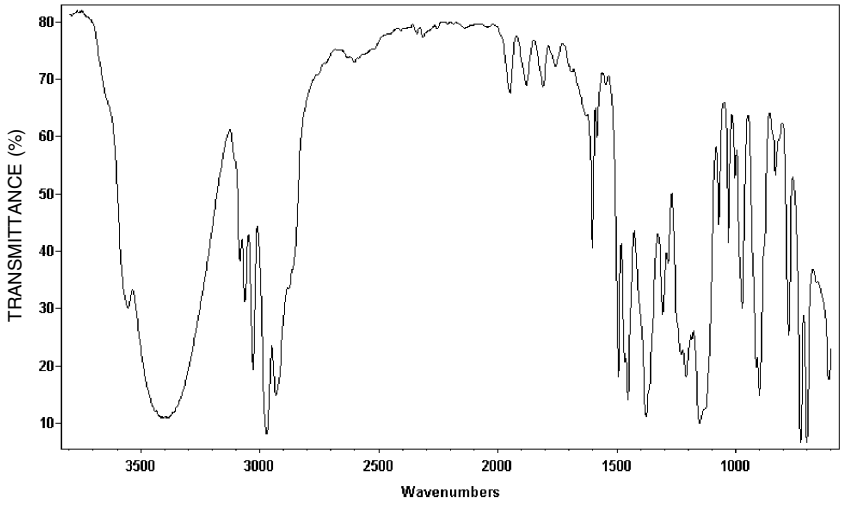
Dillweed Oil, American Type View Monograph



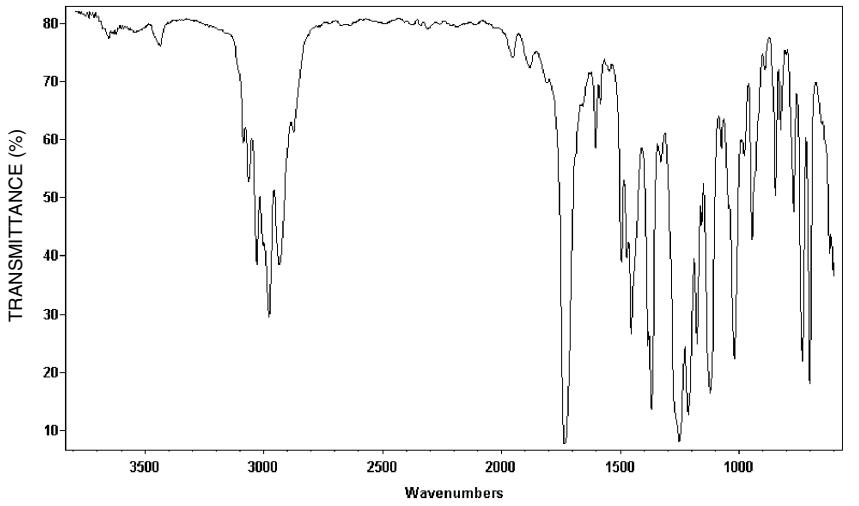
2,6-Dimethoxy Phenol View Flavor Chemical



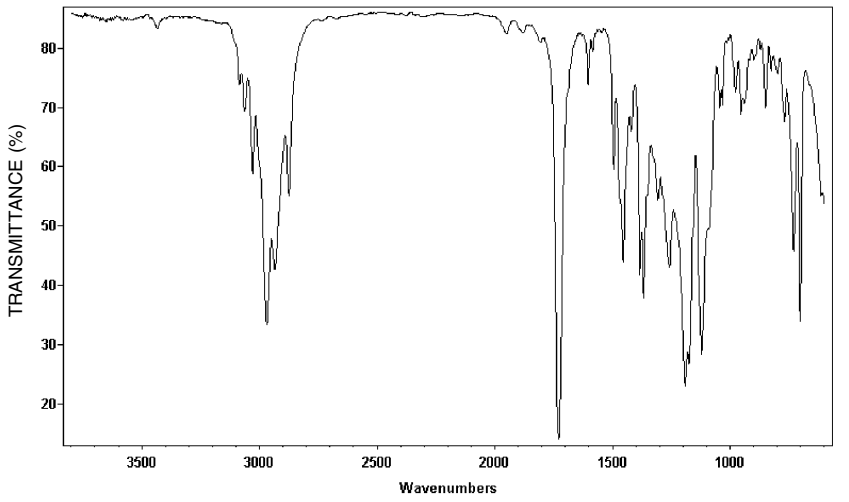
Dimethyl Benzyl Carbinol View Flavor Chemical



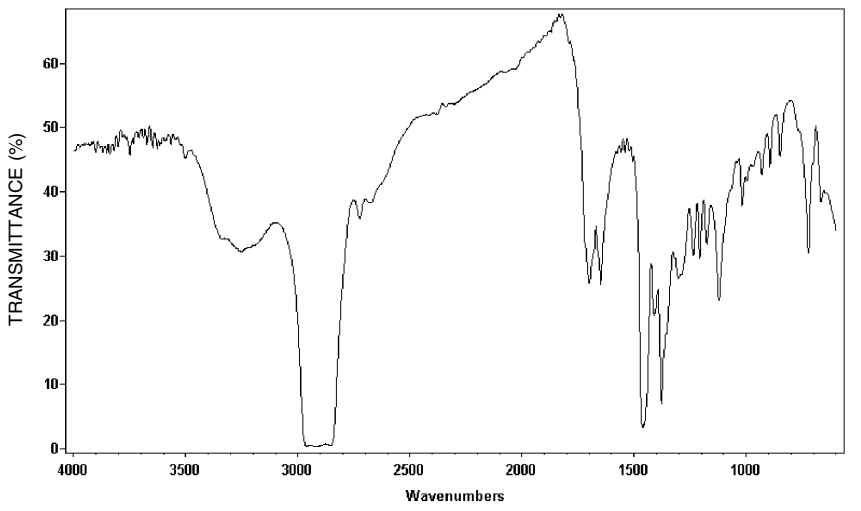
Dimethyl Benzyl Carbinyl Acetate View Flavor Chemical



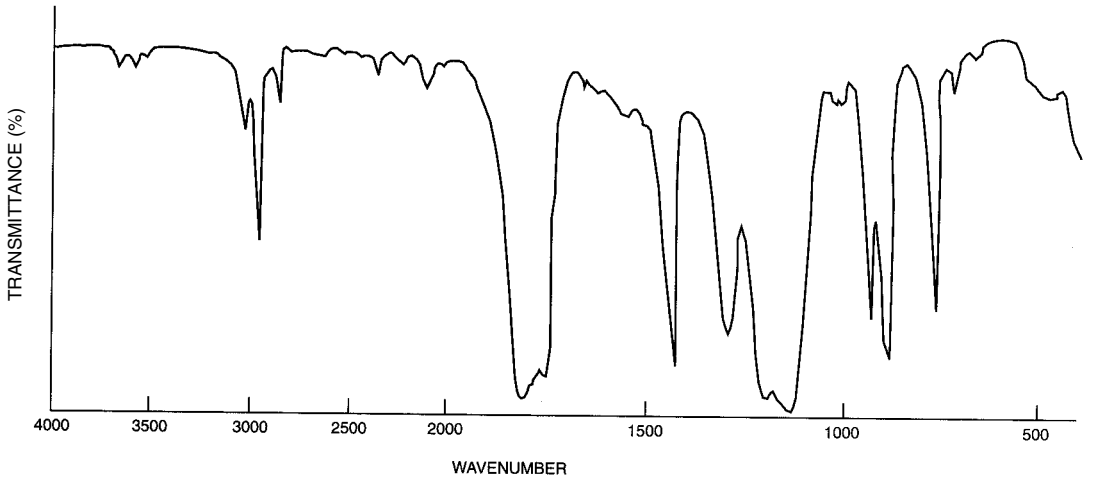
Dimethyl Benzyl Carbinyl Butyrate View Flavor Chemical



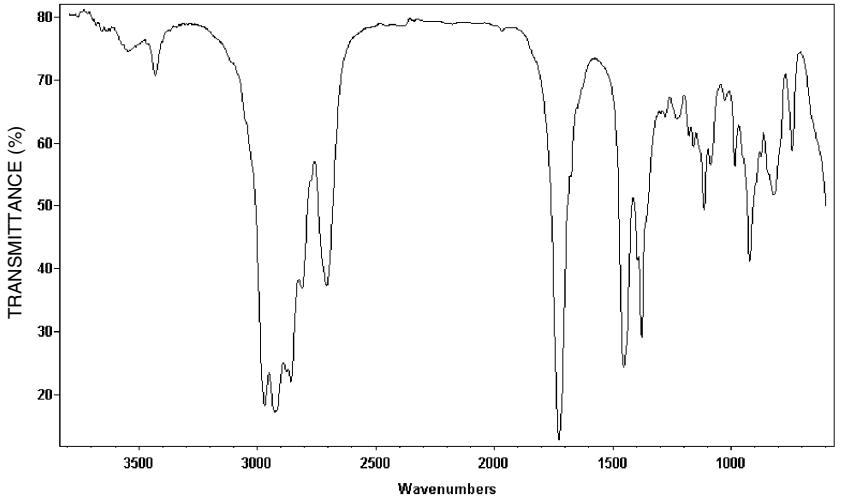
3,4-Dimethyl 1,2-Cyclopentandione View Flavor Chemical



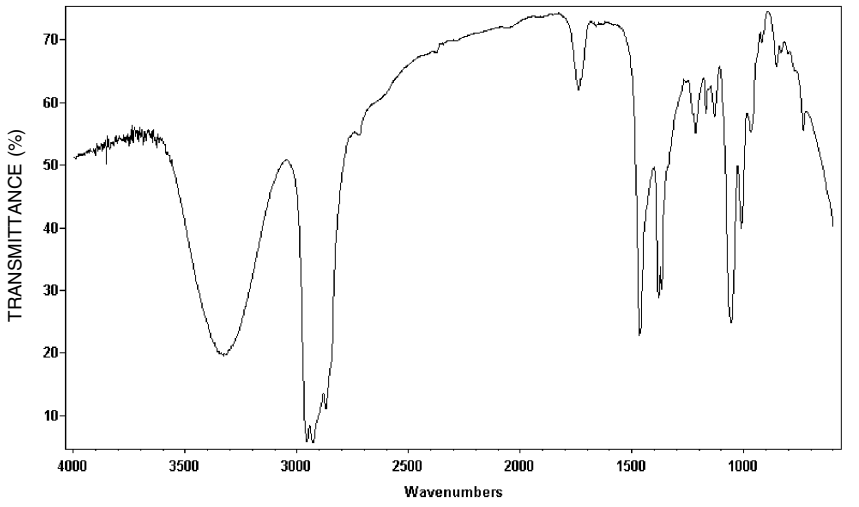
Dimethyl Dicarbonate View Monograph



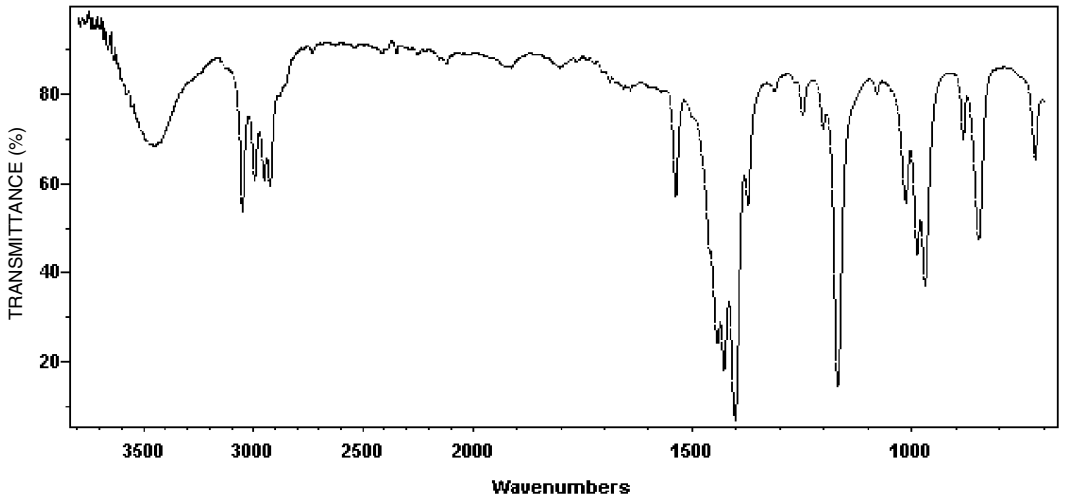
2,6-Dimethyl-5-heptenal View Flavor Chemical



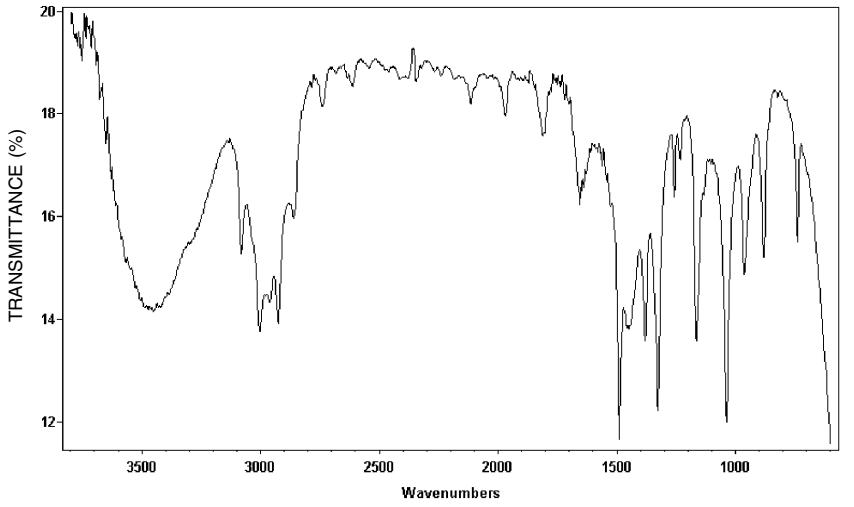
3,7-Dimethyl-1-octanol View Flavor Chemical



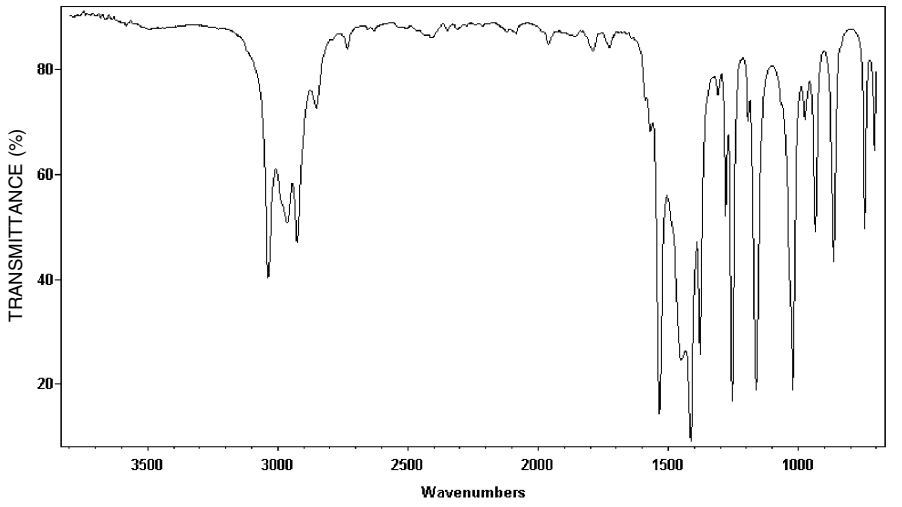
2,3-Dimethylpyrazine View Flavor Chemical



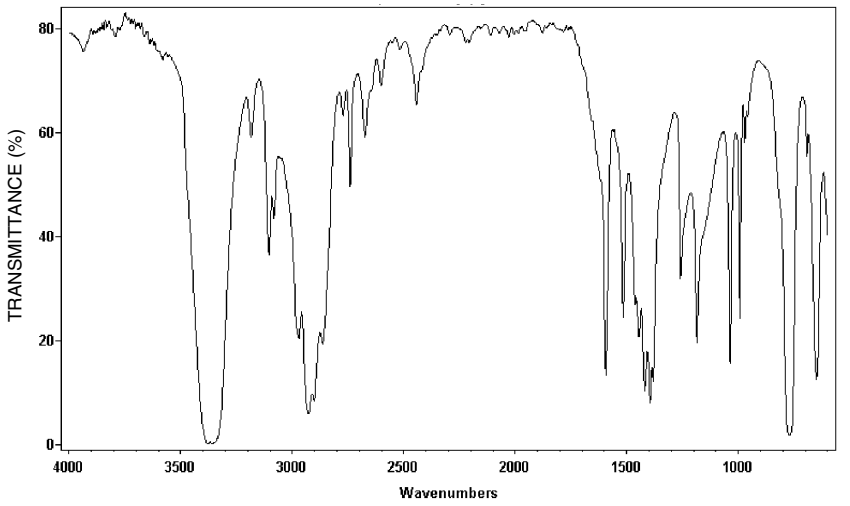
2,5-Dimethylpyrazine View Flavor Chemical



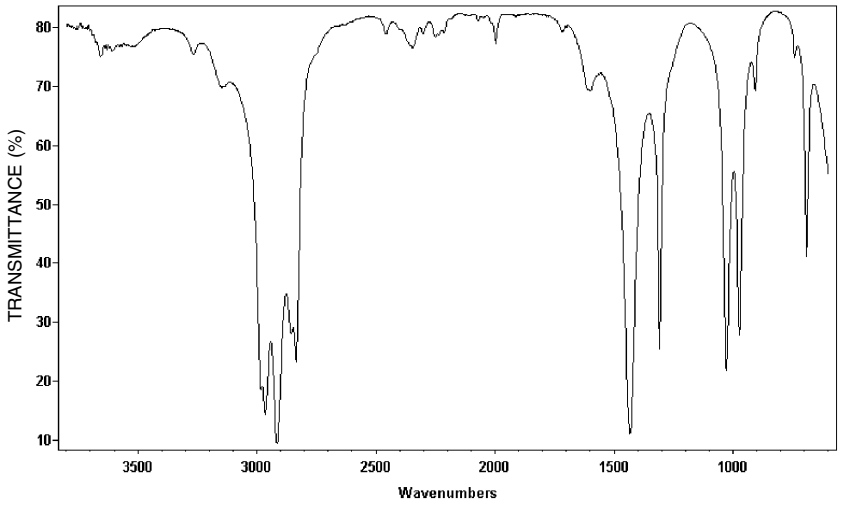
2,6-Dimethylpyrazine View Flavor Chemical



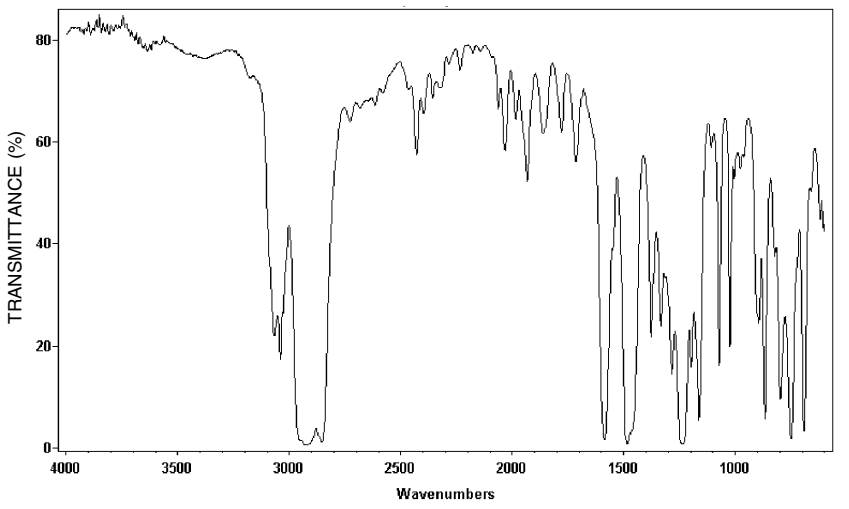
2,5-Dimethylpyrrole View Flavor Chemical



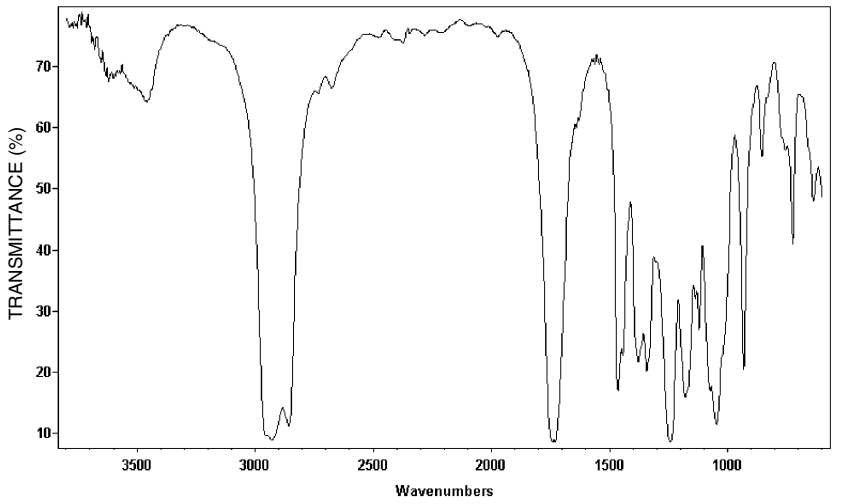
Dimethyl Sulfide View Flavor Chemical



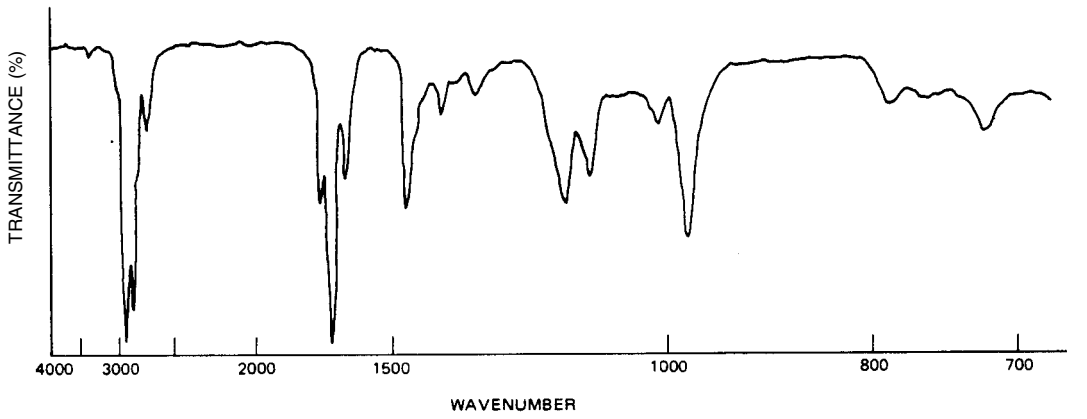
Diphenyl Ether View Flavor Chemical



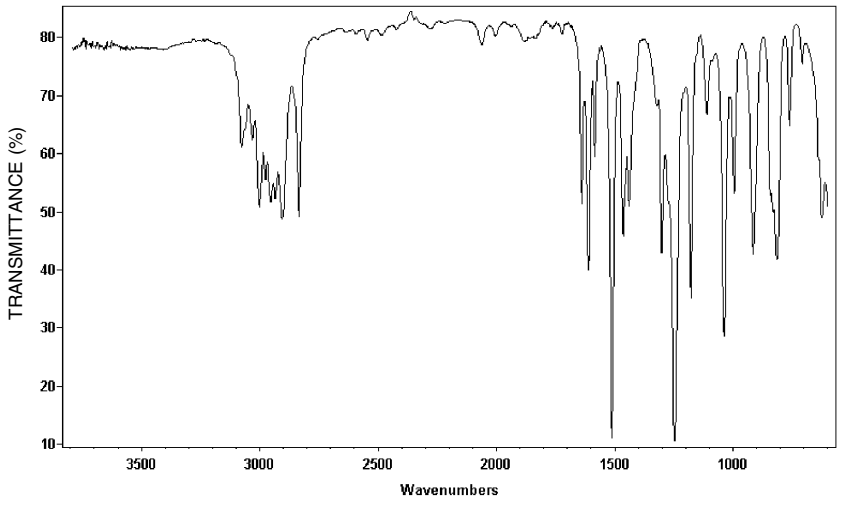
δ-Dodecalactone View Flavor Chemical



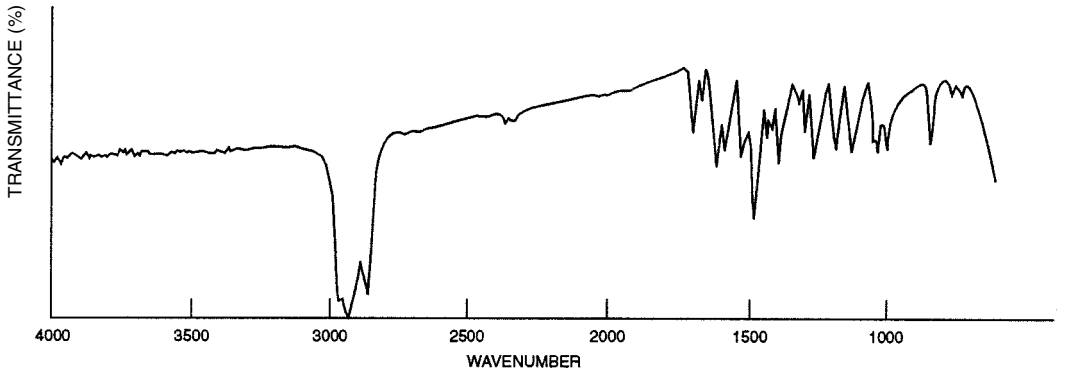
(*E*)-2-Dodecen-1-al View Flavor Chemical



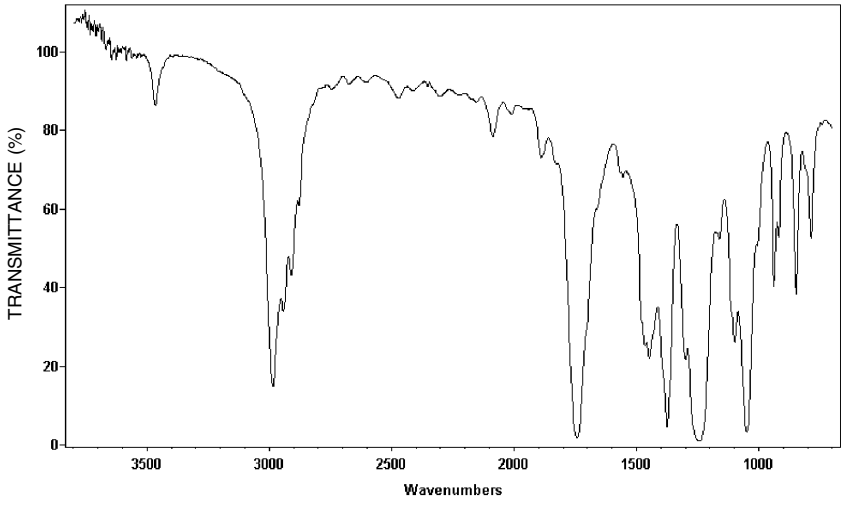
Estragole View Flavor Chemical



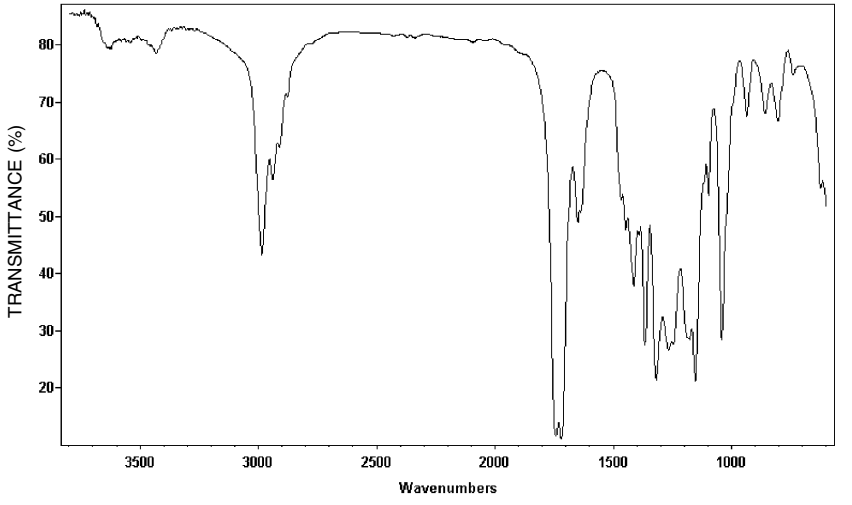
Ethone (Mineral Oil Mull) View Flavor Chemical



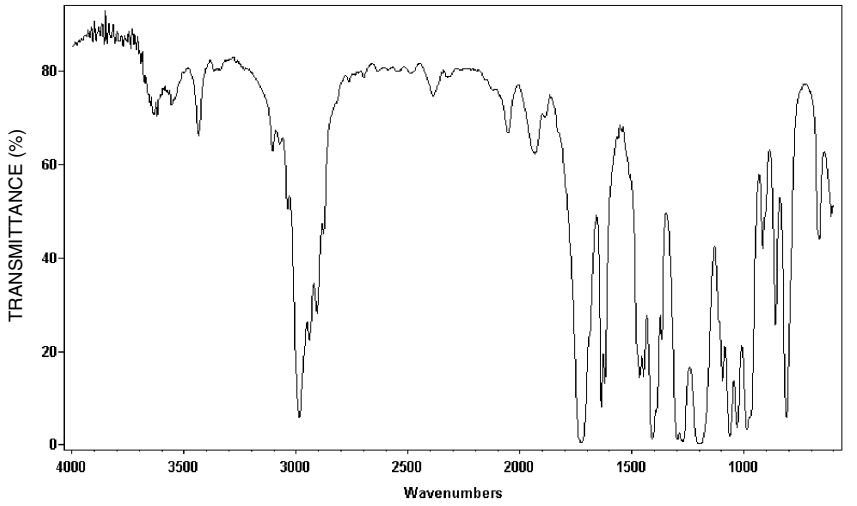
Ethyl Acetate View Flavor Chemical



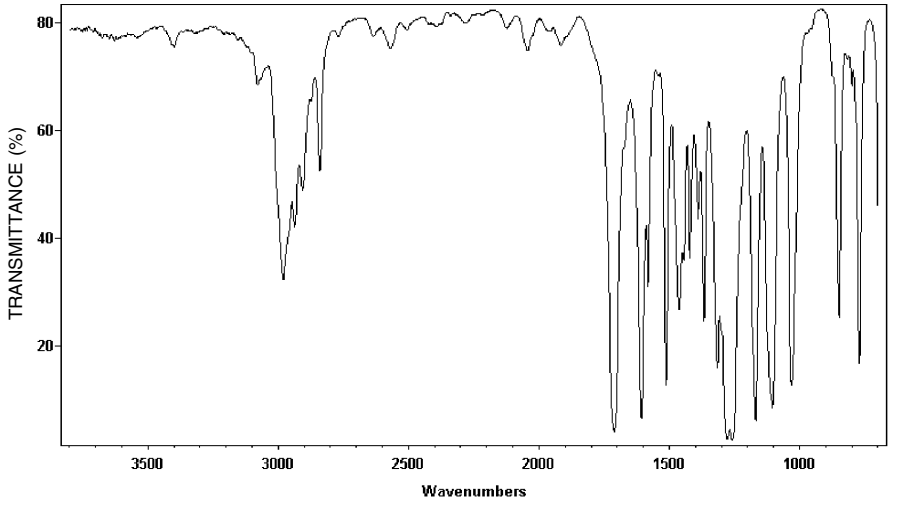
Ethyl Acetoacetate View Flavor Chemical



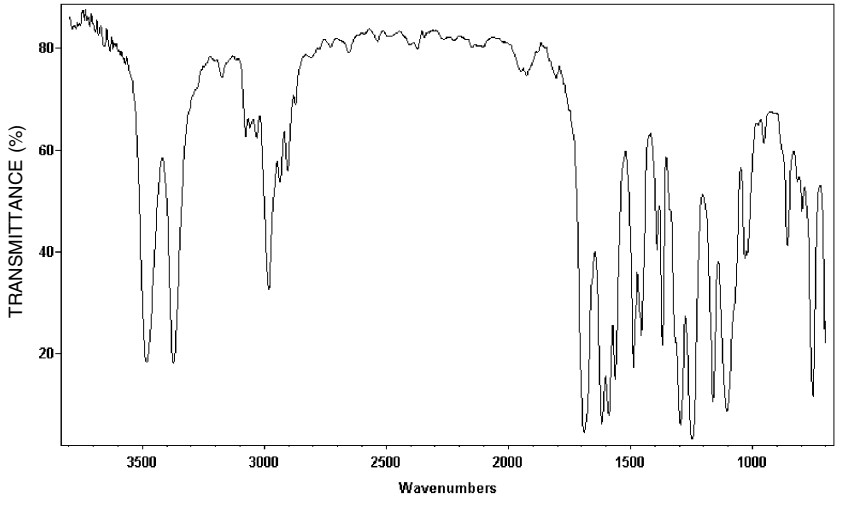
Ethyl Acrylate View Flavor Chemical



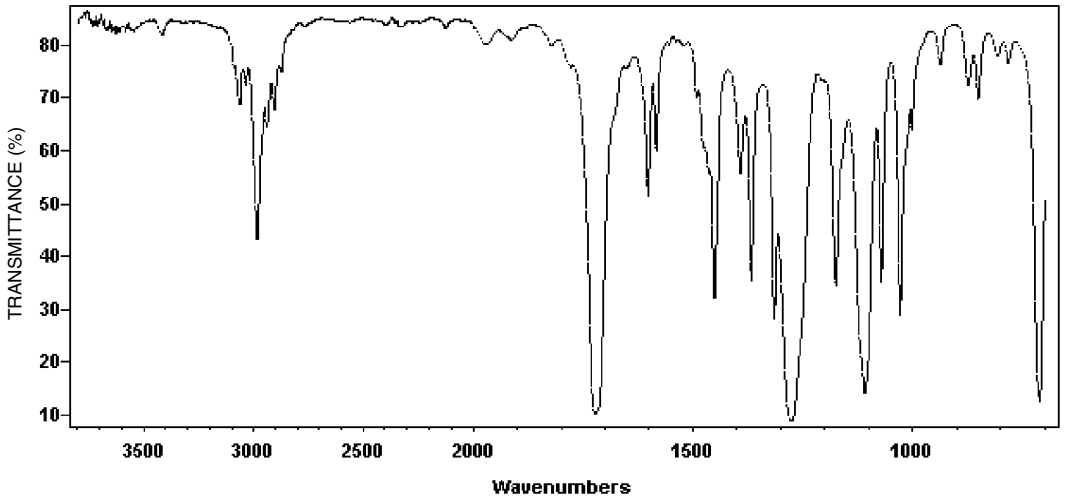
Ethyl *p*-Anisate View Flavor Chemical



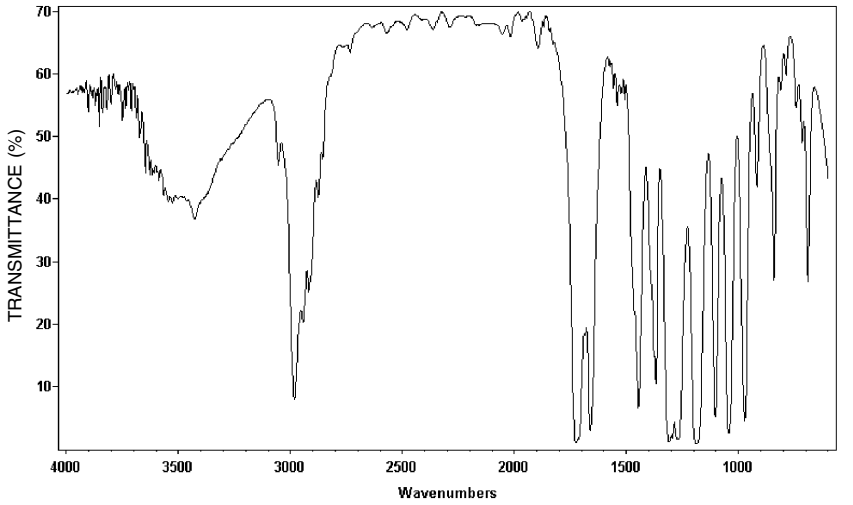
Ethyl Anthranilate View Flavor Chemical



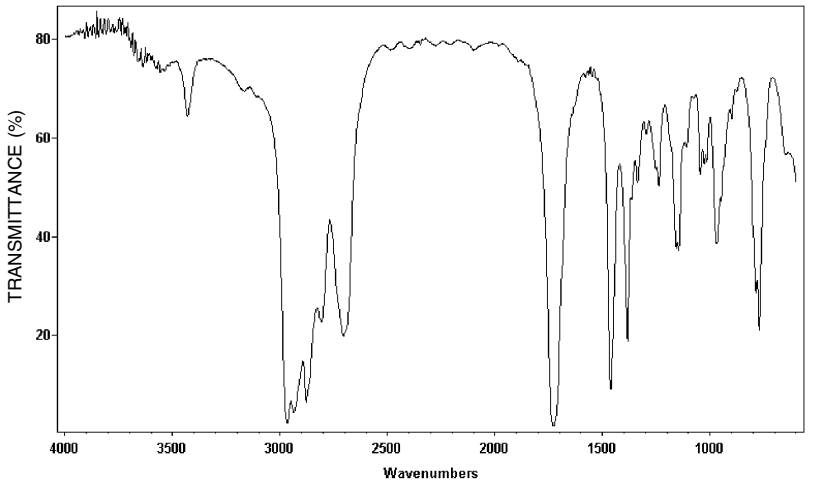
Ethyl Benzoate View Flavor Chemical



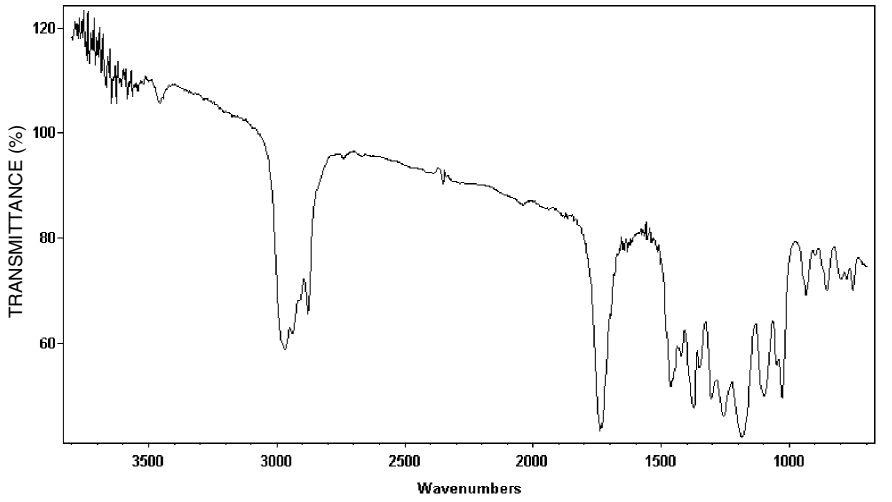
Ethyl-(*E*)-2-butenoate View Flavor Chemical



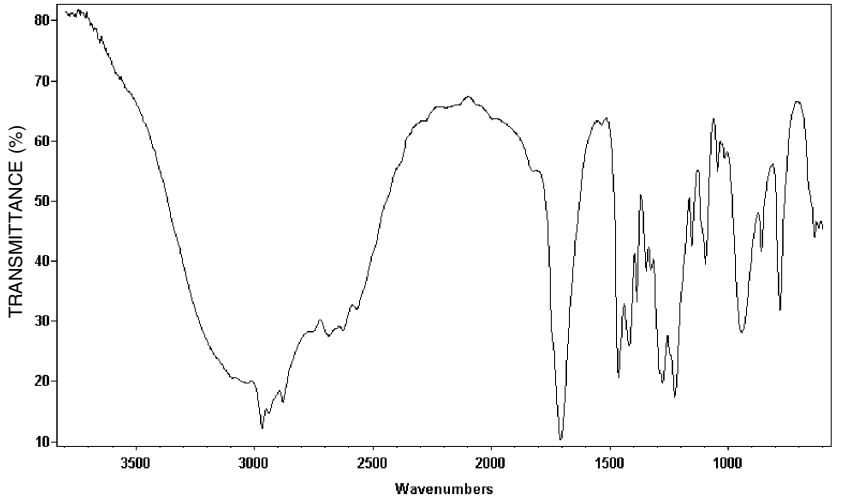
2-Ethylbutyraldehyde View Flavor Chemical



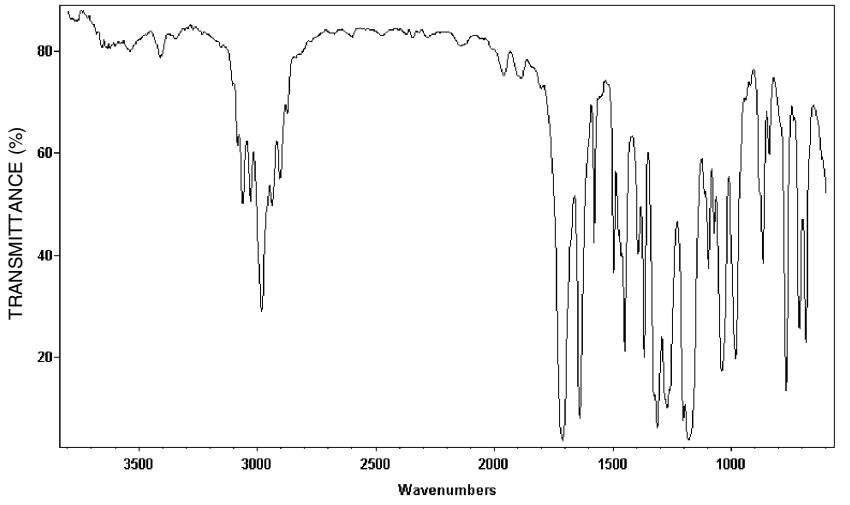
Ethyl Butyrate View Flavor Chemical



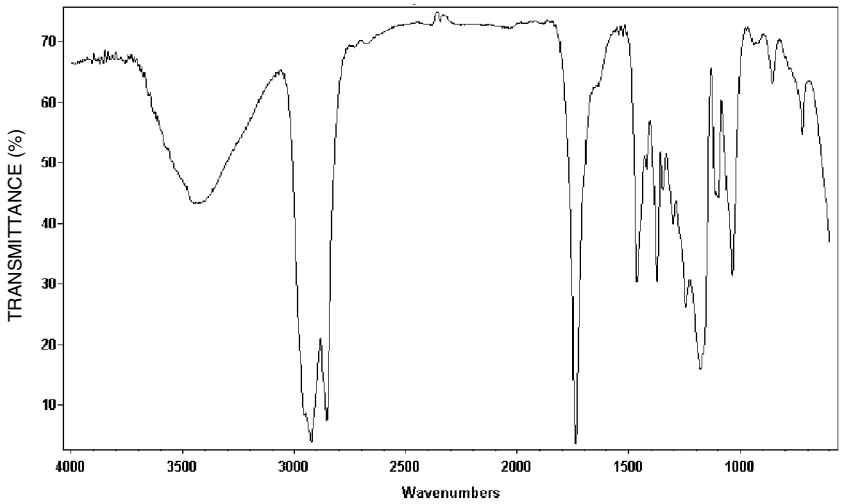
2-Ethylbutyric Acid View Flavor Chemical



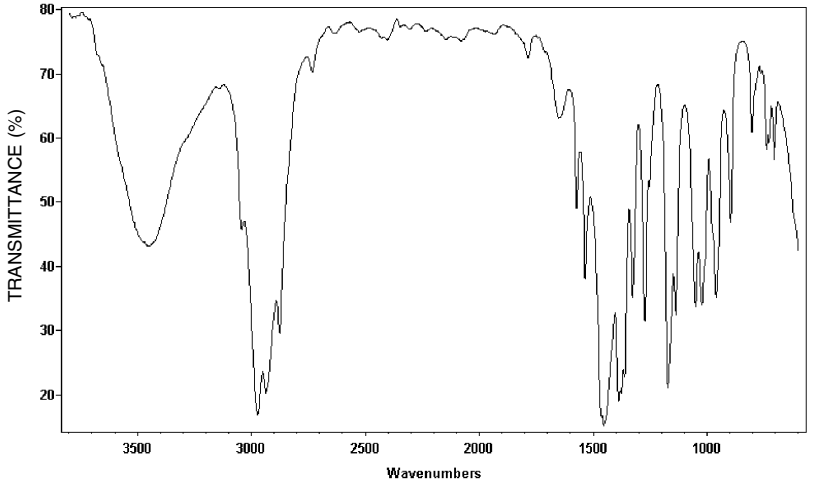
Ethyl Cinnamate View Flavor Chemical



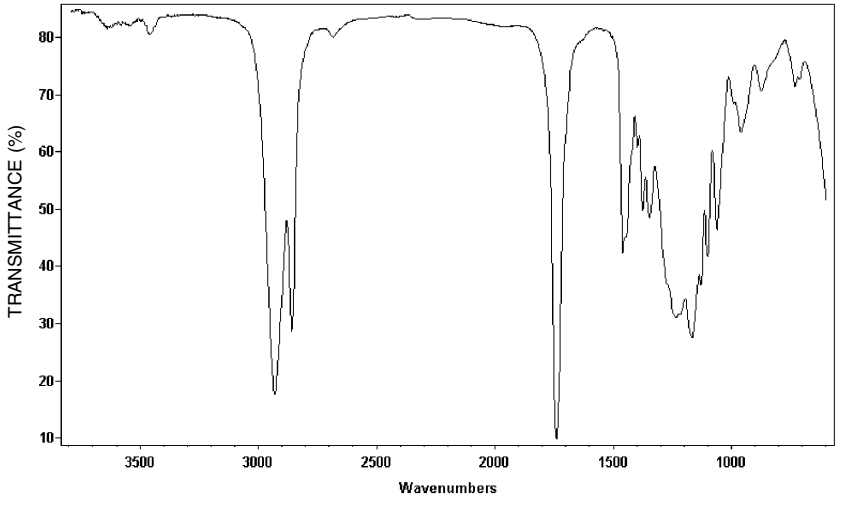
Ethyl Decanoate View Flavor Chemical



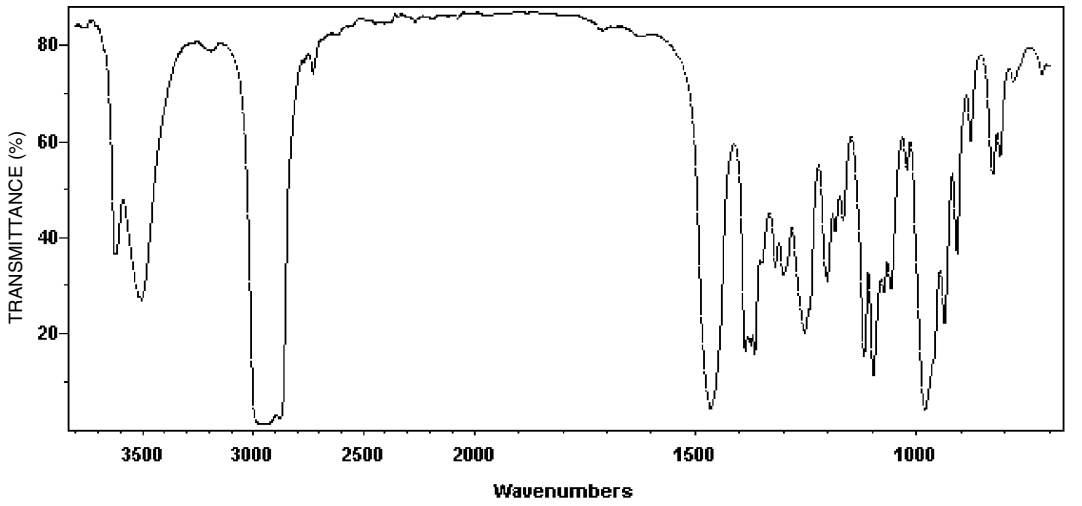
2-Ethyl-3,5(6)-dimethylpyrazine View Flavor Chemical



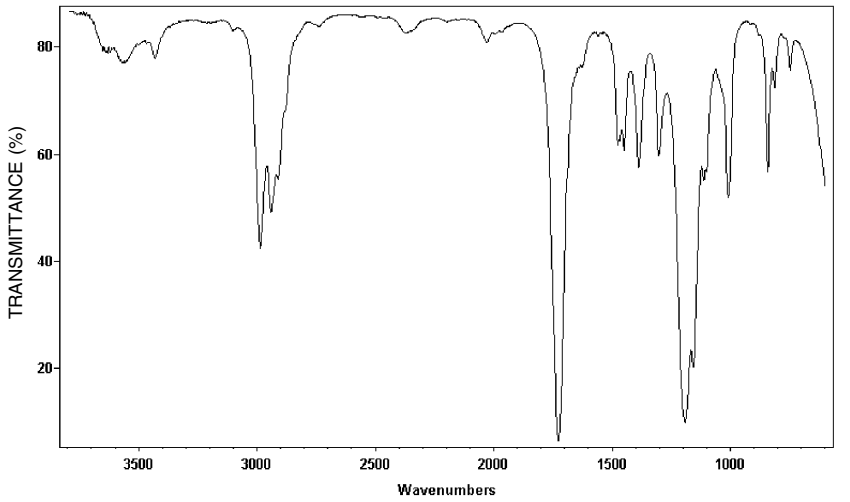
Ethylene Brassylate View Flavor Chemical



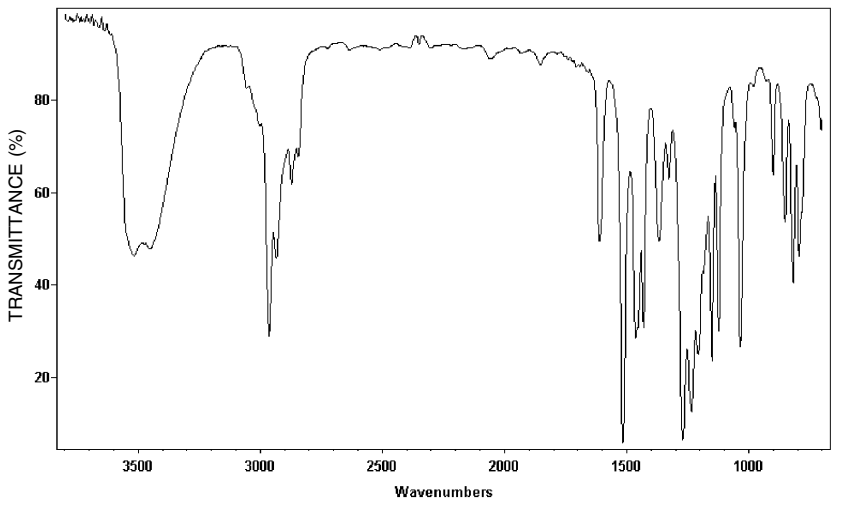
2-Ethyl Fenchol View Flavor Chemical



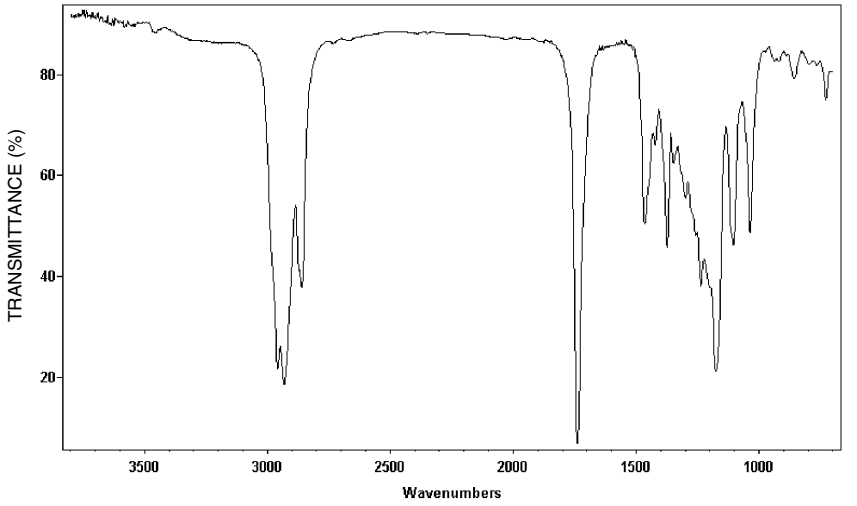
Ethyl Formate View Flavor Chemical



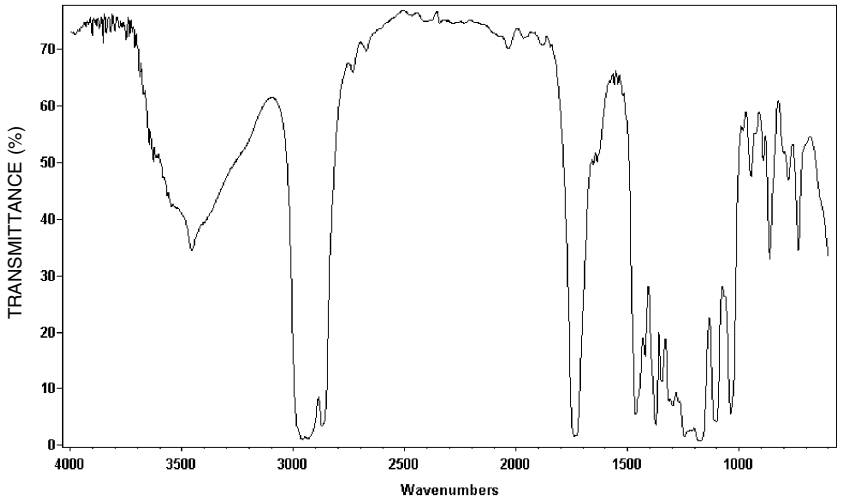
4-Ethyl Guaiacol View Flavor Chemical



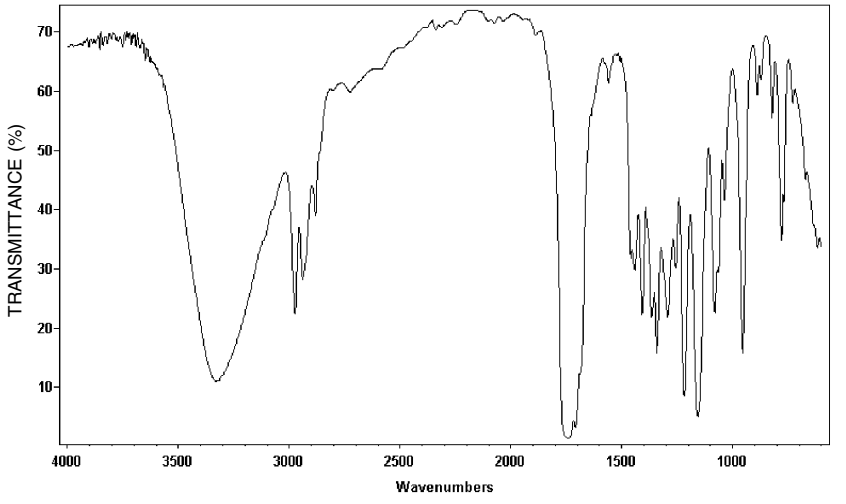
Ethyl Heptanoate View Flavor Chemical



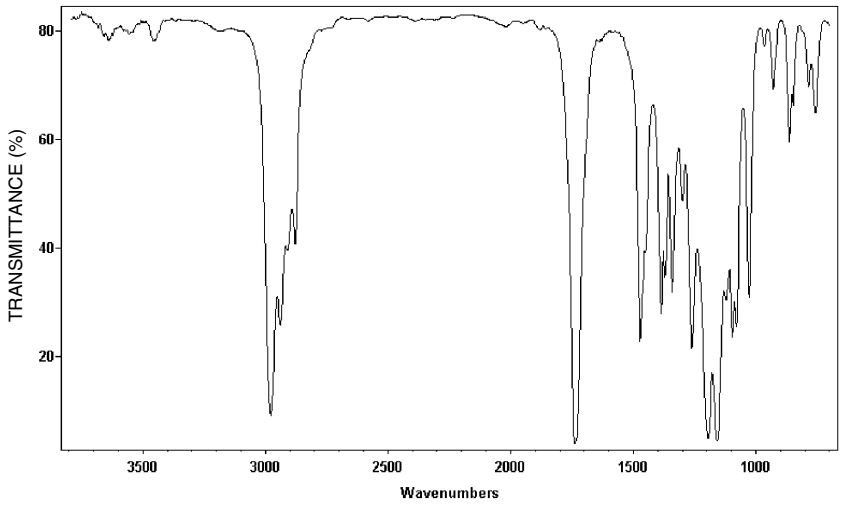
Ethyl Hexanoate View Flavor Chemical



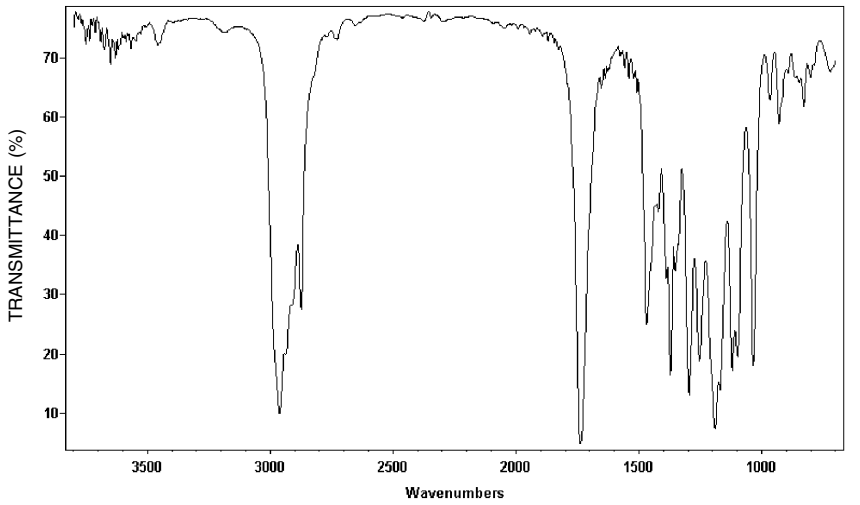
5-Ethyl 3-Hydroxy 4-Methyl 2(5H)-Furanone View Flavor Chemical



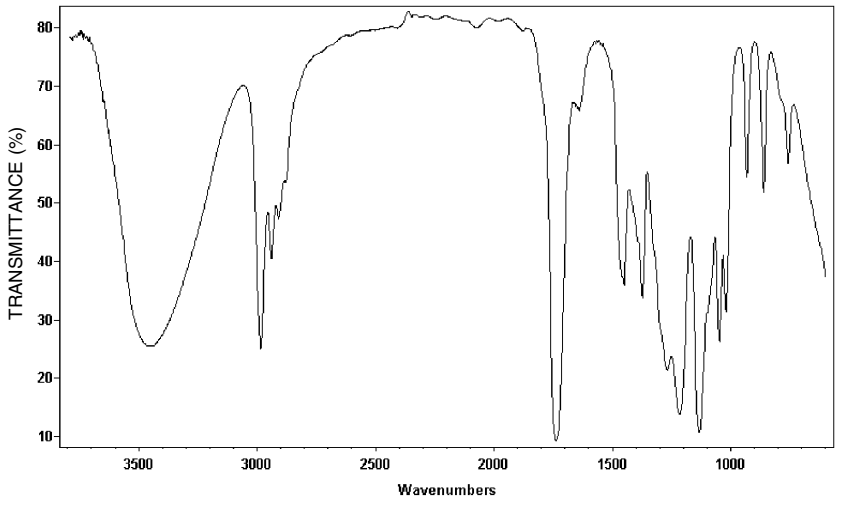
Ethyl Isobutyrate View Flavor Chemical



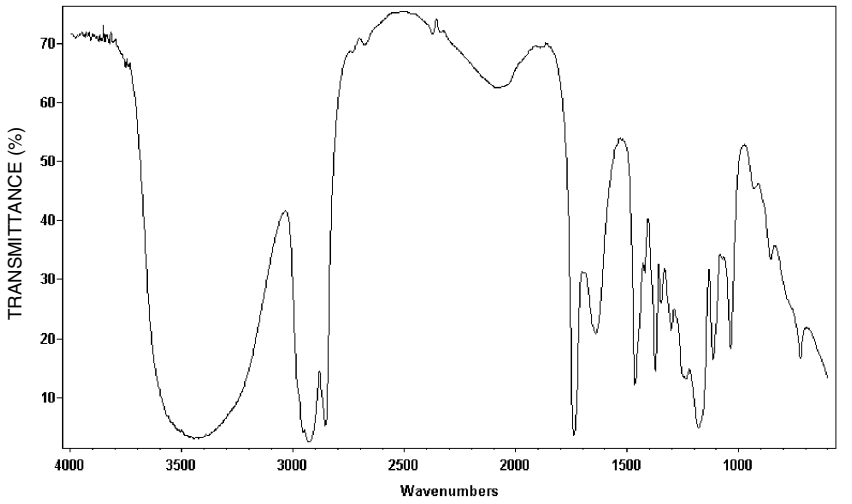
Ethyl Isovalerate View Flavor Chemical



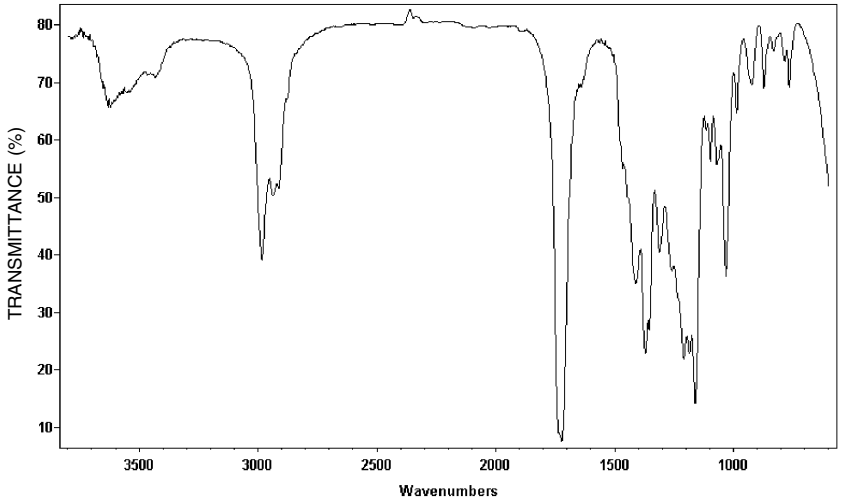
Ethyl Lactate View Flavor Chemical



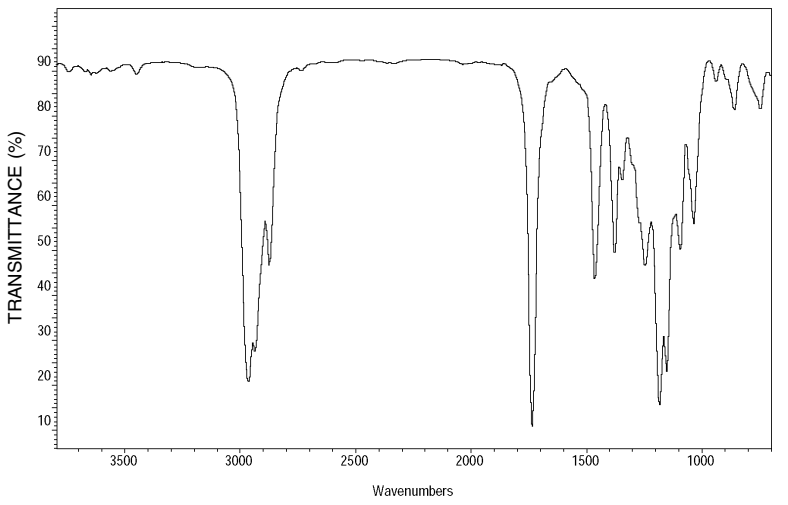
Ethyl Laurate View Flavor Chemical



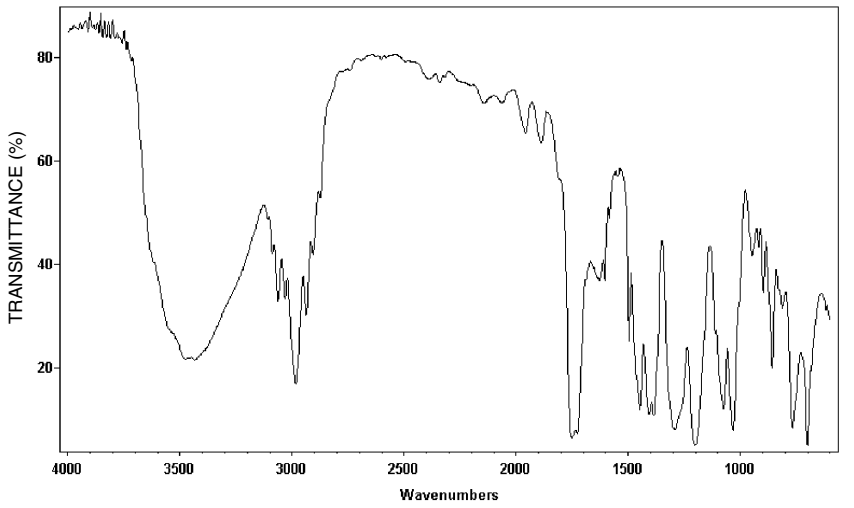
Ethyl Levulinate View Flavor Chemical



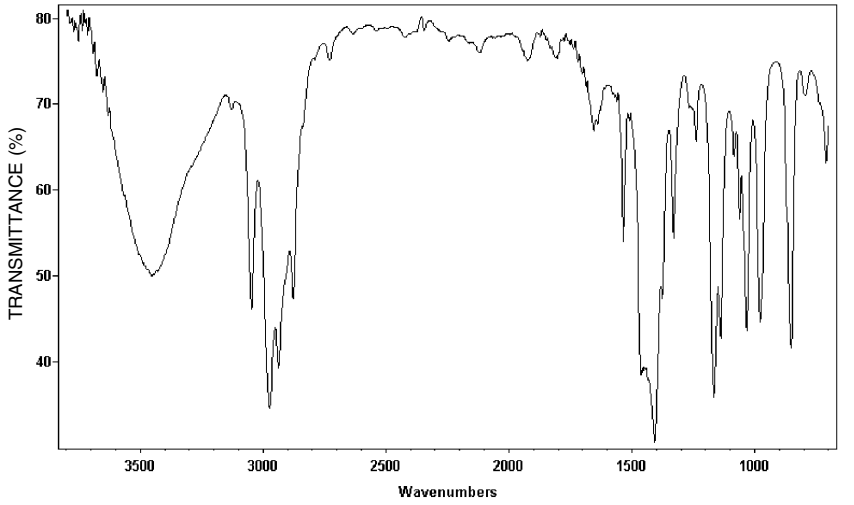
Ethyl 2-Methylpentanoate View Flavor Chemical



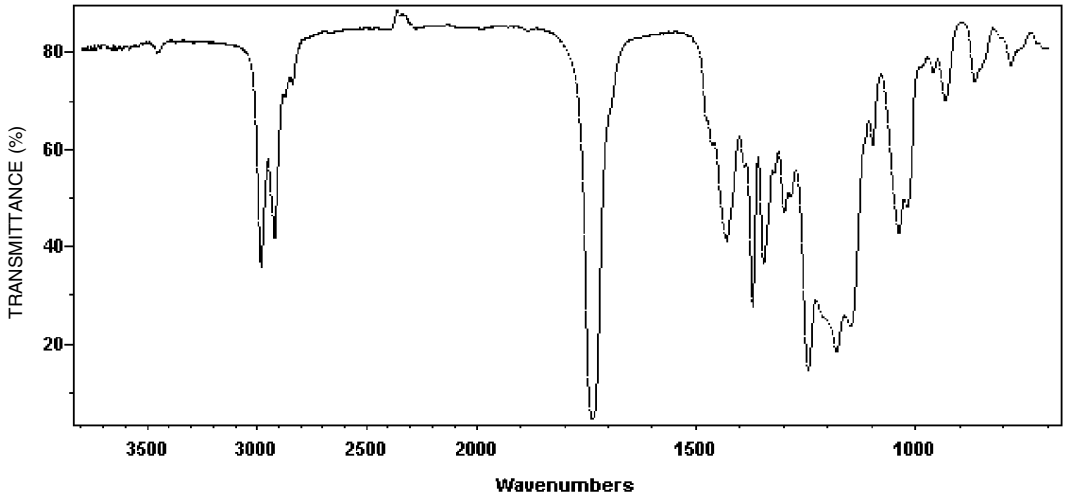
Ethyl Methylphenylglycidate View Flavor Chemical



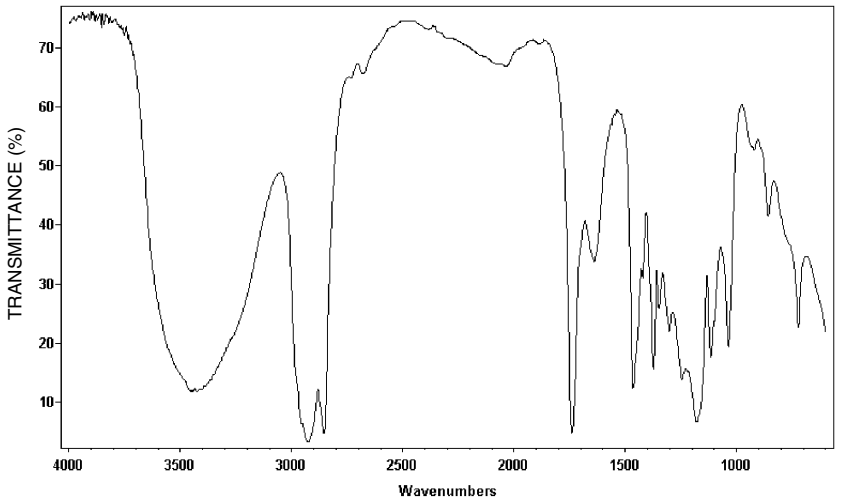
2-Ethyl-3-methylpyrazine View Flavor Chemical



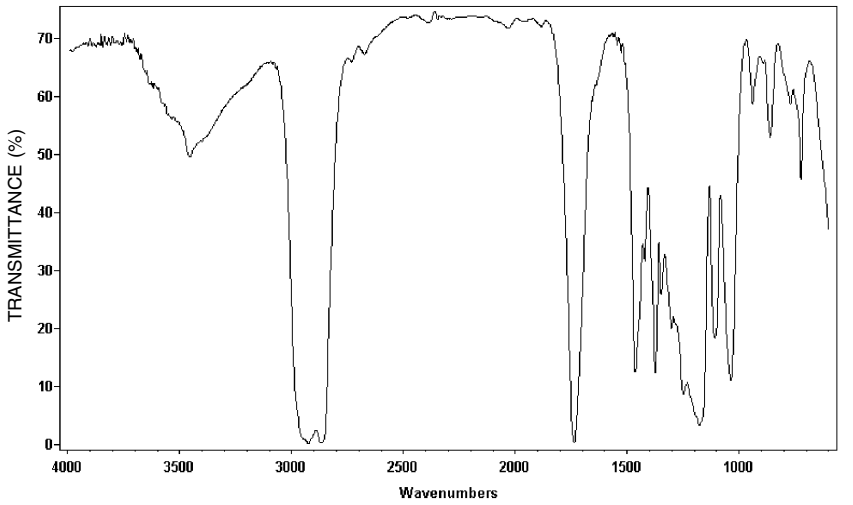
Ethyl 3-Methylthiopropionate View Flavor Chemical



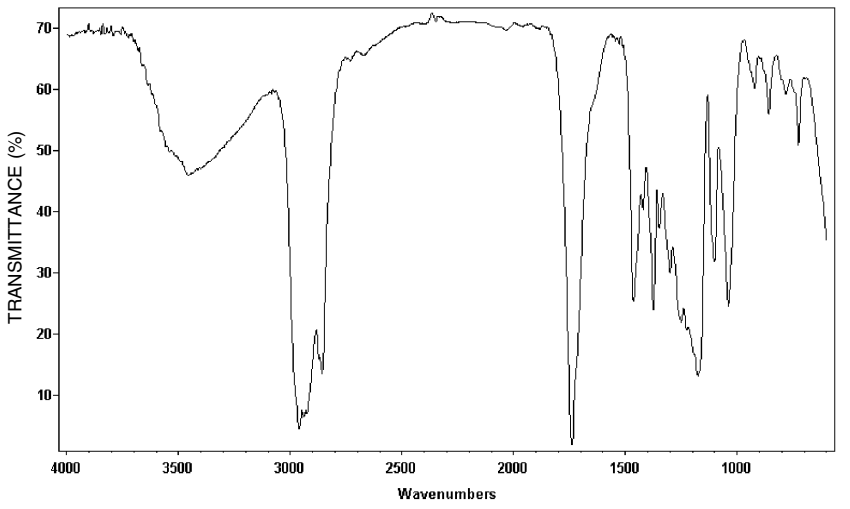
Ethyl Myristate View Flavor Chemical



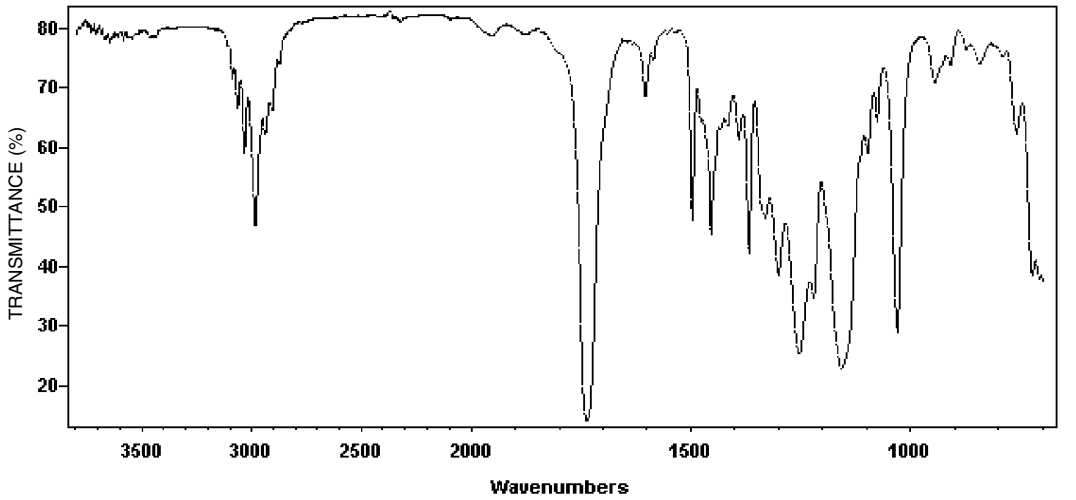
Ethyl Nonanoate View Flavor Chemical



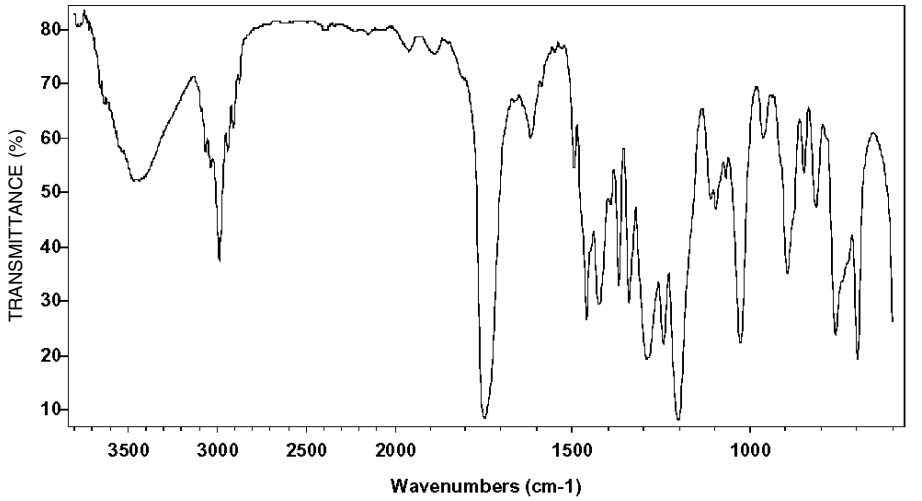
Ethyl Octanoate View Flavor Chemical



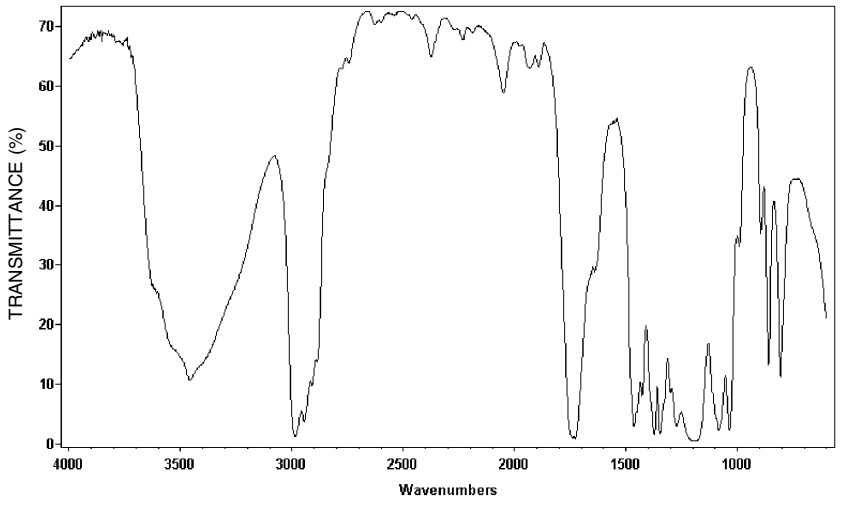
Ethyl Phenylacetate View Flavor Chemical



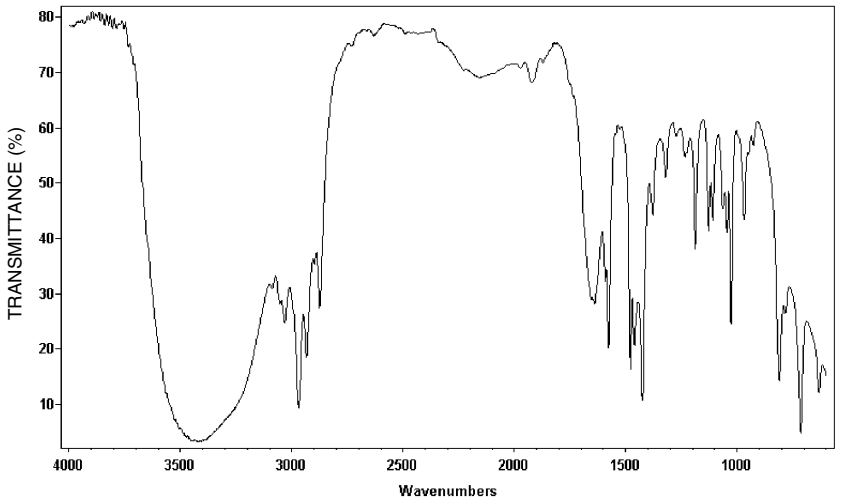
Ethyl Phenylglycidate View Flavor Chemical



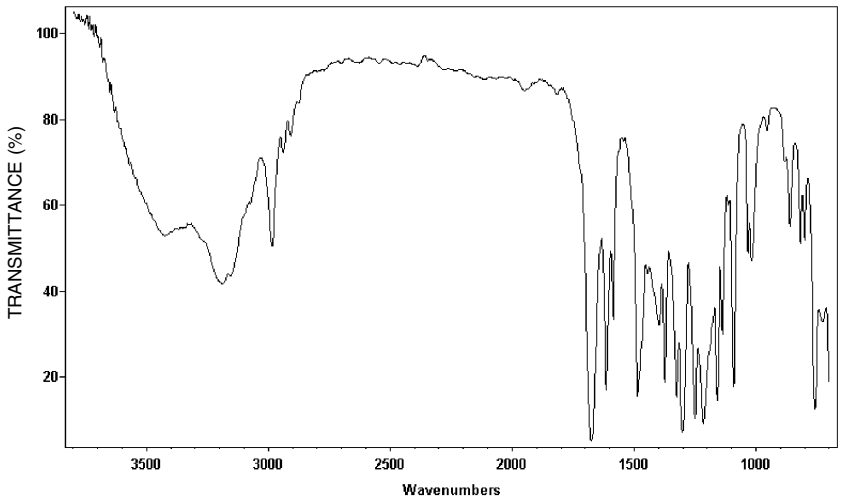
Ethyl Propionate View Flavor Chemical



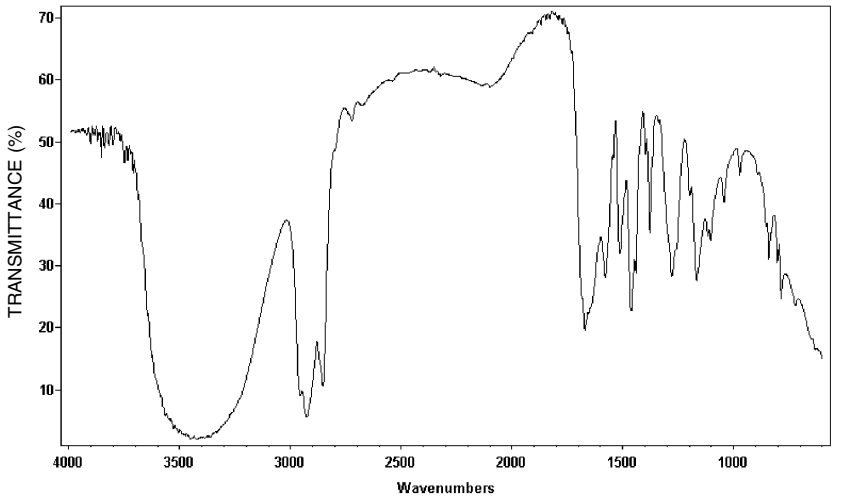
3-Ethyl Pyridine View Flavor Chemical



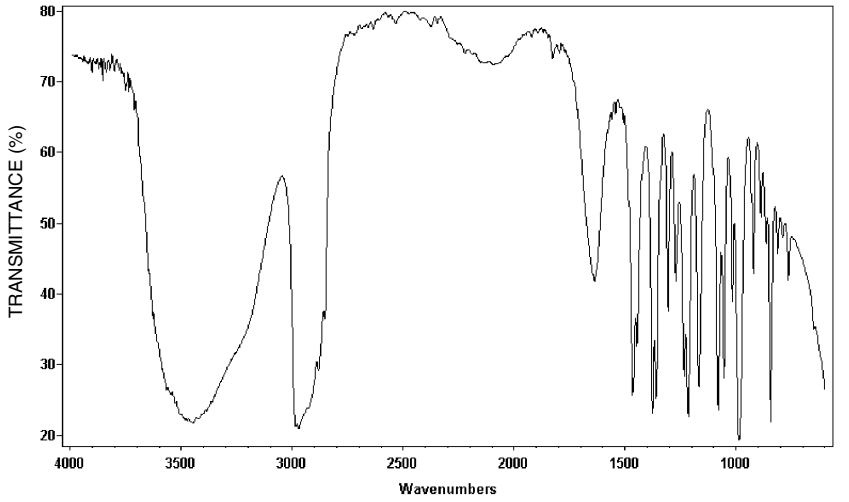
Ethyl Salicylate View Flavor Chemical



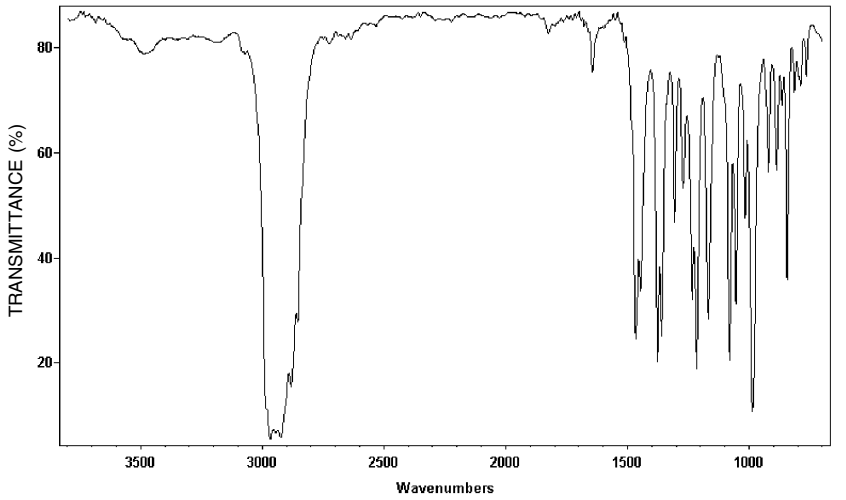
Ethyl Vanillin View Flavor Chemical



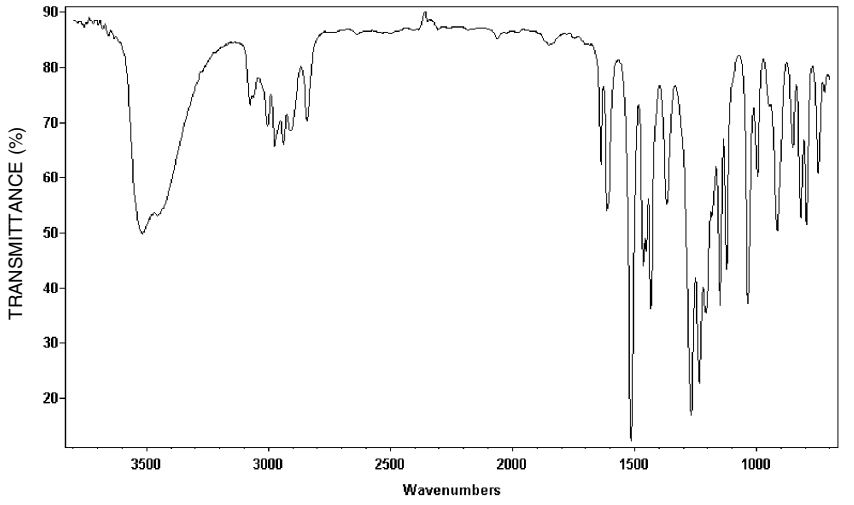
Eucalyptol View Flavor Chemical



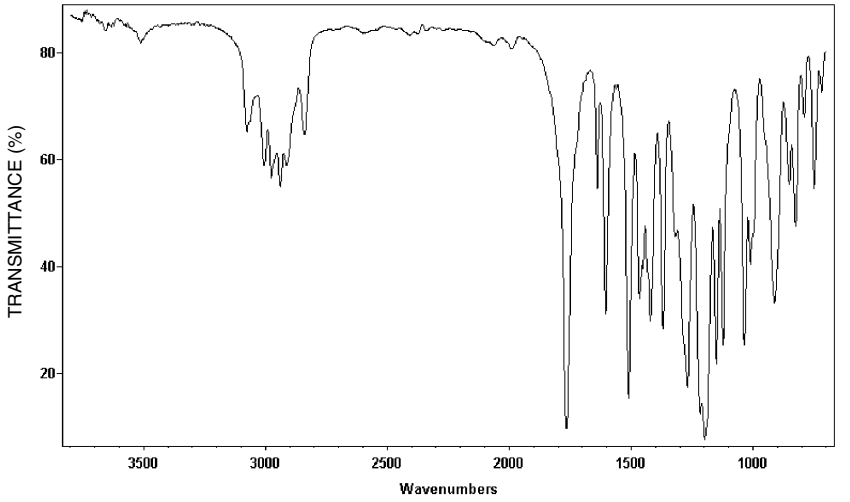
Eucalyptus Oil View Monograph



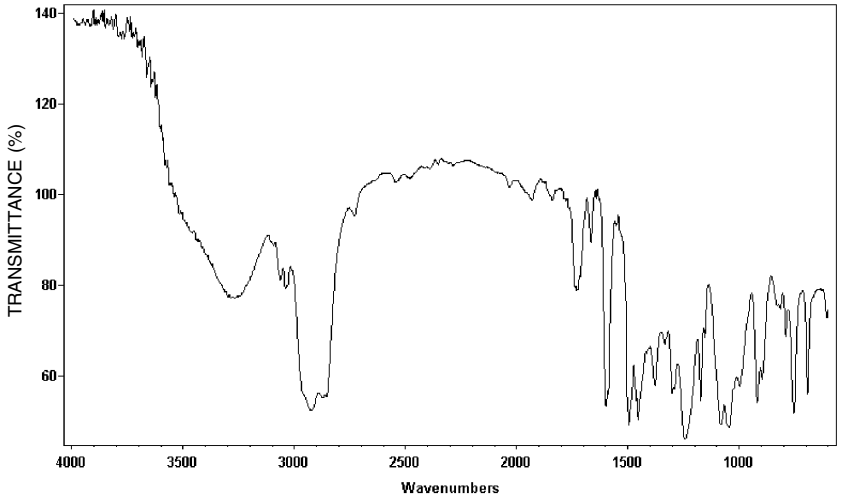
Eugenol View Flavor Chemical



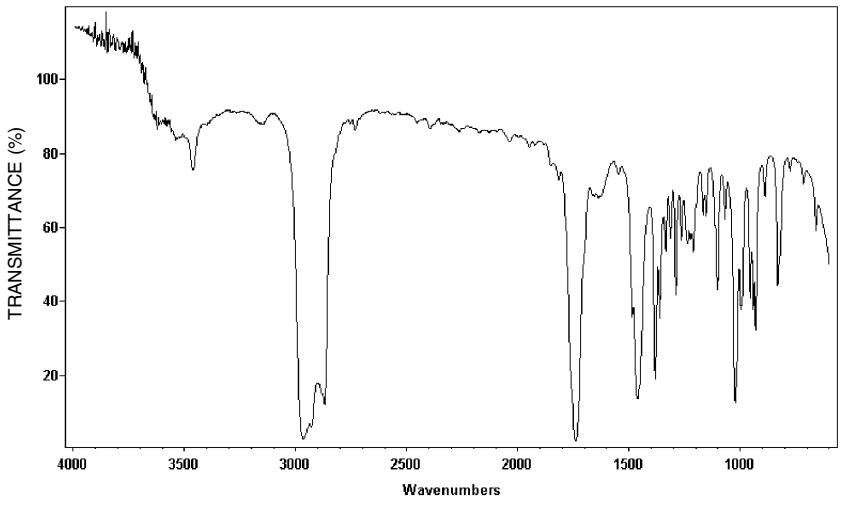
Eugenyl Acetate View Flavor Chemical



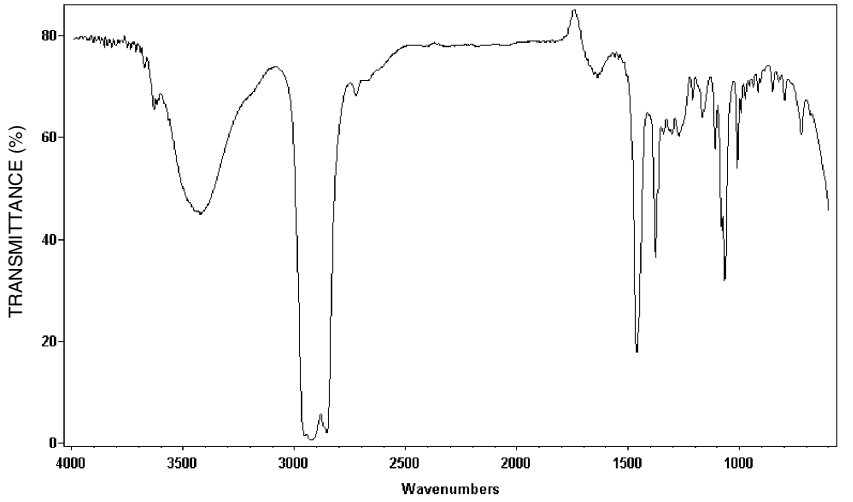
Farnesol View Flavor Chemical



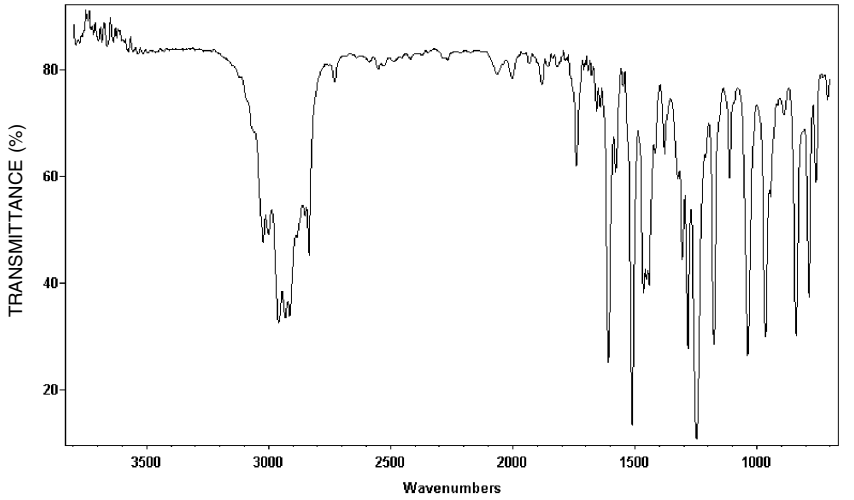
***d*-Fenchone** View Flavor Chemical



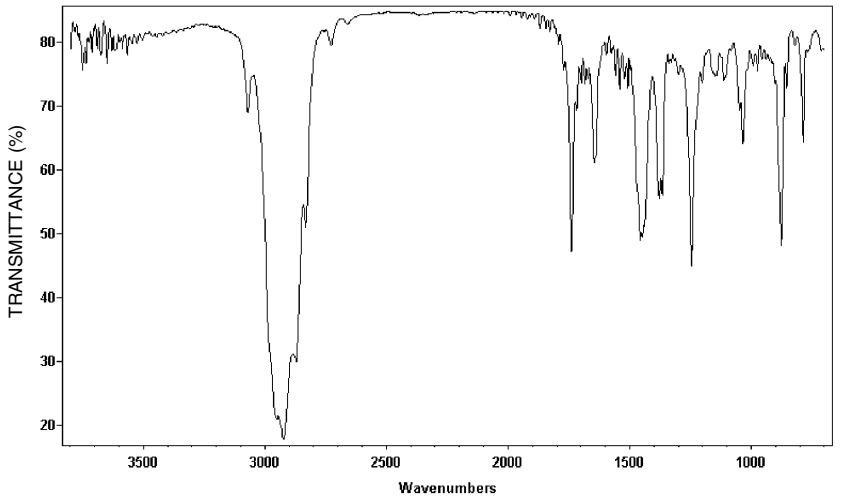
Fenchyl Alcohol View Flavor Chemical



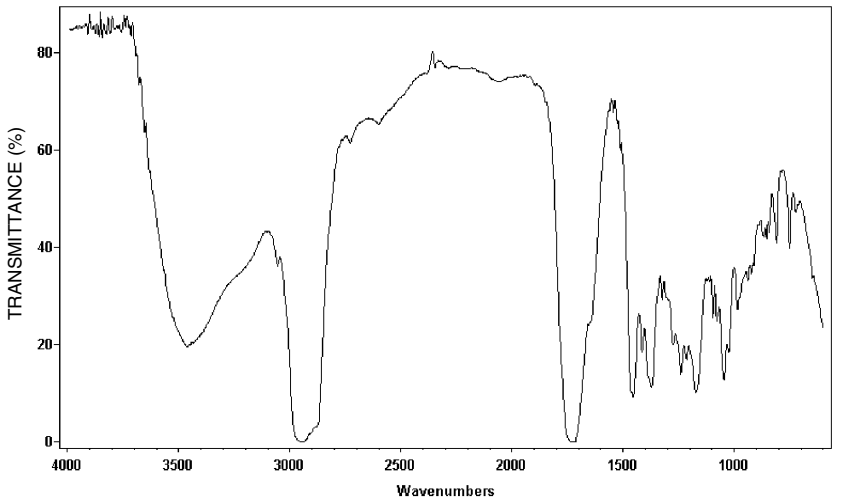
Fennel Oil View Monograph



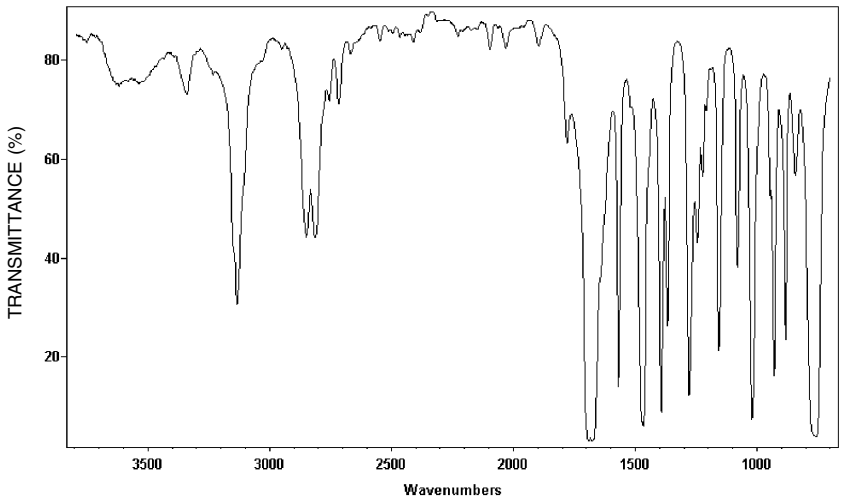
Fir Needle Oil, Canadian Type View Monograph



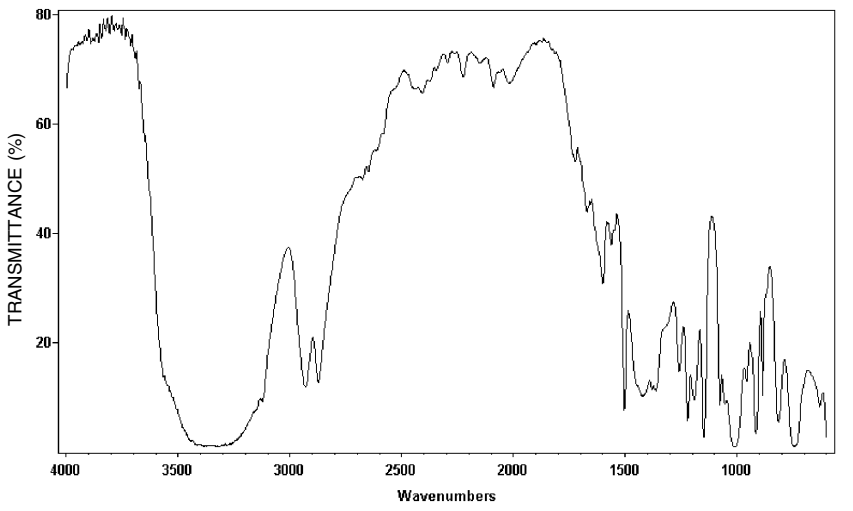
Fir Needle Oil, Siberian Type View Monograph



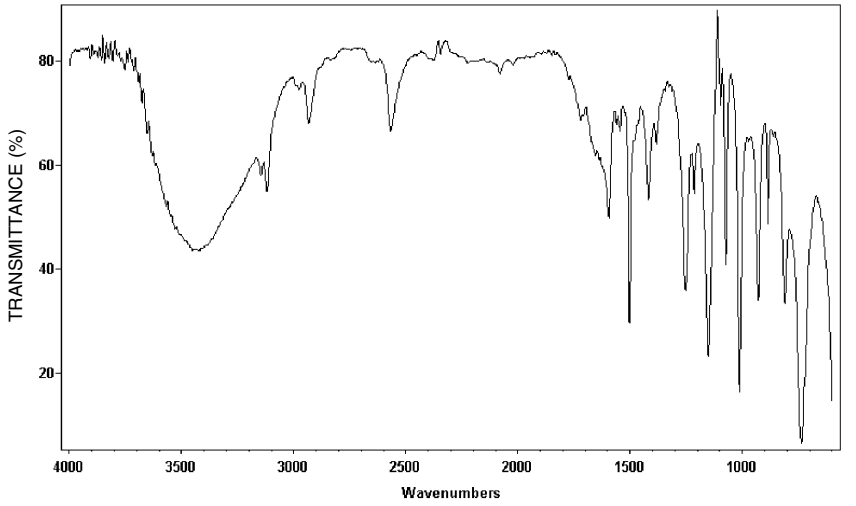
Furfural View Flavor Chemical



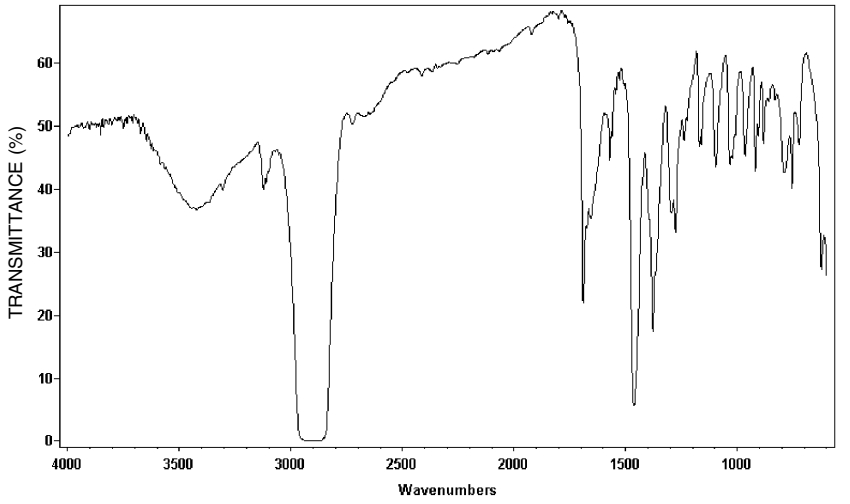
Furfuryl Alcohol View Flavor Chemical



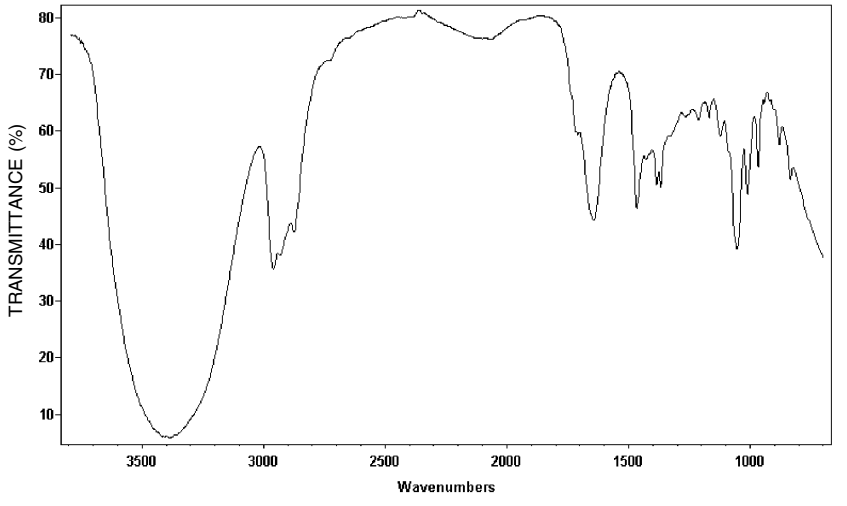
Furfuryl Mercaptan View Flavor Chemical



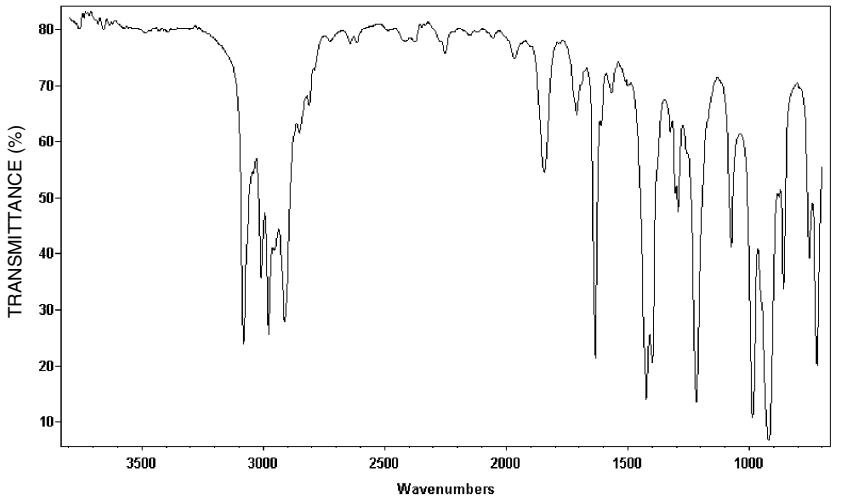
2-Furyl Methyl Ketone View Flavor Chemical



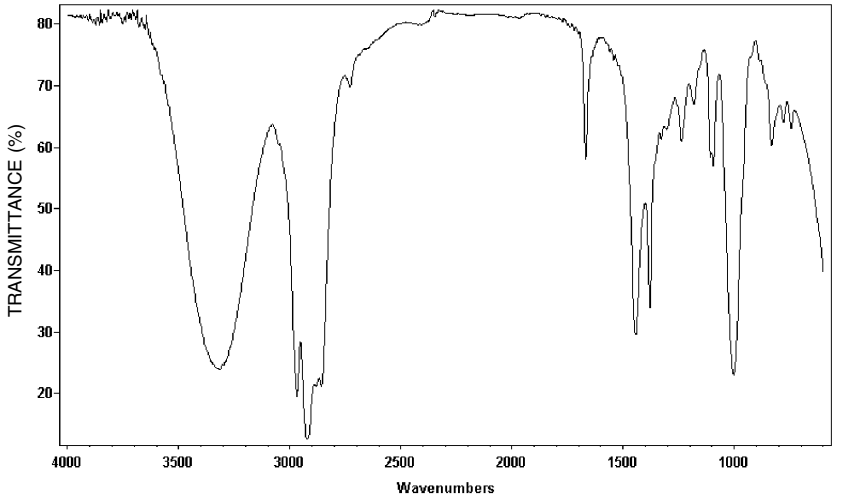
Fusel Oil, Refined View Flavor Chemical



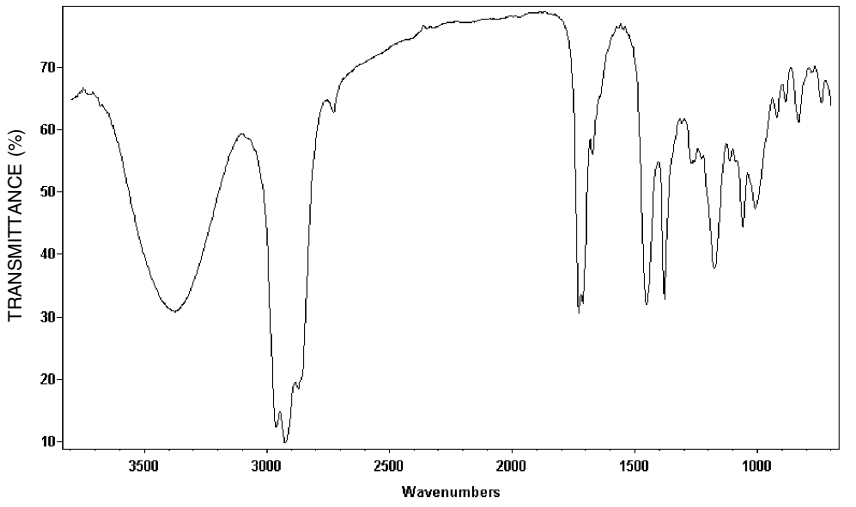
Garlic Oil View Monograph



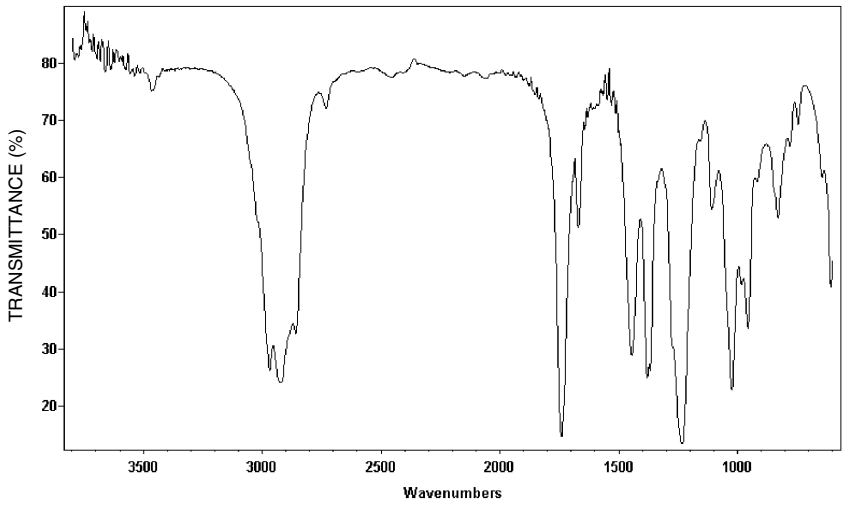
Geraniol View Flavor Chemical



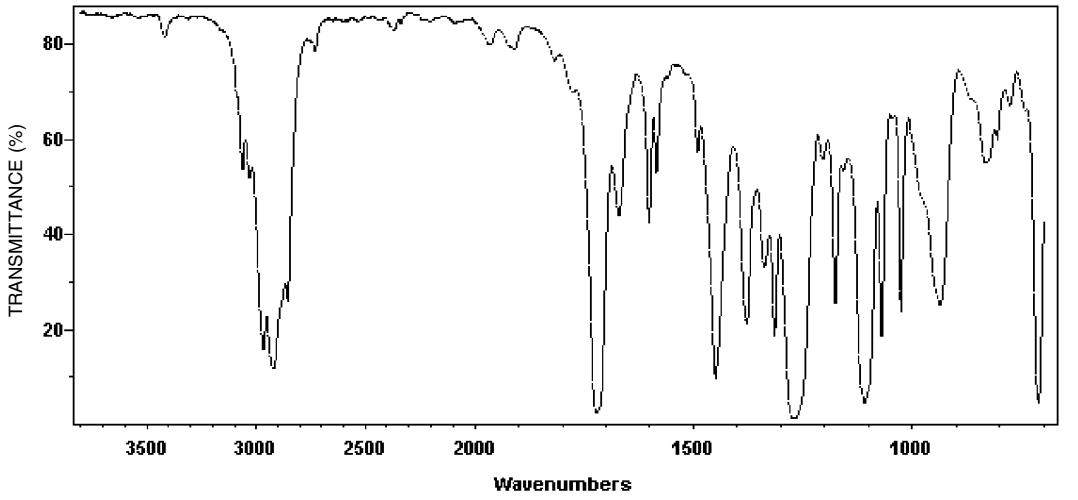
Geranium Oil, Algerian Type View Monograph



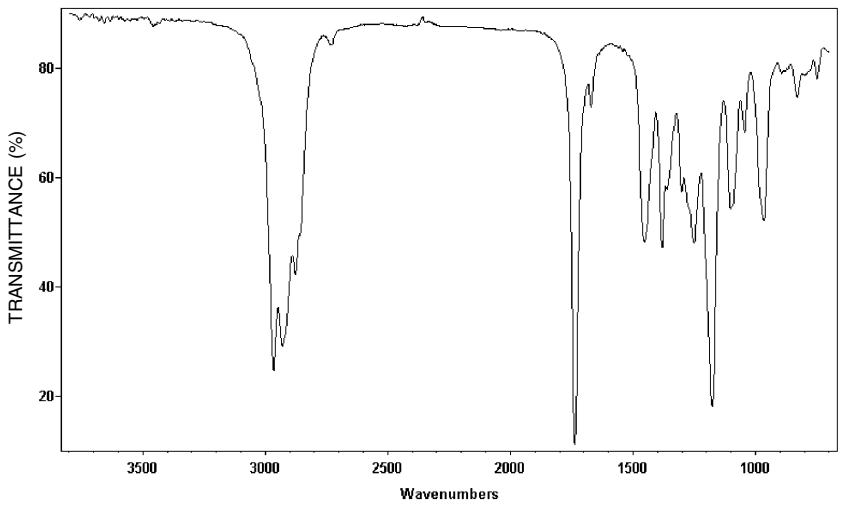
Geranyl Acetate View Flavor Chemical



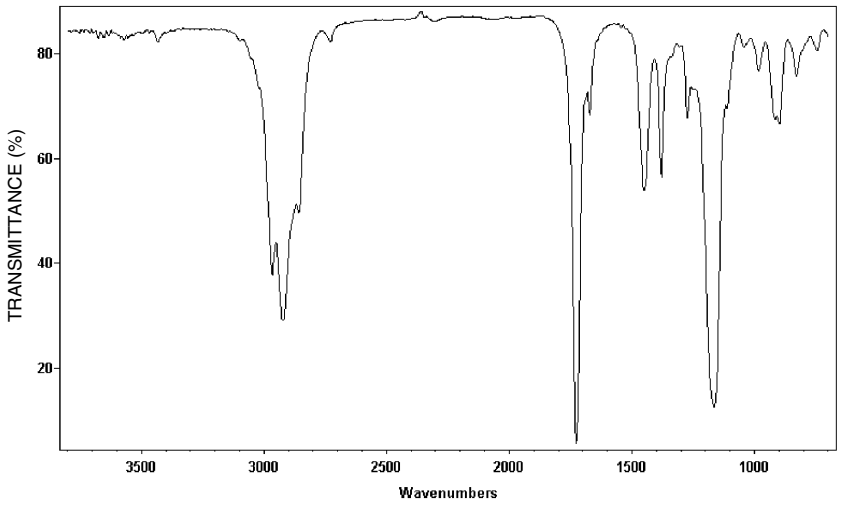
Geranyl Benzoate View Flavor Chemical



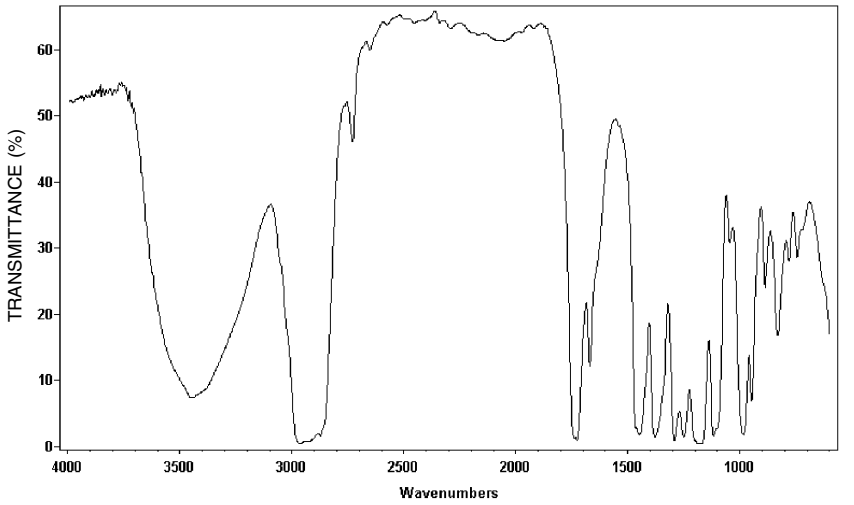
Geranyl Butyrate View Flavor Chemical



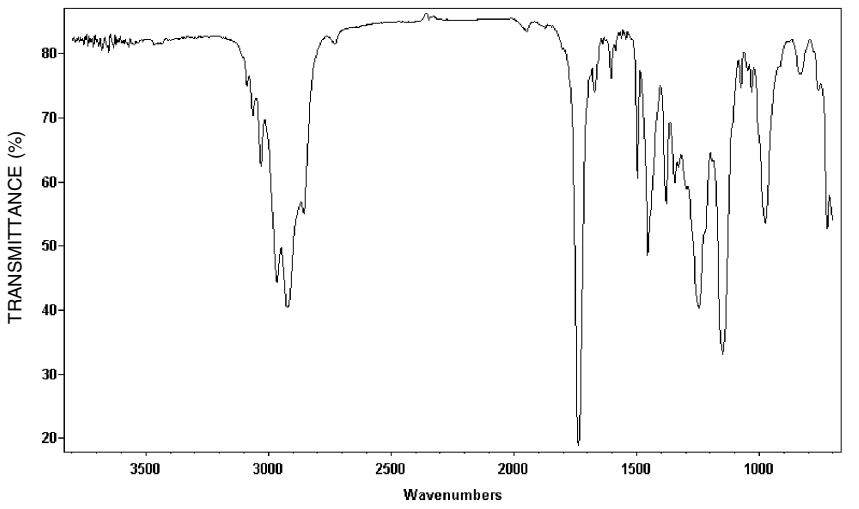
Geranyl Formate View Flavor Chemical



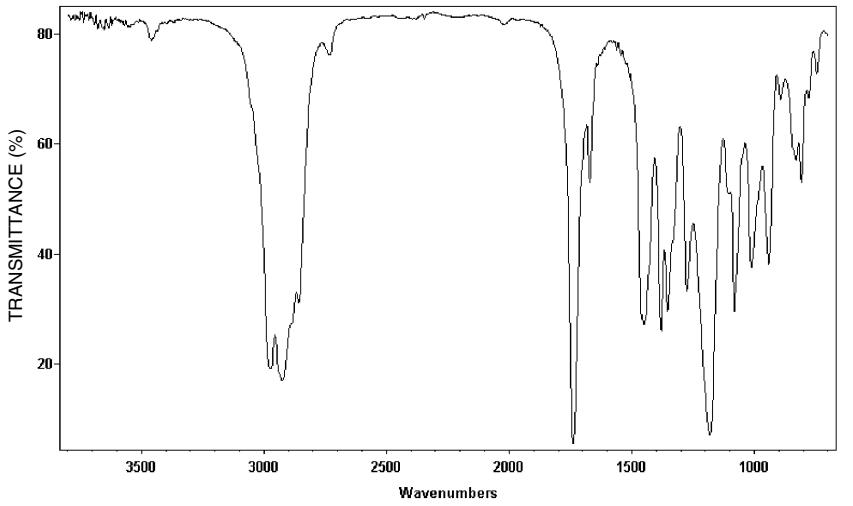
Geranyl Isovalerate View Flavor Chemical



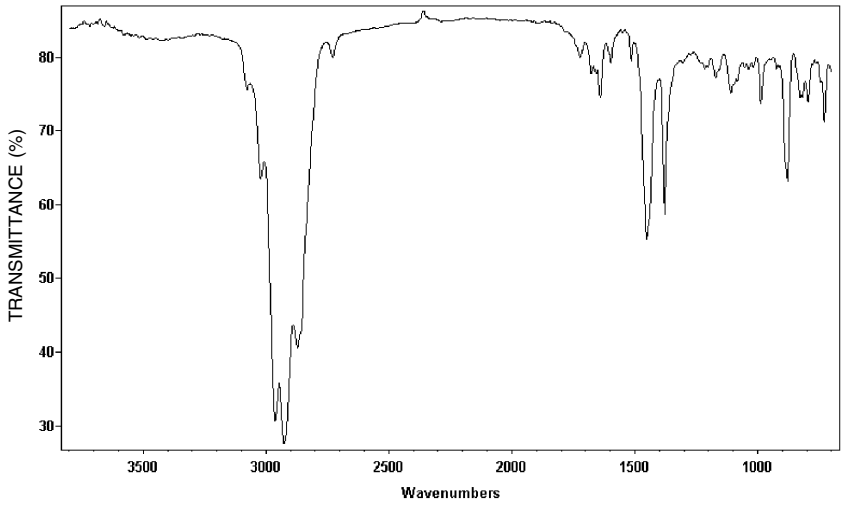
Geranyl Phenylacetate View Flavor Chemical



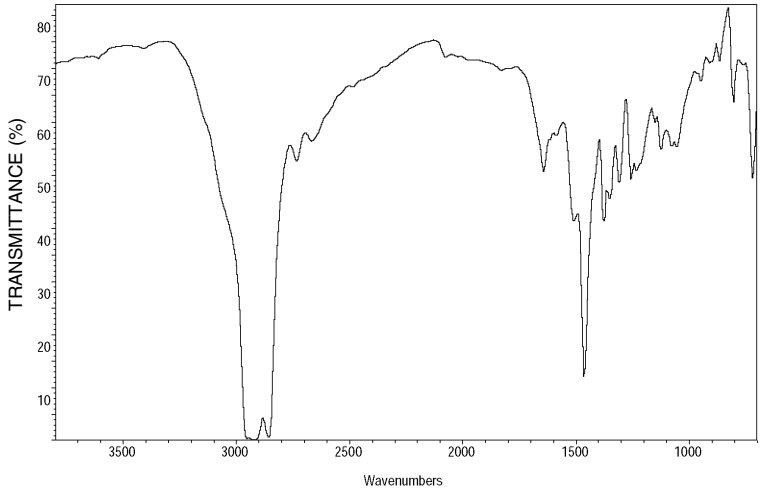
Geranyl Propionate **View Flavor Chemical**



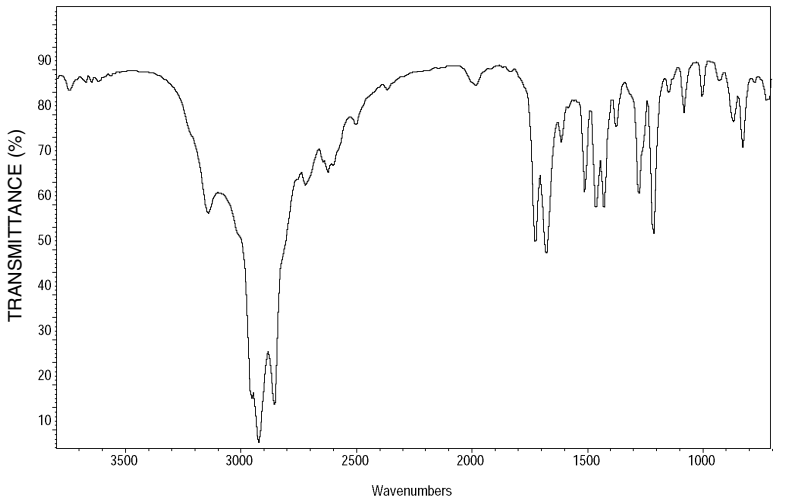
Ginger Oil **View Monograph**



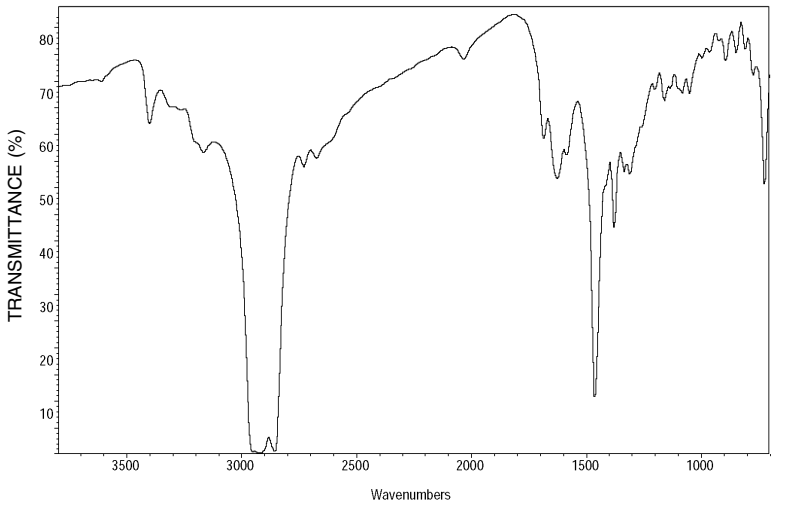
L-Glutamic Acid (Mineral Oil Mull) **View Monograph**



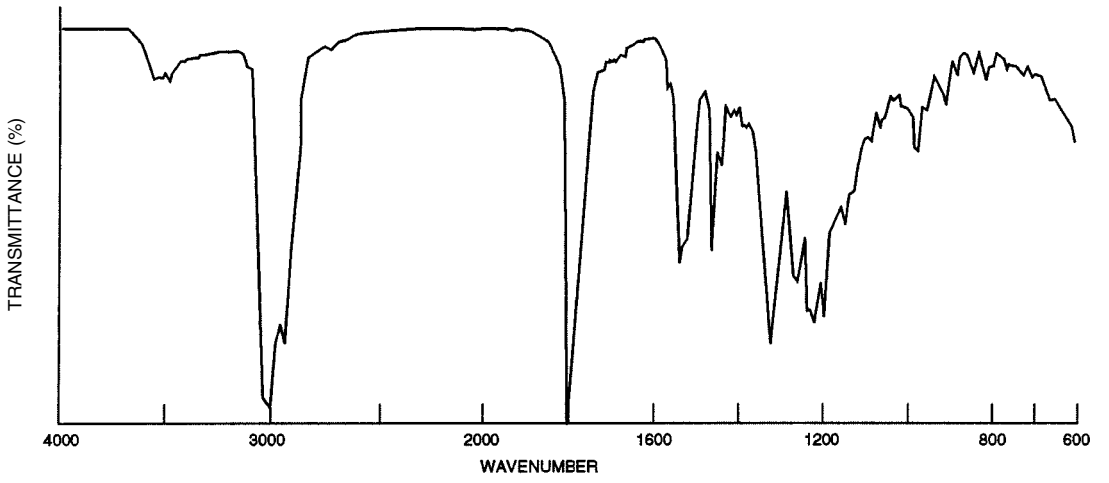
L-Glutamic Acid Hydrochloride View Monograph



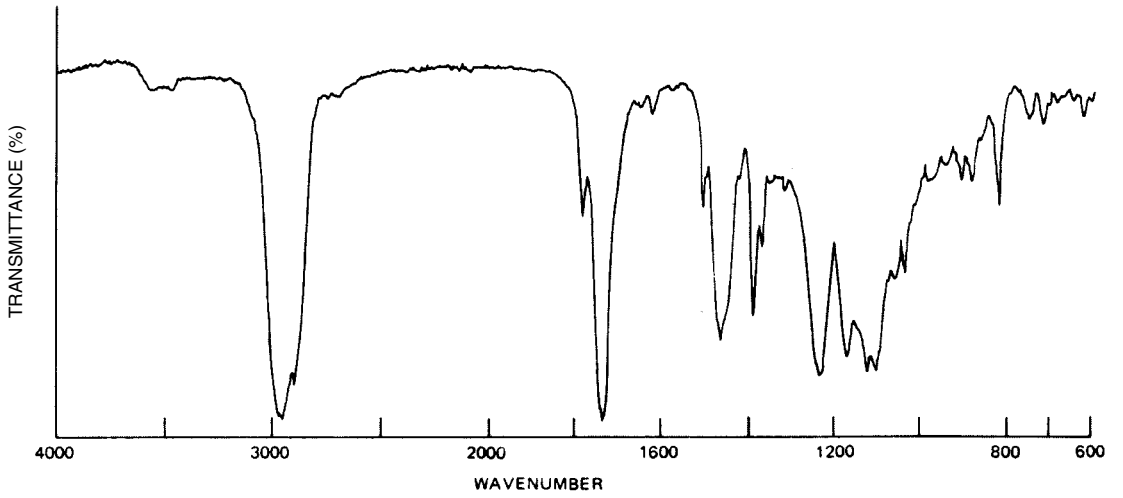
L-Glutamine (Mineral Oil Mull) View Monograph



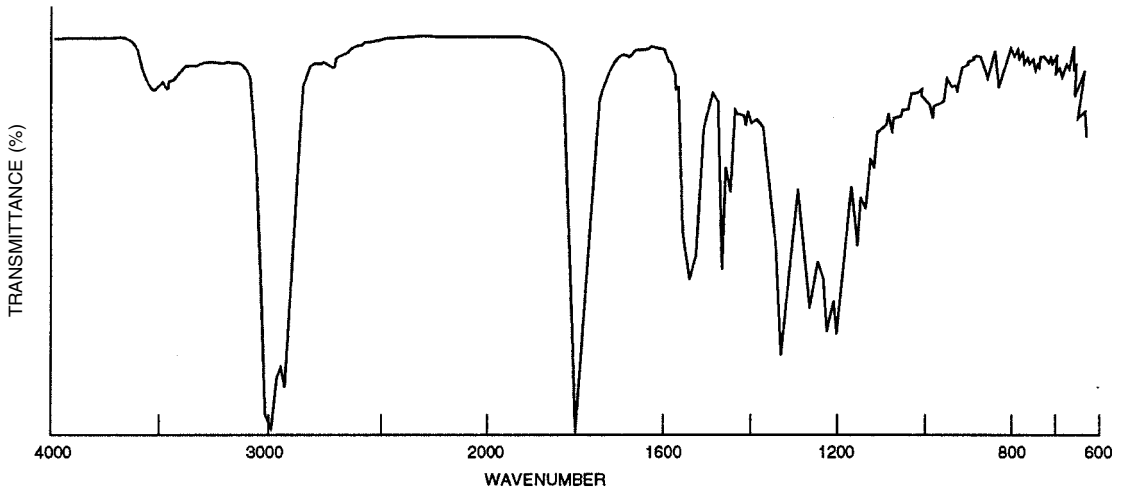
Glycerol Ester of Gum Rosin View Monograph



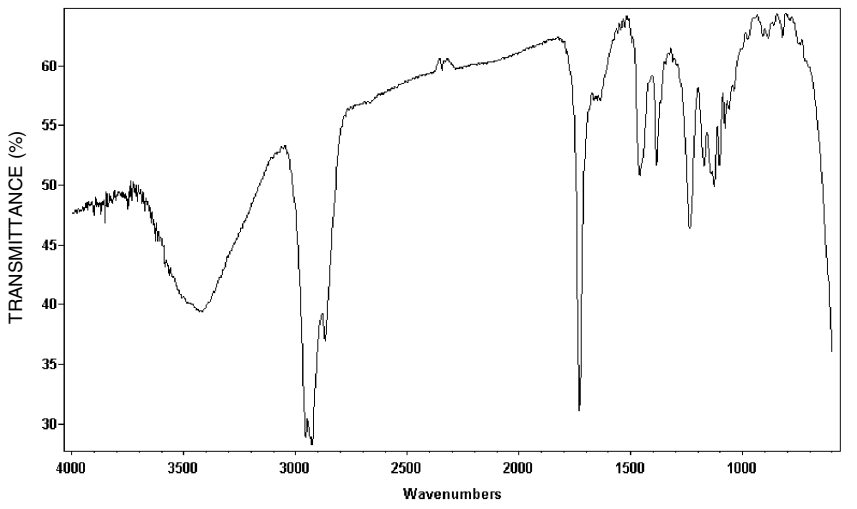
Glycerol Ester of Partially Dimerized Rosin View Monograph



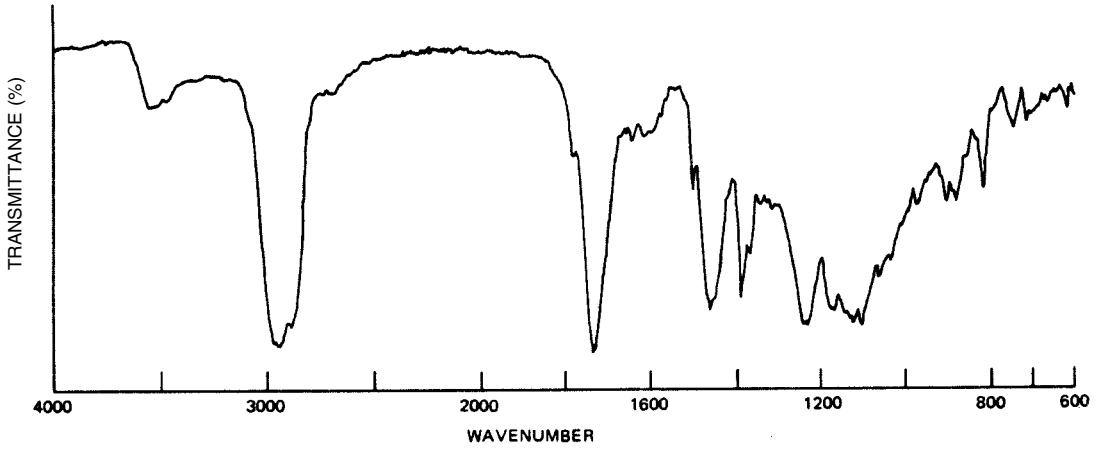
Glycerol Ester of Partially Hydrogenated Gum Rosin View Monograph



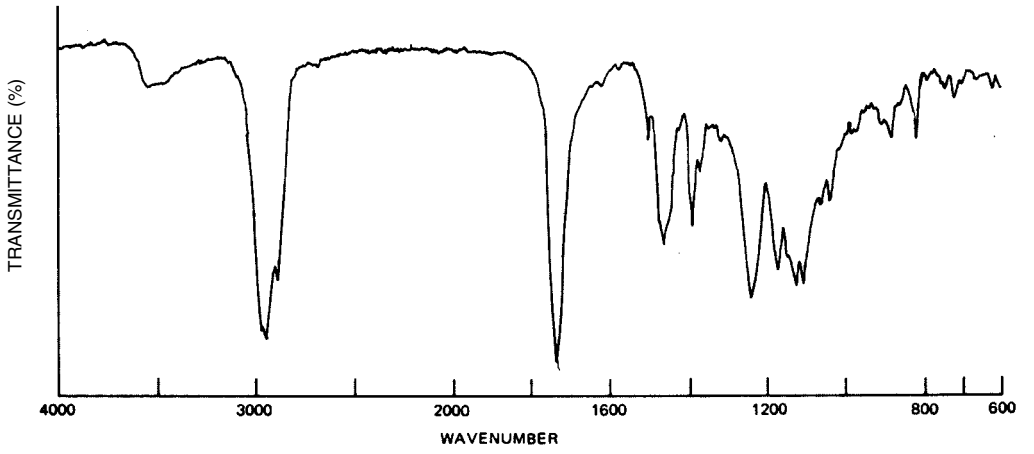
Glycerol Ester of Partially Hydrogenated Wood Rosin View Monograph



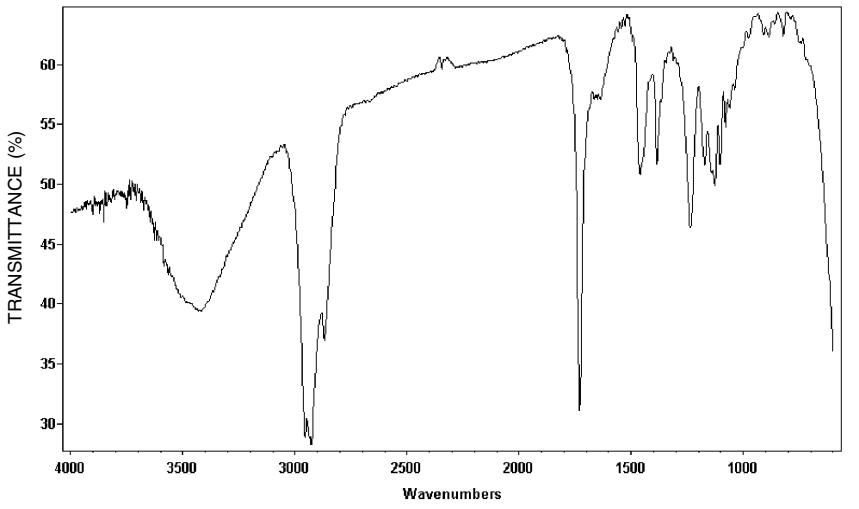
Glycerol Ester of Polymerized Rosin View Monograph



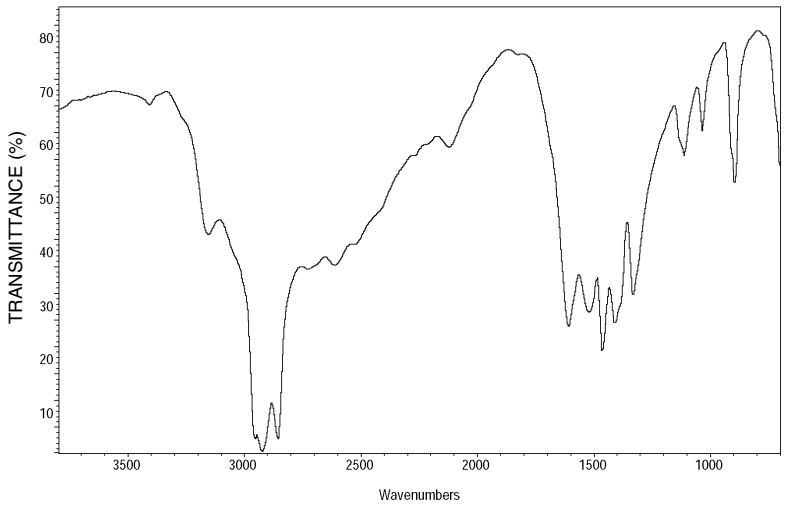
Glycerol Ester of Tall Oil Rosin View Monograph



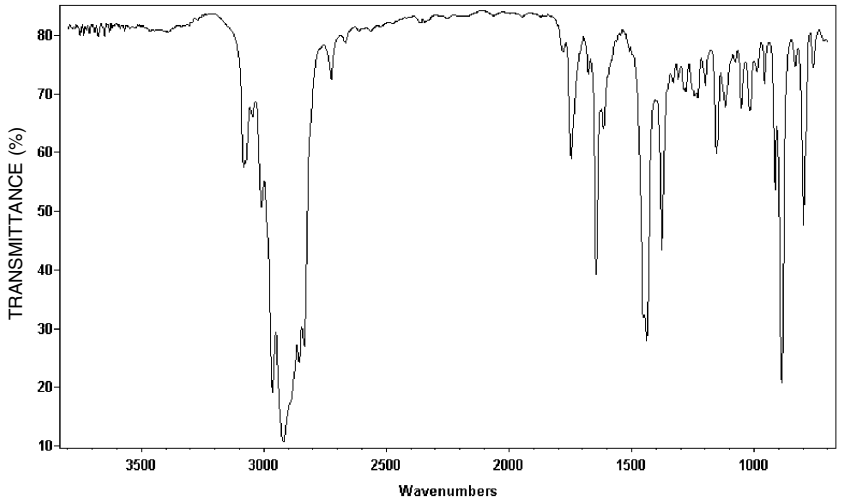
Glycerol Ester of Wood Rosin View Monograph



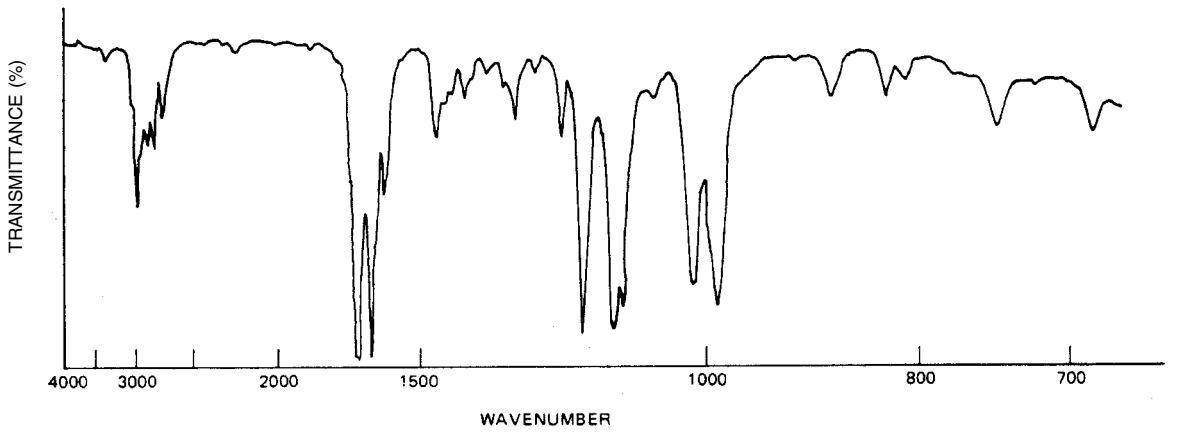
Glycine (Mineral Oil Mull) View Monograph



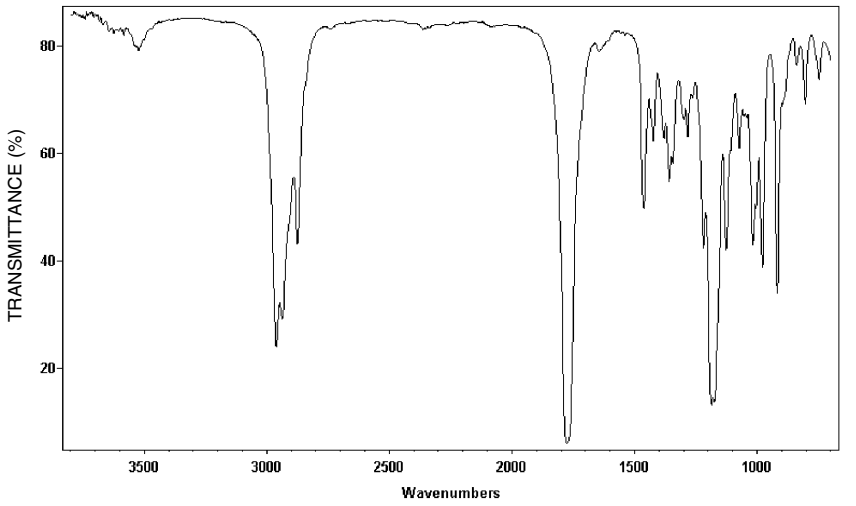
Grapefruit Oil, Coldpressed View Monograph



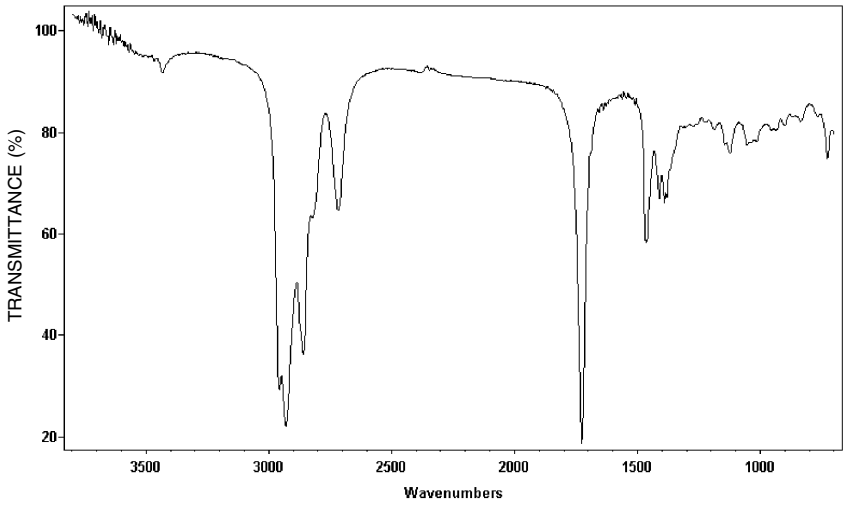
(*E*),(*E*)-2,4-Heptadienal View Flavor Chemical



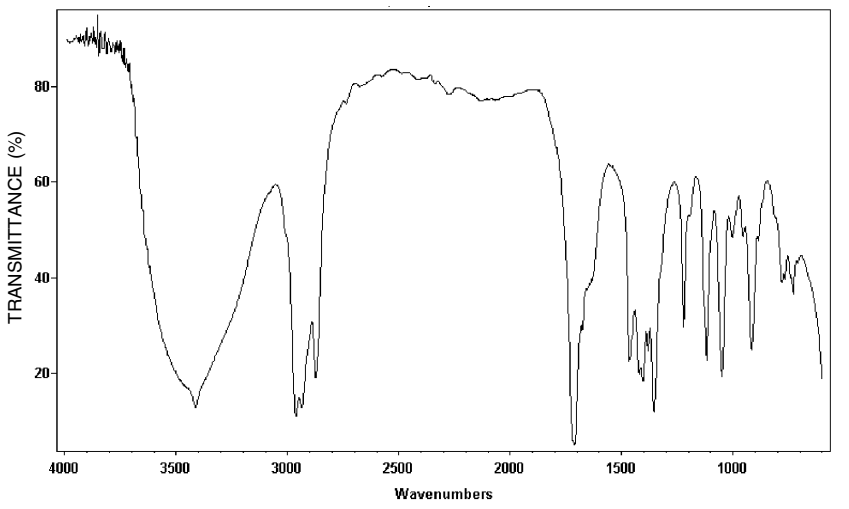
γ-Heptalactone View Flavor Chemical



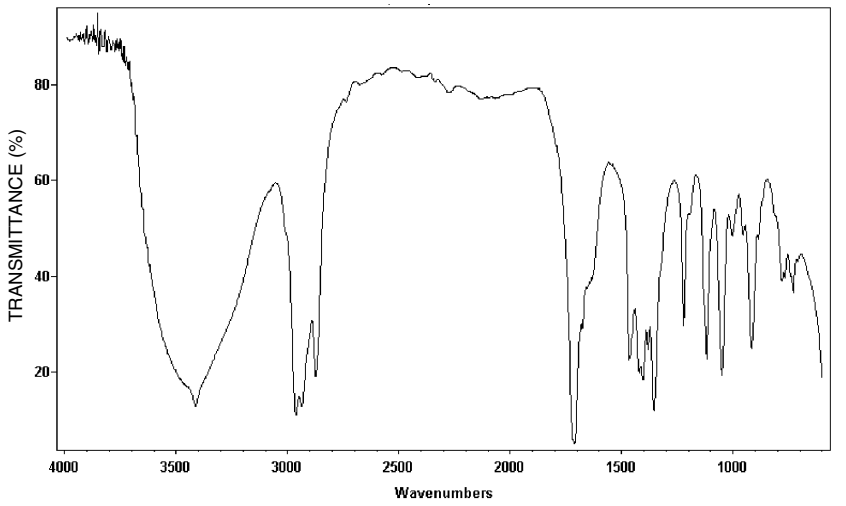
Heptanal View Flavor Chemical



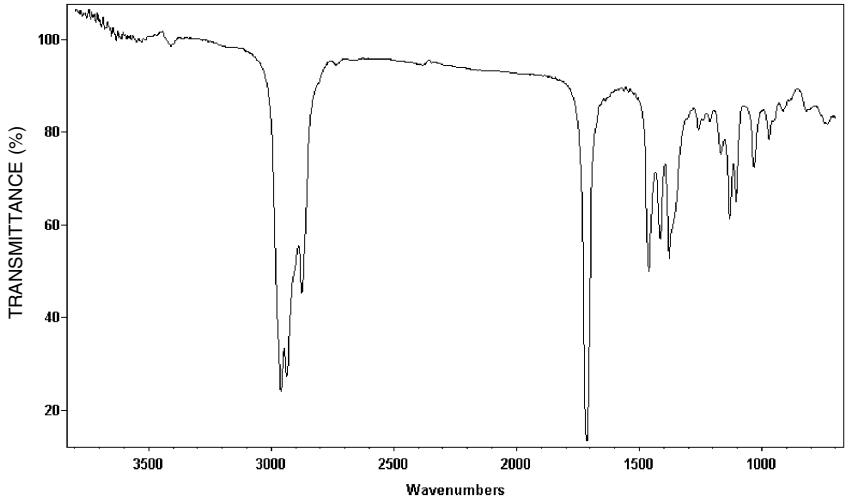
2,3-Heptandione View Flavor Chemical



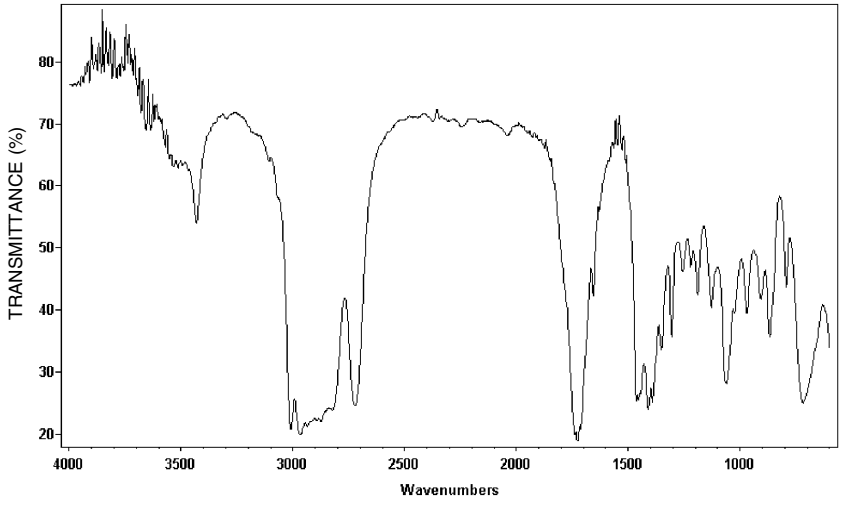
2-Heptanone View Flavor Chemical



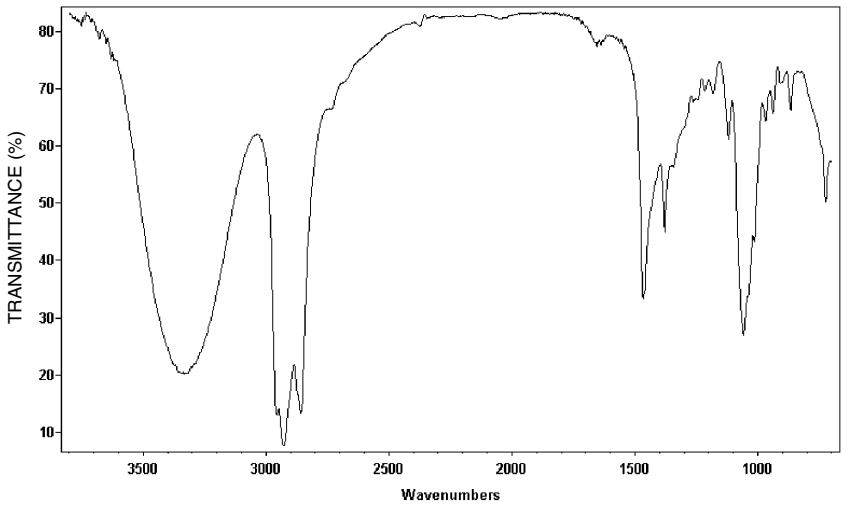
3-Heptanone View Flavor Chemical



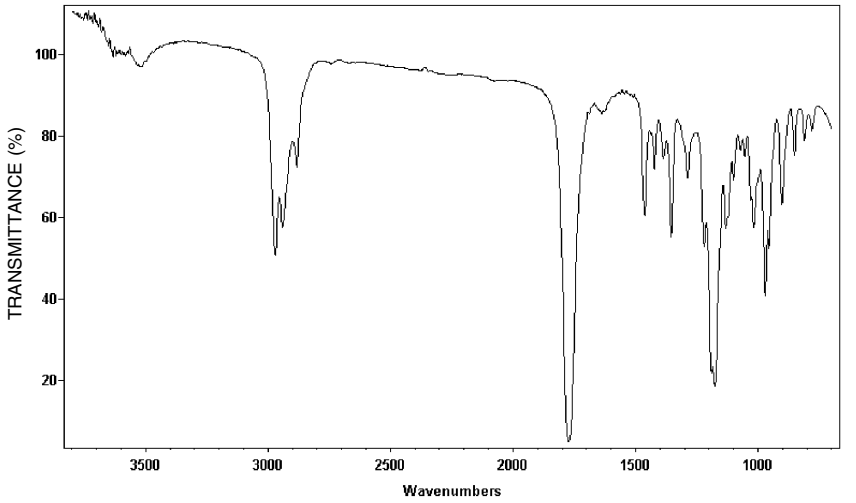
(Z)-4-Hepten-1-al View Flavor Chemical



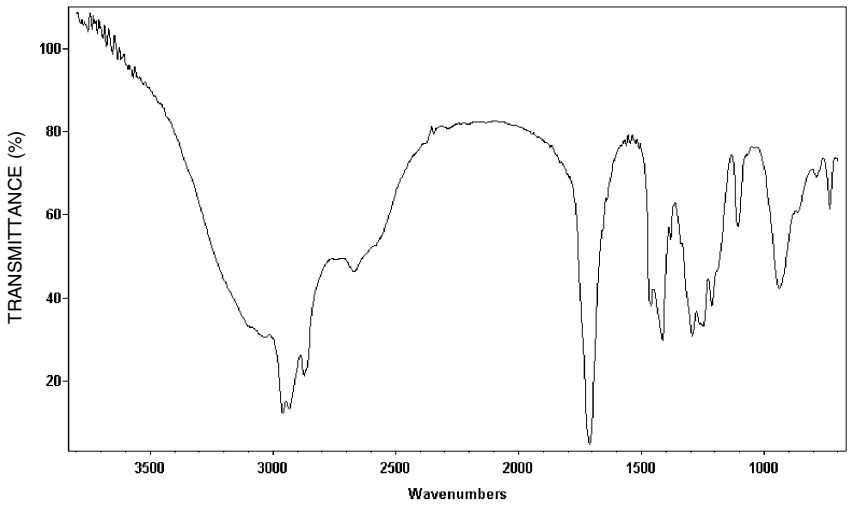
Heptyl Alcohol **View Flavor Chemical**



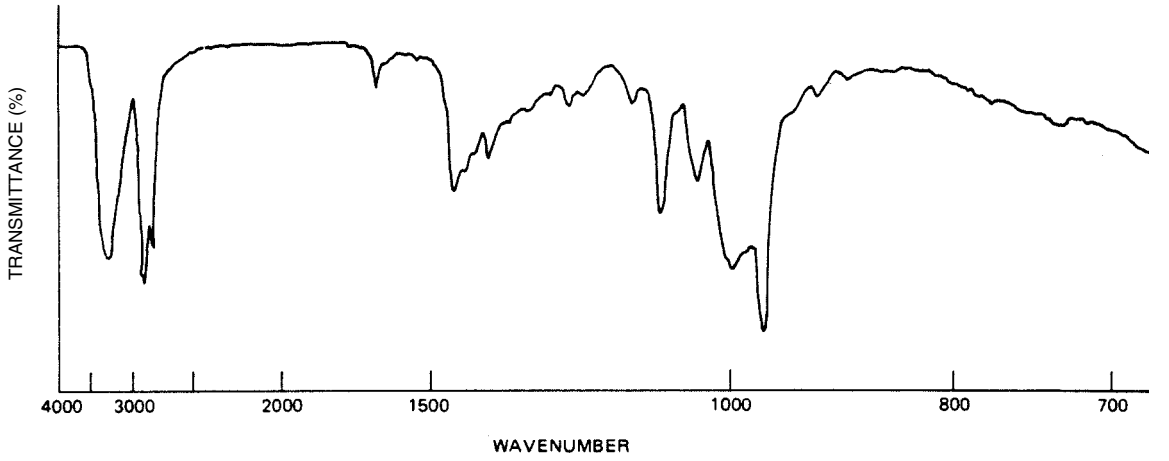
γ-Hexalactone **View Flavor Chemical**



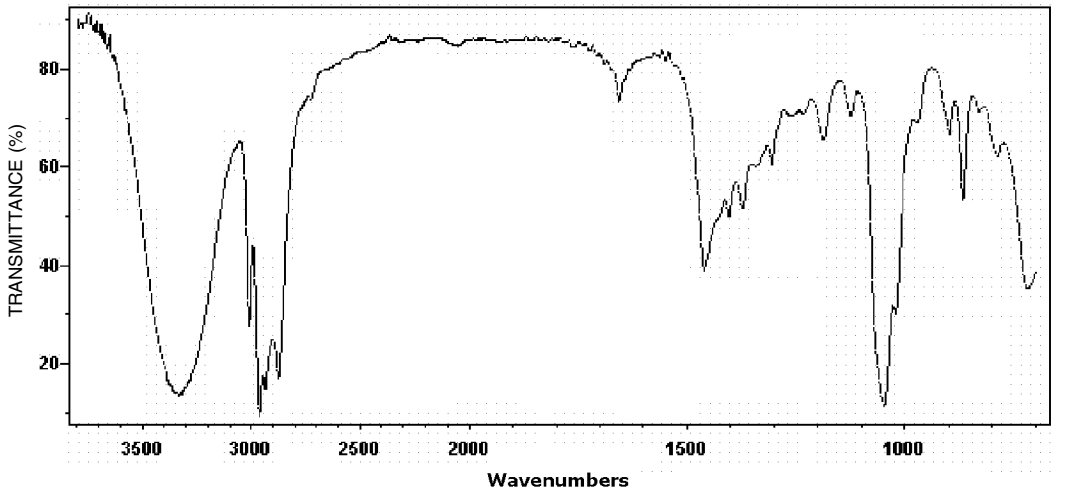
Hexanoic Acid **View Flavor Chemical**



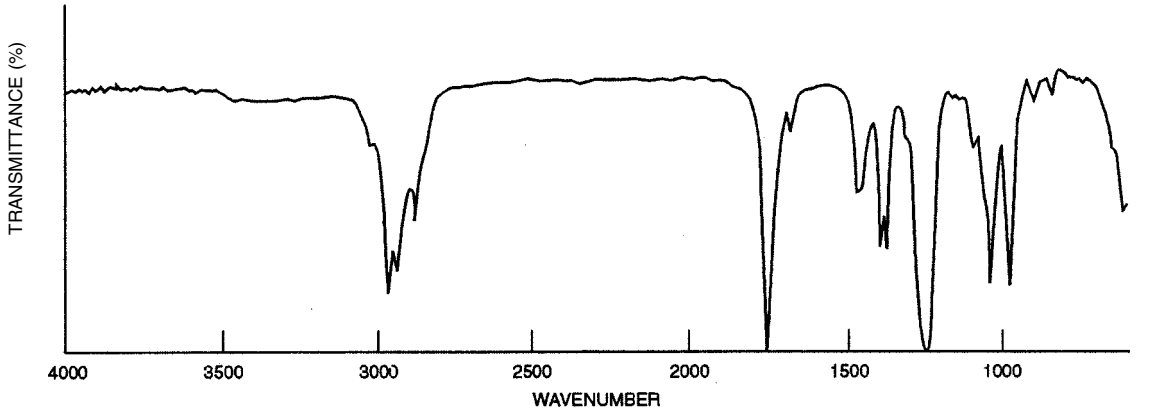
(*E*)-2-Hexen-1-ol View Flavor Chemical



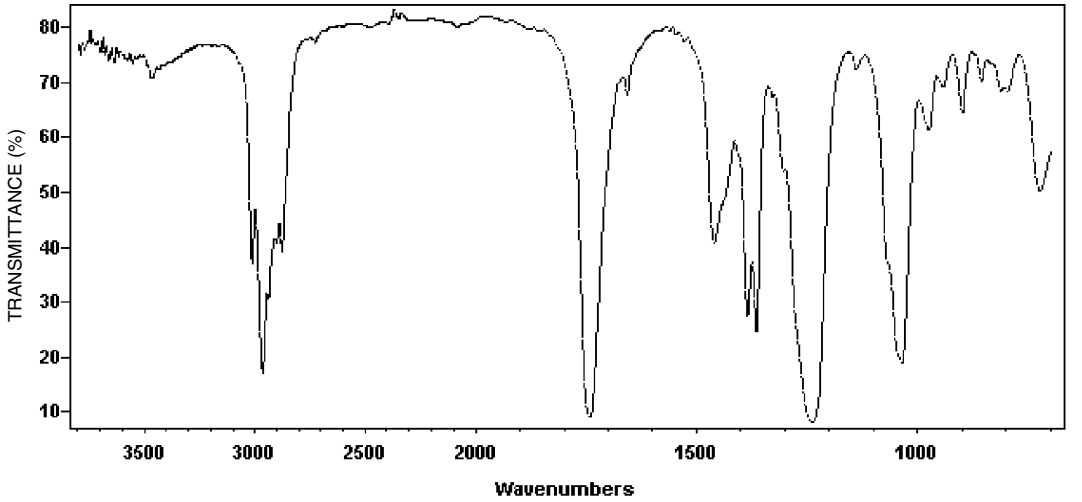
(*Z*)-3-Hexenol View Flavor Chemical



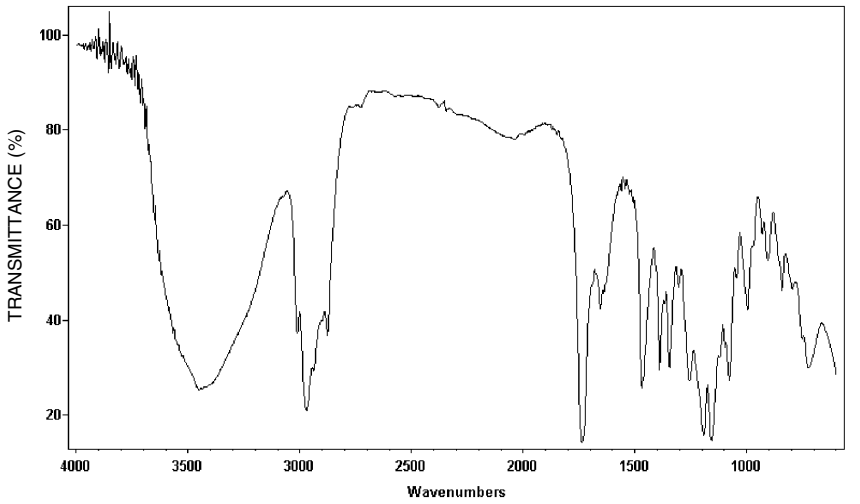
(*E*)-2-Hexenyl Acetate View Flavor Chemical



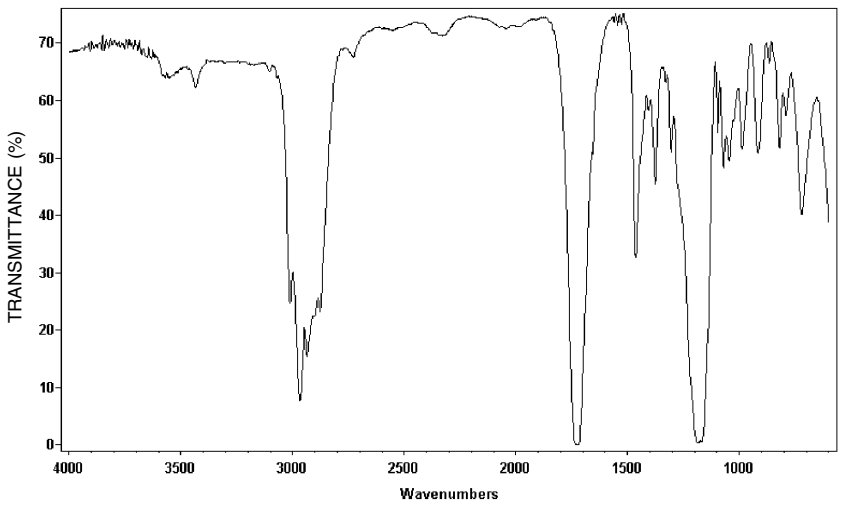
(Z)-3-Hexenyl Acetate View Flavor Chemical



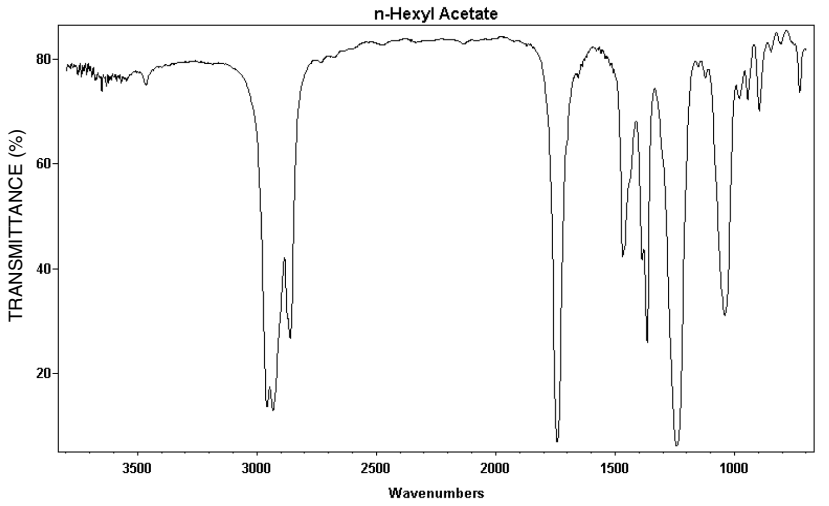
(Z)-3-Hexenyl Butyrate View Flavor Chemical



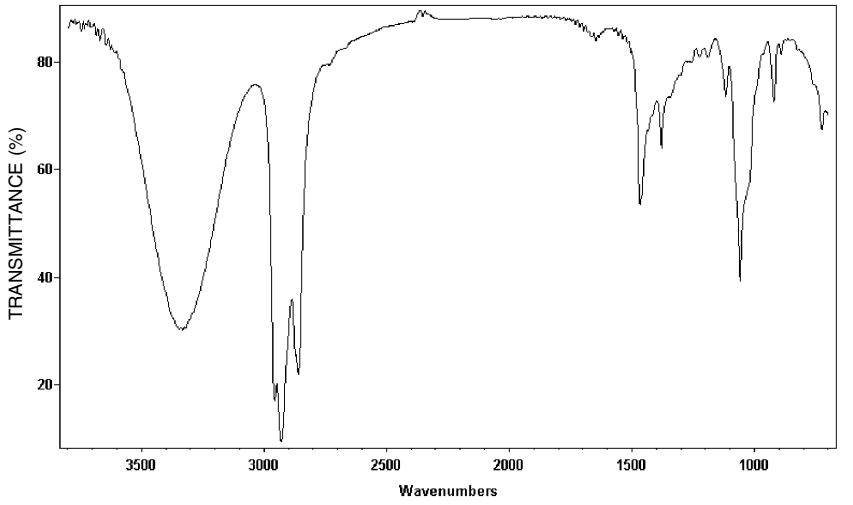
(Z)-3-Hexenyl Formate View Flavor Chemical



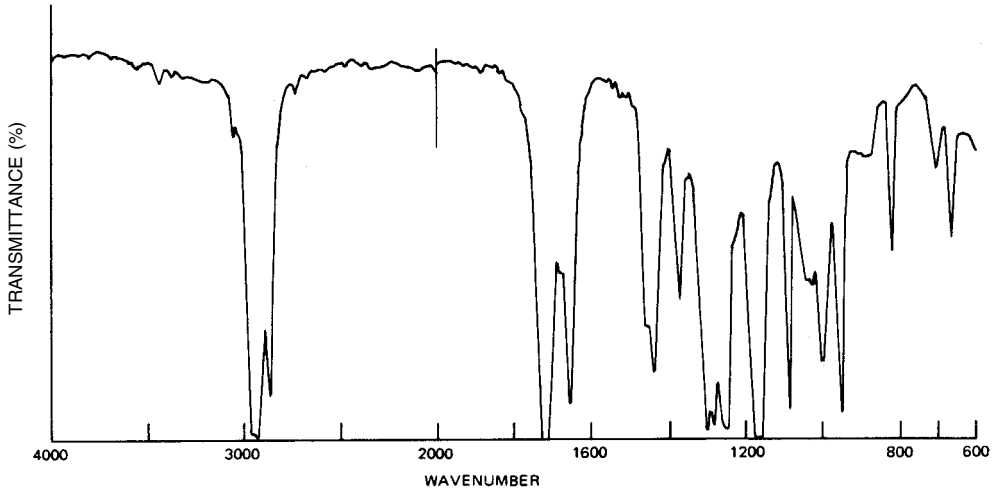
Hexyl Acetate **View Flavor Chemical**



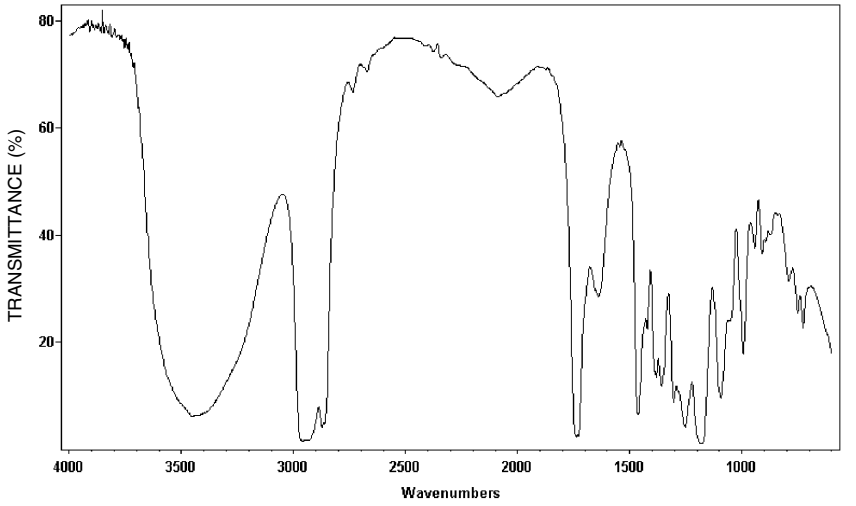
Hexyl Alcohol **View Flavor Chemical**



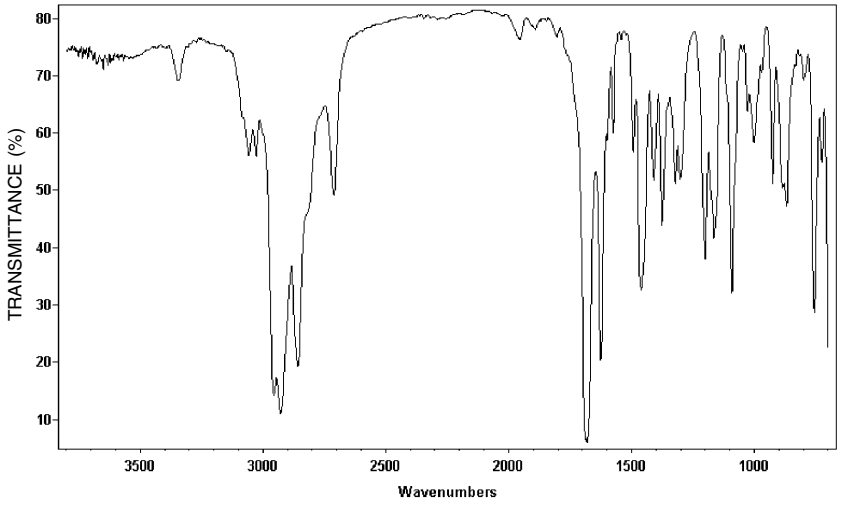
Hexyl-2-butenoate **View Flavor Chemical**



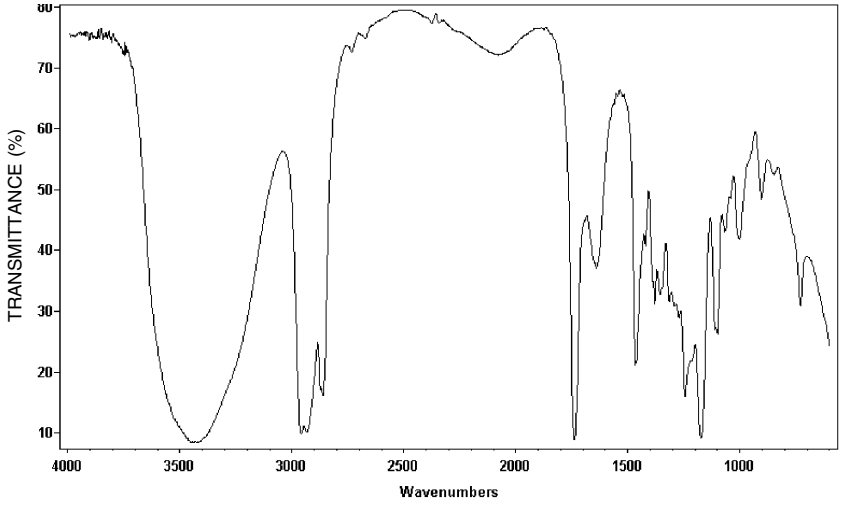
Hexyl Butyrate View Flavor Chemical



α-Hexylcinnamaldehyde View Flavor Chemical

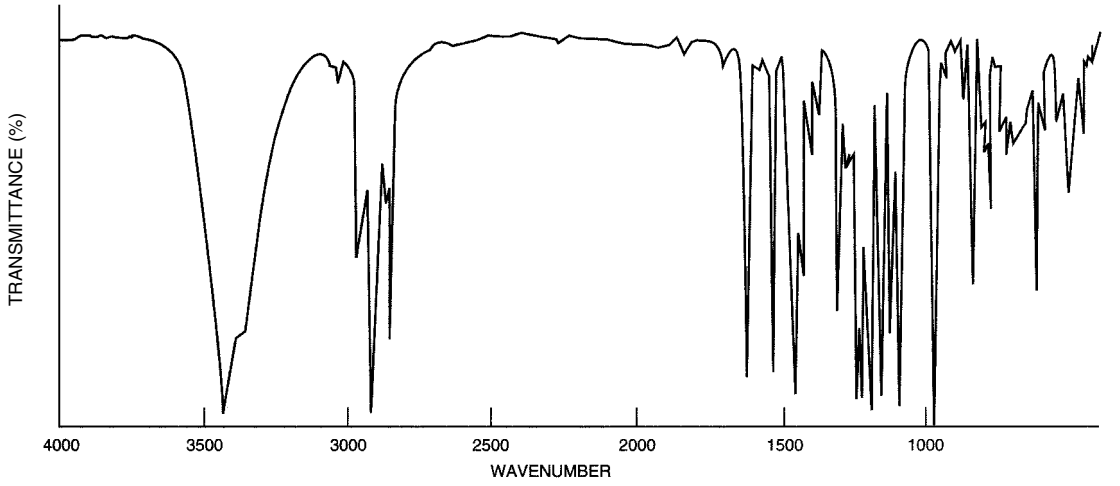


Hexyl Hexanoate View Flavor Chemical



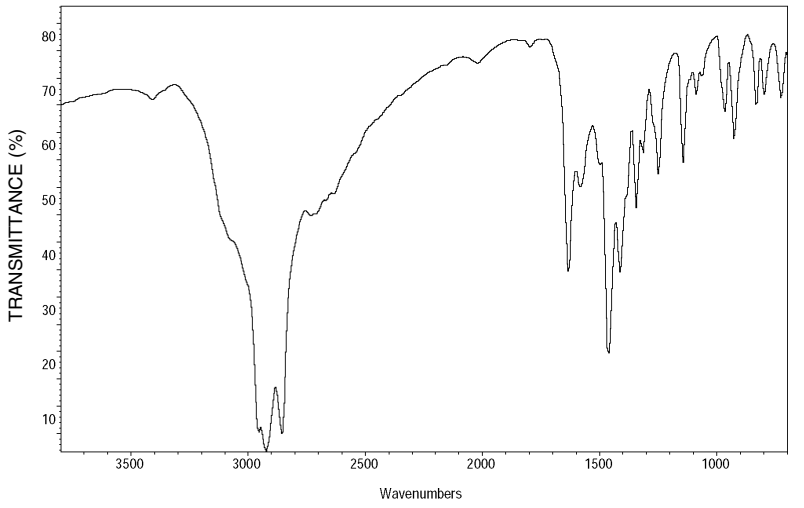
4-Hexylresorcinol

View Monograph



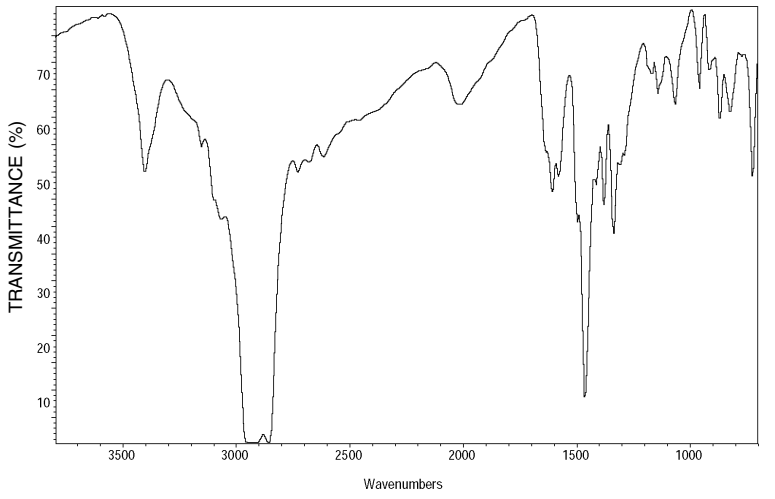
L-Histidine (Mineral Oil Mull)

View Monograph

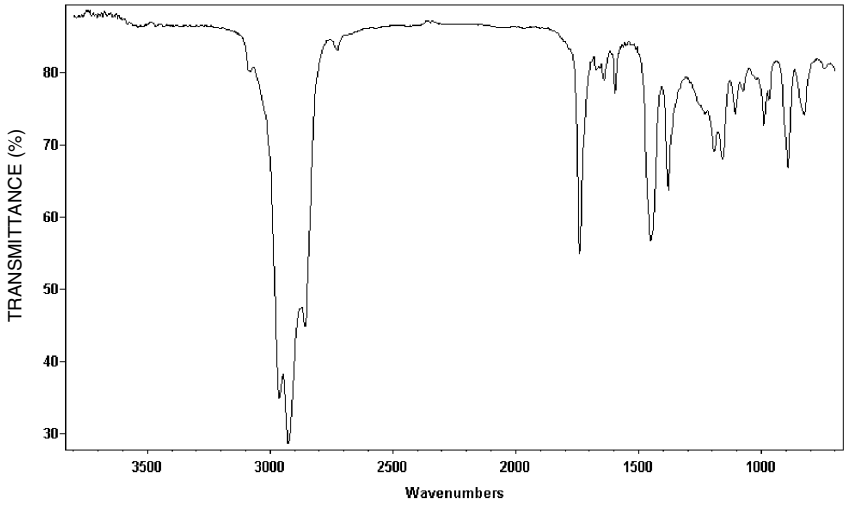


L-Histidine Monohydrochloride

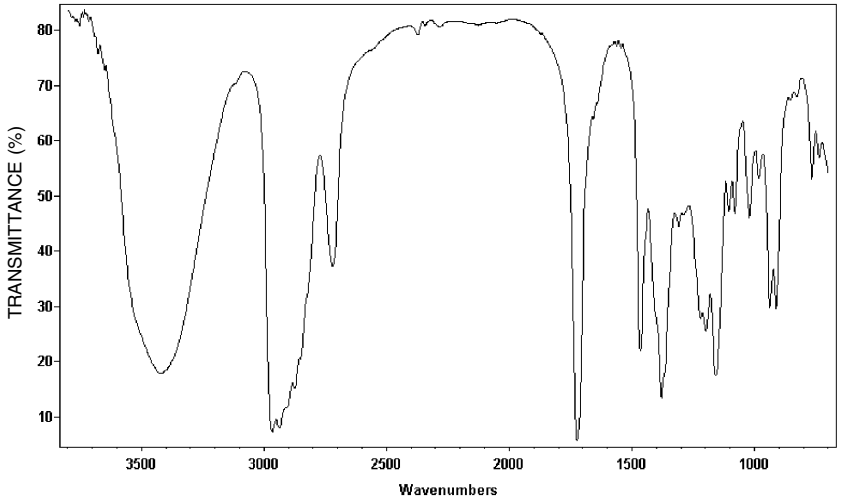
View Monograph



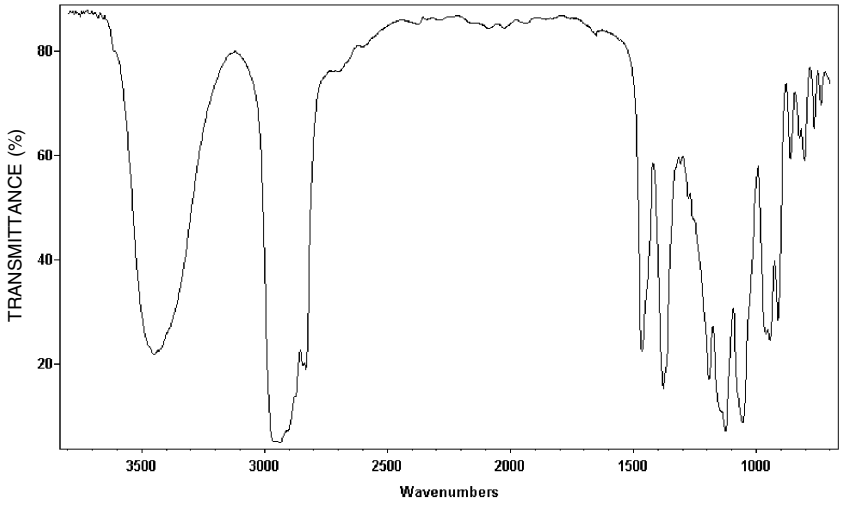
Hops Oil View Monograph



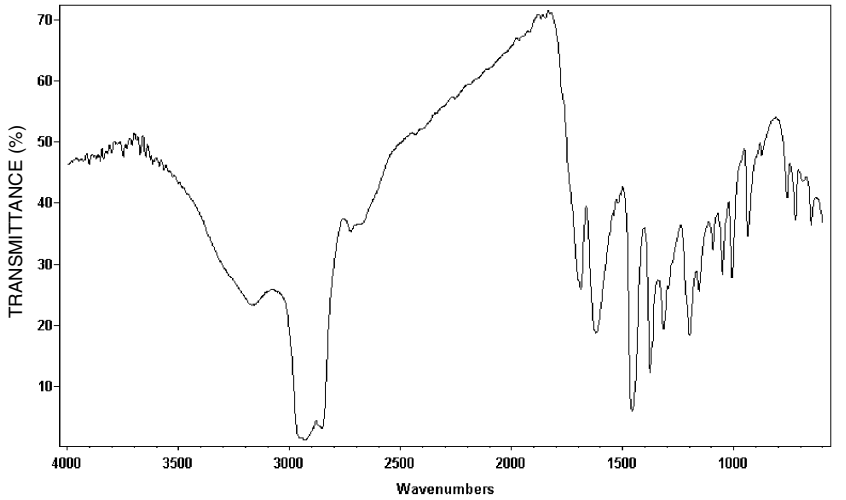
Hydroxycitronellal View Flavor Chemical



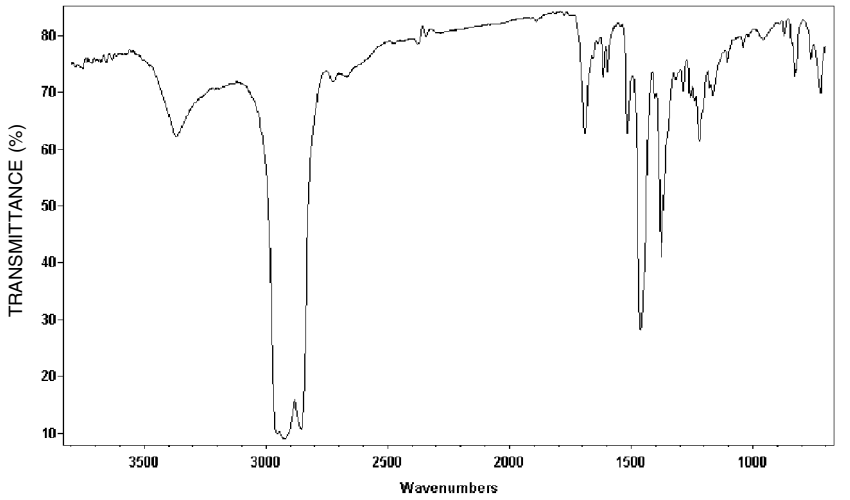
Hydroxycitronellal Dimethyl Acetal View Flavor Chemical



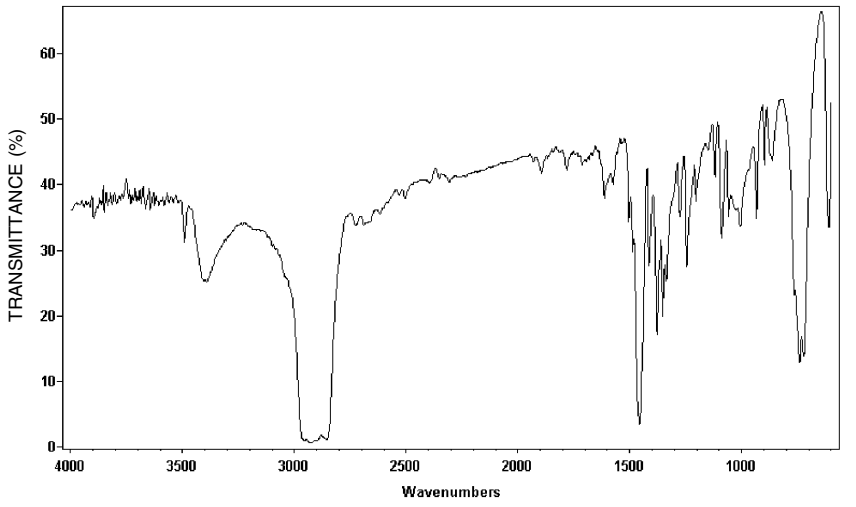
4-Hydroxy-2,5-dimethyl-3(2H)-furanone View Flavor Chemical



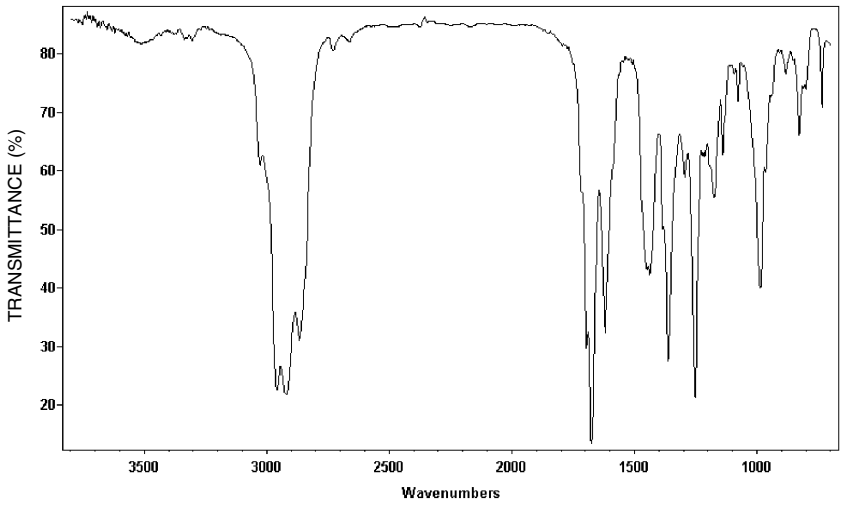
4-(*p*-Hydroxyphenyl)-2-butanone View Flavor Chemical



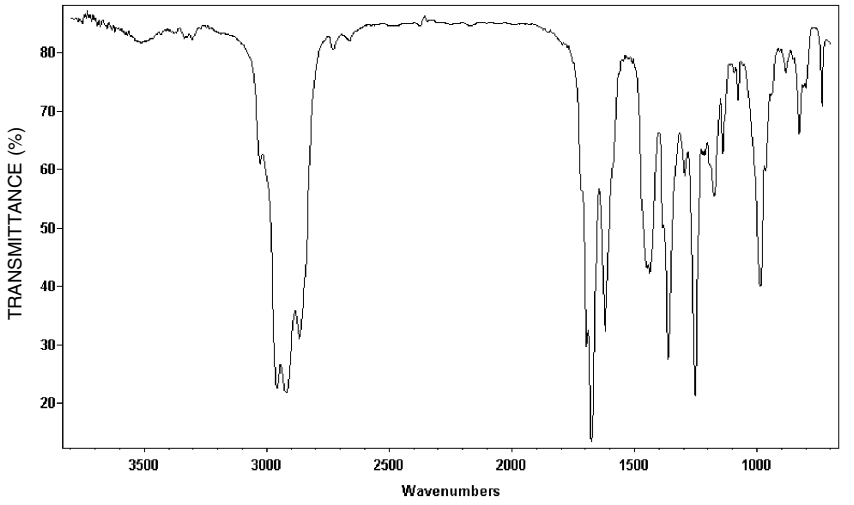
Indole (Mineral Oil Mull) View Flavor Chemical



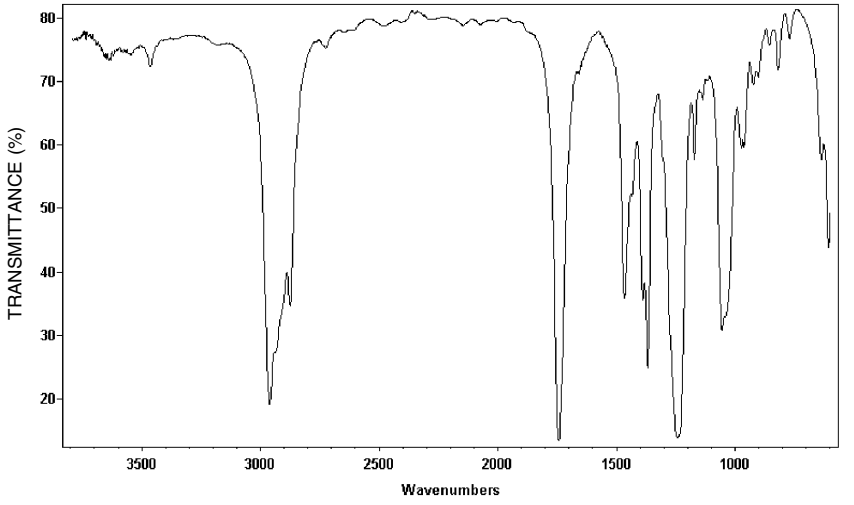
α-Ionone View Flavor Chemical



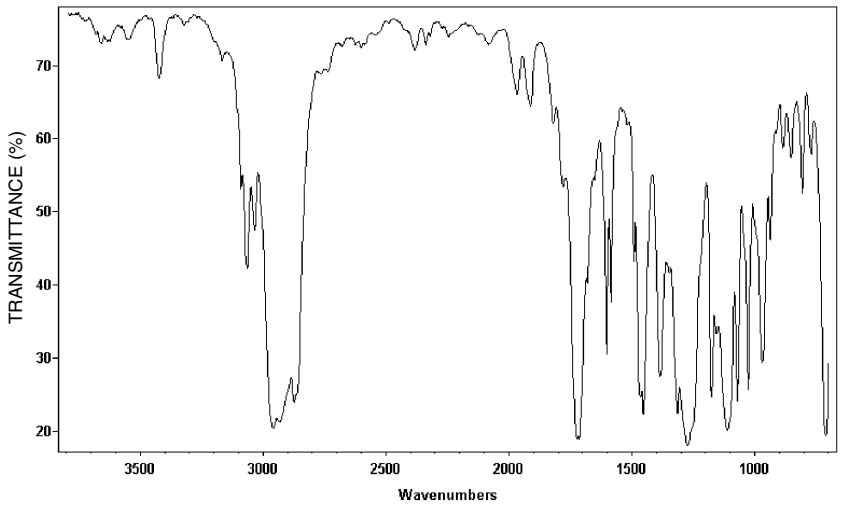
β-Ionone View Flavor Chemical



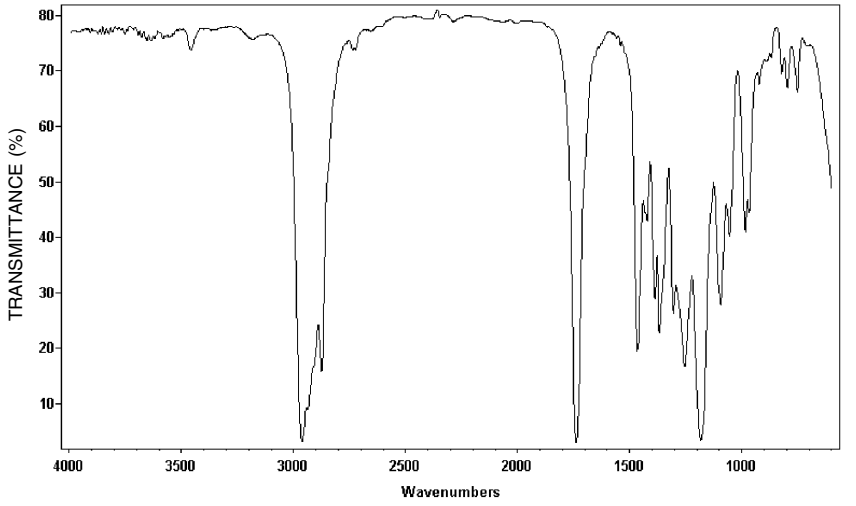
Isoamyl Acetate View Flavor Chemical



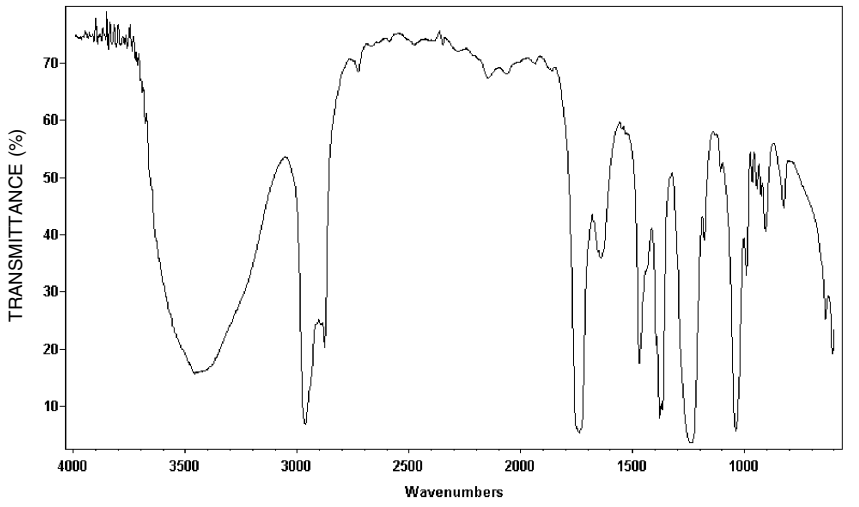
Isoamyl Benzoate View Flavor Chemical



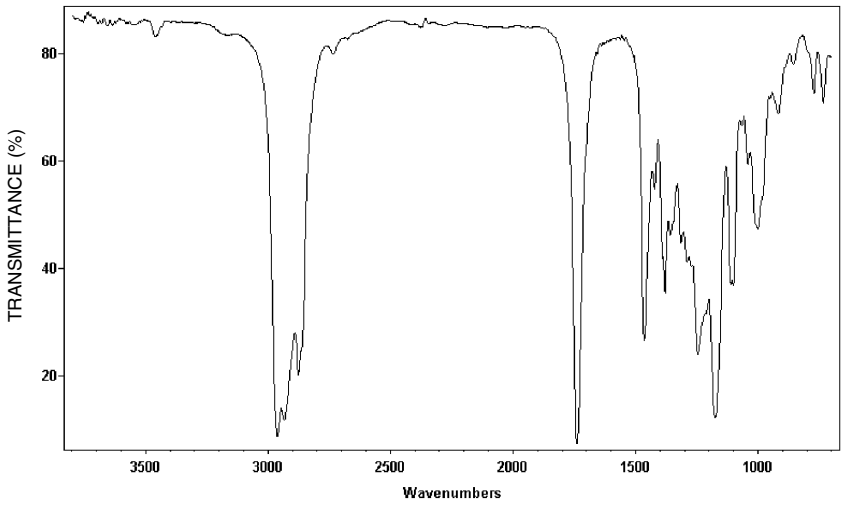
Isoamyl Butyrate View Flavor Chemical



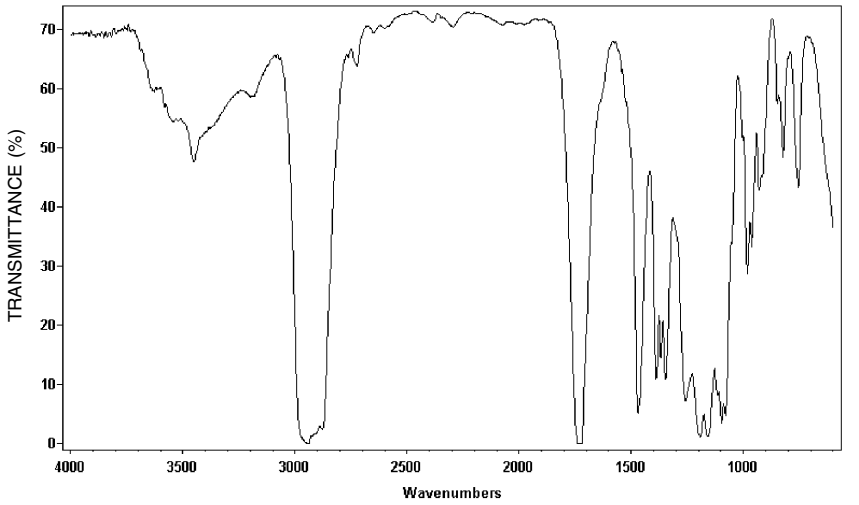
Isoamyl Formate View Flavor Chemical



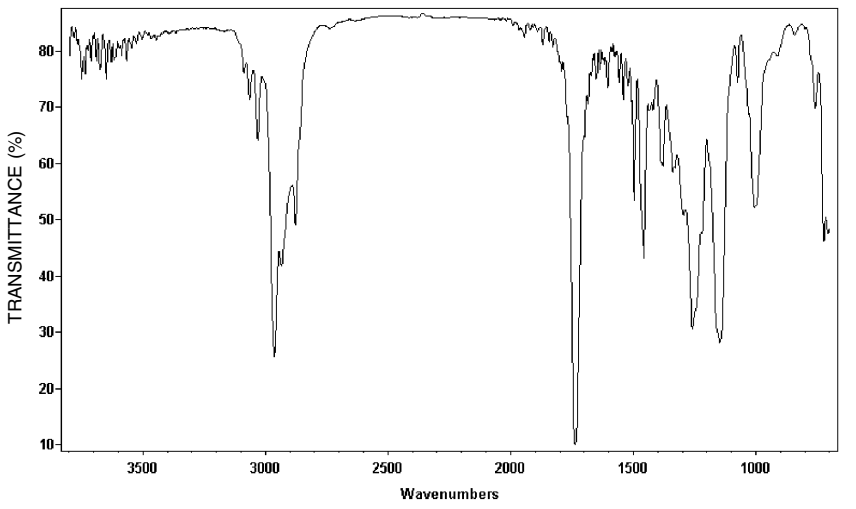
Isoamyl Hexanoate View Flavor Chemical



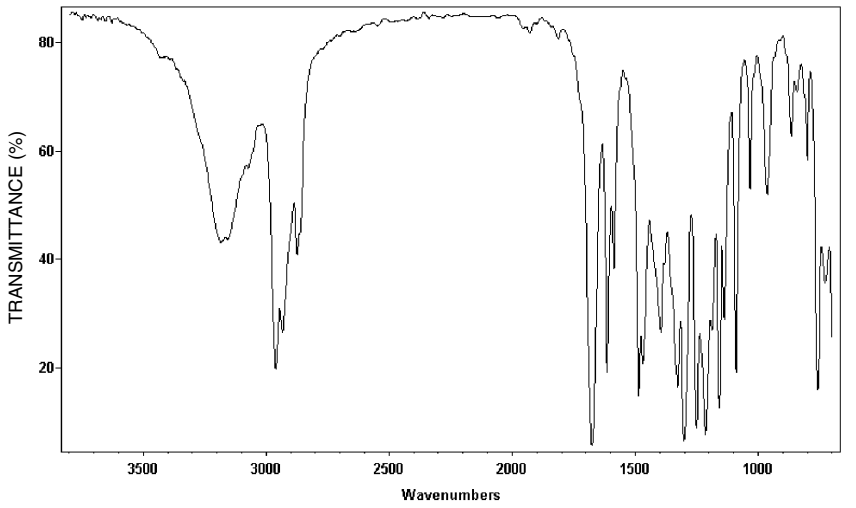
Isoamyl Isobutyrate View Flavor Chemical



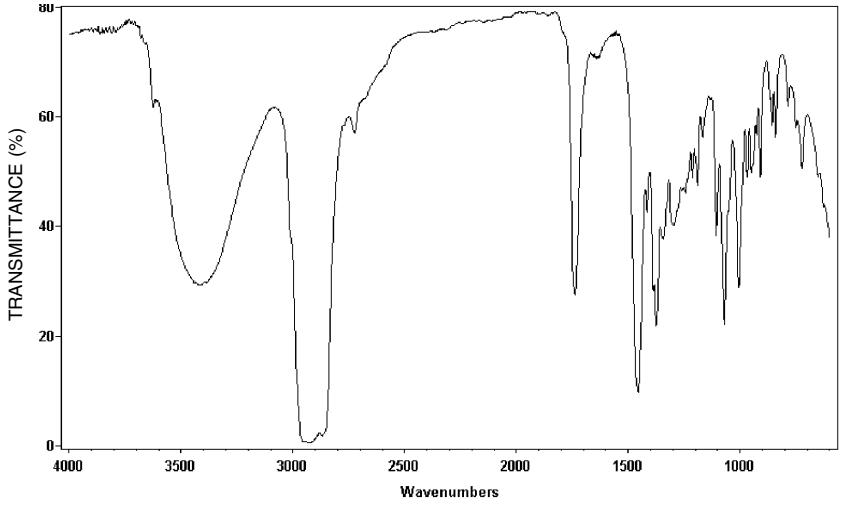
Isoamyl Phenyl Acetate View Flavor Chemical



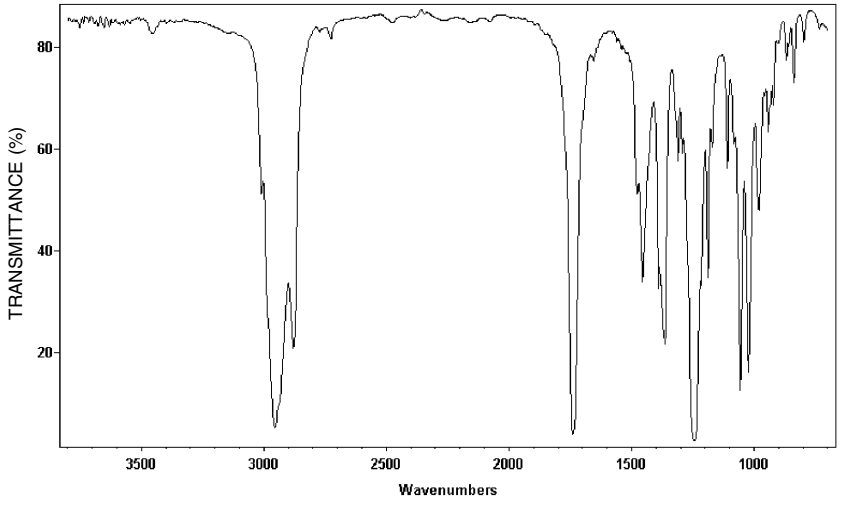
Isoamyl Salicylate **View Flavor Chemical**



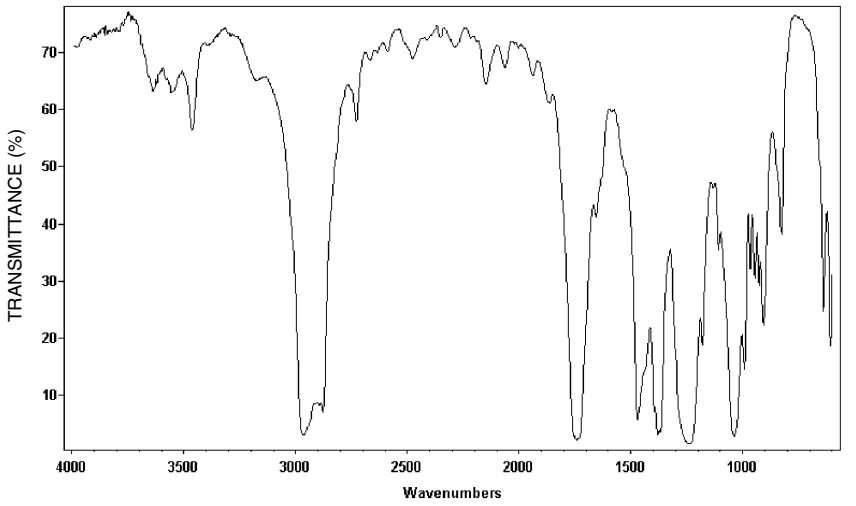
Isoborneol (Mineral Oil Mull) **View Flavor Chemical**



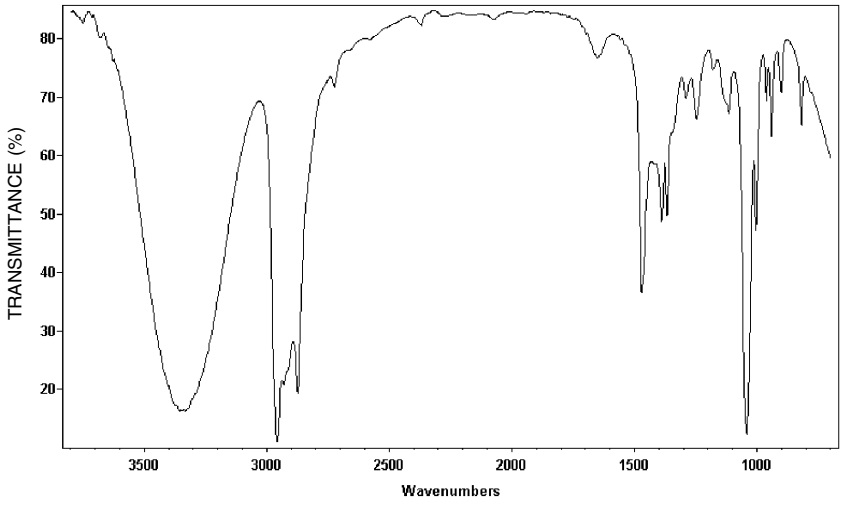
Isobornyl Acetate **View Flavor Chemical**



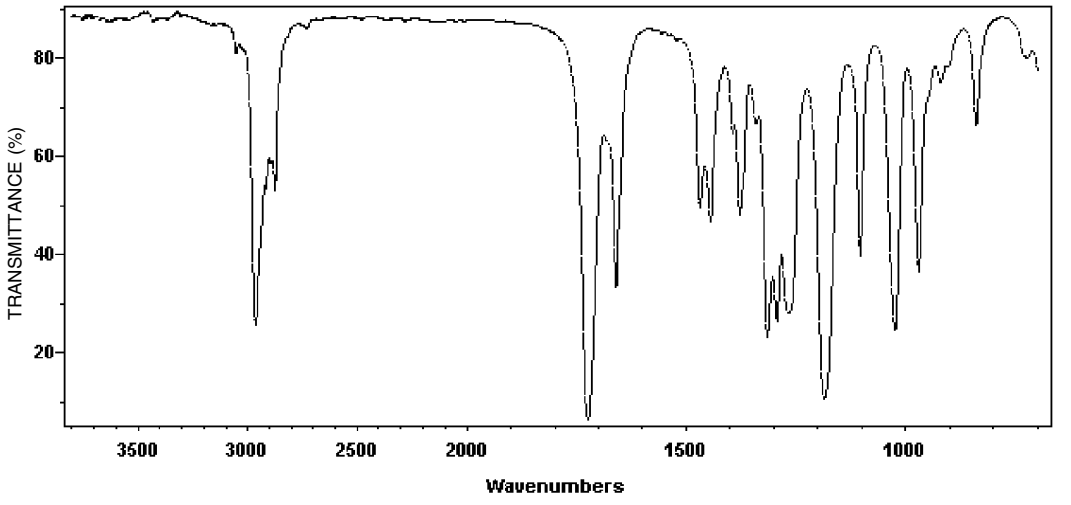
Isobutyl Acetate View Flavor Chemical



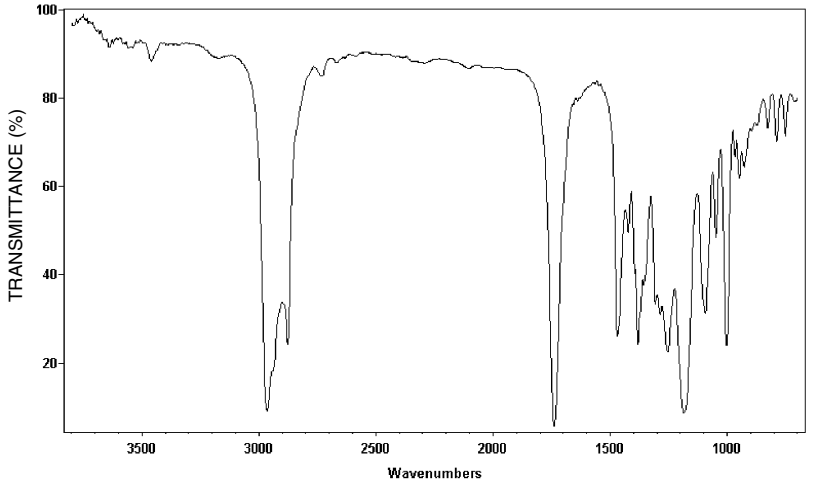
Isobutyl Alcohol View Flavor Chemical



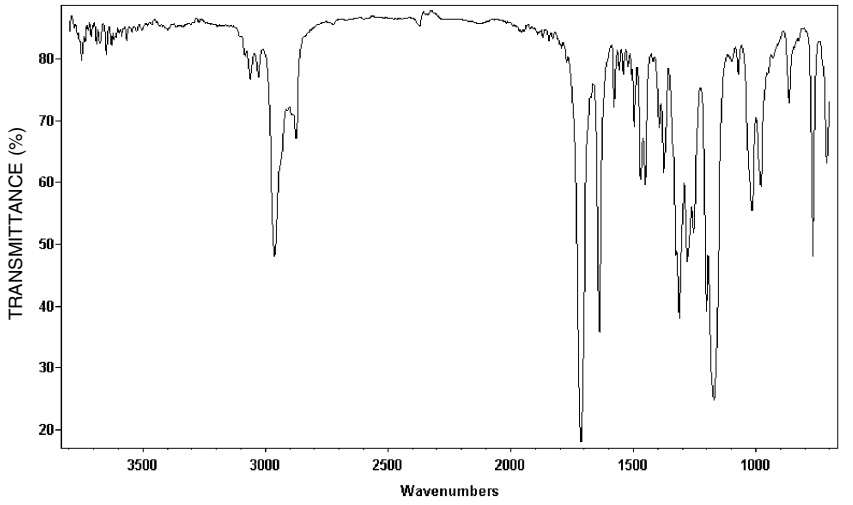
Isobutyl-2-butenoate View Flavor Chemical



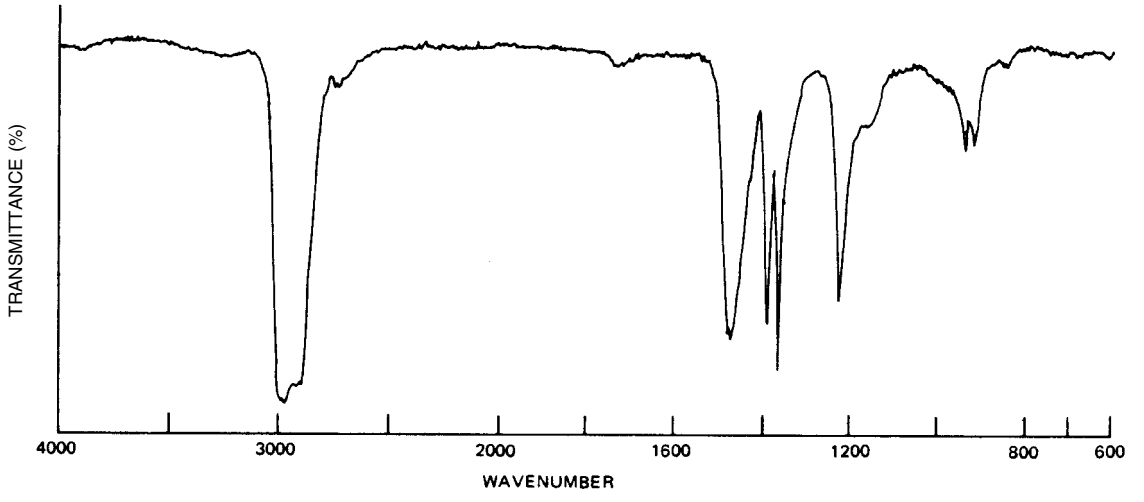
Isobutyl Butyrate View Flavor Chemical



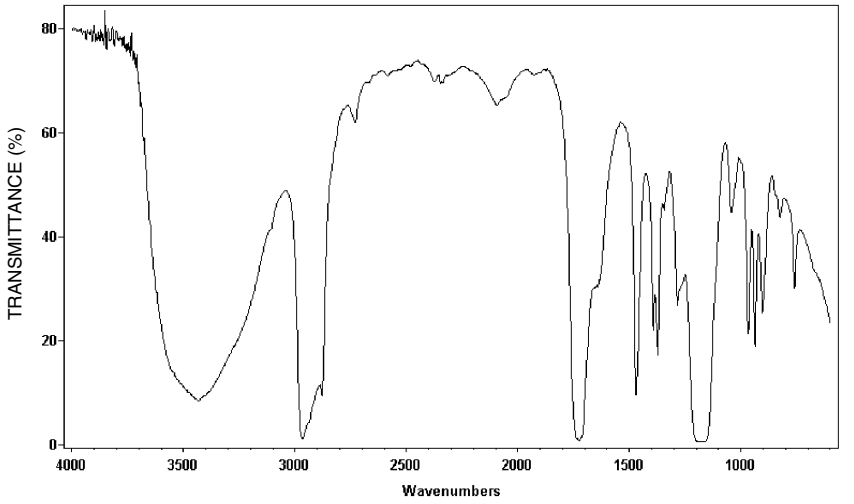
Isobutyl Cinnamate View Flavor Chemical



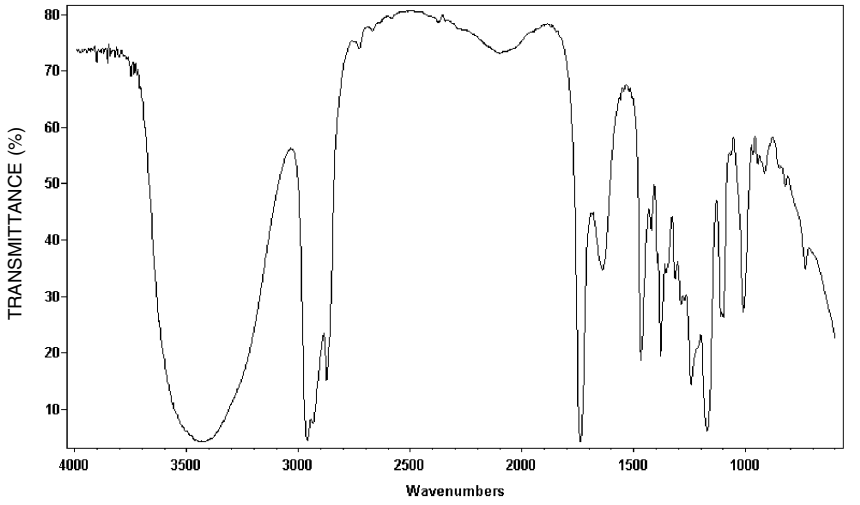
Isobutylene-Isoprene Copolymer View Monograph



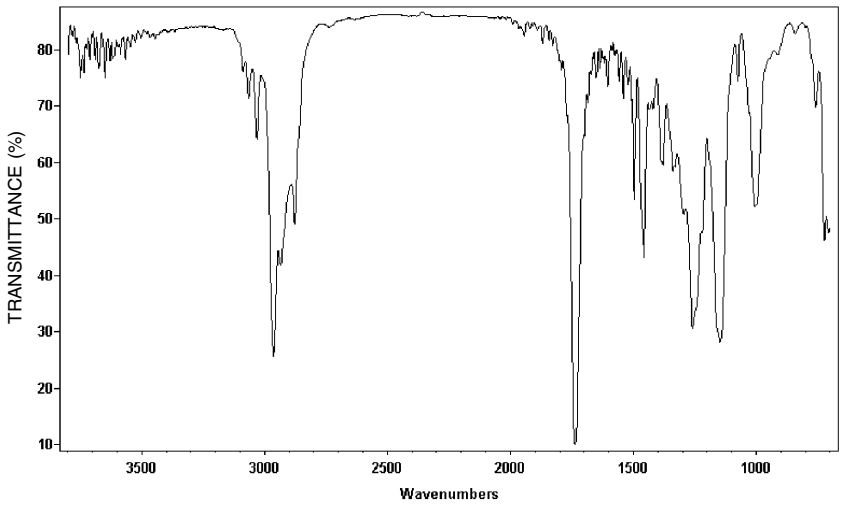
Isobutyl Formate View Flavor Chemical



Isobutyl Hexanoate View Flavor Chemical

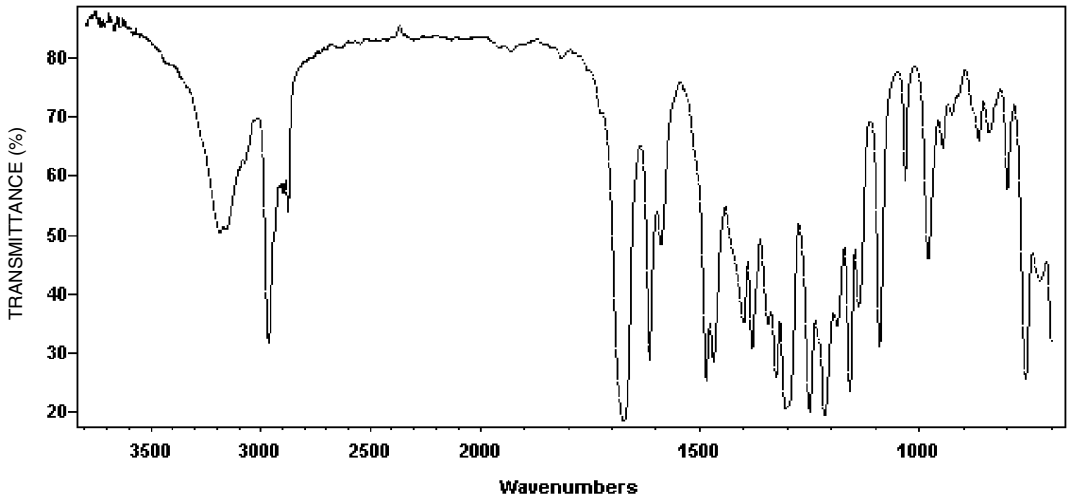


Isobutyl Phenylacetate View Flavor Chemical



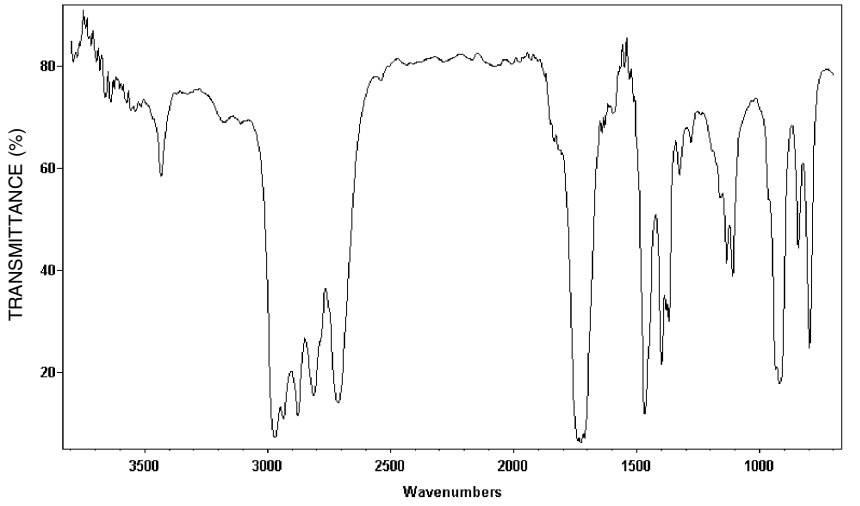
Isobutyl Salicylate

View Flavor Chemical



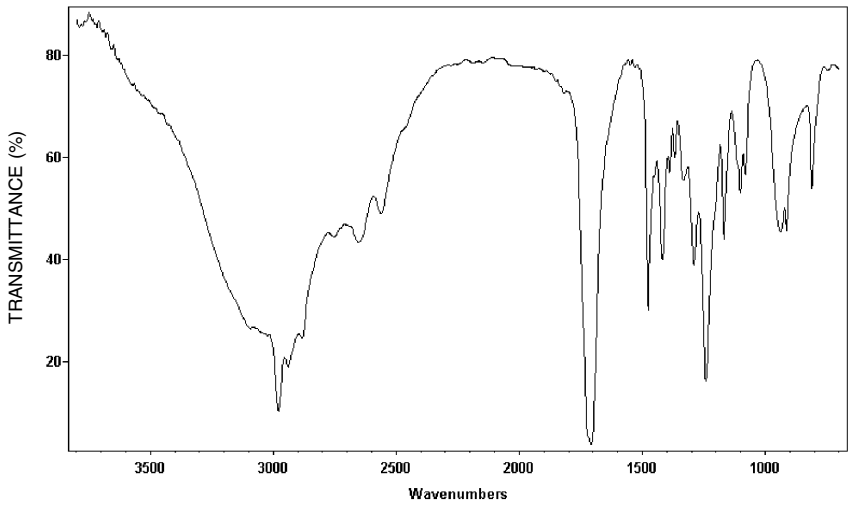
Isobutyraldehyde

View Flavor Chemical

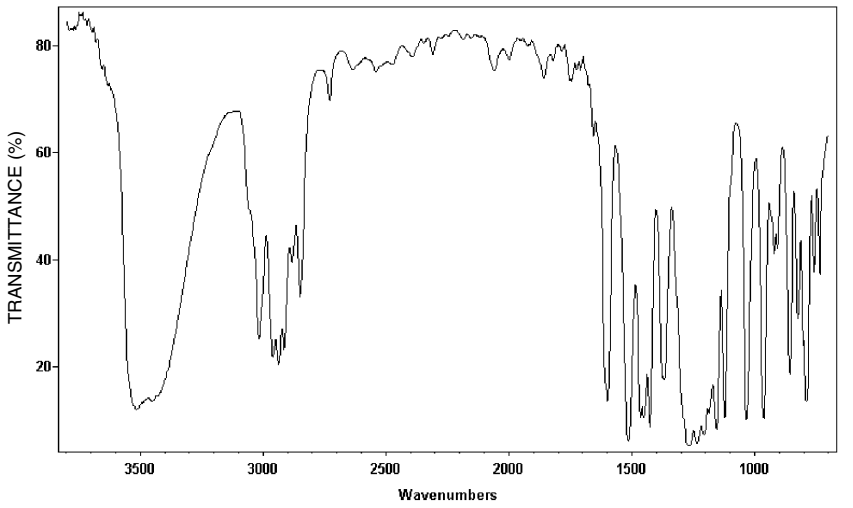


Isobutyric Acid

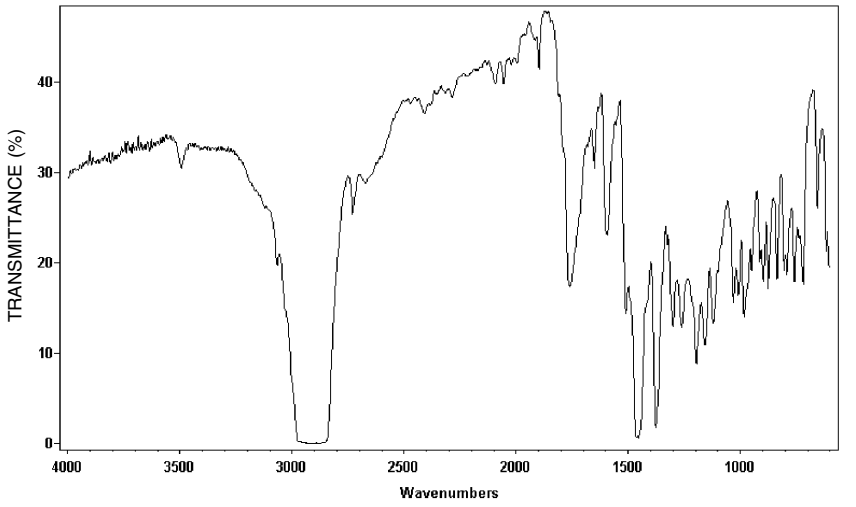
View Flavor Chemical



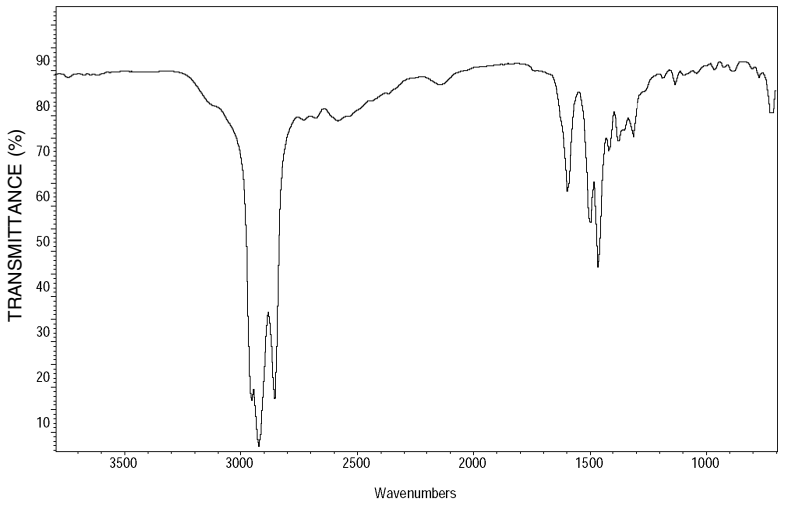
Isoeugenol View Flavor Chemical



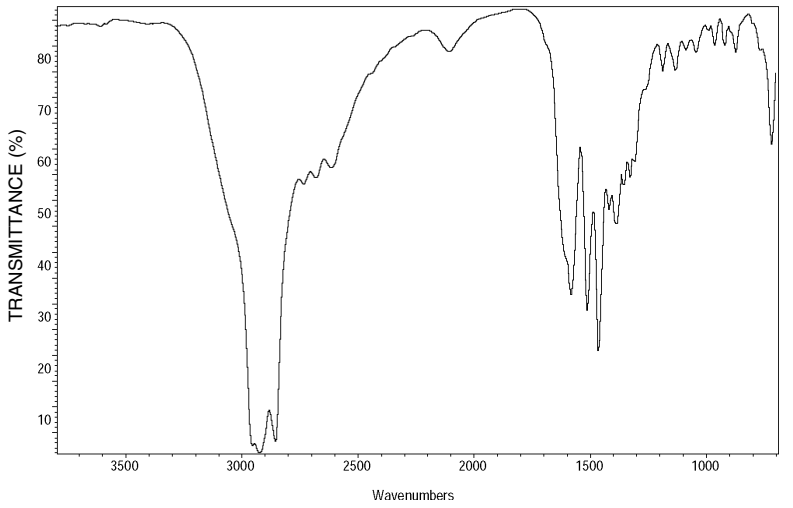
Isoeugenyl Acetate (Mineral Oil Mull) View Flavor Chemical



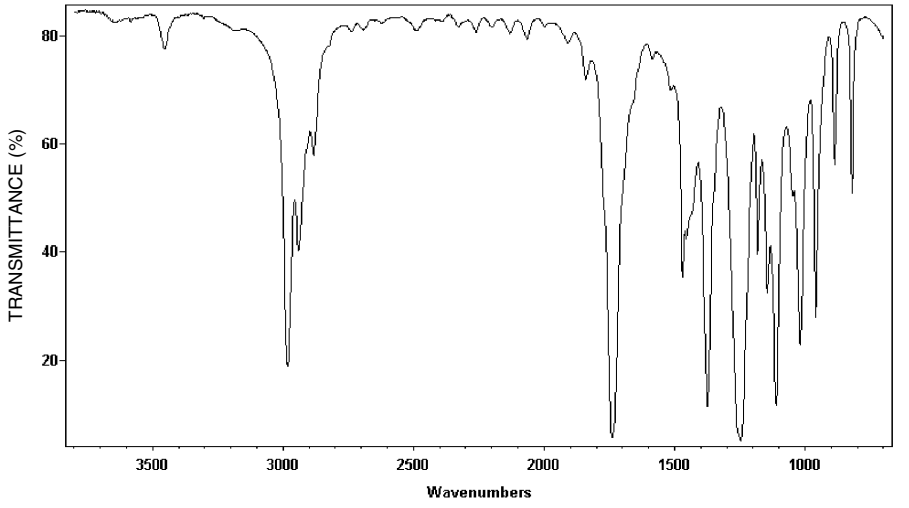
DL-Isoleucine (Mineral Oil Mull) View Monograph



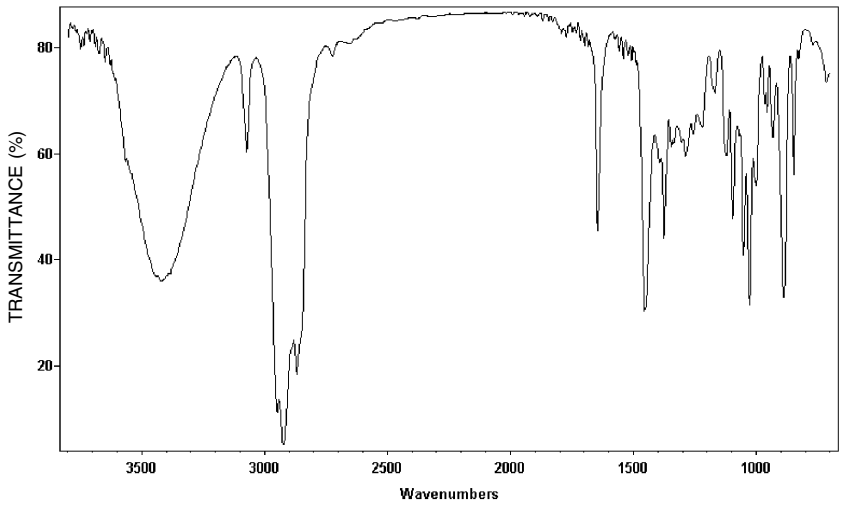
L-Isoleucine (Mineral Oil Mull) View Monograph



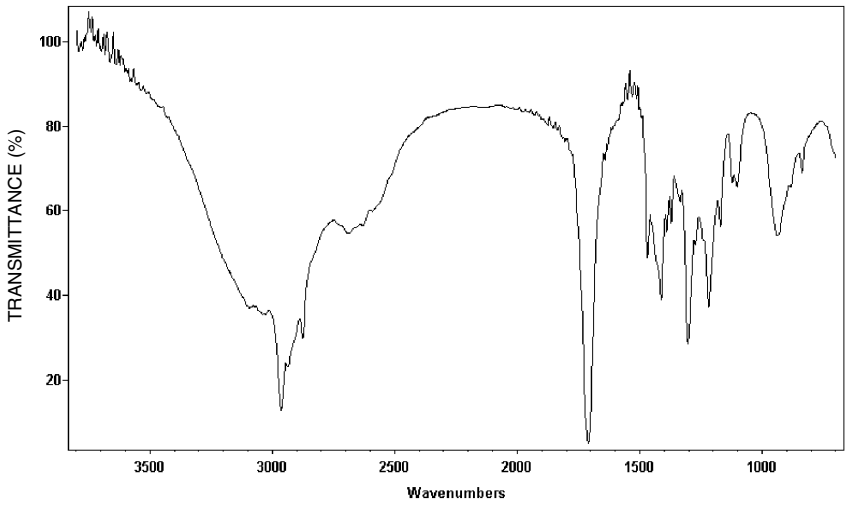
Isopropyl Acetate View Flavor Chemical



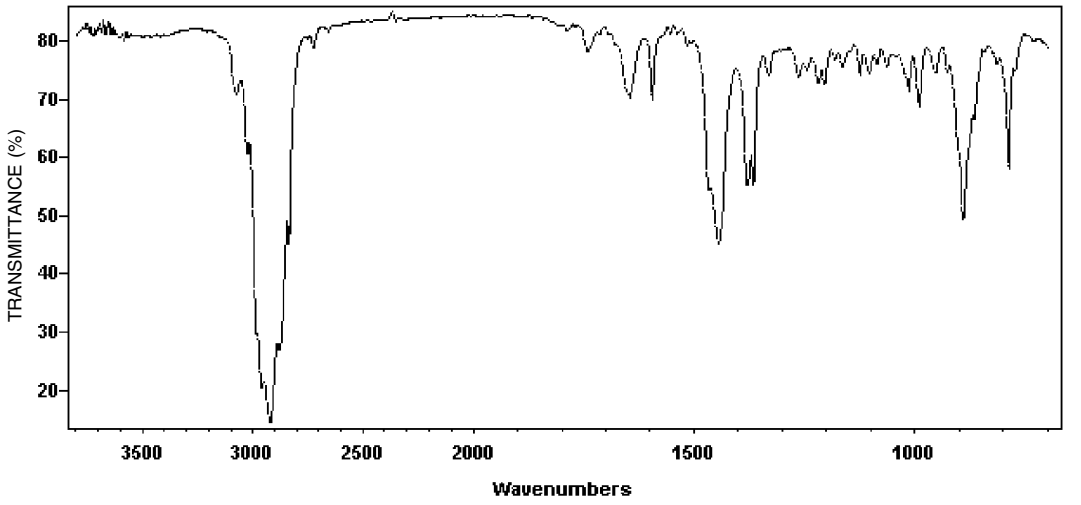
Isopulegol View Flavor Chemical



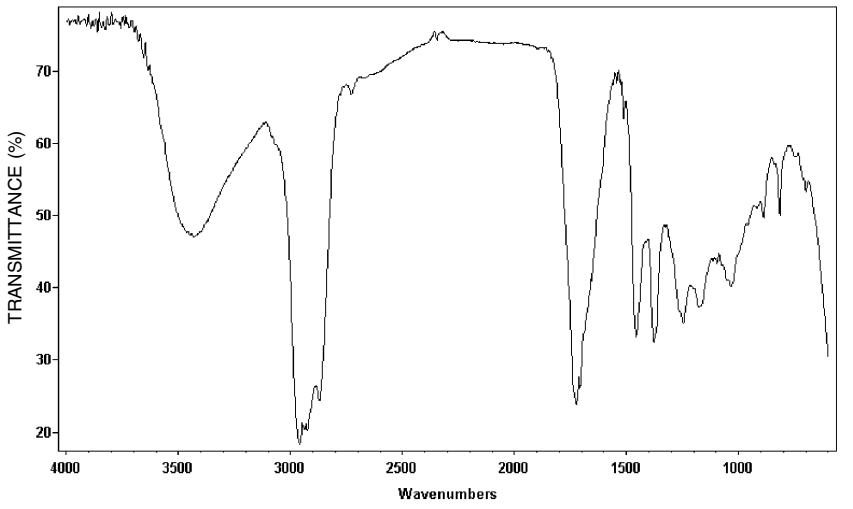
Isovaleric Acid **View Flavor Chemical**



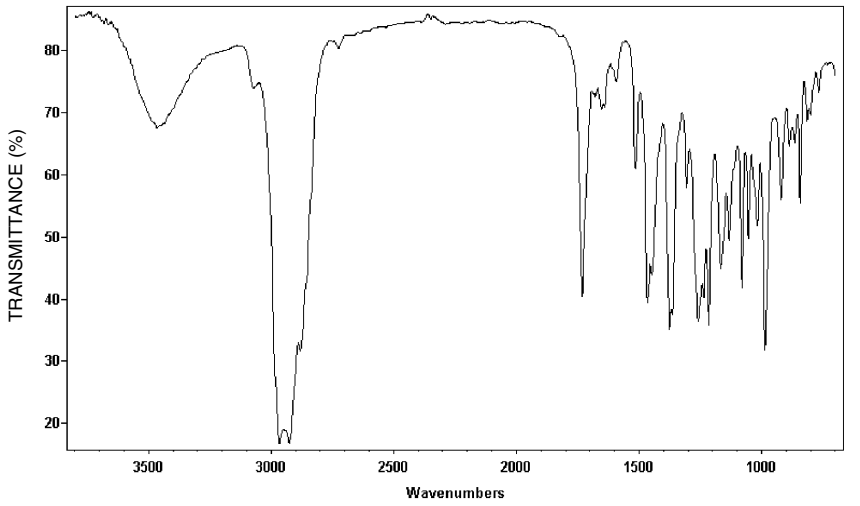
Juniper Berries Oil **View Monograph**



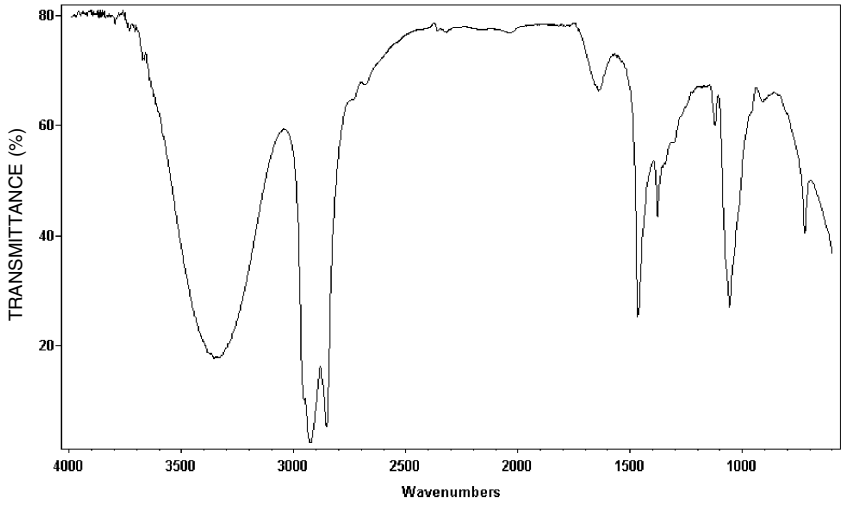
Labdanum Oil **View Monograph**



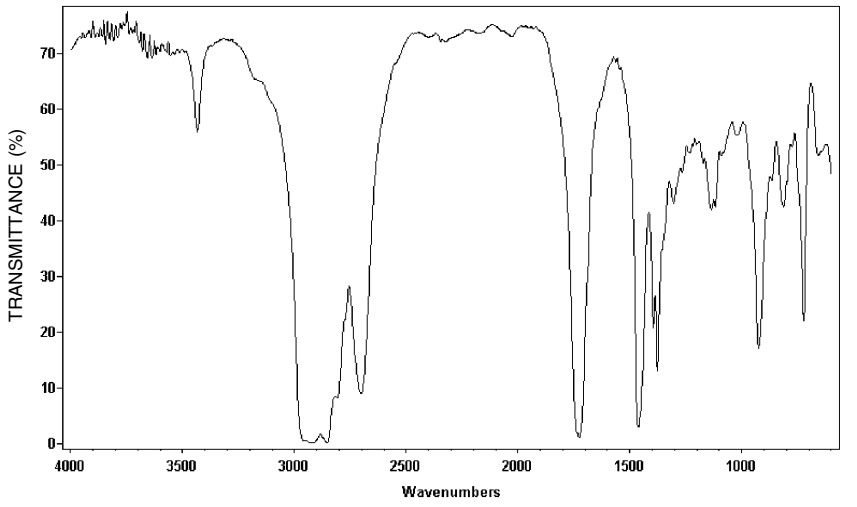
Laurel Leaf Oil View Monograph



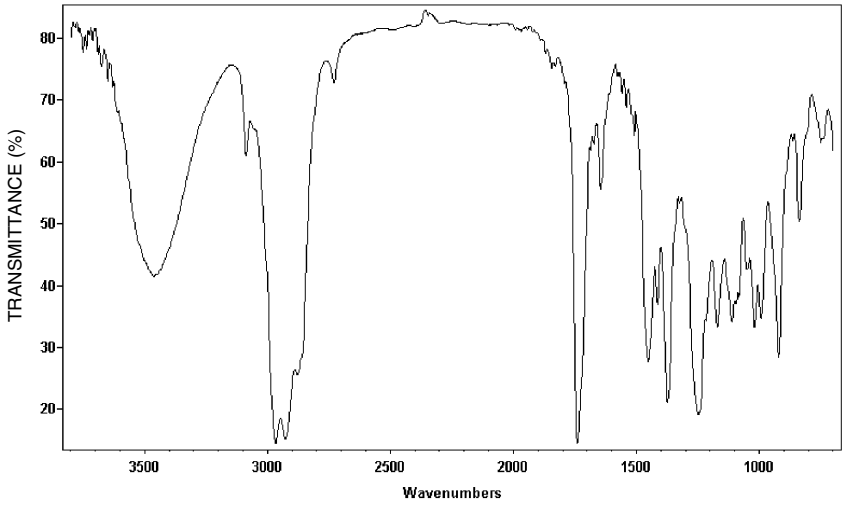
Lauryl Alcohol View Flavor Chemical



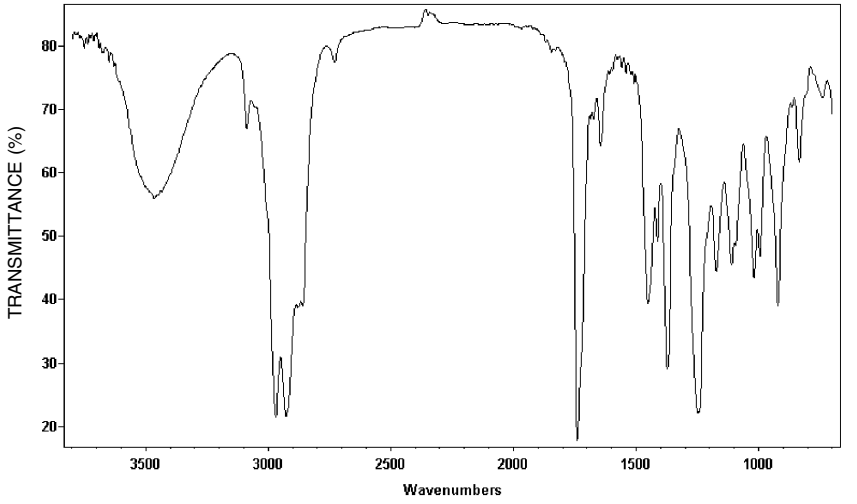
Lauryl Aldehyde View Flavor Chemical



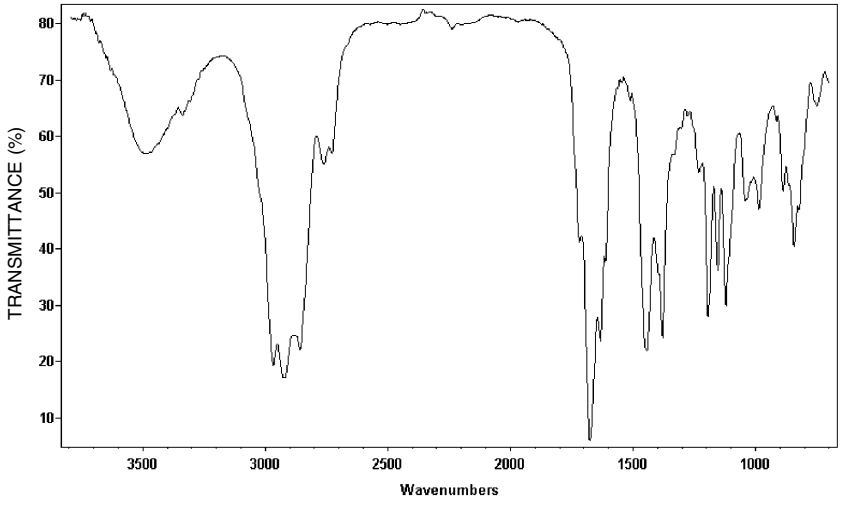
Lavandin Oil, Abrial Type View Monograph



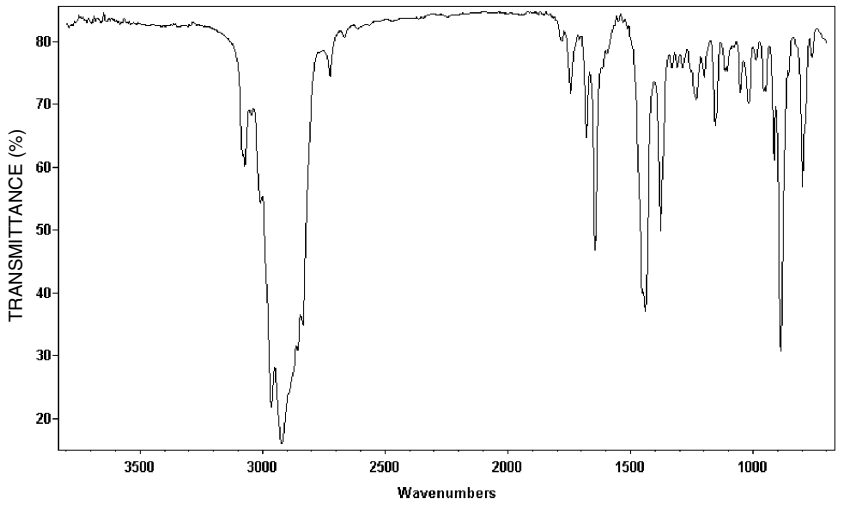
Lavender Oil View Monograph



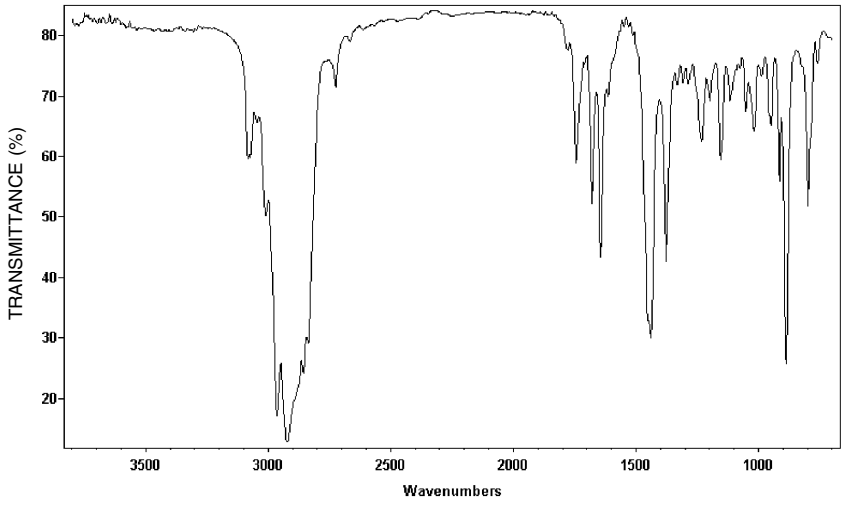
Lemongrass Oil View Monograph



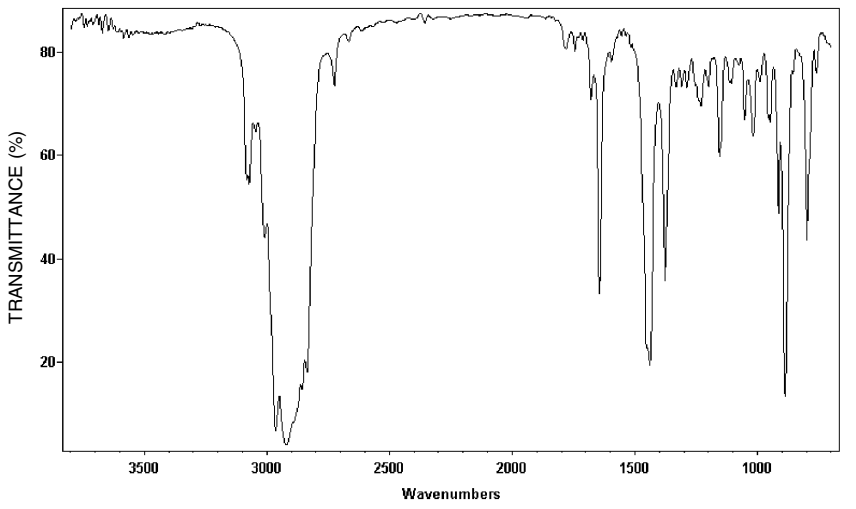
Lemon Oil, Coldpressed View Monograph



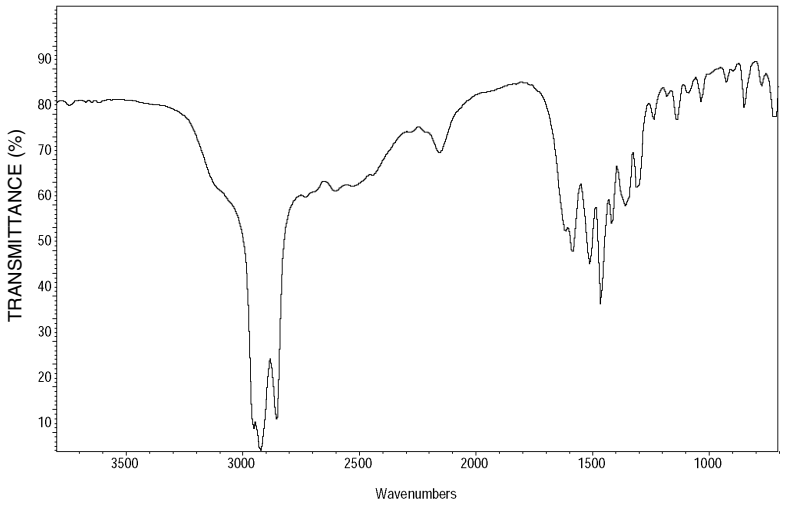
Lemon Oil, Desert Type, Coldpressed View Monograph



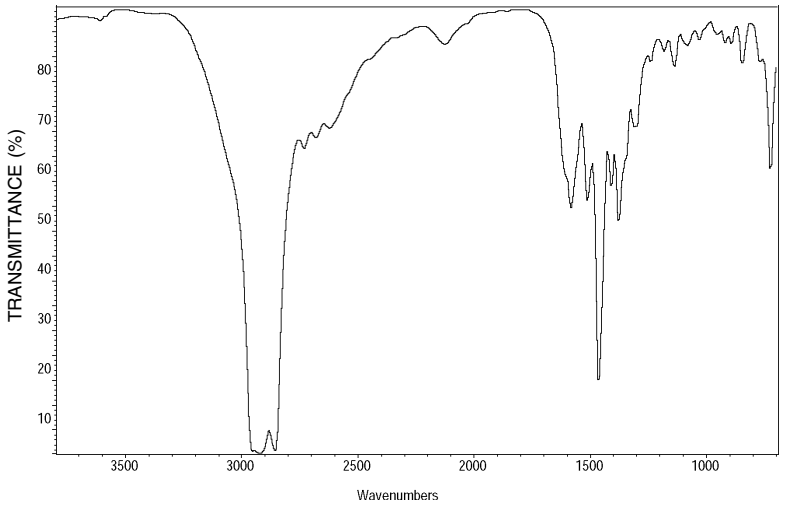
Lemon Oil, Distilled, Brazil Type View Monograph



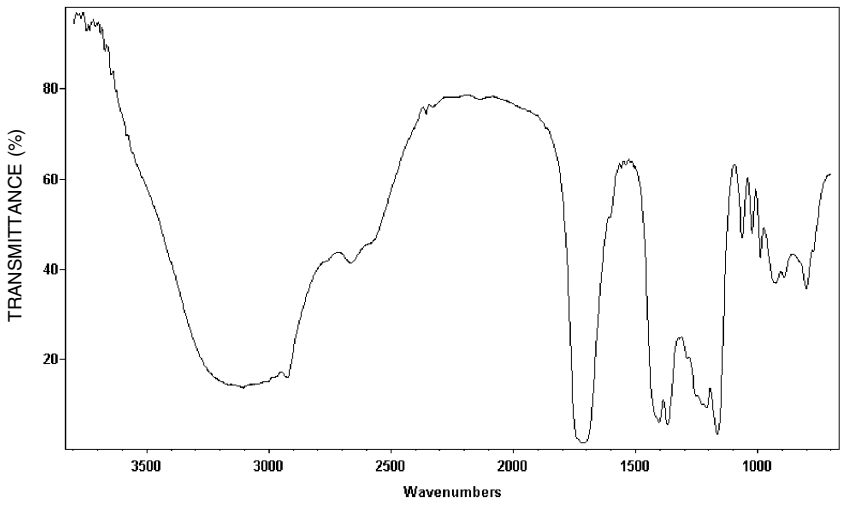
DL-Leucine (Mineral Oil Mull) View Monograph



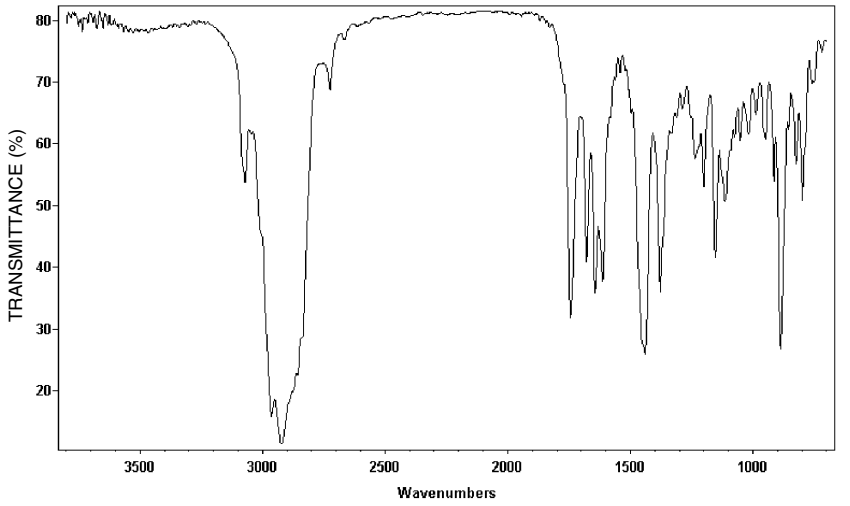
L-Leucine (Mineral Oil Mull) View Monograph



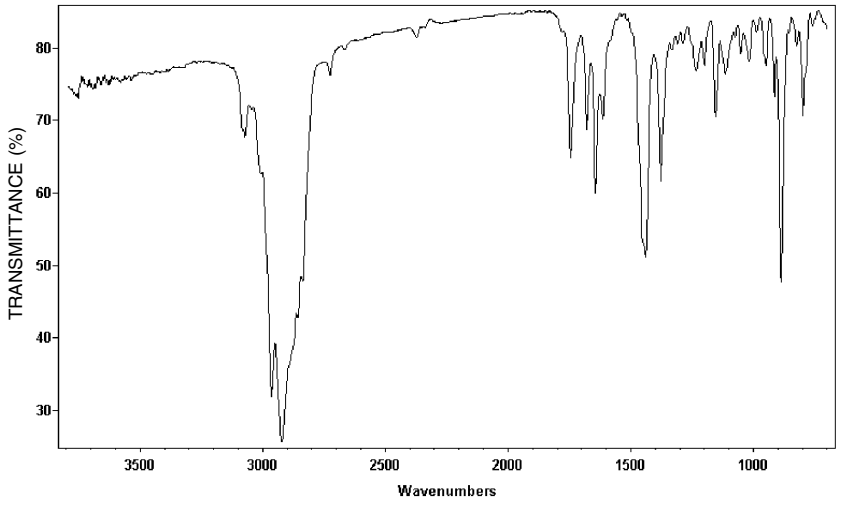
Levulinic Acid View Flavor Chemical



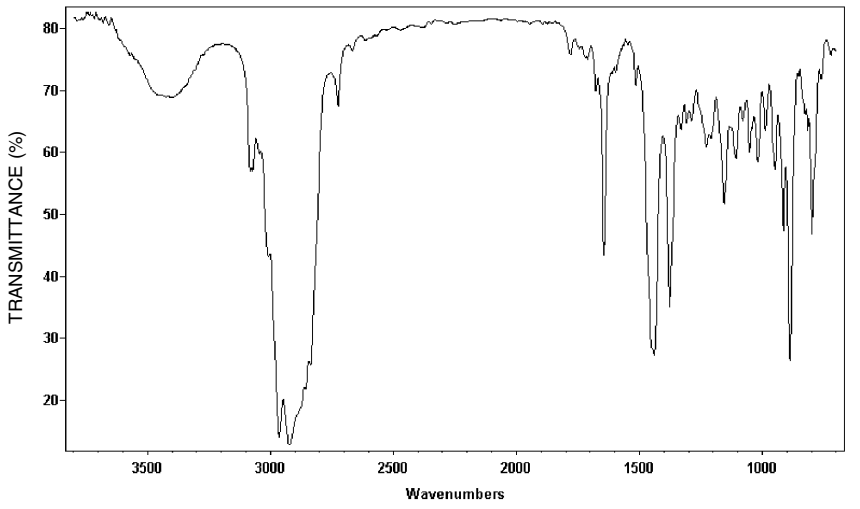
Lime Oil, Coldpressed, Mexican Type View Monograph



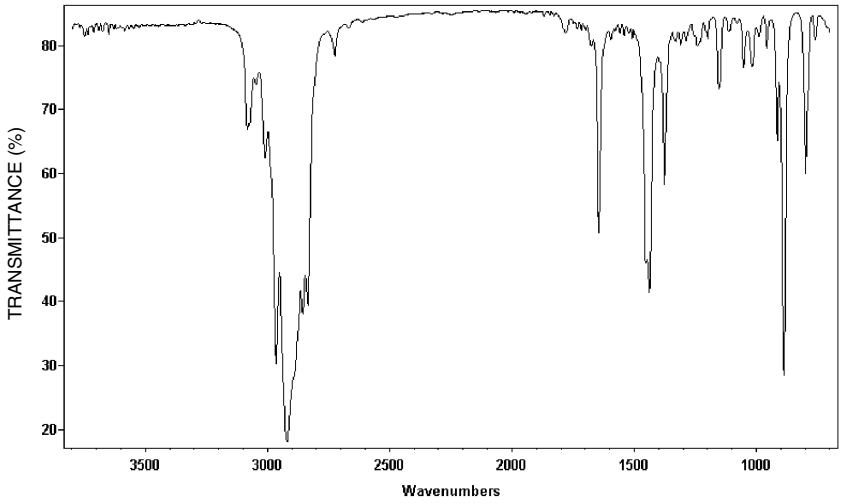
Lime Oil, Coldpressed, Tahitian Type View Monograph



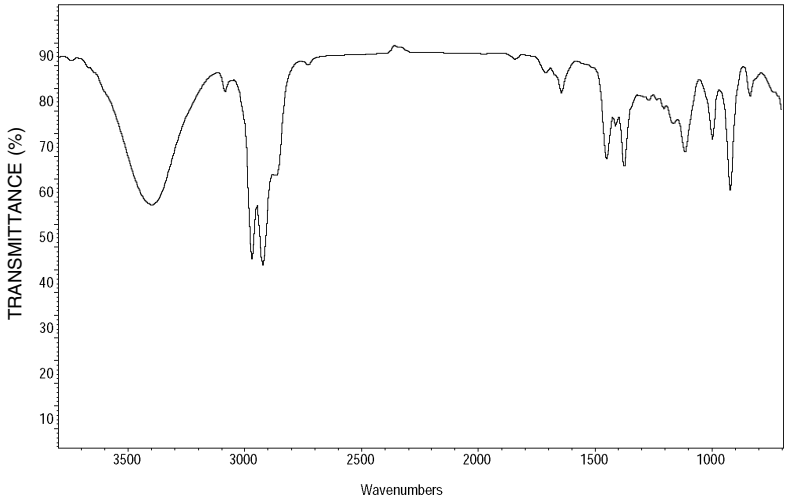
Lime Oil, Distilled View Monograph



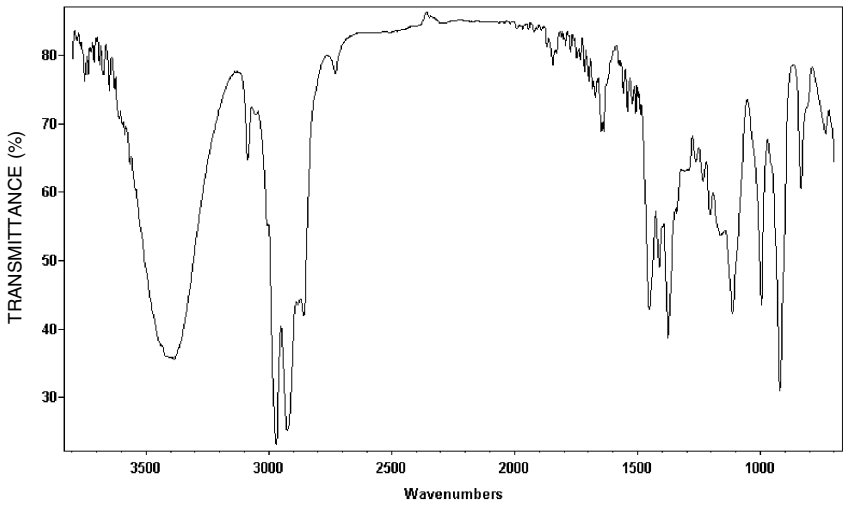
_d_-Limonene View Flavor Chemical



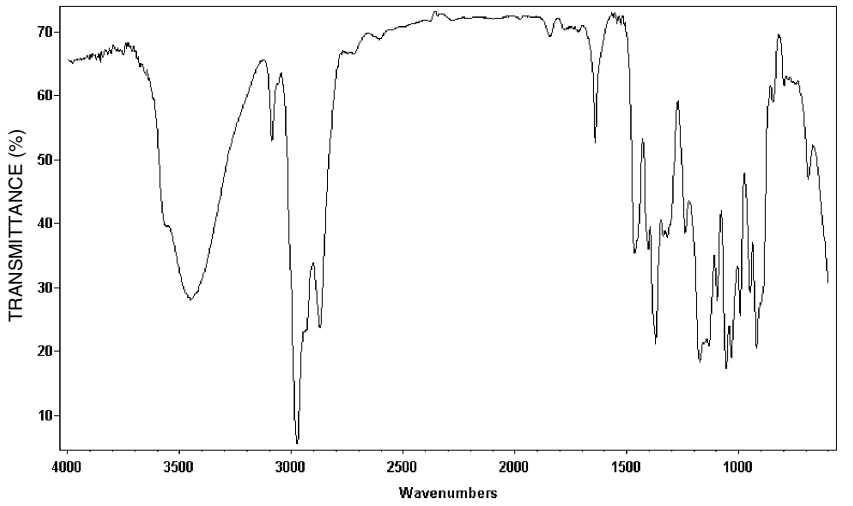
Linaloe Wood Oil View Monograph



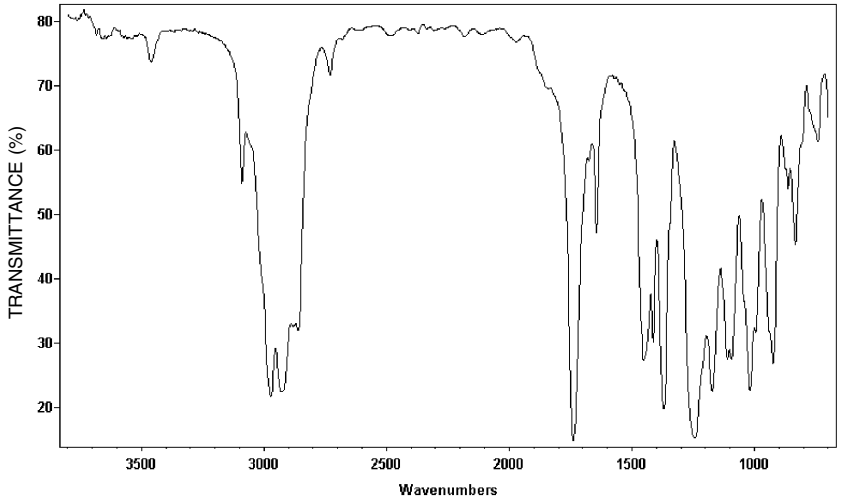
Linalool View Flavor Chemical



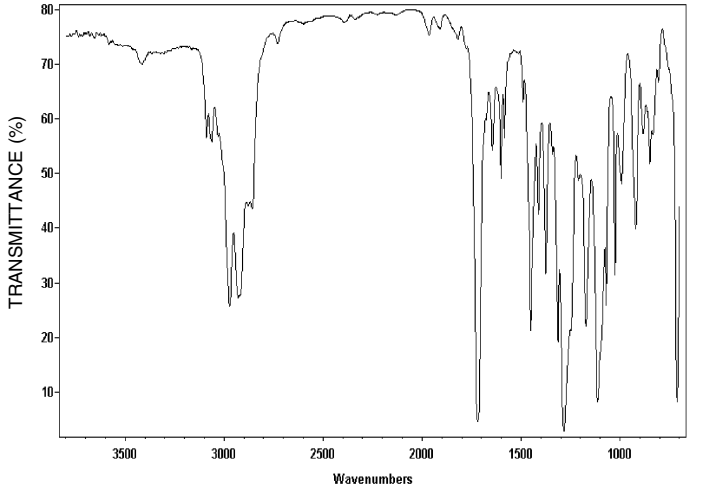
Linalool Oxide View Flavor Chemical



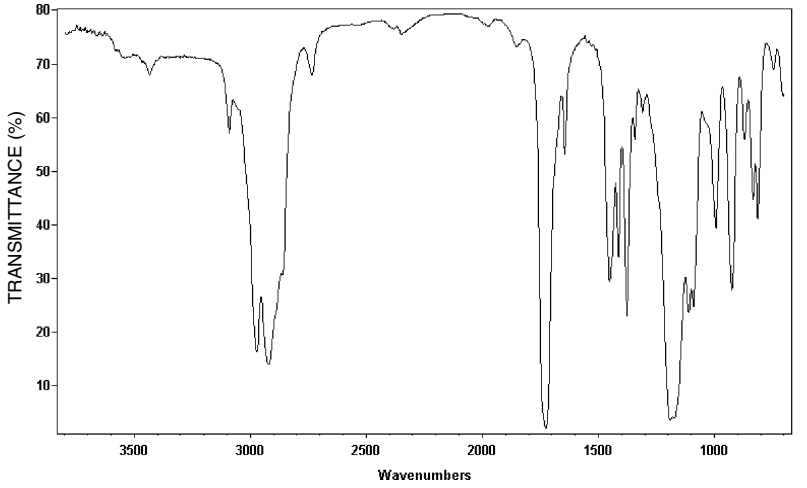
Linalyl Acetate View Flavor Chemical



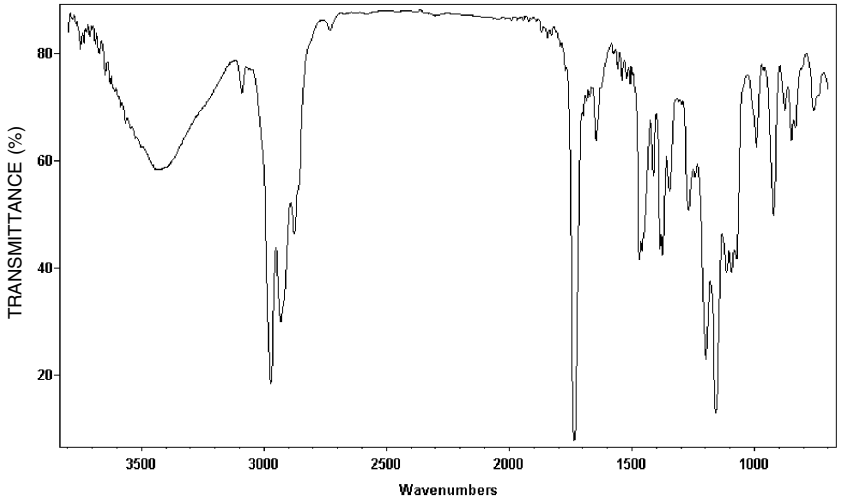
Linalyl Benzoate View Flavor Chemical



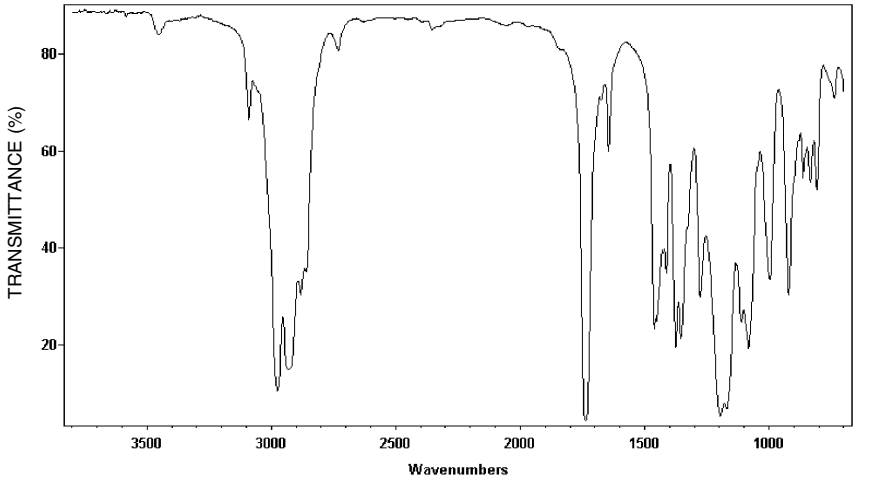
Linalyl Formate View Flavor Chemical



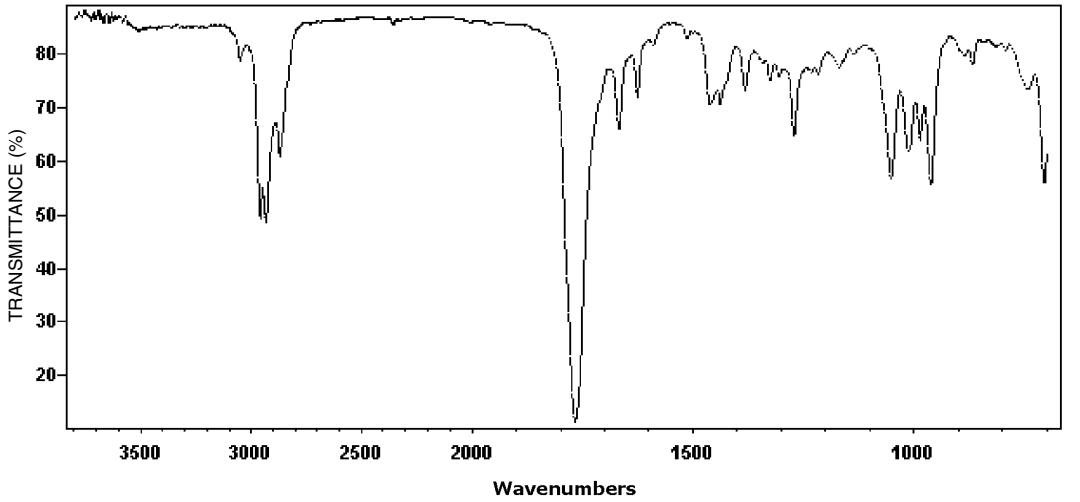
Linalyl Isobutyrate View Flavor Chemical



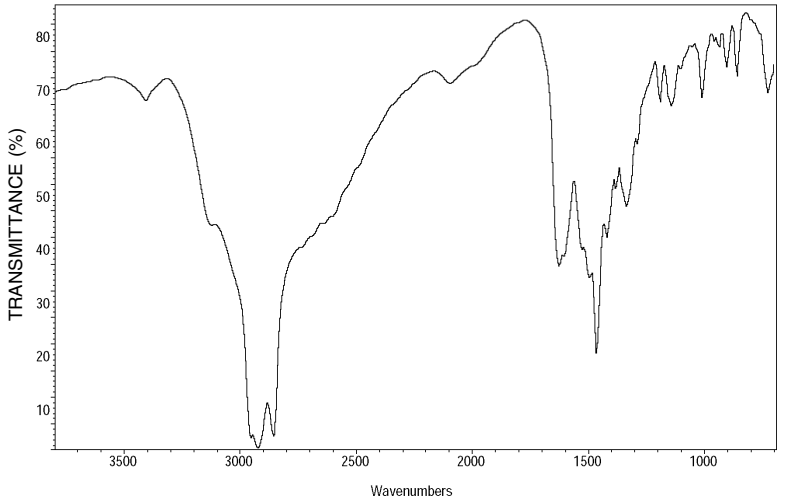
Linalyl Propionate View Flavor Chemical



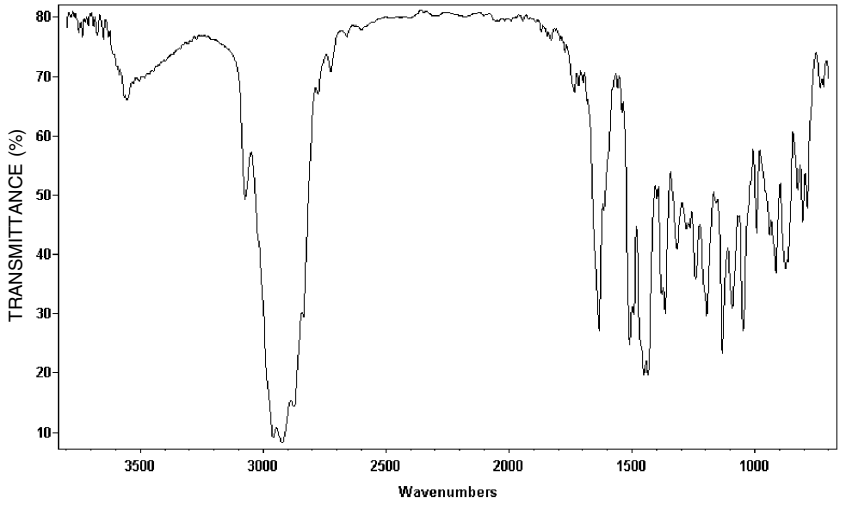
Lovage Oil View Monograph



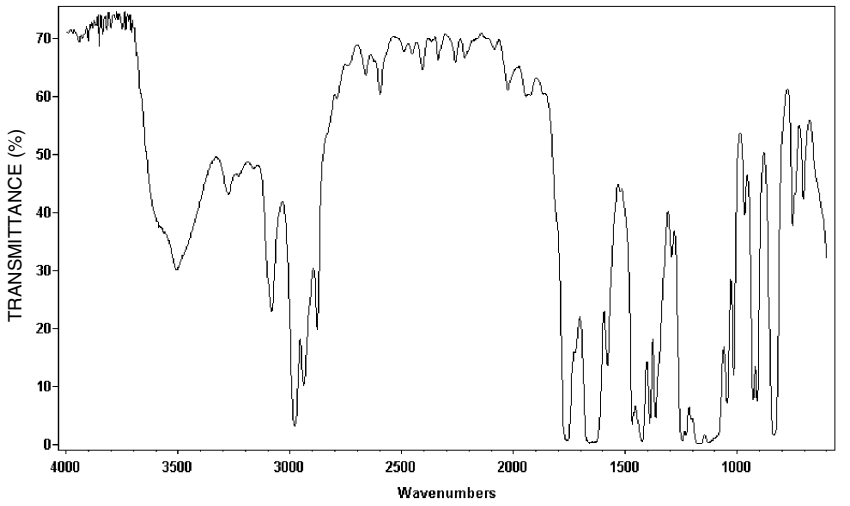
L-Lysine Monohydrochloride View Monograph



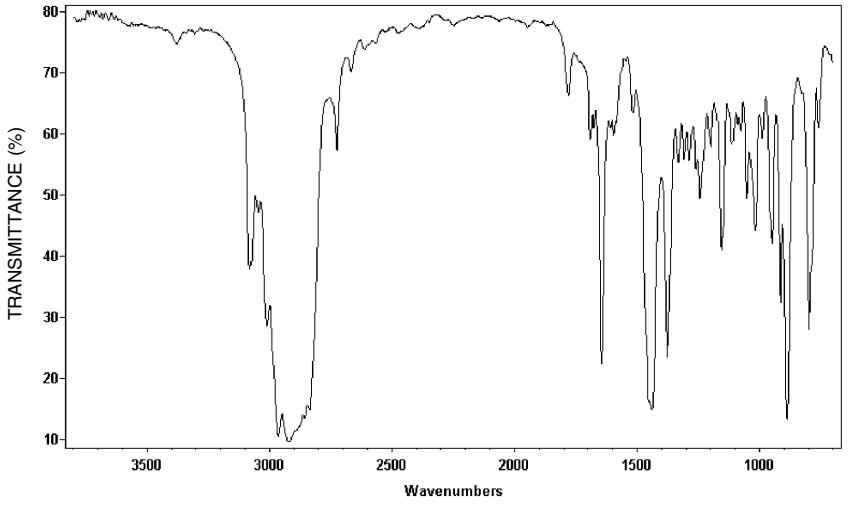
Mace Oil View Monograph



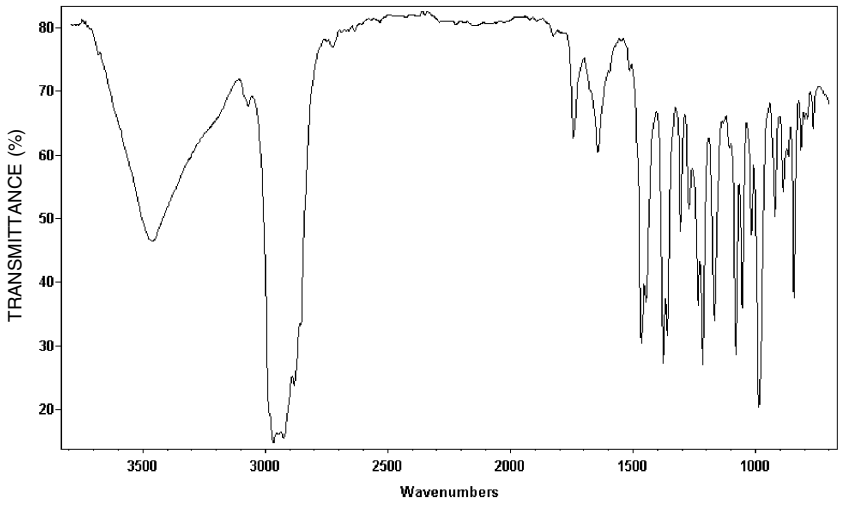
Maltol Isobutyrate View Flavor Chemical



Mandarin Oil, Coldpressed View Monograph

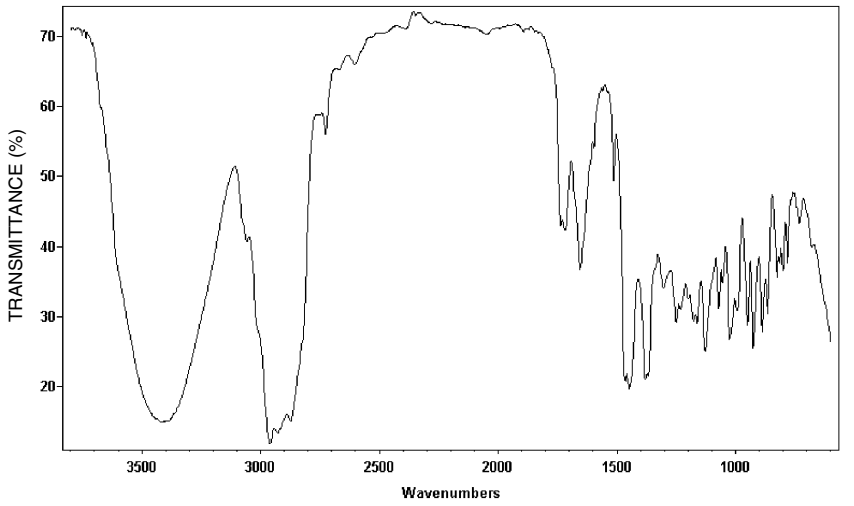


Marjoram Oil, Spanish Type View Monograph



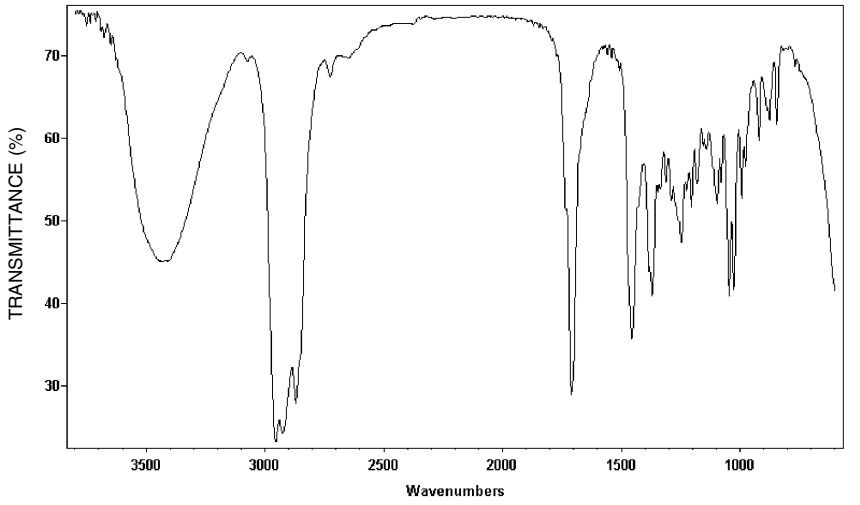
Marjoram Oil, Sweet

View Monograph



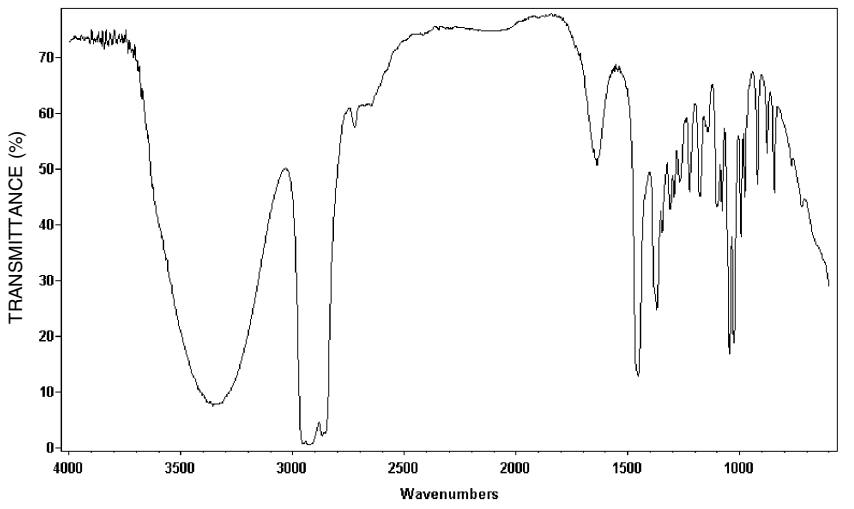
Mentha Avensis Oil, Partially Dementholized

View Monograph

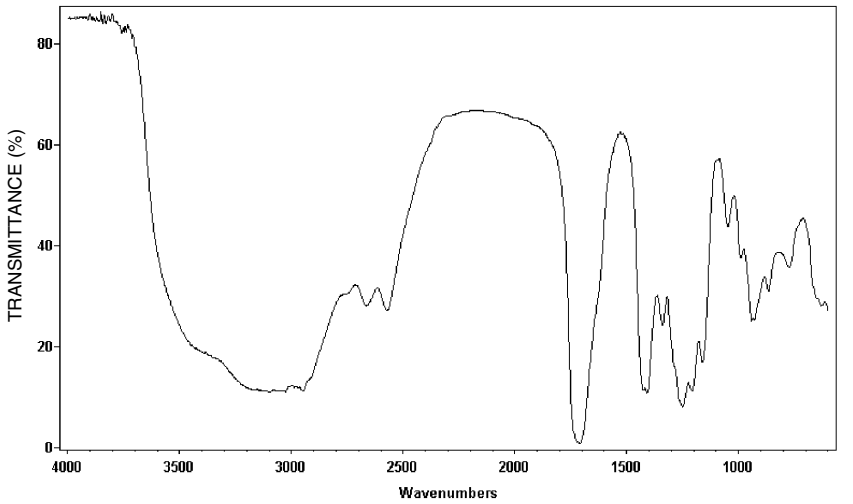


Menthol

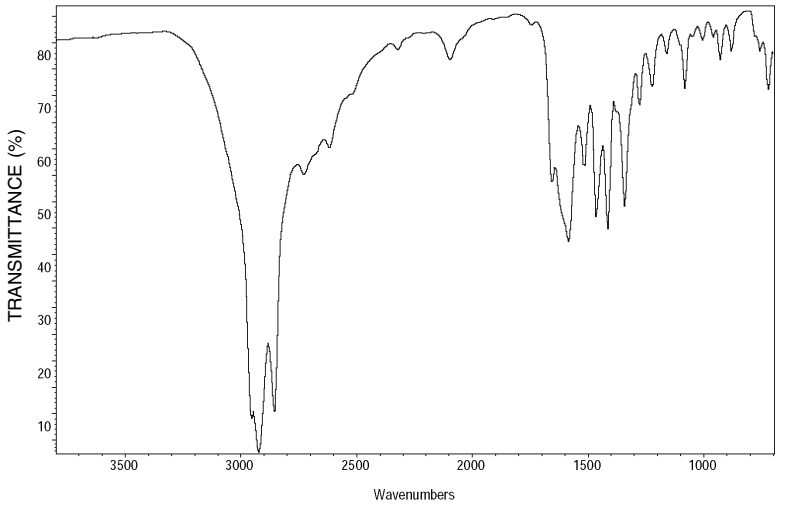
View Flavor Chemical



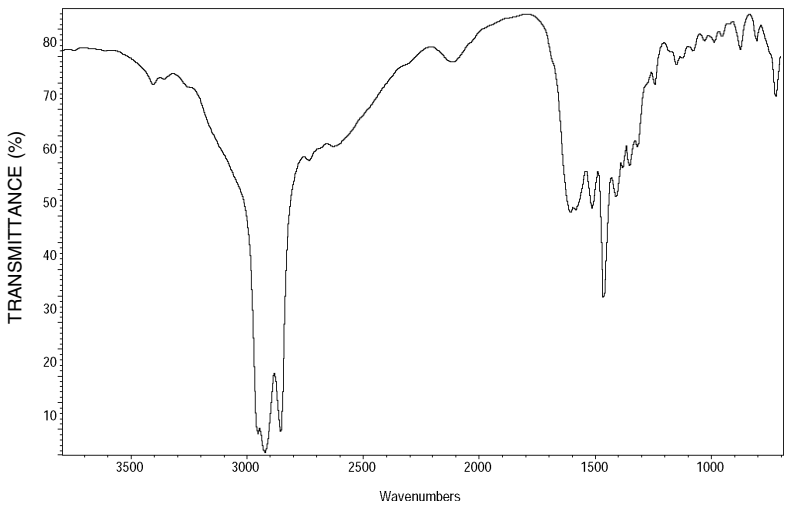
2-Mercaptopropionic Acid View Flavor Chemical



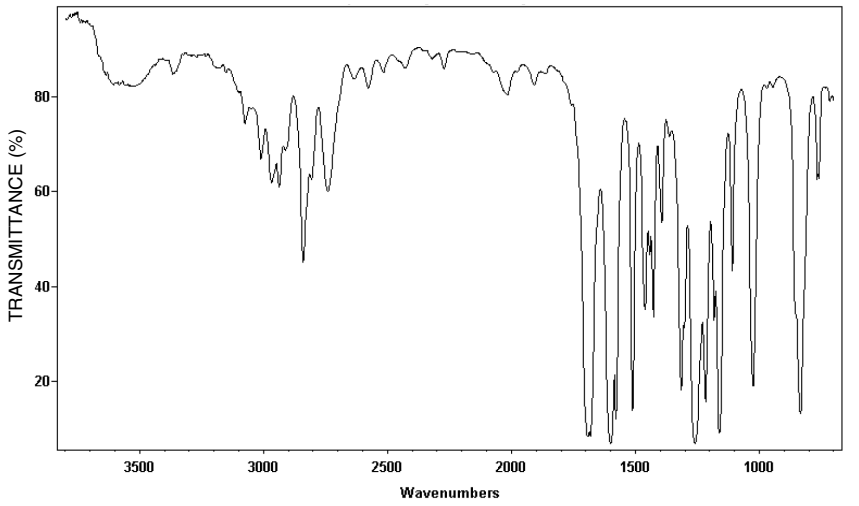
DL-Methionine (Mineral Oil Mull) View Monograph



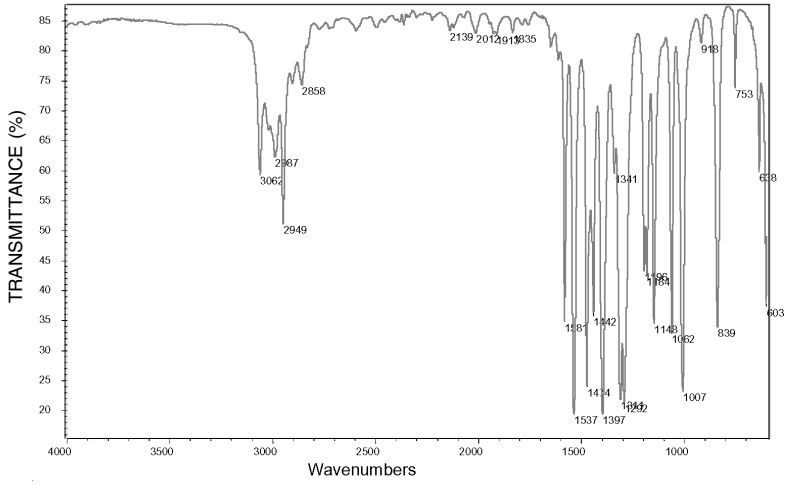
L-Methionine (Mineral Oil Mull) View Monograph



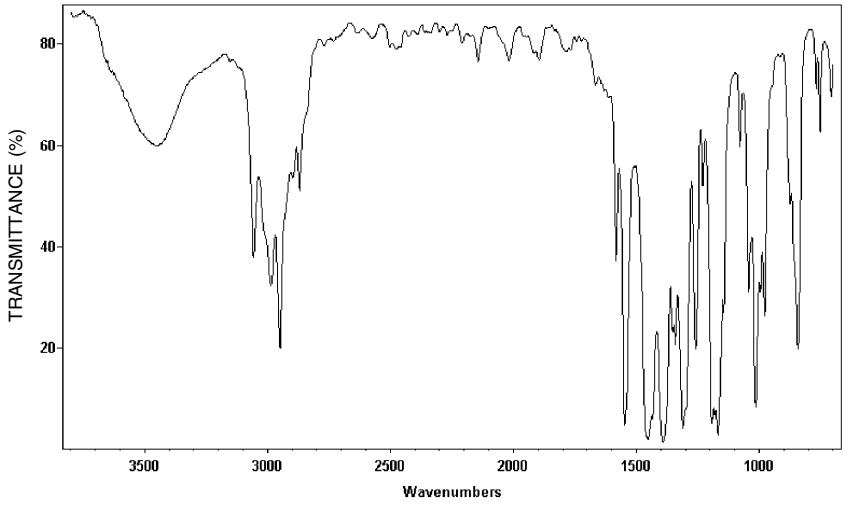
p-Methoxybenzaldehyde View Flavor Chemical



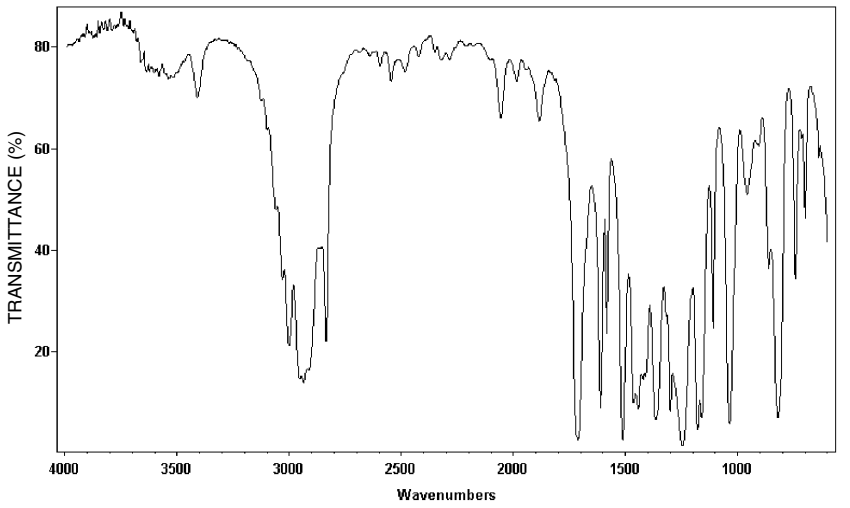
2-Methoxy 3- (or 5- or 6-) Isopropyl Pyrazine View Flavor Chemical



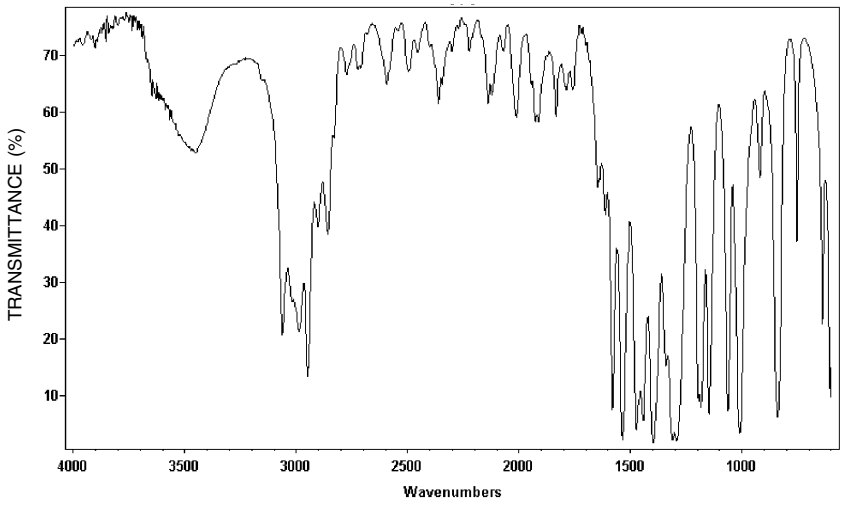
2-Methoxy-3(5)-methylpyrazine View Flavor Chemical



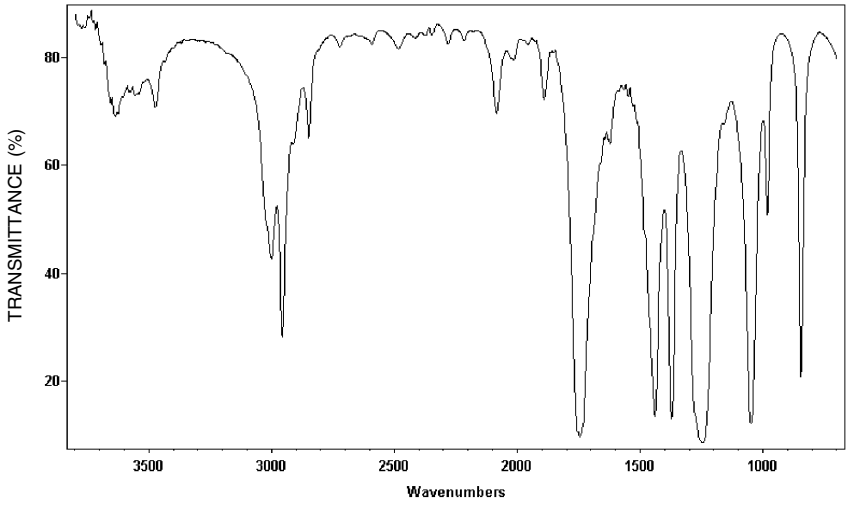
4-*p*-Methoxyphenyl-2-butanone View Flavor Chemical



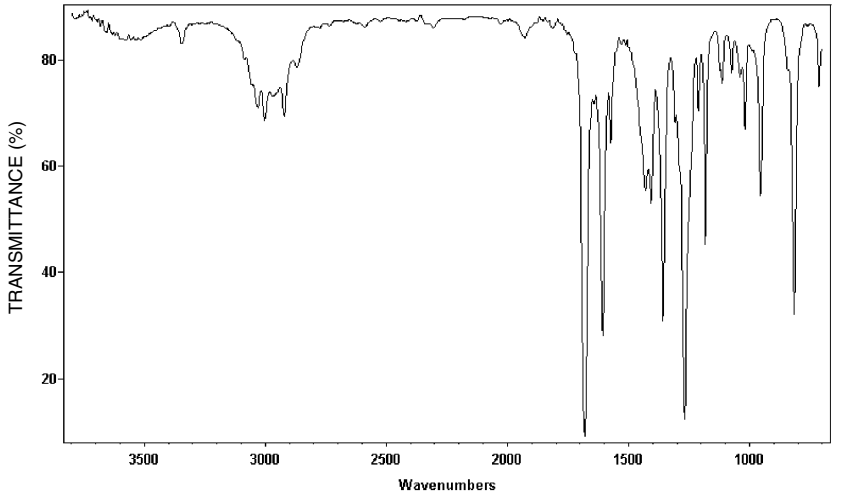
2-Methoxypyrazine View Flavor Chemical



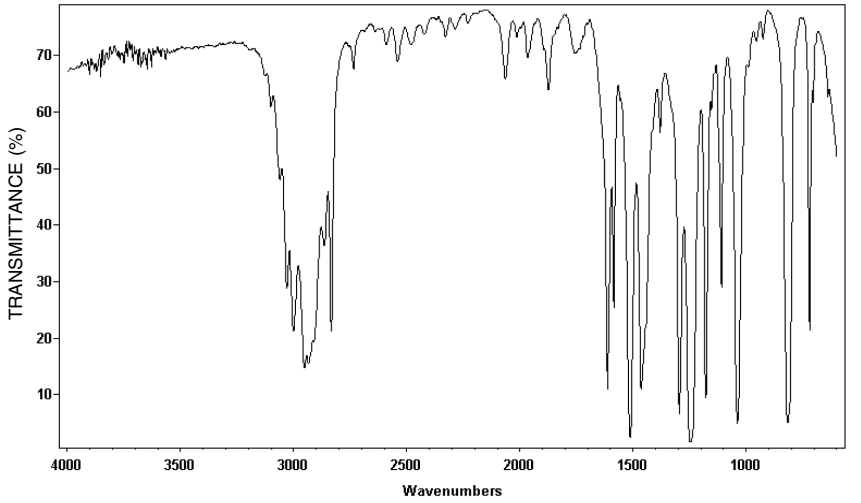
Methyl Acetate View Flavor Chemical



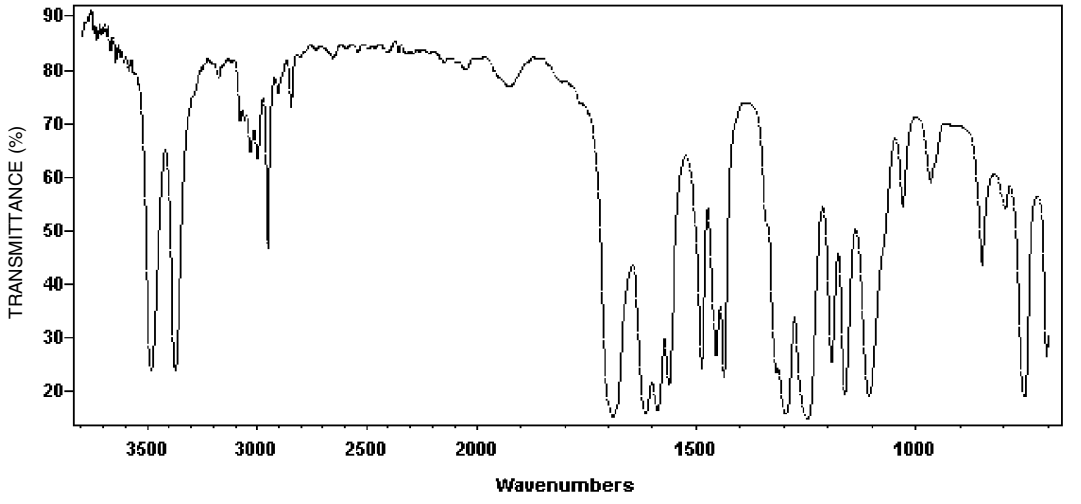
4-Methyl Acetophenone View Flavor Chemical



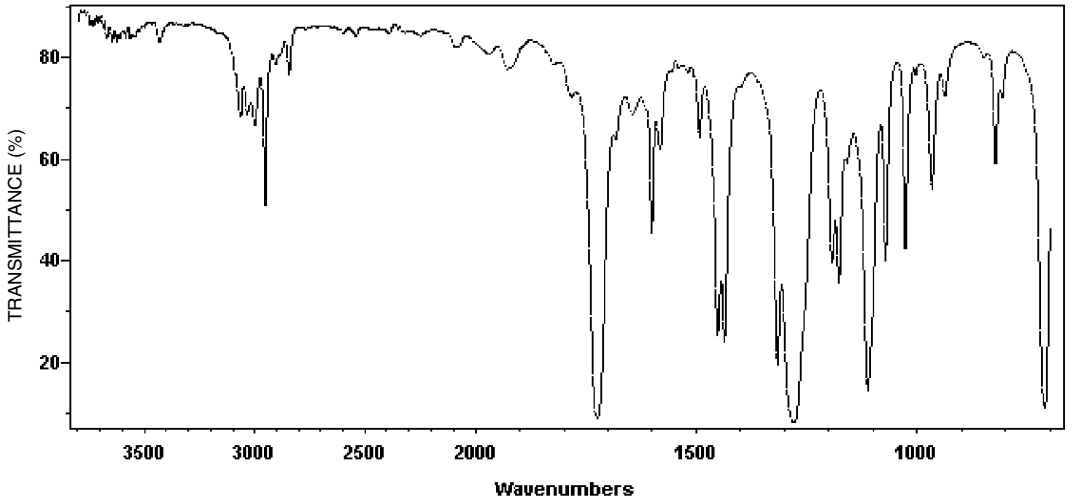
***p*-Methyl Anisole** View Flavor Chemical



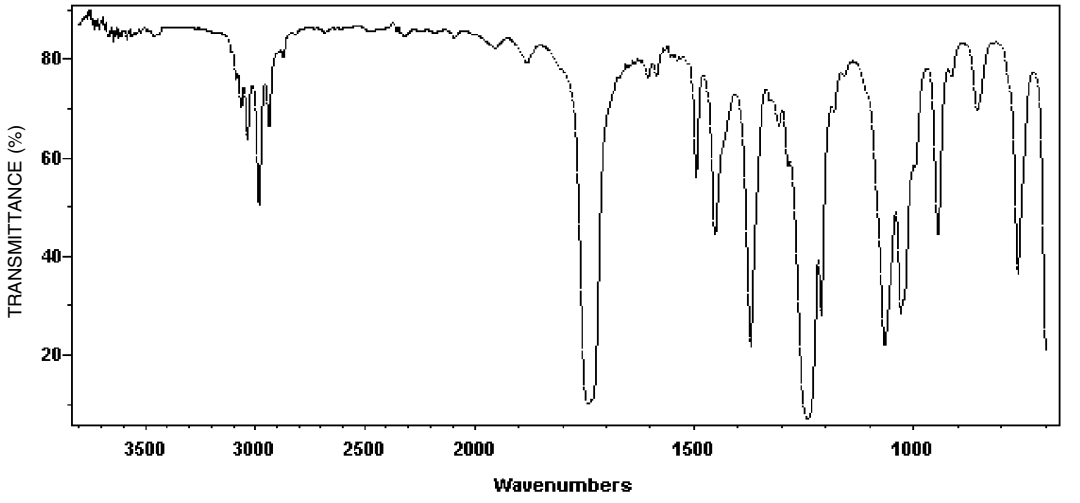
Methyl Anthranilate View Flavor Chemical



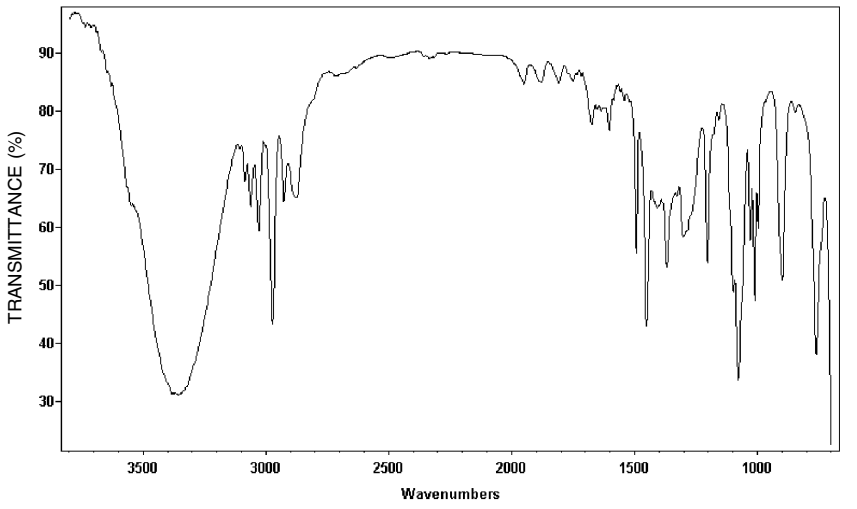
Methyl Benzoate View Flavor Chemical



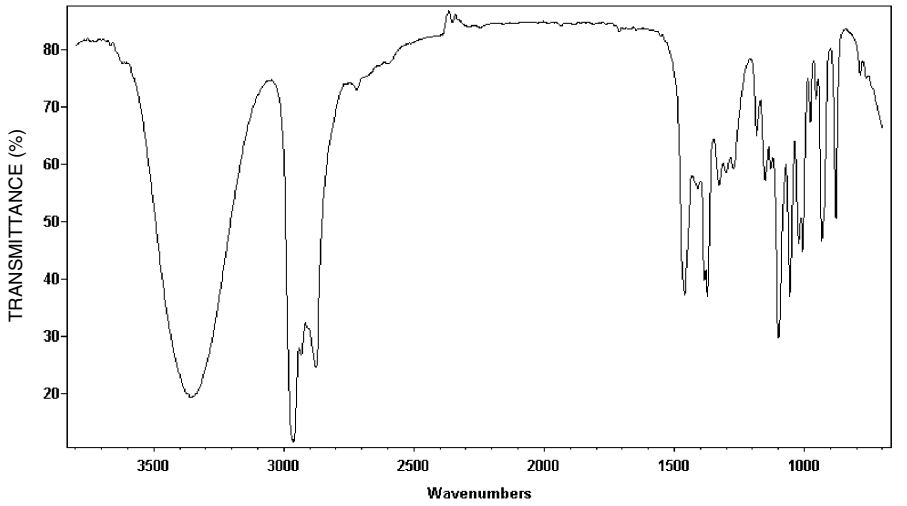
α-Methylbenzyl Acetate View Flavor Chemical



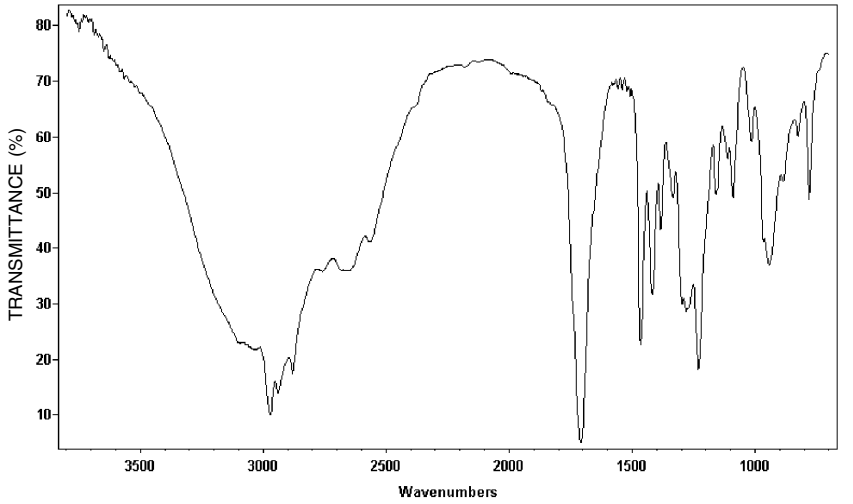
α-Methylbenzyl Alcohol View Flavor Chemical



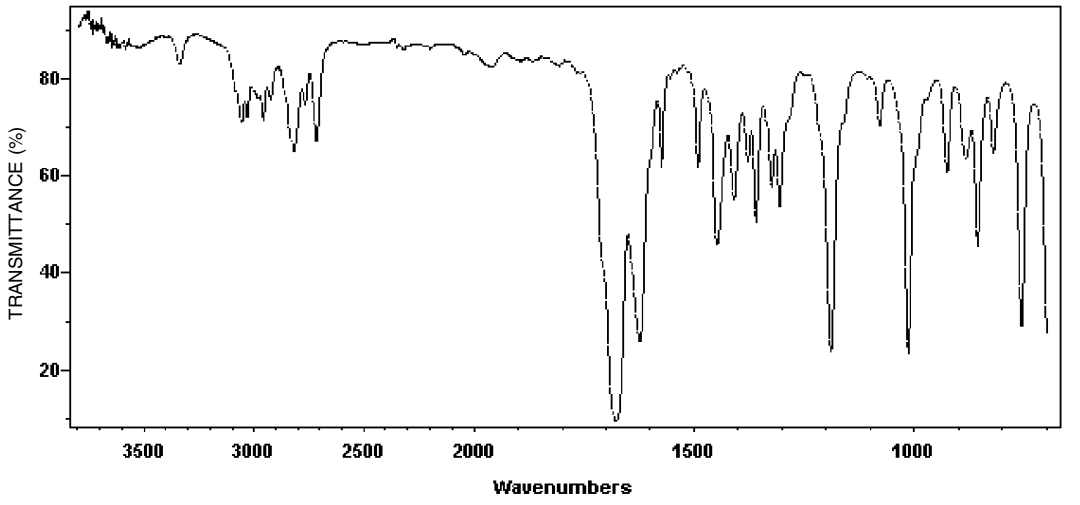
3-Methyl Butanal View Flavor Chemical



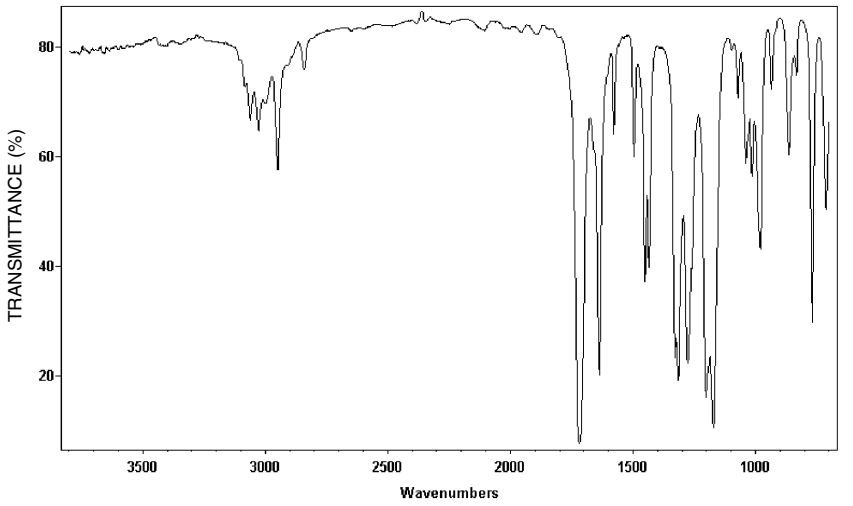
2-Methylbutyric Acid View Flavor Chemical



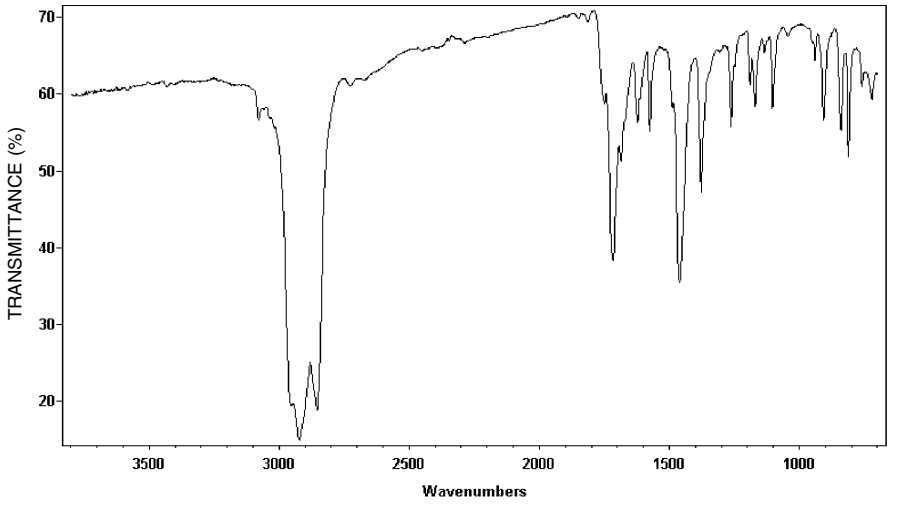
α-Methylcinnamaldehyde View Flavor Chemical



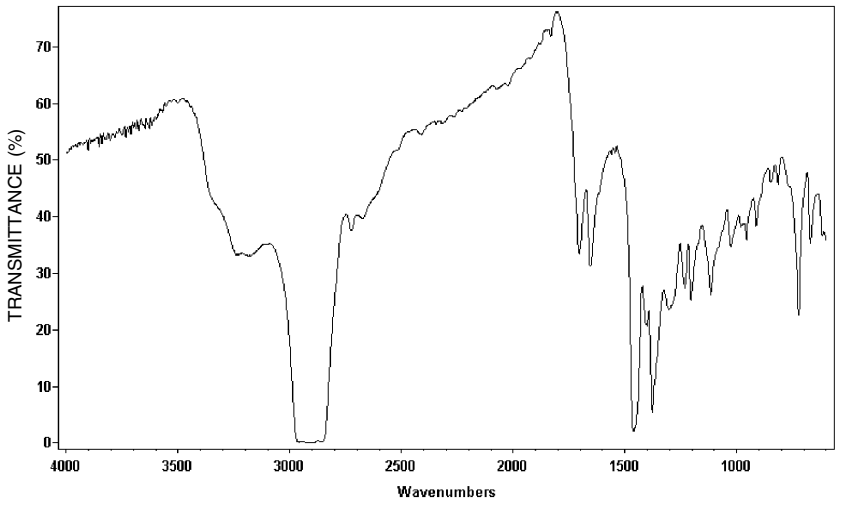
Methyl Cinnamate View Flavor Chemical



6-Methylcoumarin (Mineral Oil Mull) View Flavor Chemical

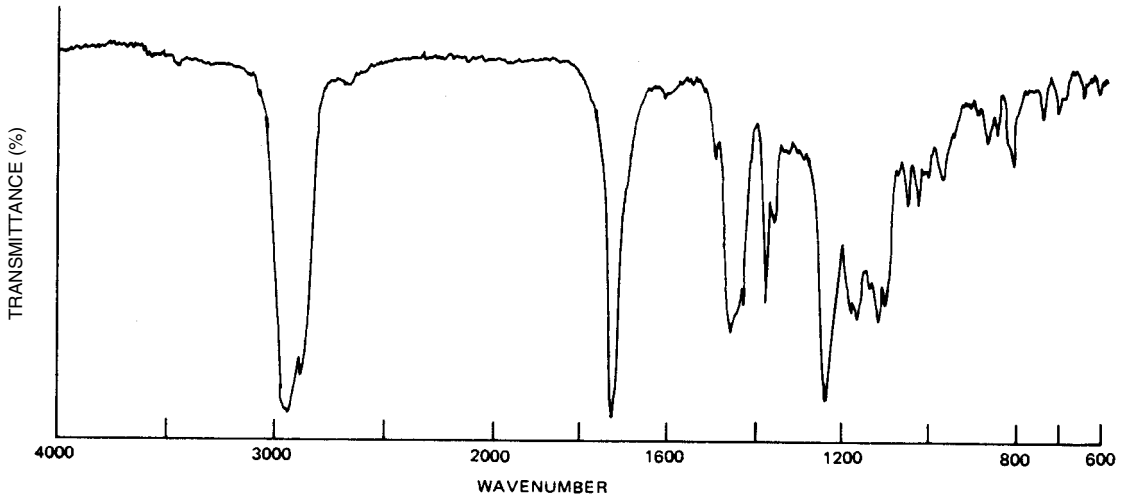


Methyl Cyclopentenolone View Flavor Chemical



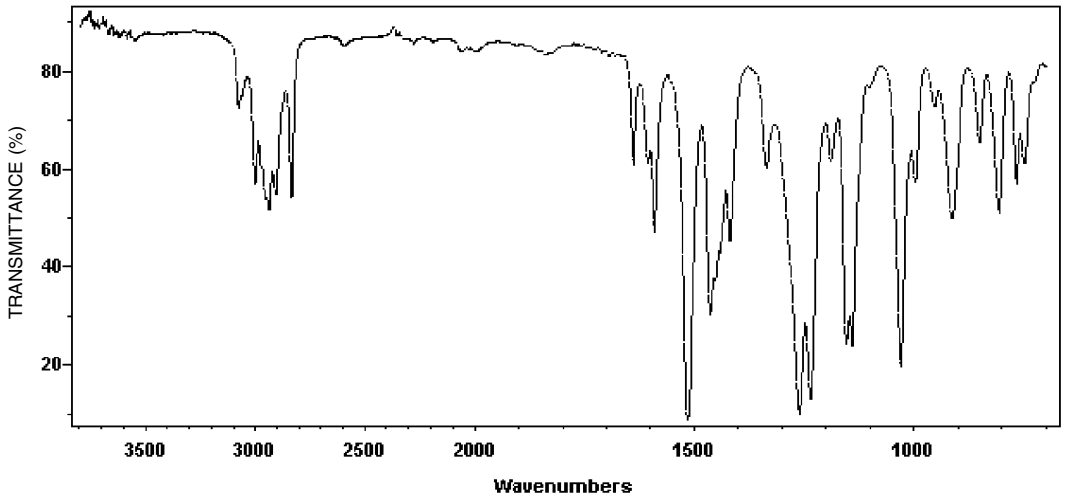
Methyl Ester of Rosin, Partially Hydrogenated

View Monograph



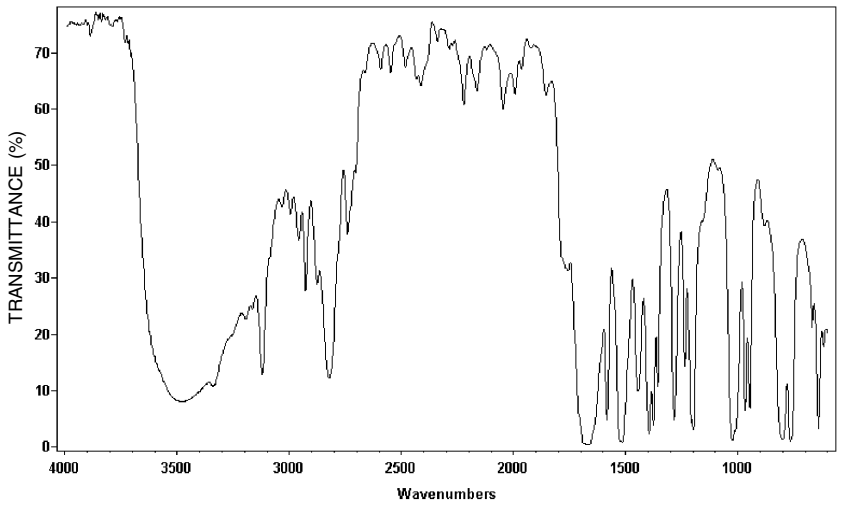
Methyl Eugenol

View Flavor Chemical

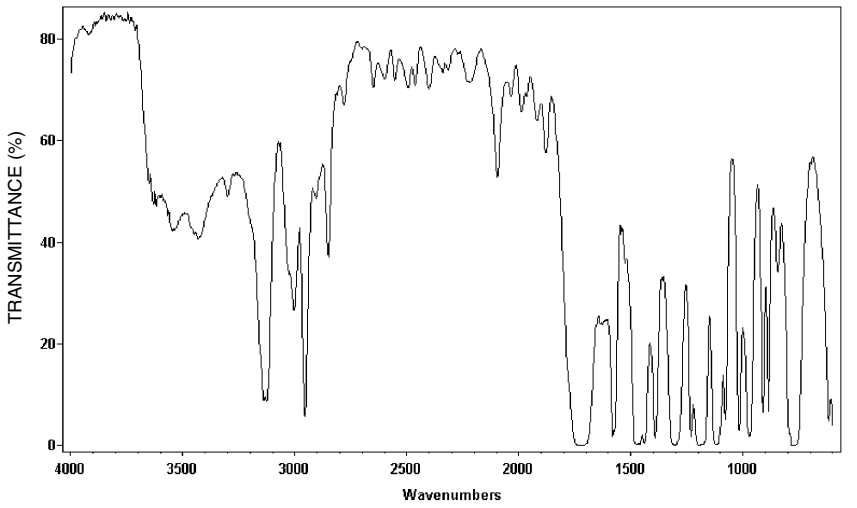


5-Methyl Furfural

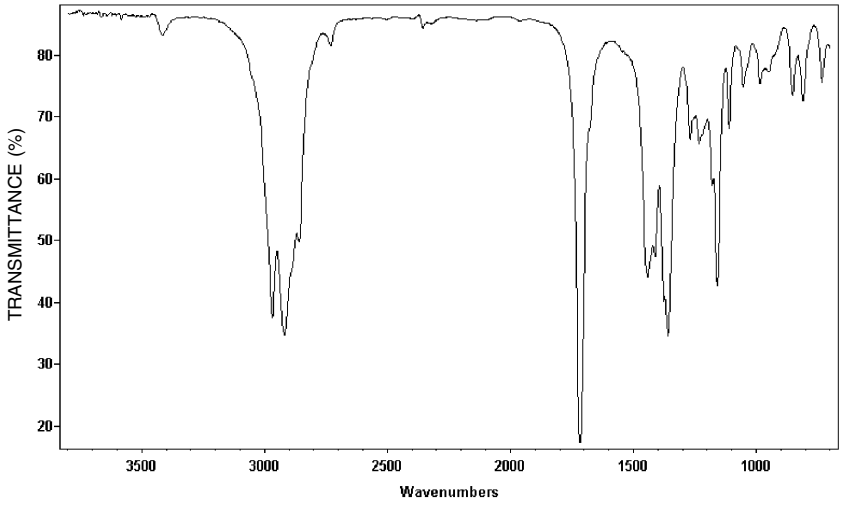
View Flavor Chemical



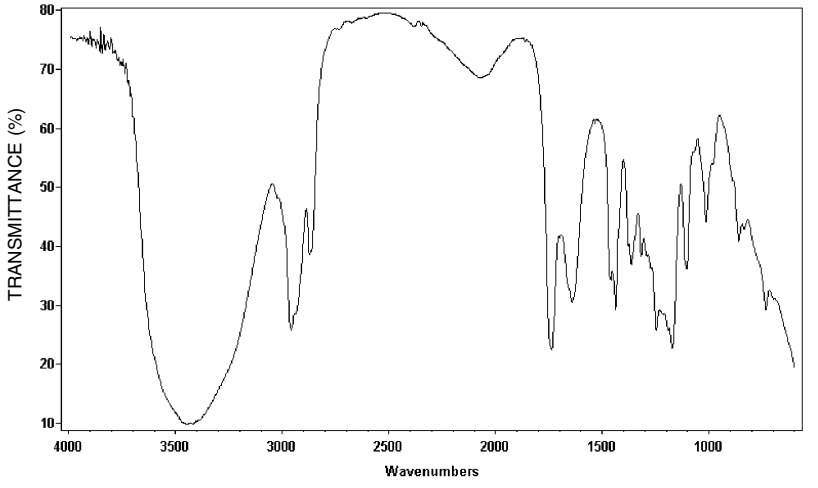
Methyl Furoate View Flavor Chemical



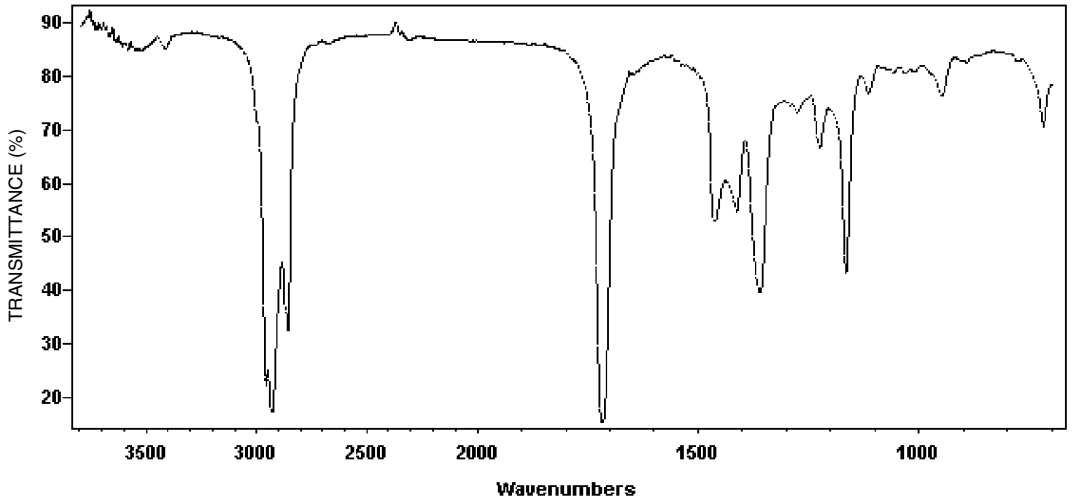
6-Methyl-5-hepten-2-one View Flavor Chemical



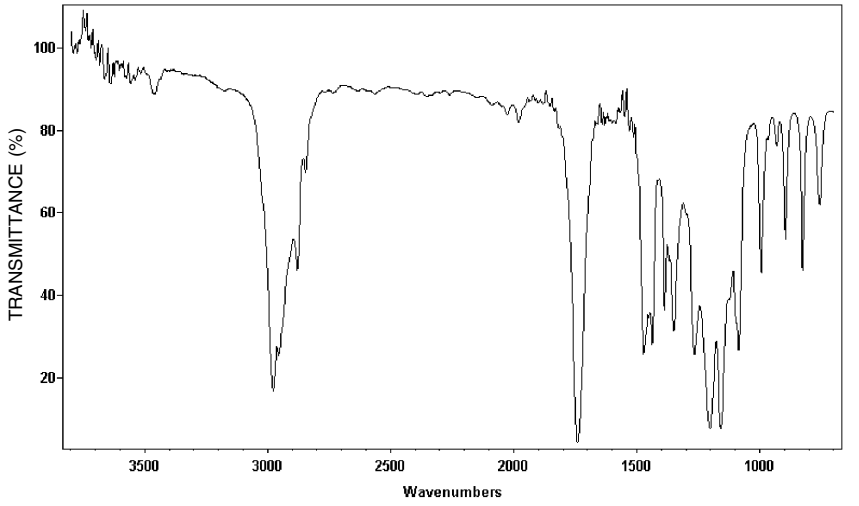
Methyl Hexanoate View Flavor Chemical



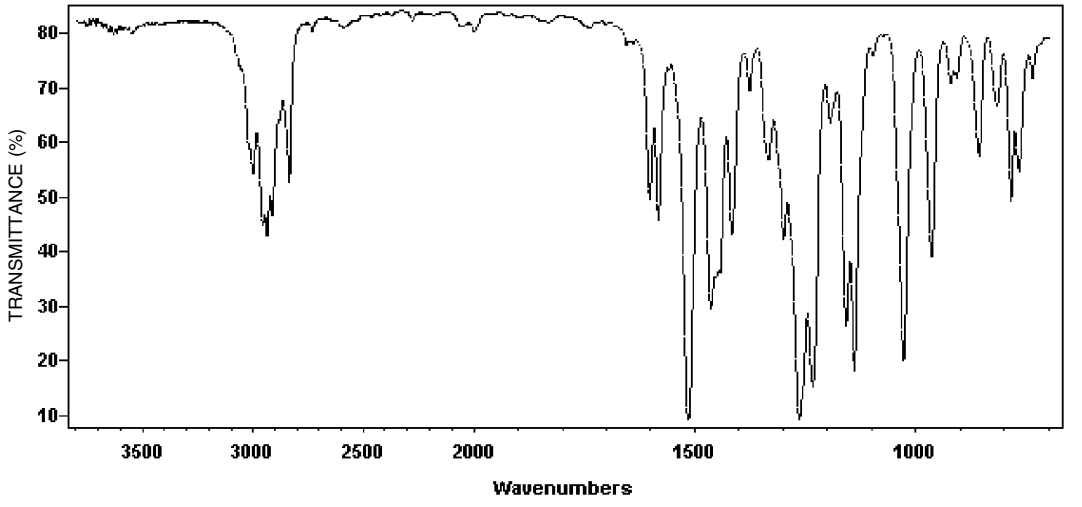
Methyl Hexyl Ketone View Flavor Chemical



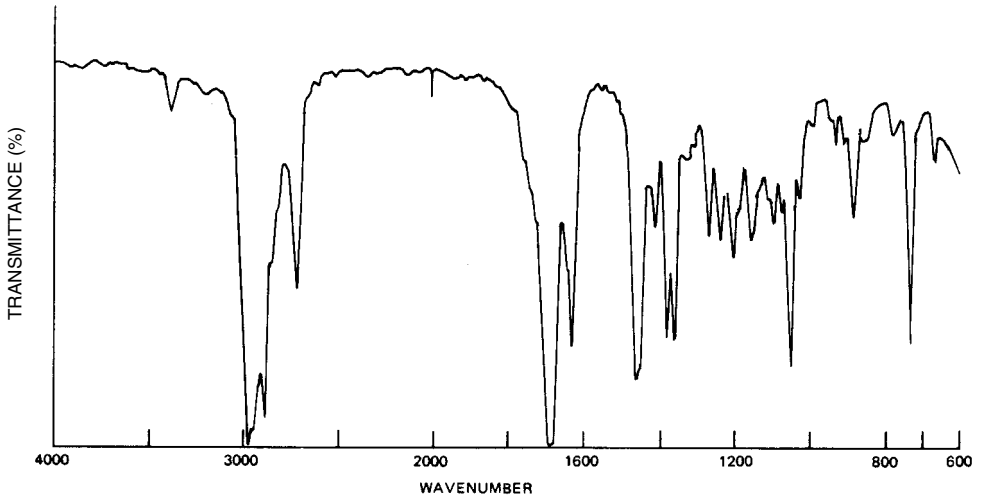
Methyl Isobutyrate View Flavor Chemical



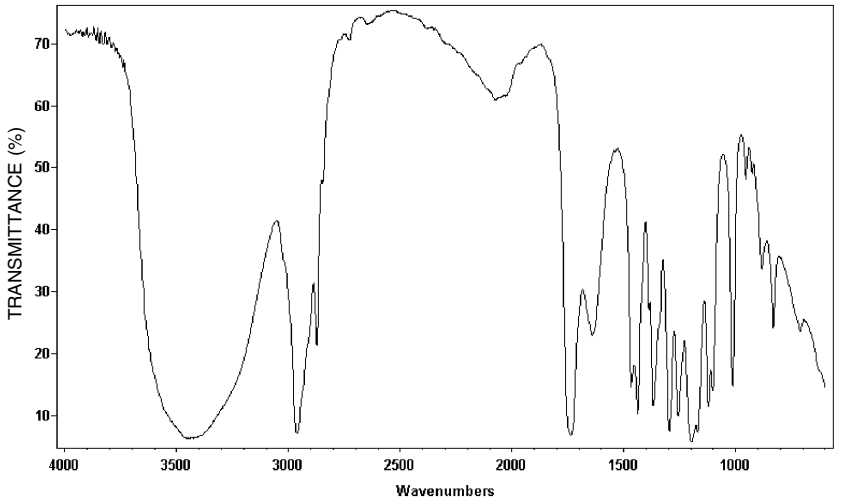
Methyl Isoeugenol View Flavor Chemical



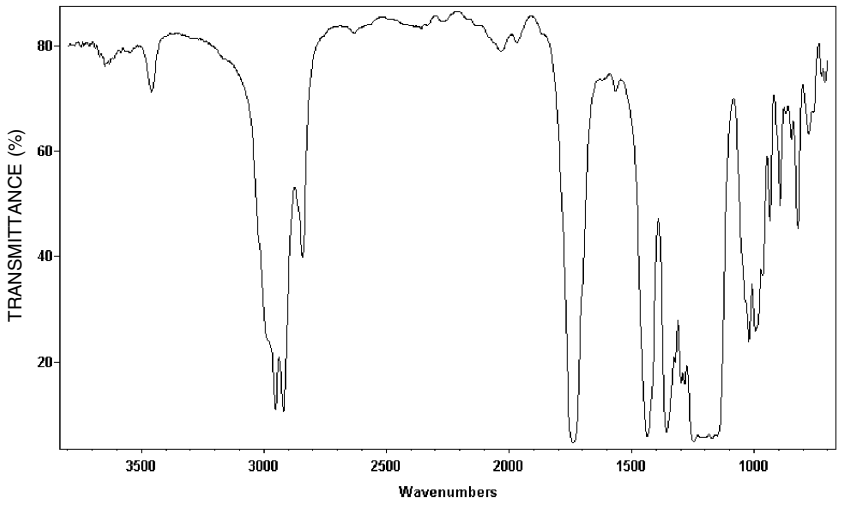
5-Methyl-2-isopropyl-2-hexenal View Flavor Chemical



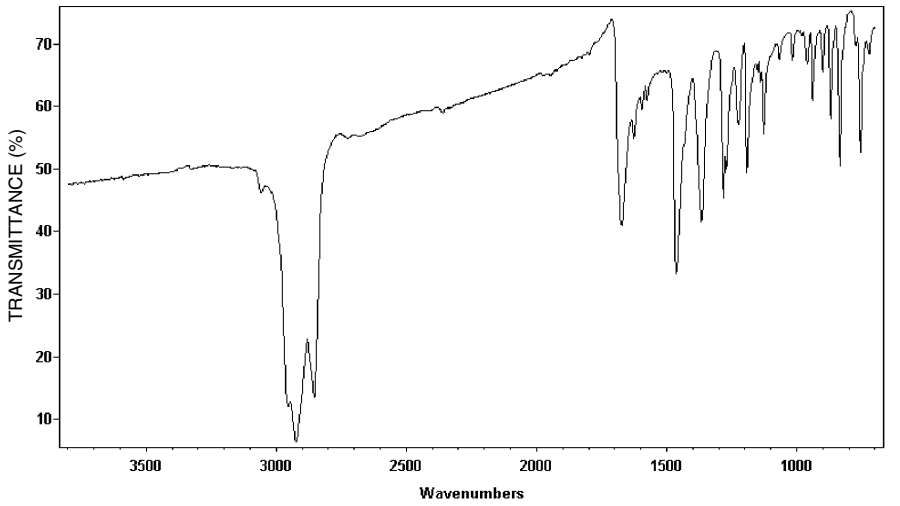
Methyl Isovalerate View Flavor Chemical



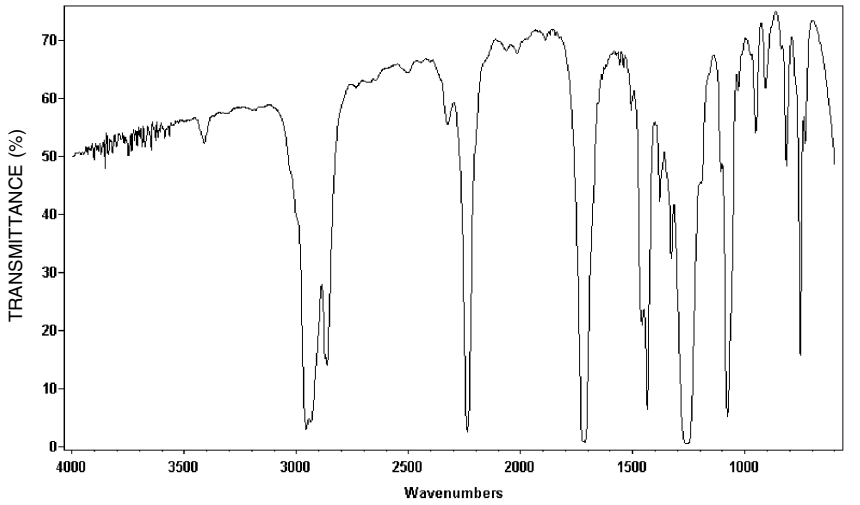
Methyl-3-methylthiopropionate View Flavor Chemical



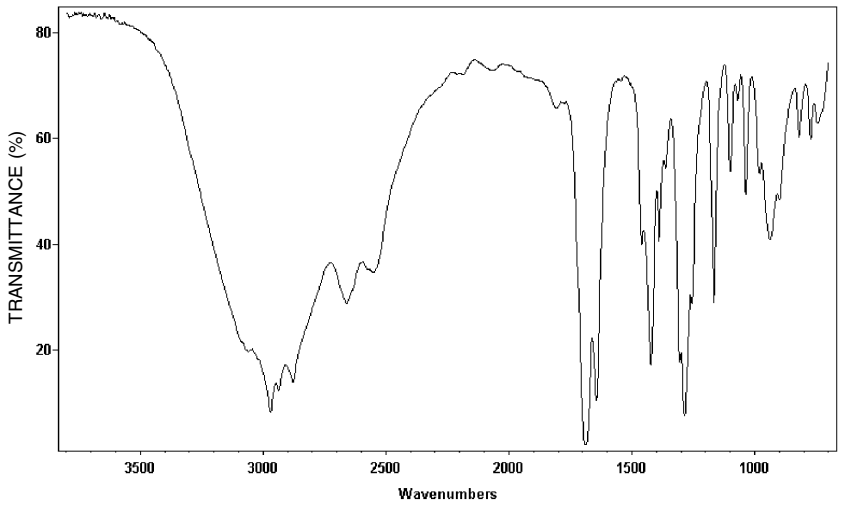
Methyl β-Naphthyl Ketone (Mineral Oil Mull) View Flavor Chemical



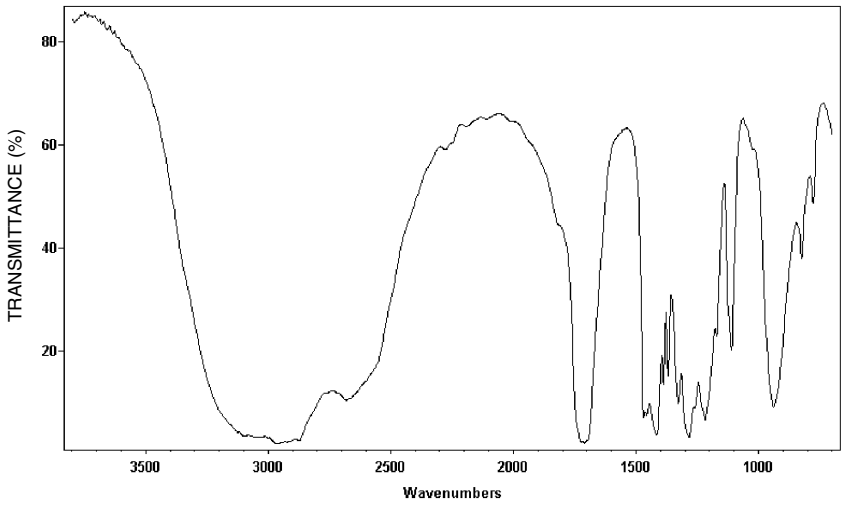
Methyl 2-Octynoate View Flavor Chemical



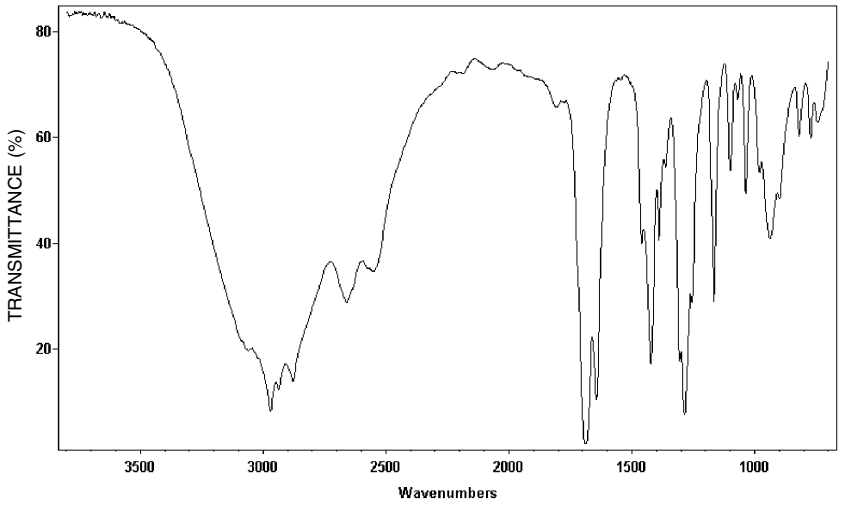
2-Methylpentanoic Acid View Flavor Chemical



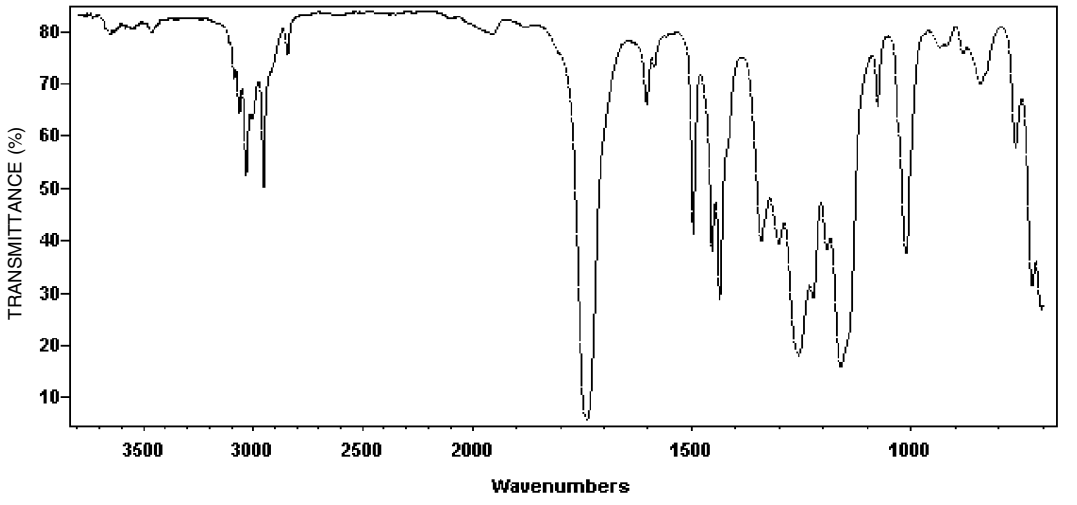
4-Methylpentanoic Acid View Flavor Chemical



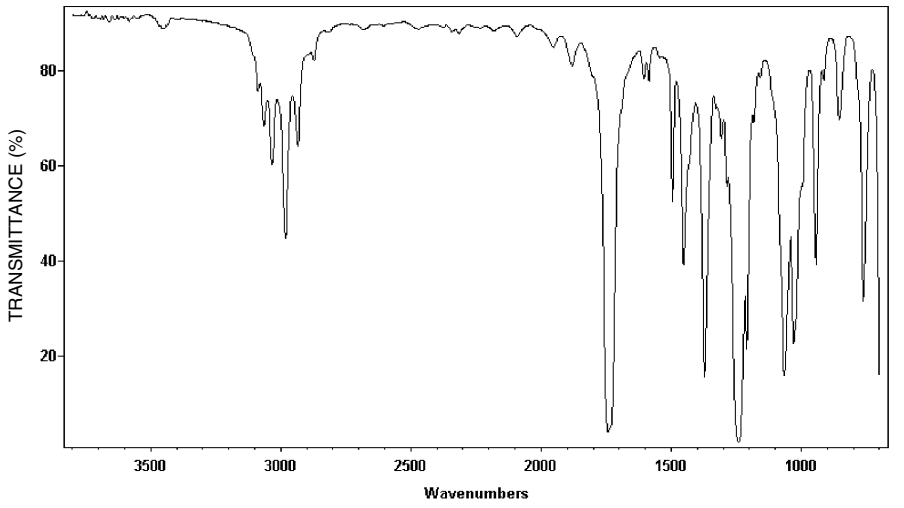
2-Methyl-2-pentenoic Acid View Flavor Chemical



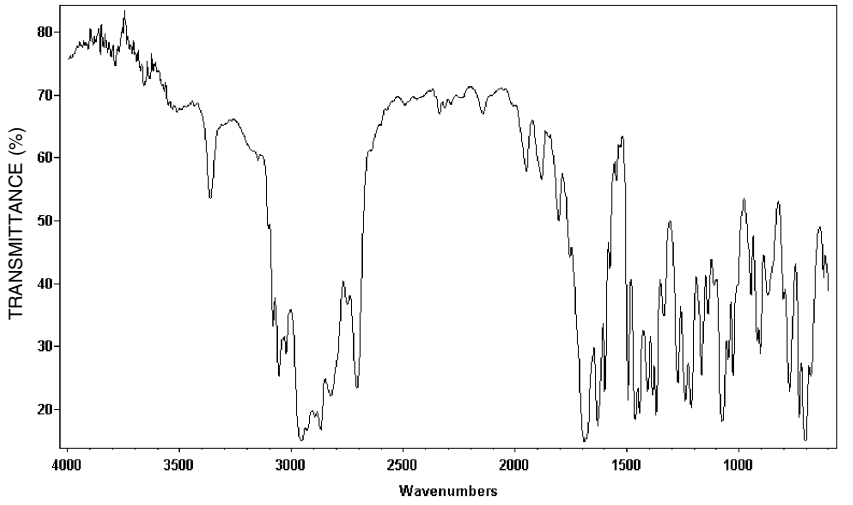
Methyl Phenylacetate View Flavor Chemical



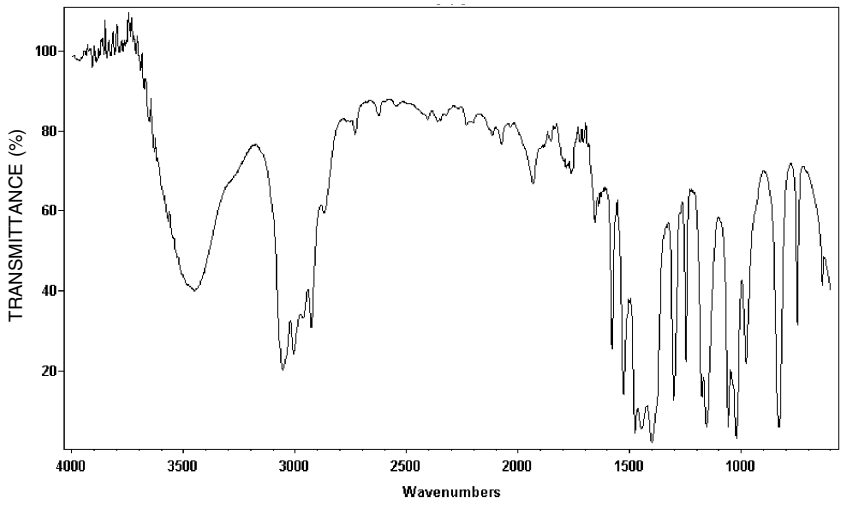
Methyl Phenylcarbinyl Acetate View Flavor Chemical



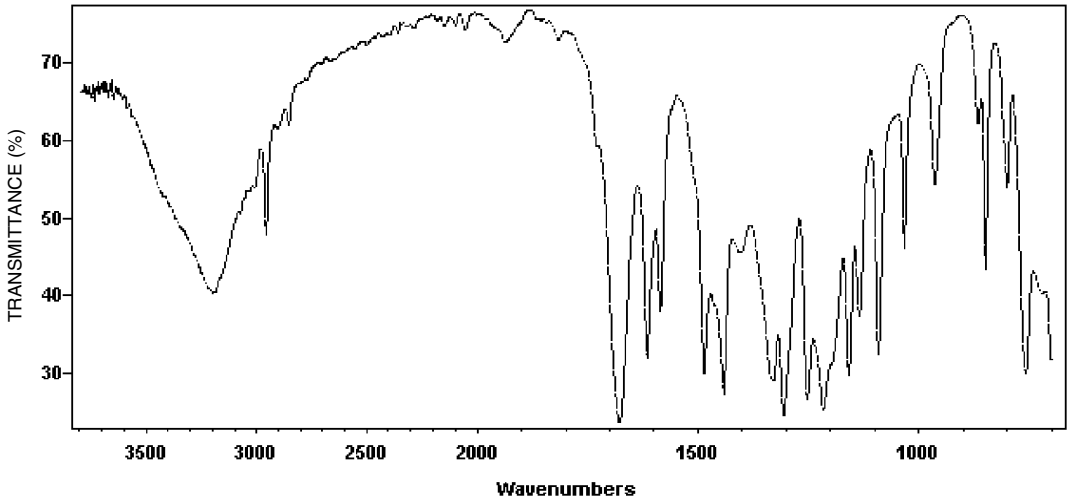
5-Methyl 2-Phenyl 2-Hexenal View Flavor Chemical



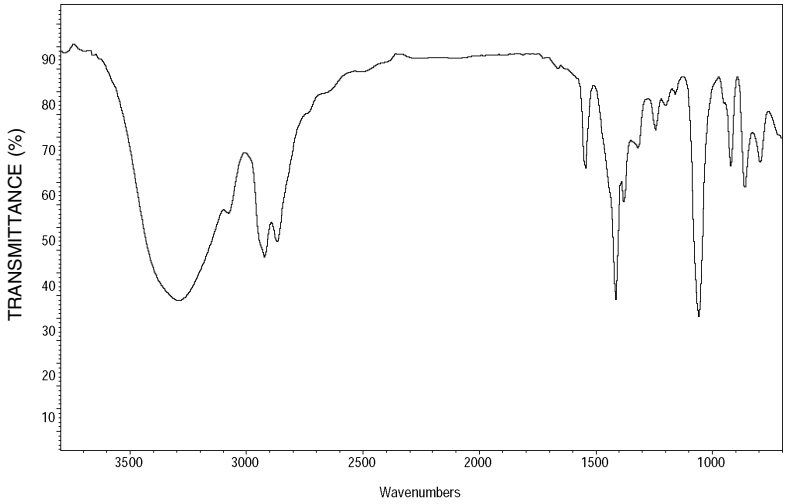
2-Methylpyrazine View Flavor Chemical



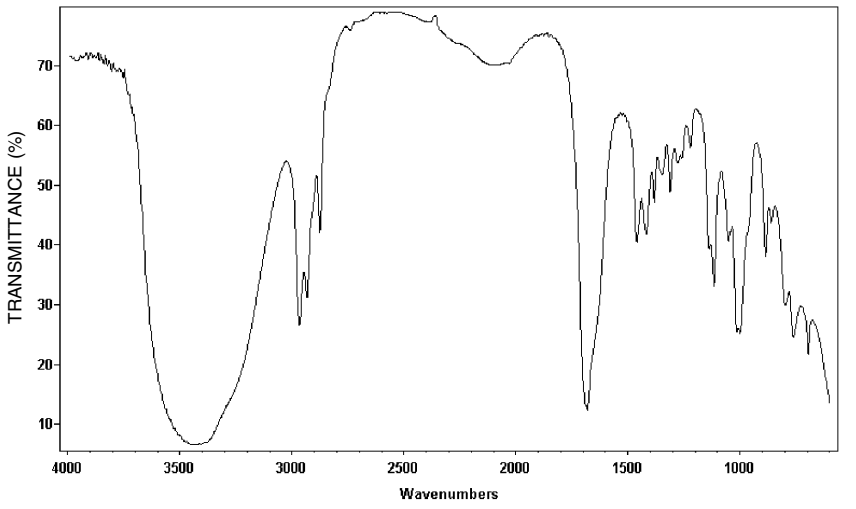
Methyl Salicylate View Flavor Chemical



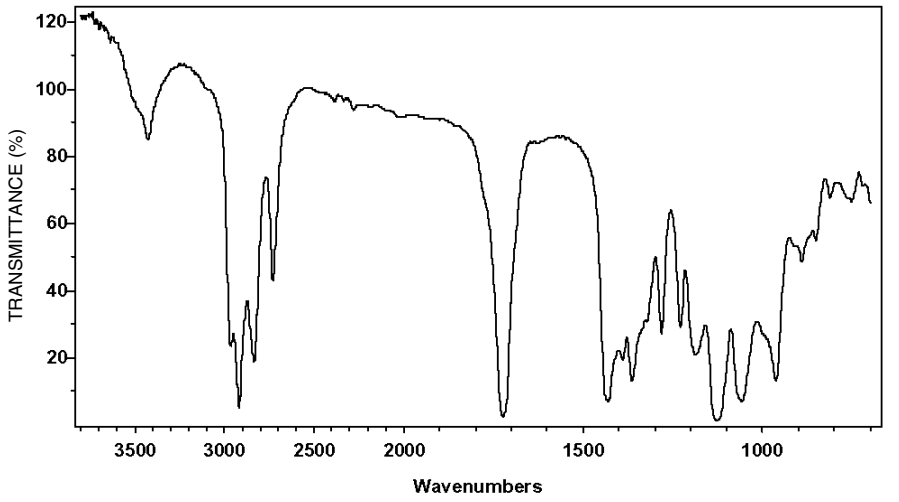
4-Methyl-5-thiazole Ethanol View Flavor Chemical



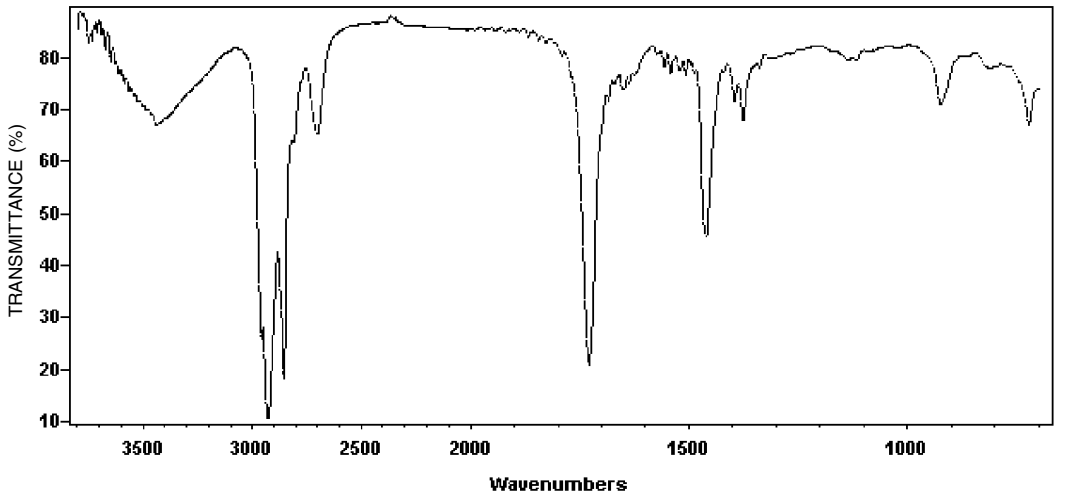
Methyl Thiobutyrate View Flavor Chemical



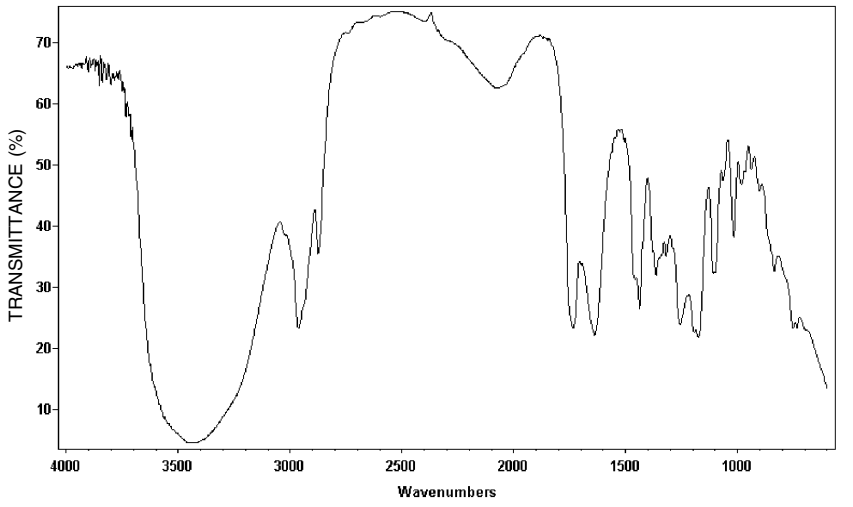
3-Methylthiopropionaldehyde View Flavor Chemical



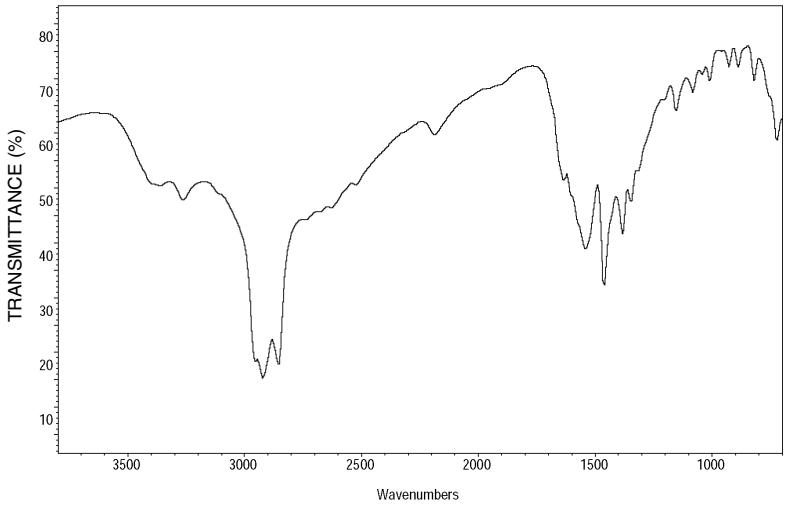
2-Methylundecanal View Flavor Chemical



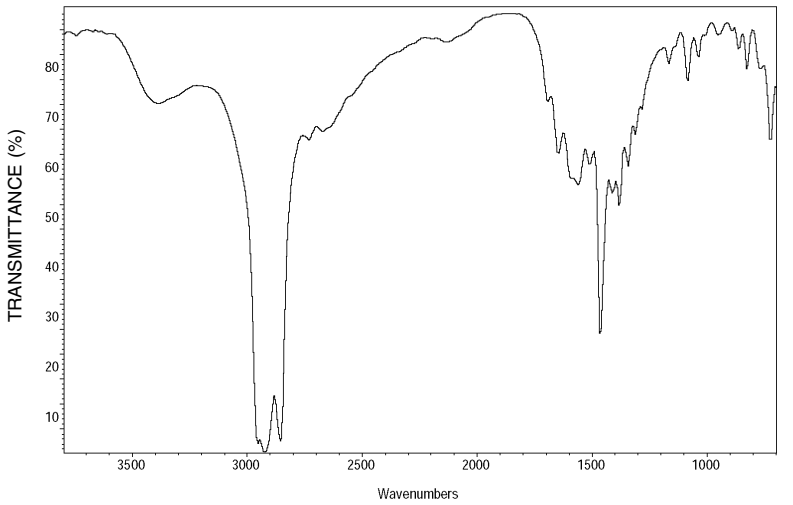
Methyl Valerate View Flavor Chemical



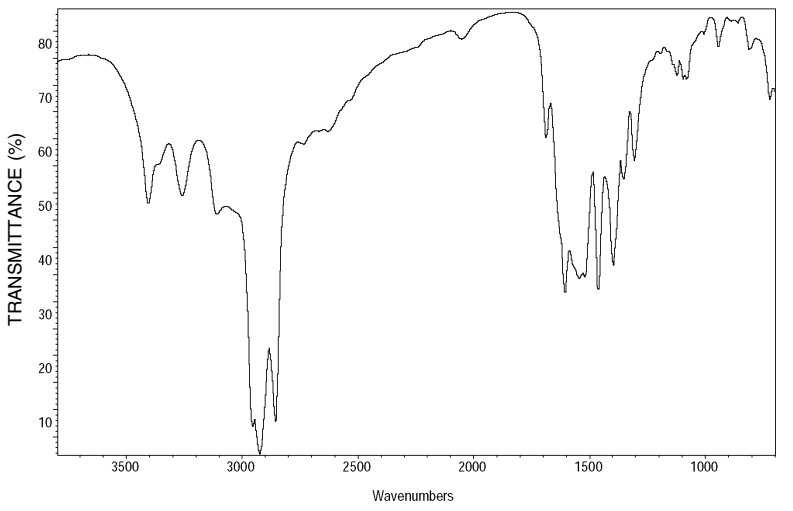
Monoammonium L-Glutamate (Mineral Oil Mull) View Monograph



Monopotassium L-Glutamate (Mineral Oil Mull) View Monograph

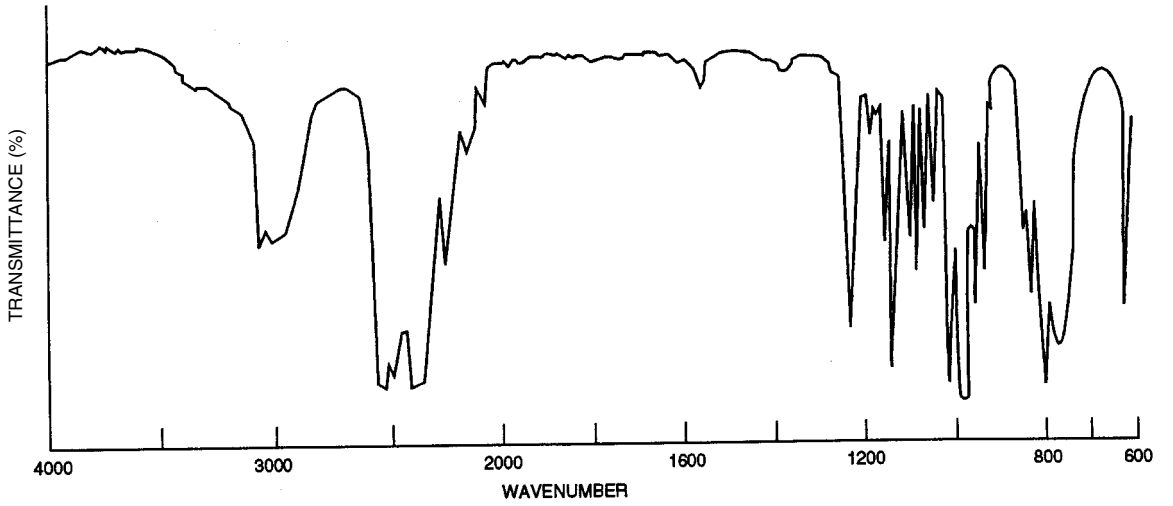


Monosodium L-Glutamate (Mineral Oil Mull) View Monograph



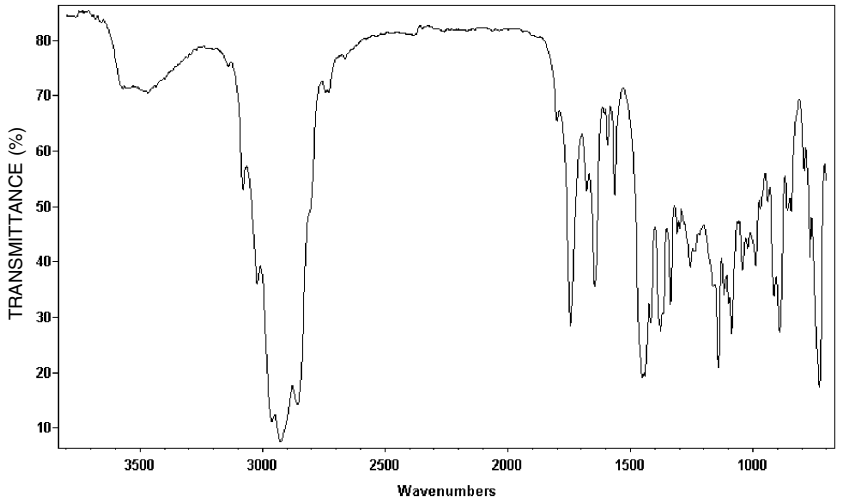
Morpholine

View Monograph



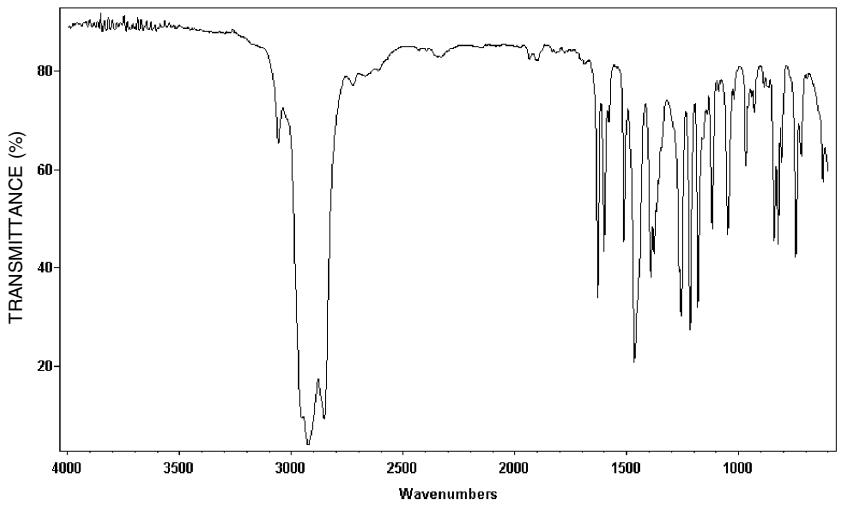
Myrrh Oil

View Monograph

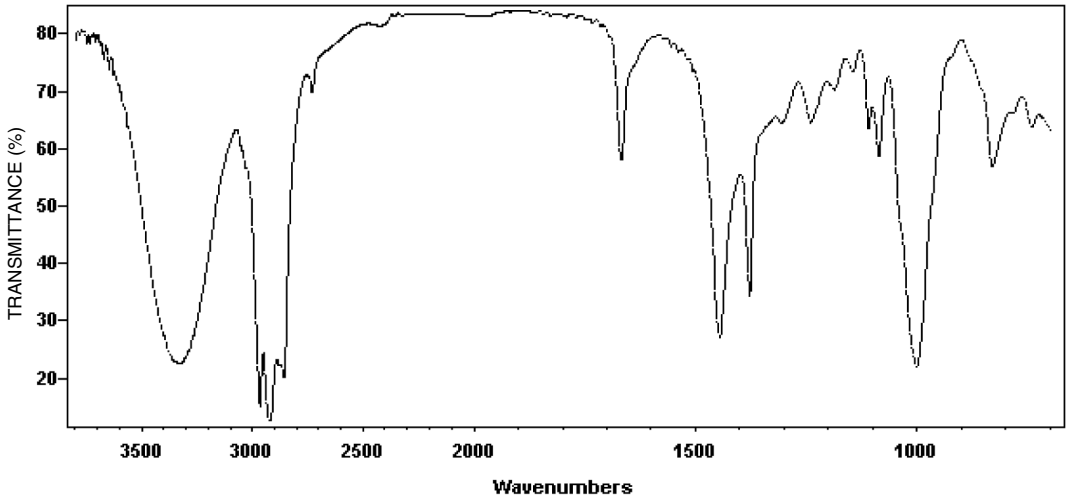


β-Naphthyl Ethyl Ether

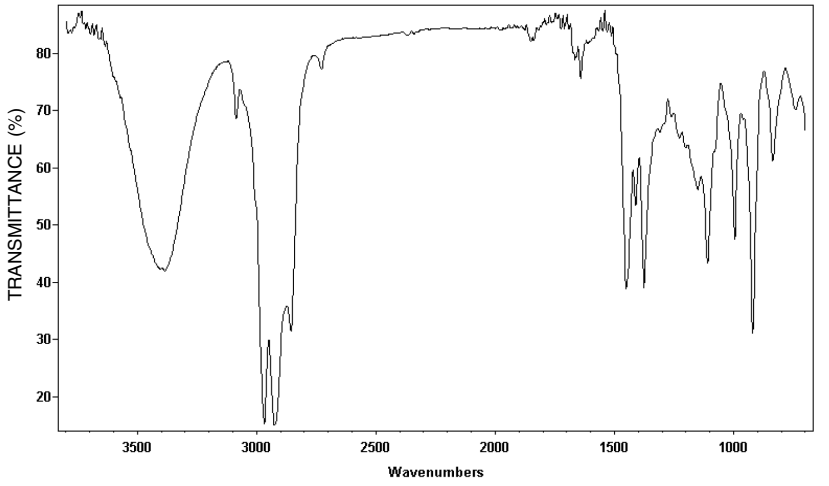
View Flavor Chemical



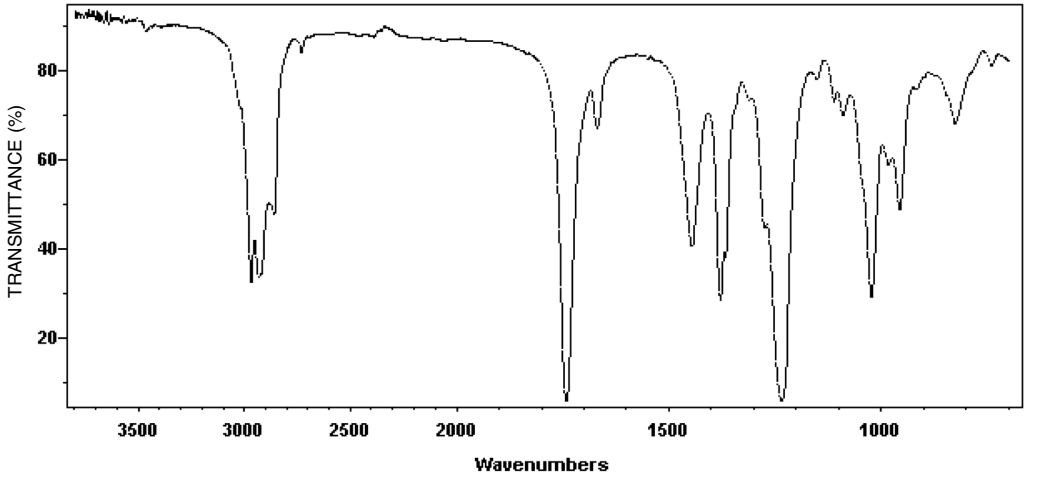
Nerol View Flavor Chemical



Nerolidol View Flavor Chemical

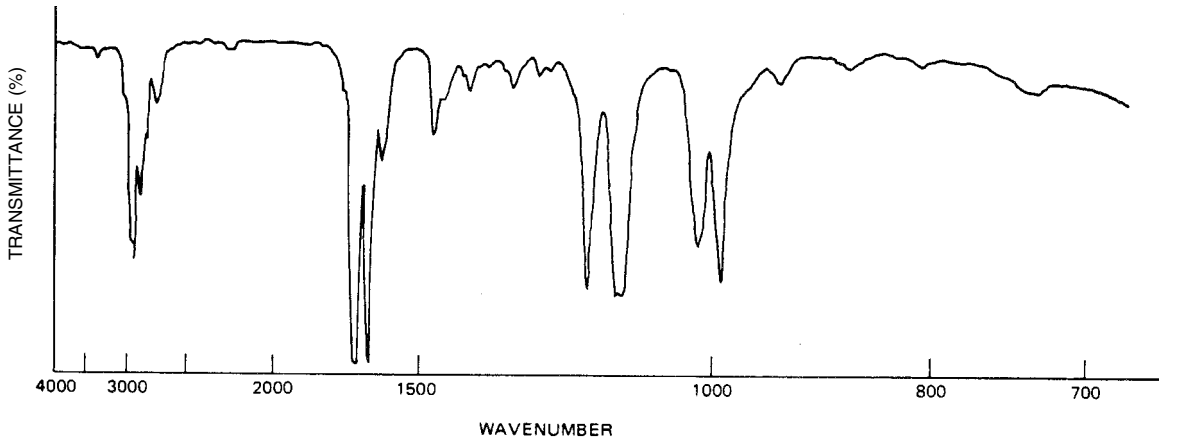


Neryl Acetate View Flavor Chemical



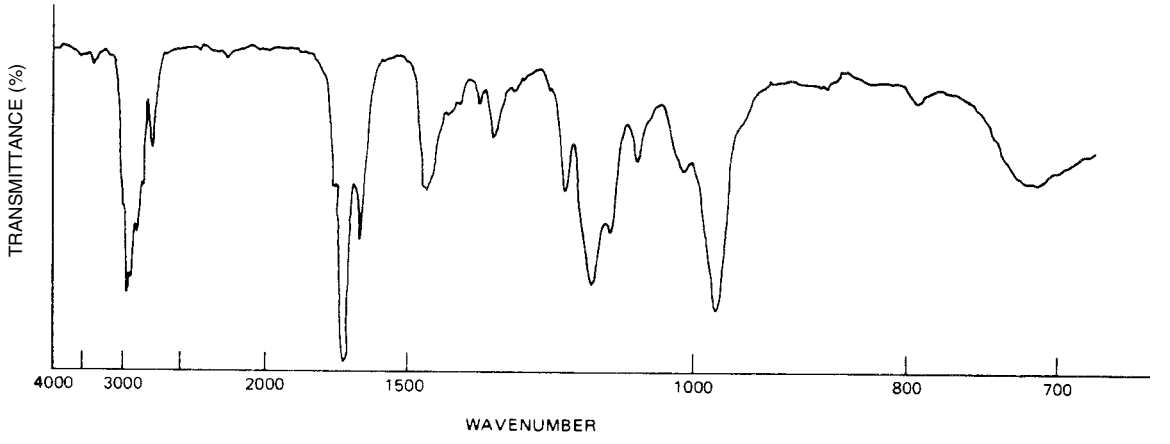
(*E*),(*E*)-2,4-Nonadienal

View Flavor Chemical



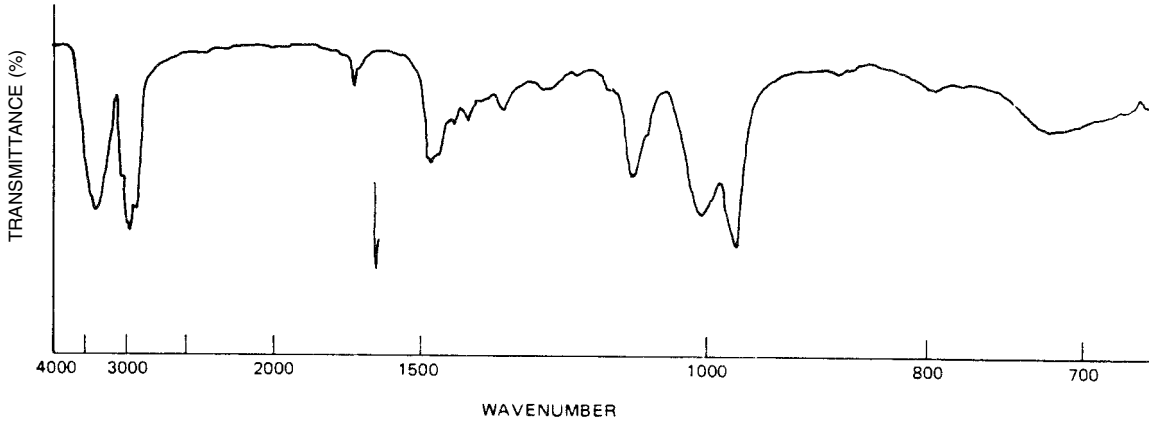
(*E*),(*Z*)-2,6-Nonadienal

View Flavor Chemical

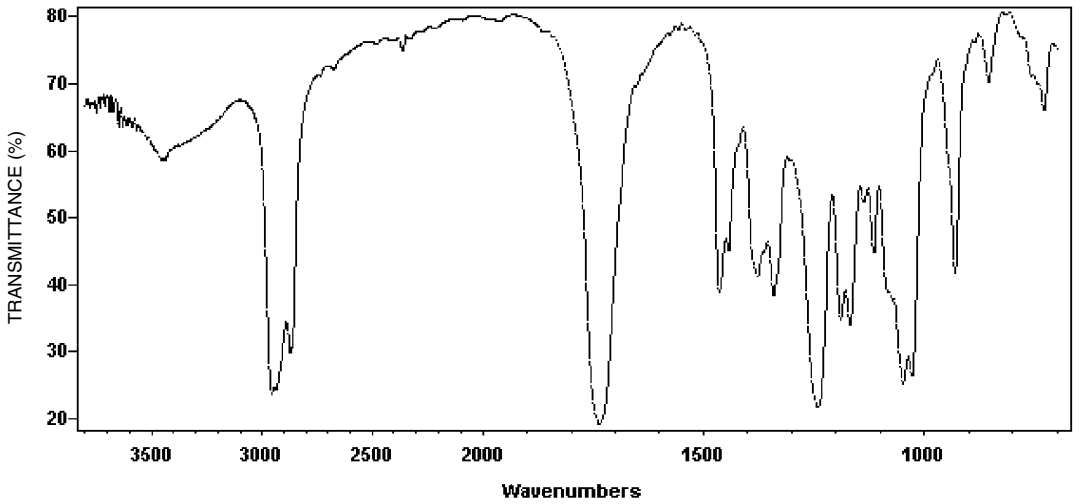


(*E*),(*Z*)-2,6-Nonadienol

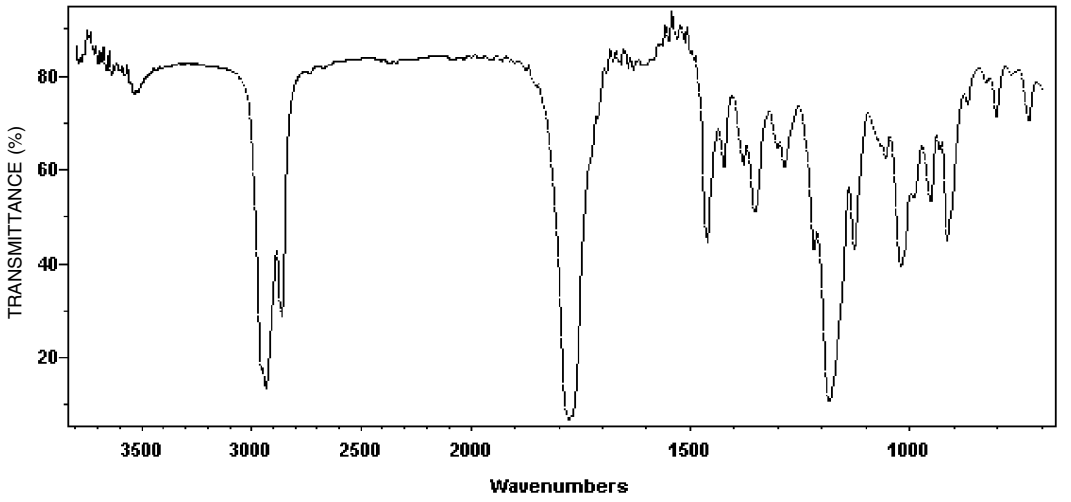
View Flavor Chemical



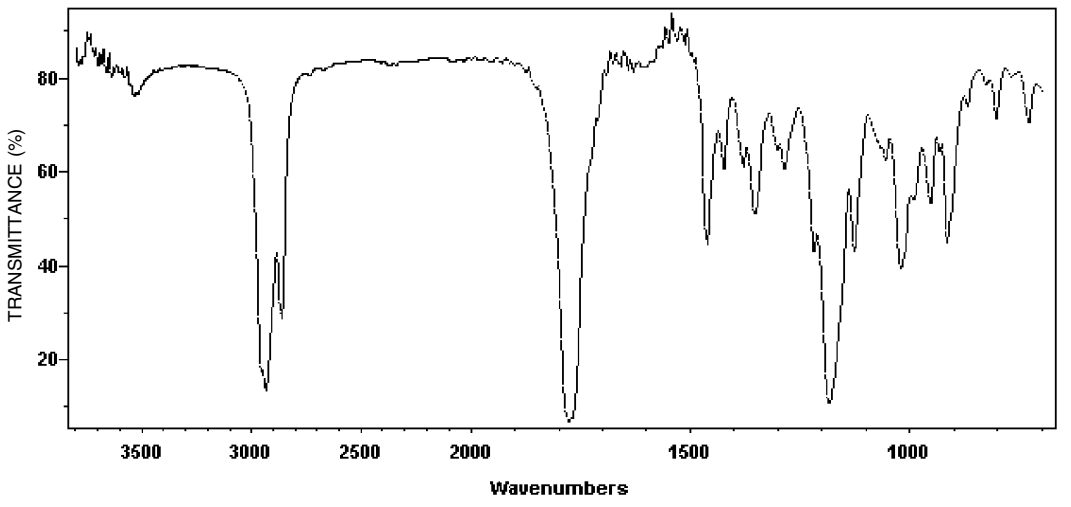
δ-Nonalactone View Flavor Chemical



γ-Nonalactone View Flavor Chemical

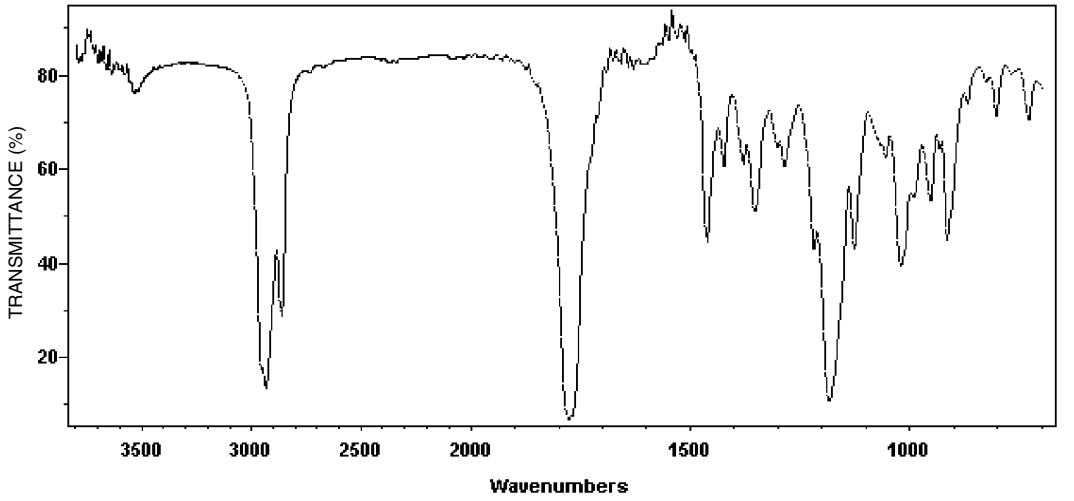


Nonanal View Flavor Chemical



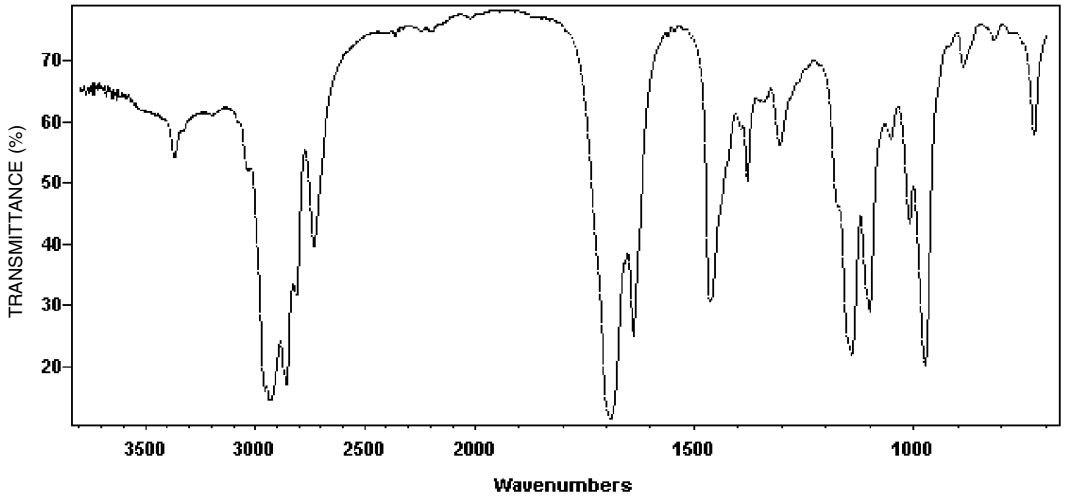
2-Nonanone

View Flavor Chemical



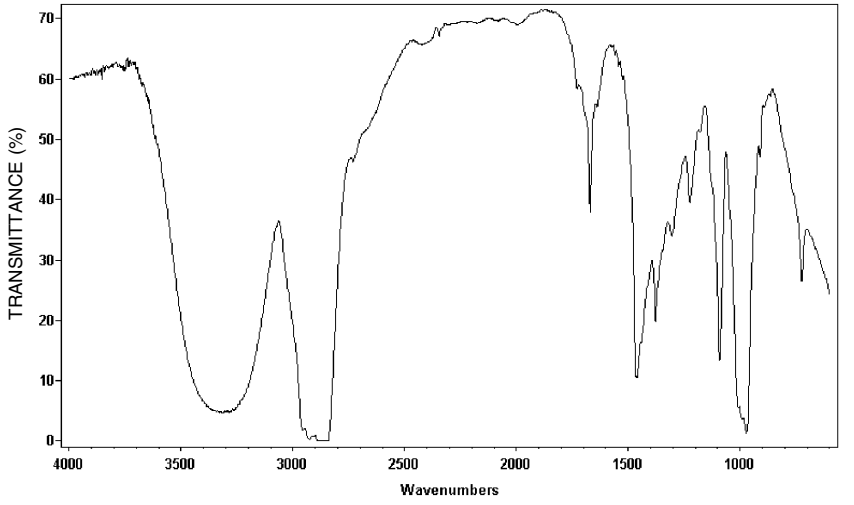
(*E*)-2-Nonenal

View Flavor Chemical

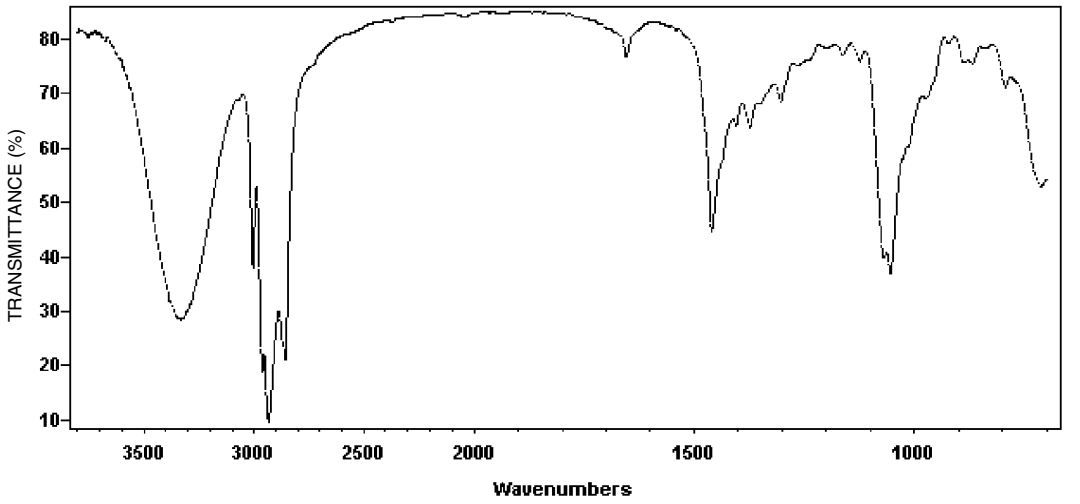


(*E*)-2-Nonen-1-ol

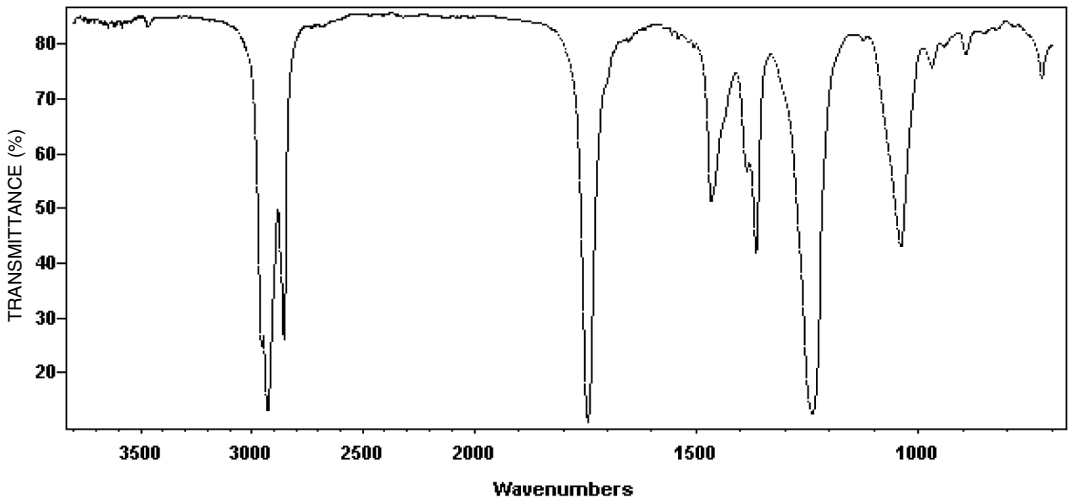
View Flavor Chemical



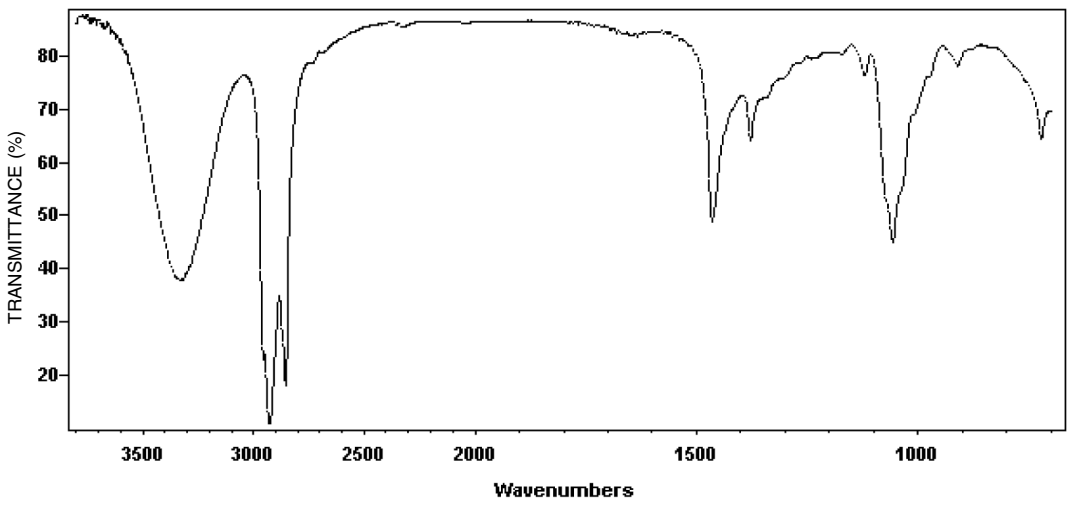
(Z)-6-Nonen-1-ol View Flavor Chemical



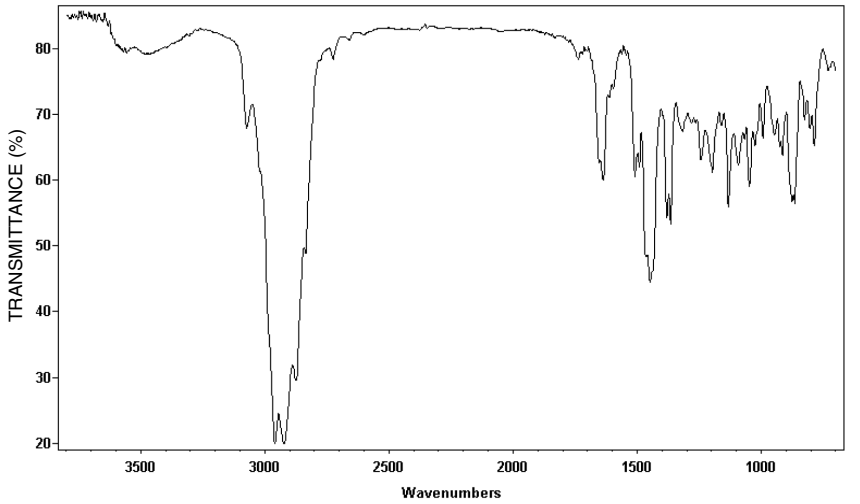
Nonyl Acetate View Flavor Chemical



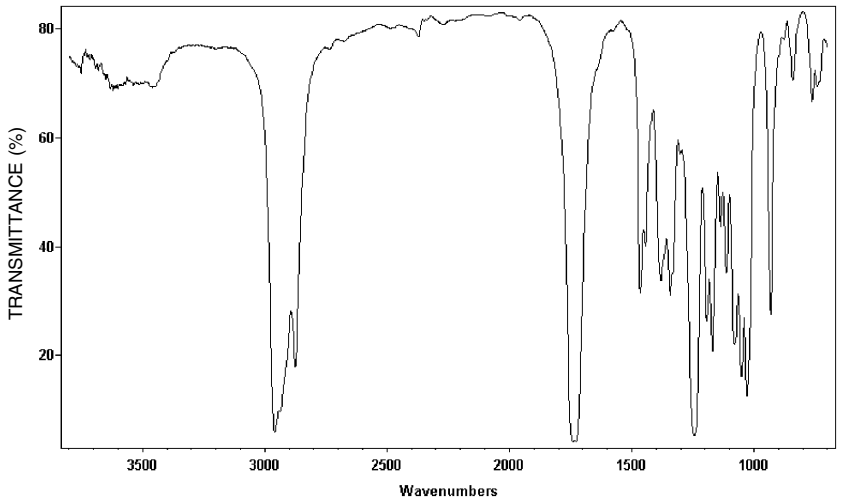
Nonyl Alcohol View Flavor Chemical



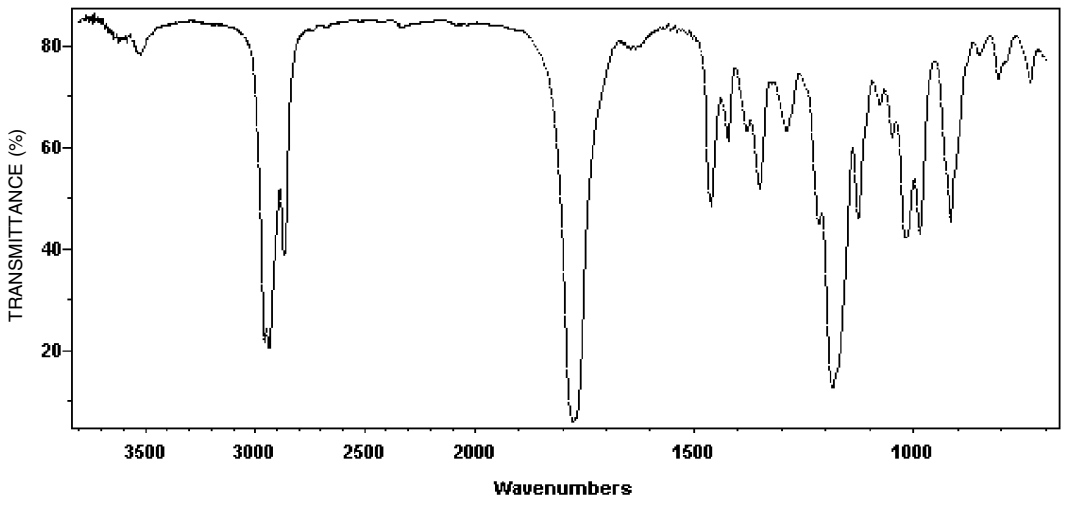
Nutmeg Oil View Monograph



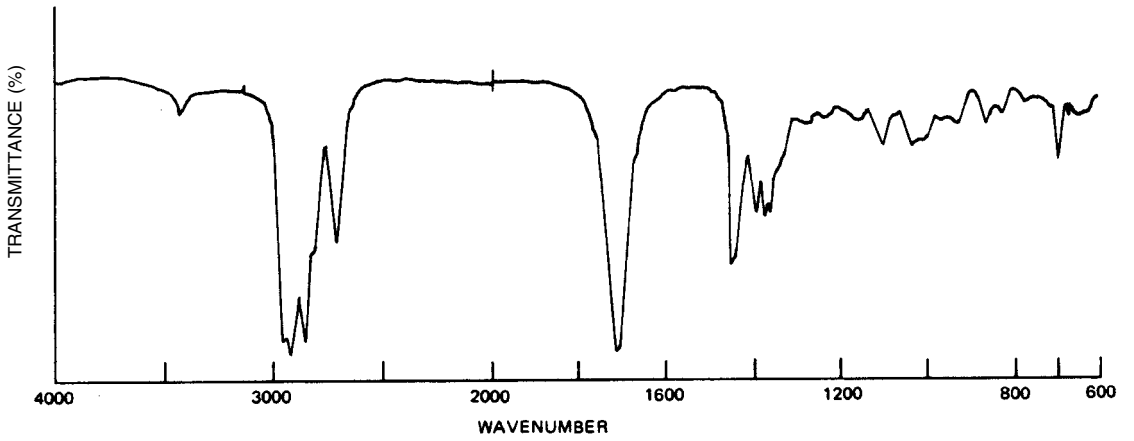
δ-Octalactone View Flavor Chemical



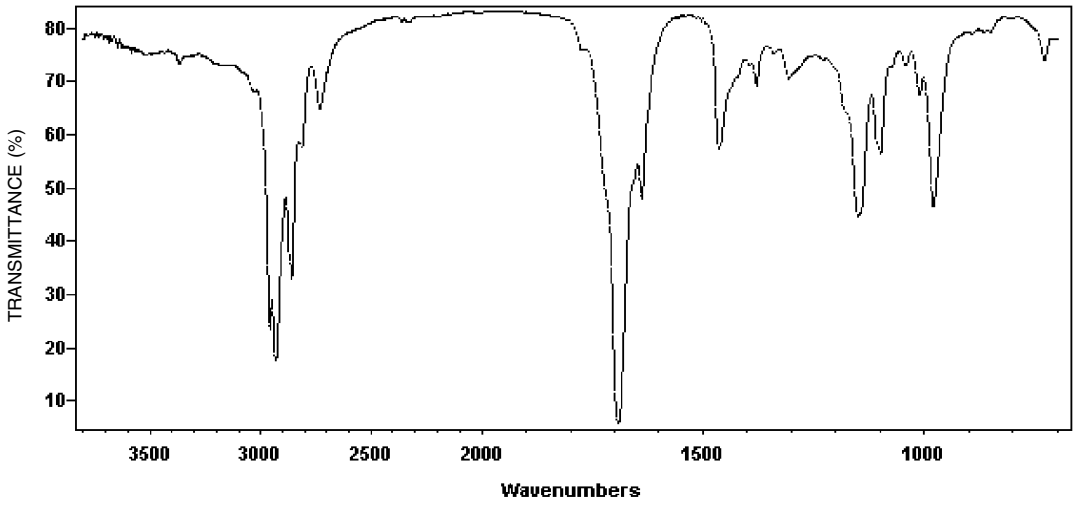
γ-Octalactone View Flavor Chemical



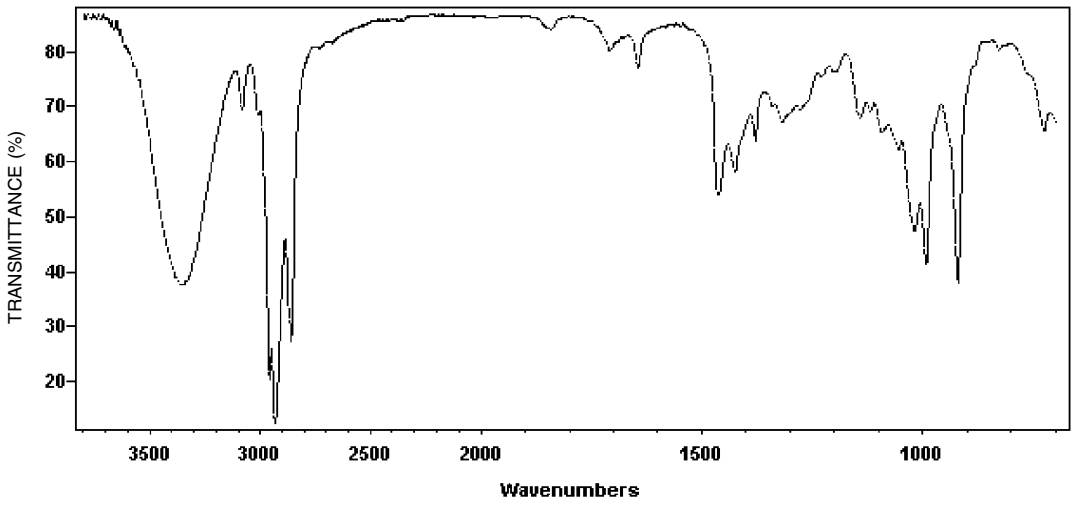
Octanal View Flavor Chemical



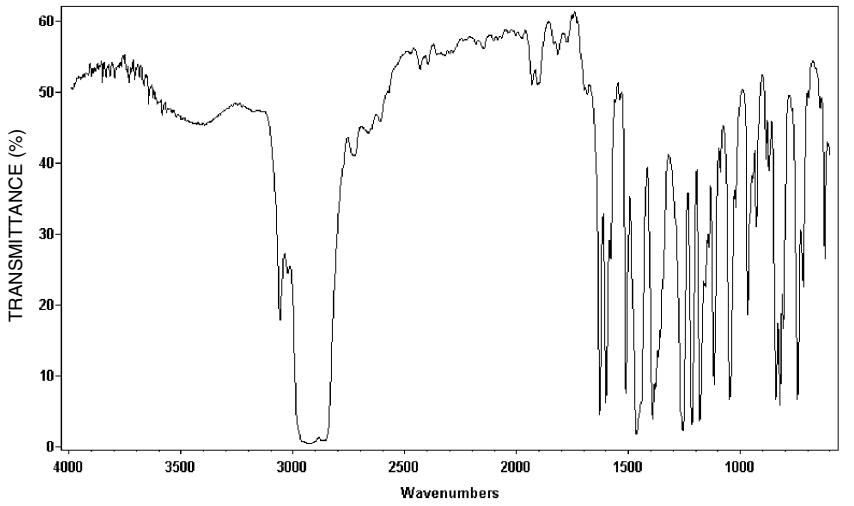
(*E*)-2-Octen-1-al View Flavor Chemical



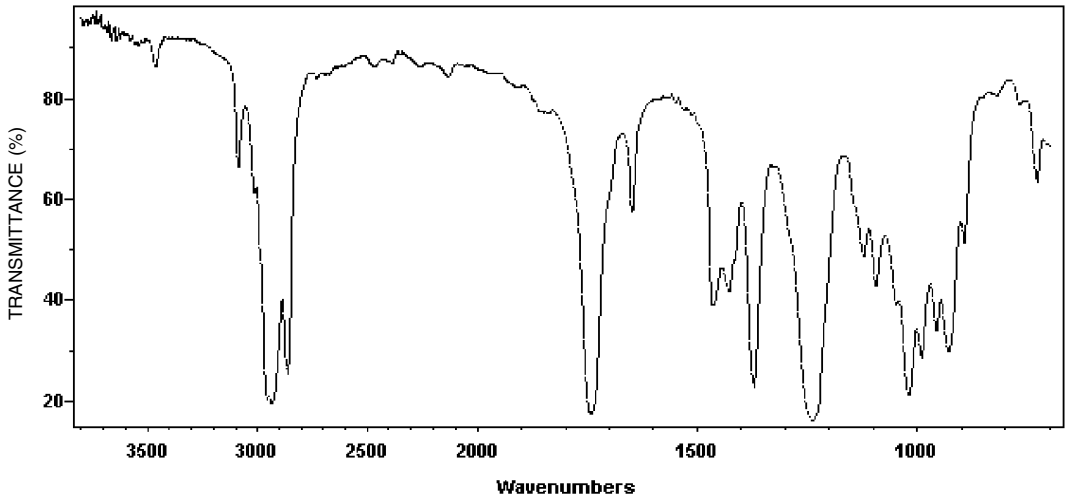
1-Octen-3-ol View Flavor Chemical



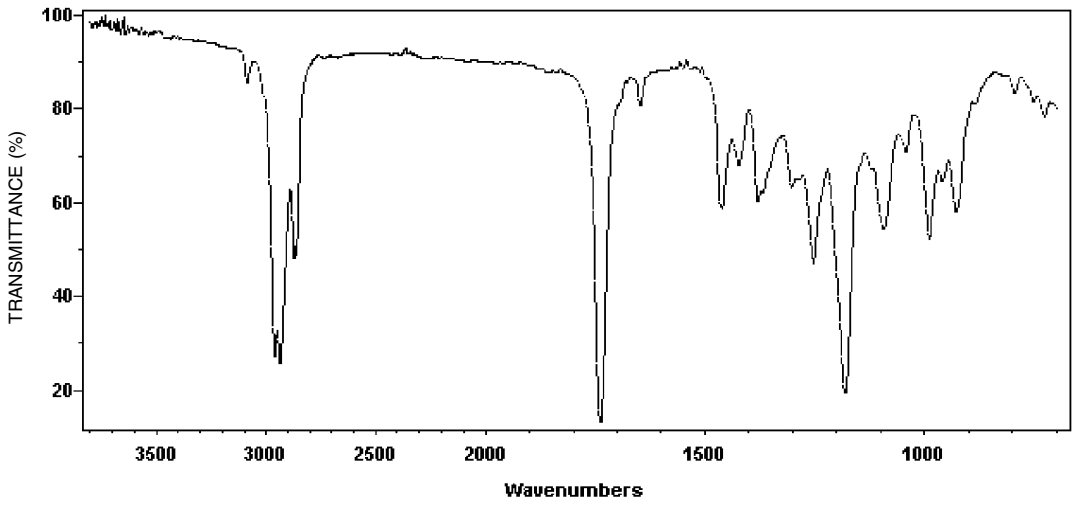
(Z)-3-Octen-1-ol View Flavor Chemical



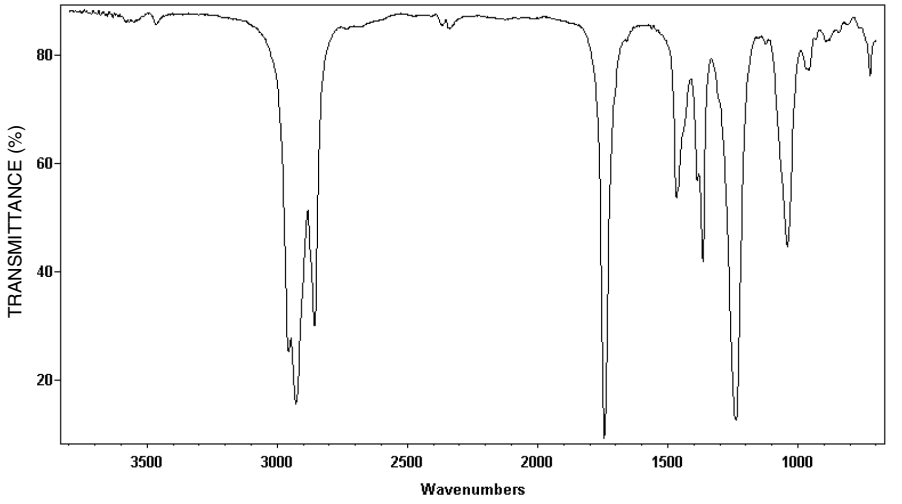
1-Octen-3-yl Acetate View Flavor Chemical



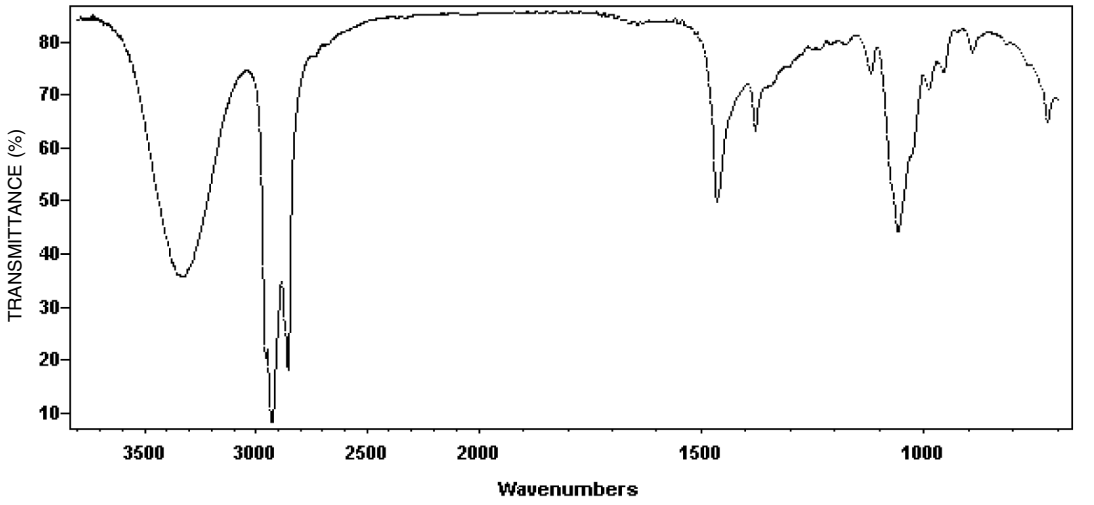
1-Octen-3-yl Butyrate View Flavor Chemical



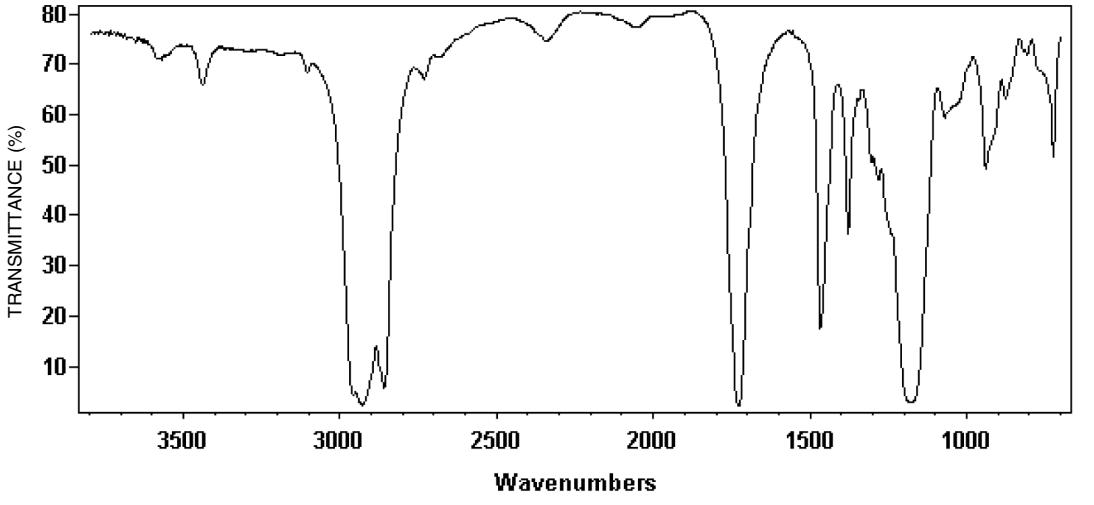
Octyl Acetate View Flavor Chemical



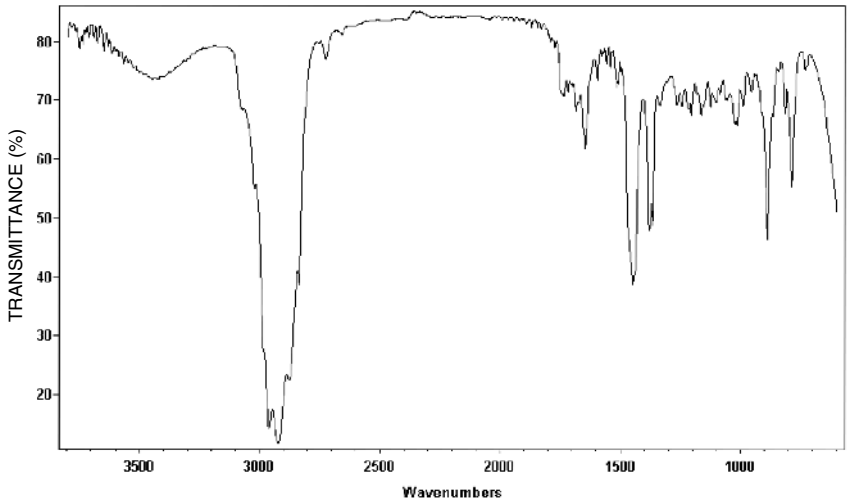
Octyl Alcohol View Flavor Chemical



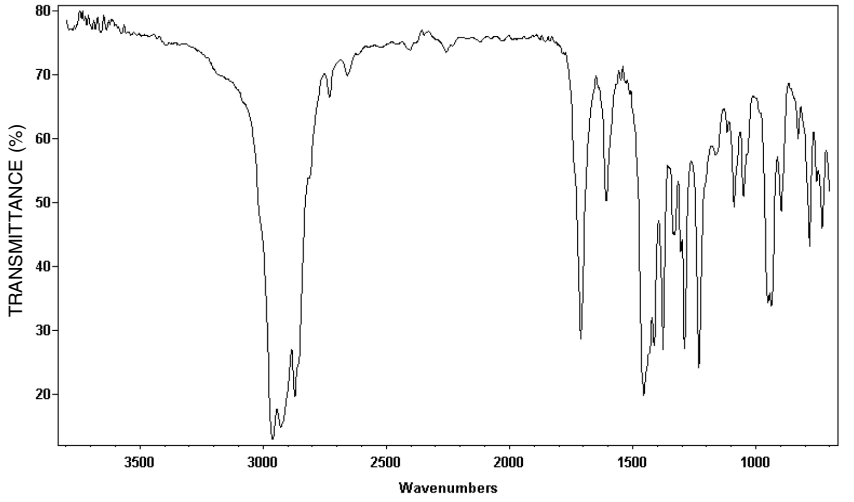
Octyl Formate View Flavor Chemical



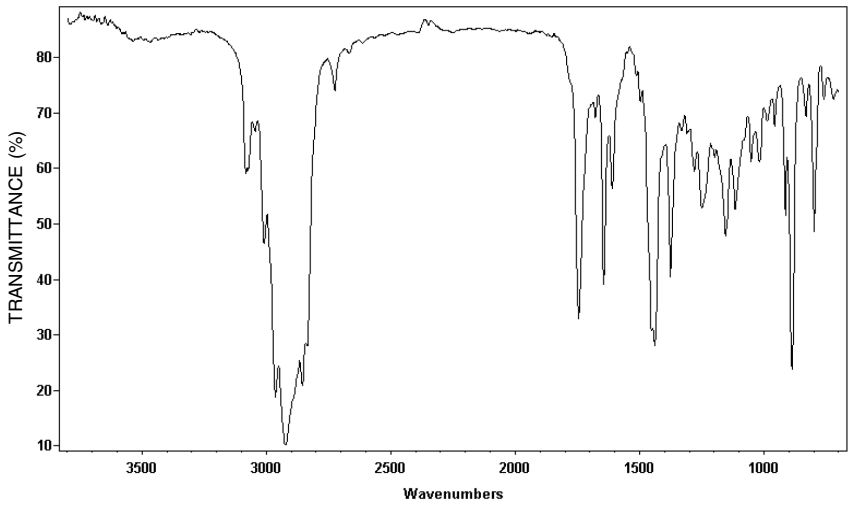
Olibanum Oil View Monograph



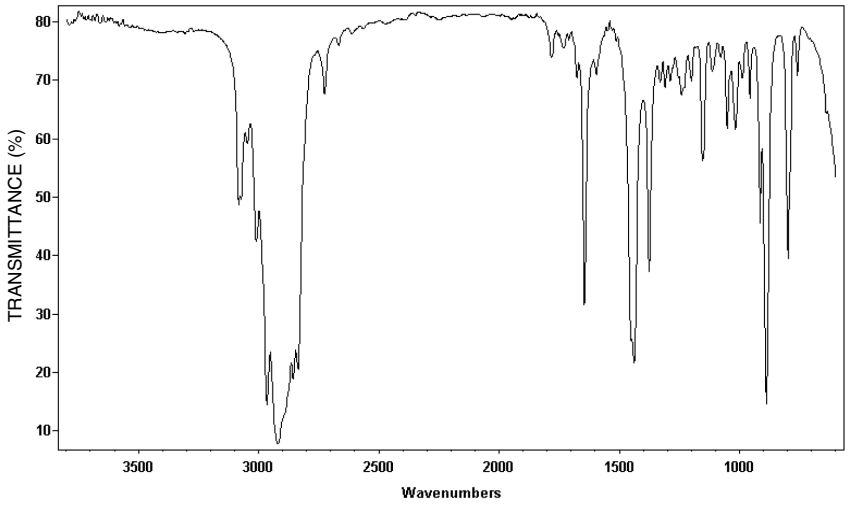
Onion Oil View Monograph



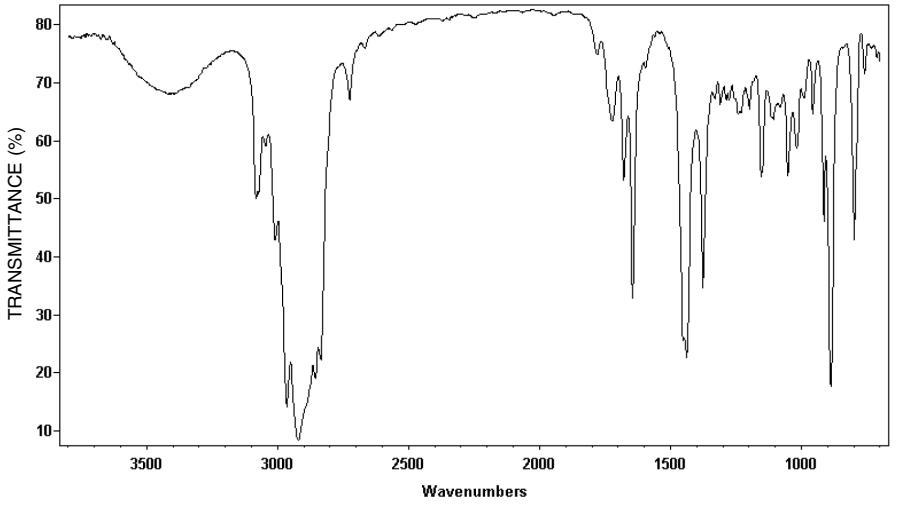
Orange Oil, Bitter, Coldpressed View Monograph



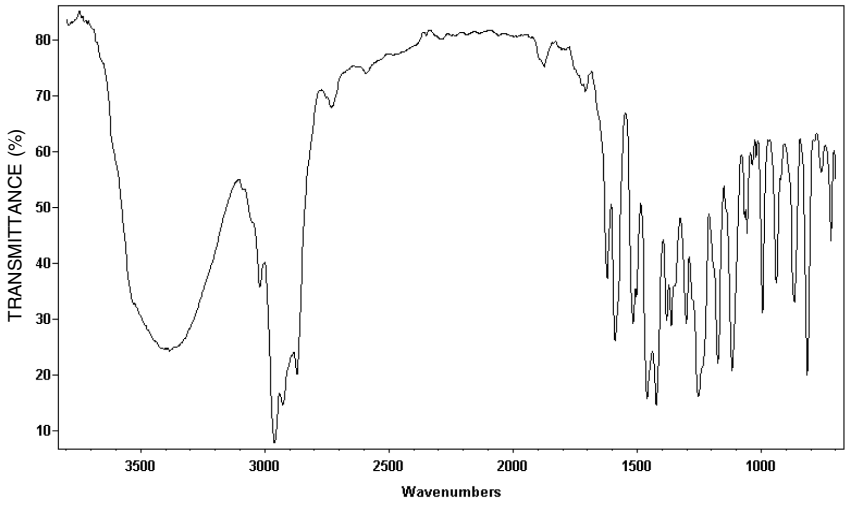
Orange Oil, Coldpressed View Monograph



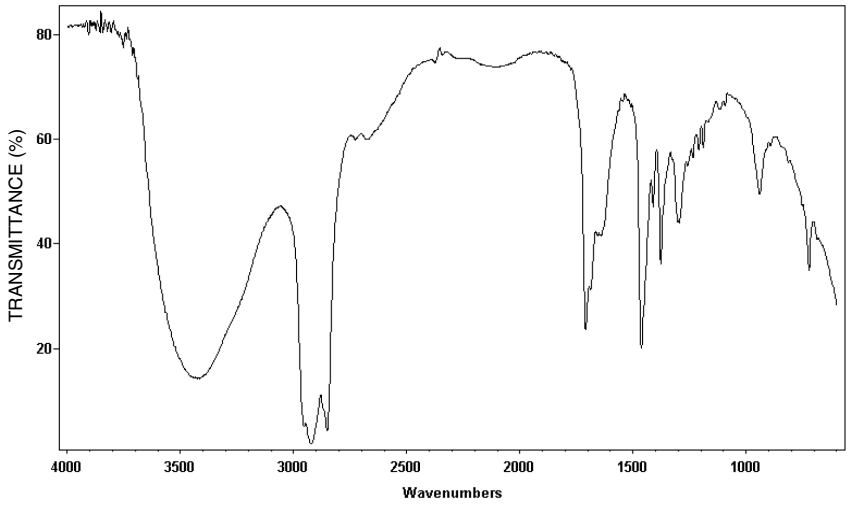
Orange Oil, Distilled View Monograph



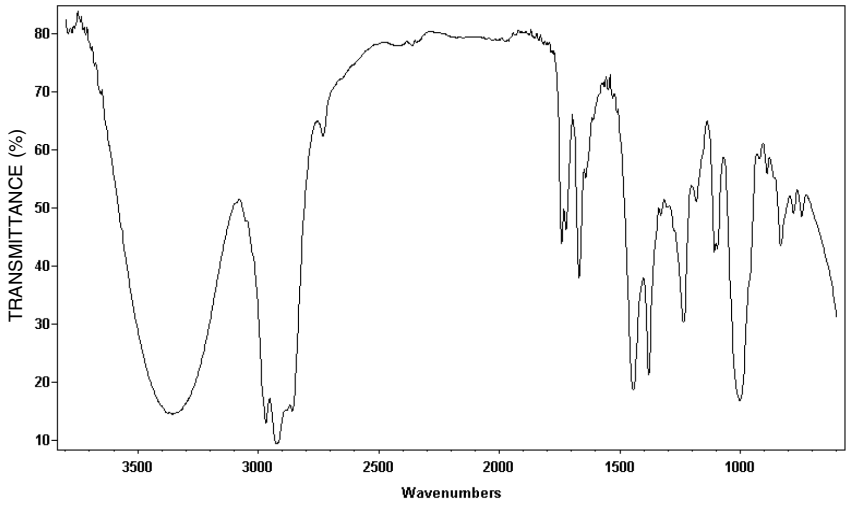
Origanum Oil, Spanish Type View Monograph



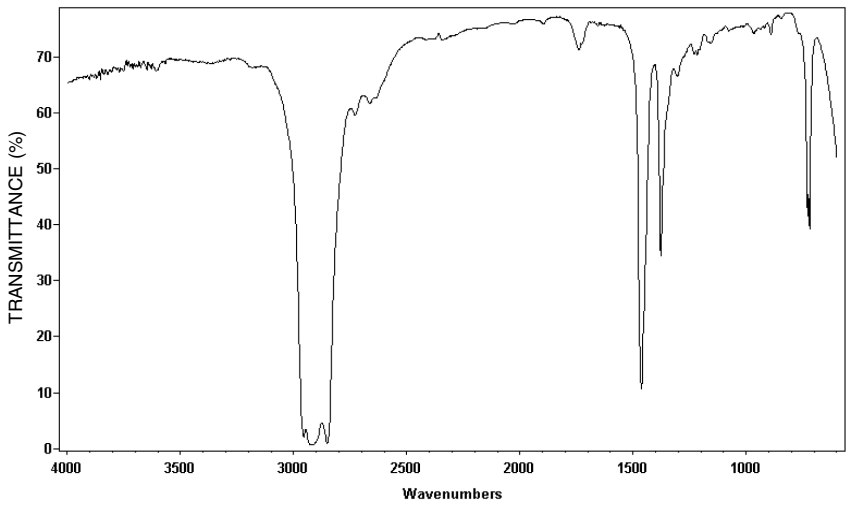
Orris Root Oil **View Monograph**



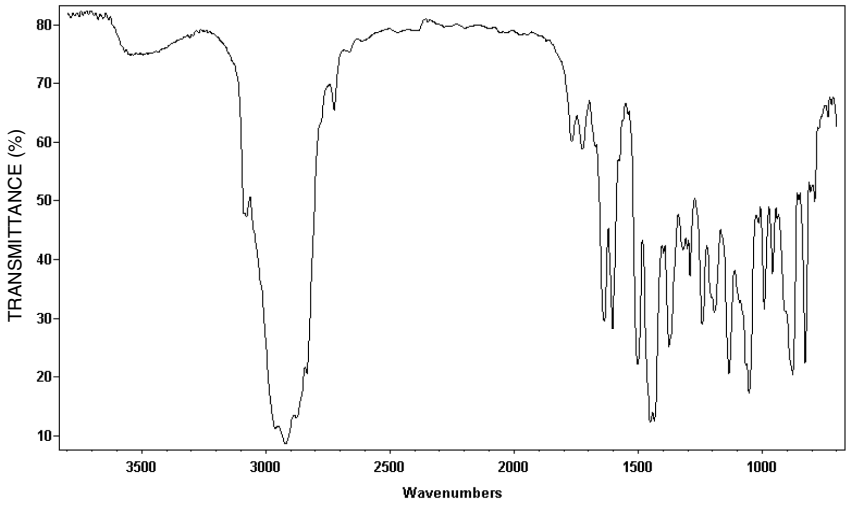
Palmarosa Oil **View Monograph**



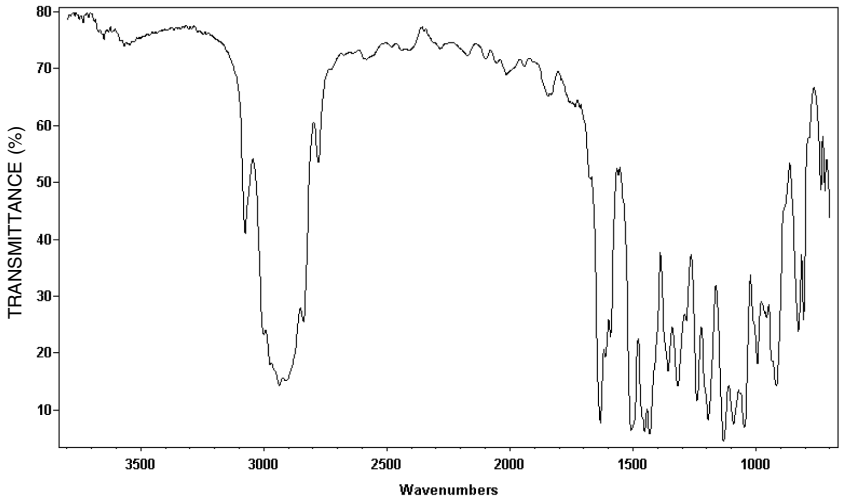
Paraffin, Synthetic (Mineral Oil Mull) **View Monograph**



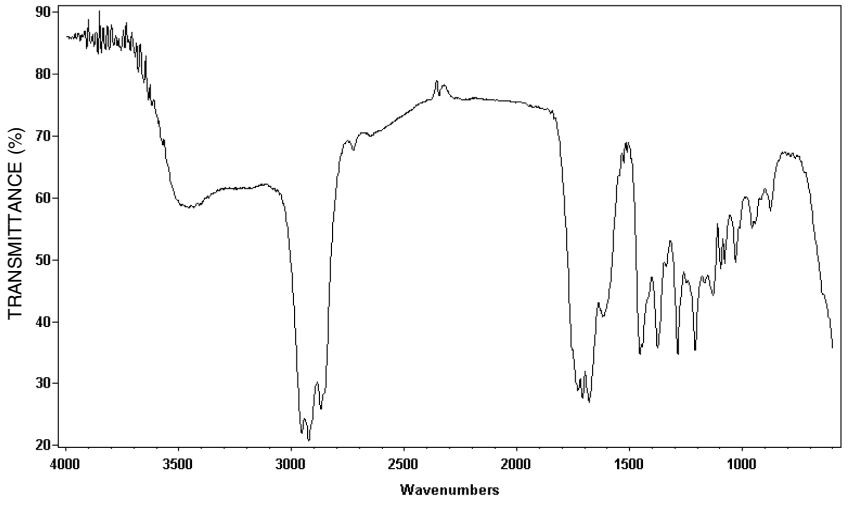
Parsley Herb Oil View Monograph



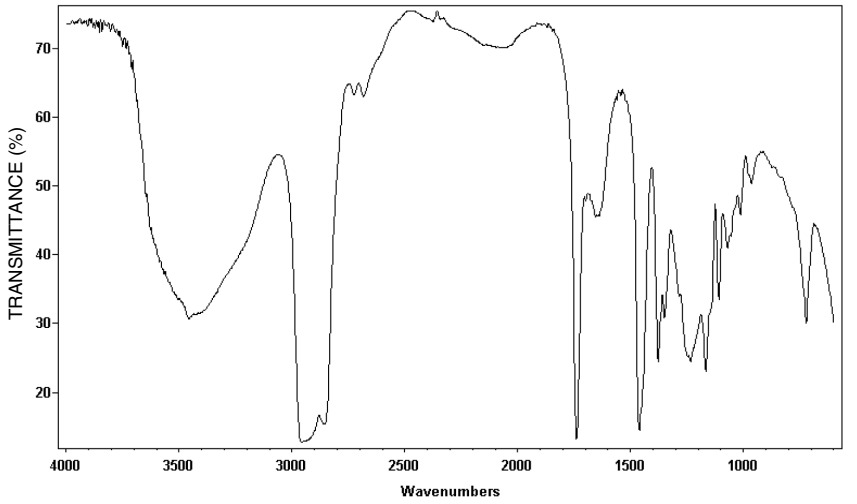
Parsley Seed Oil View Monograph



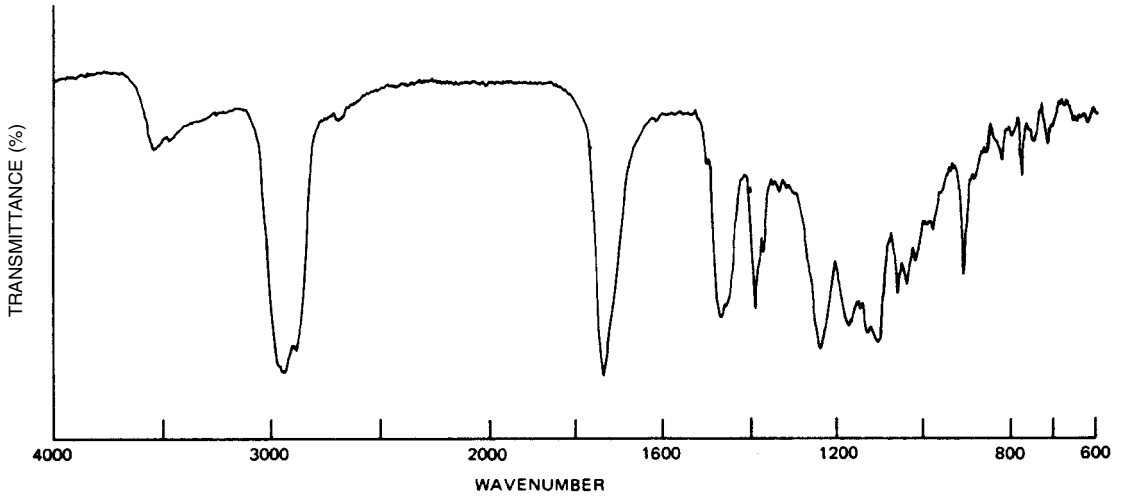
Pennyroyal Oil View Monograph



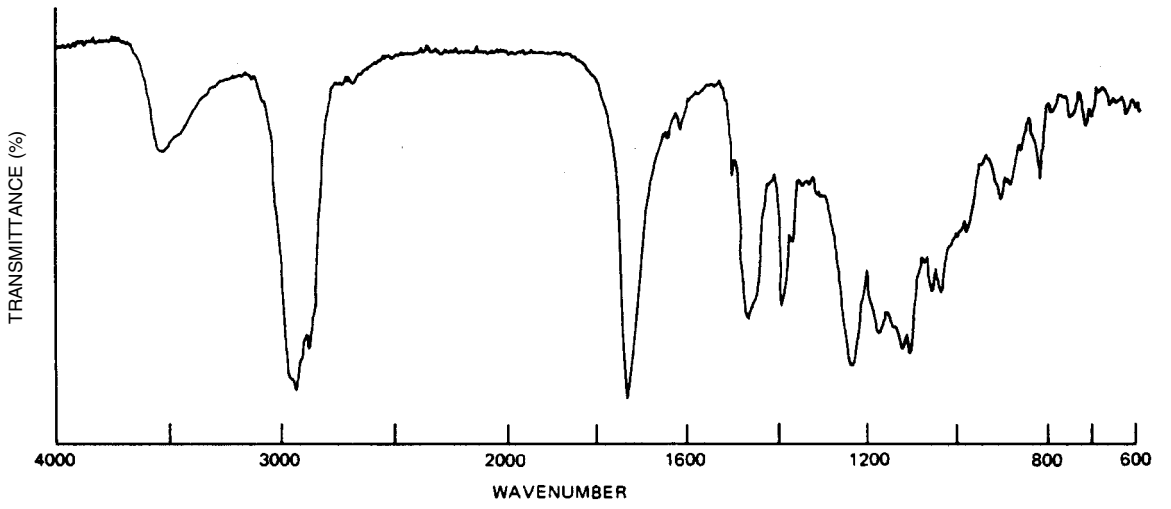
ω-Pentadecalactone View Flavor Chemical



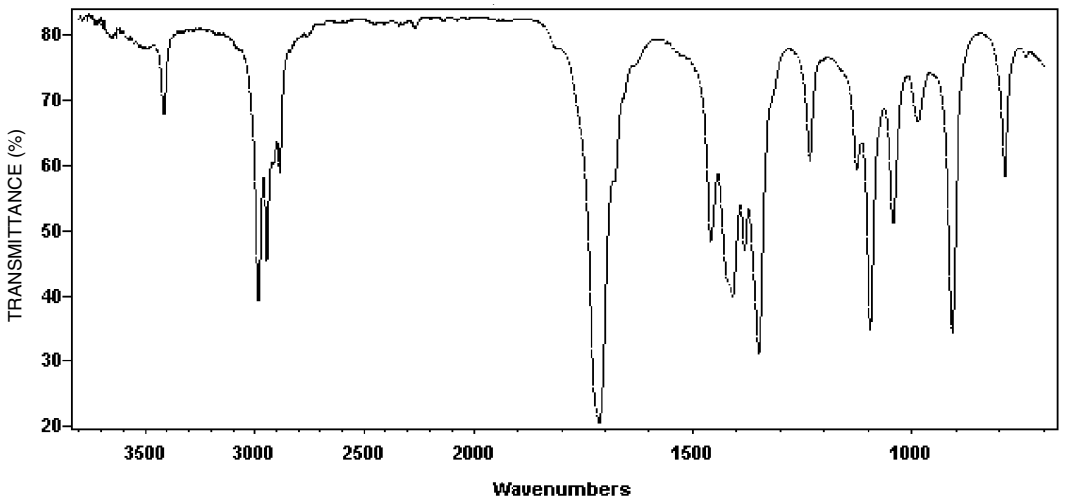
Pentaerythritol Ester of Partially Hydrogenated Wood Rosin View Monograph



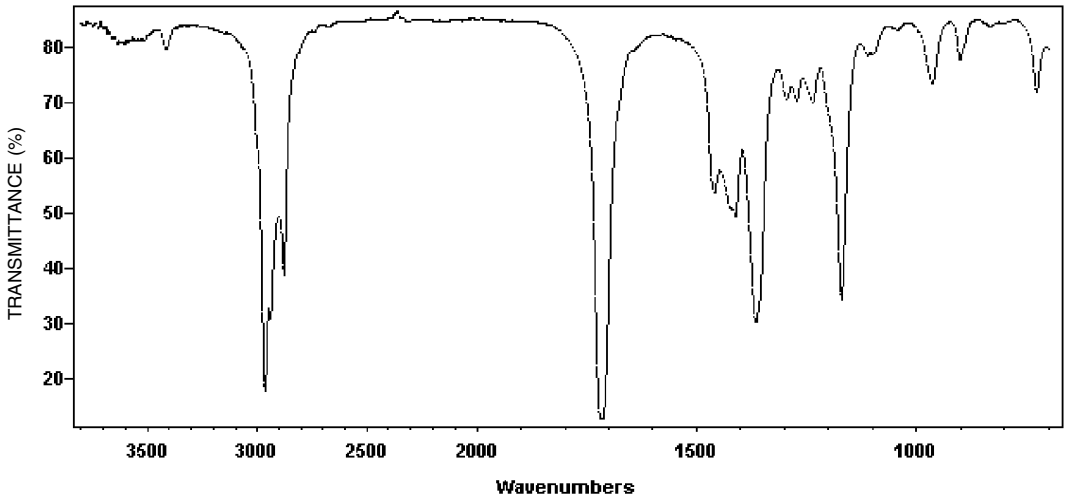
Pentaerythritol Ester of Wood Rosin View Monograph



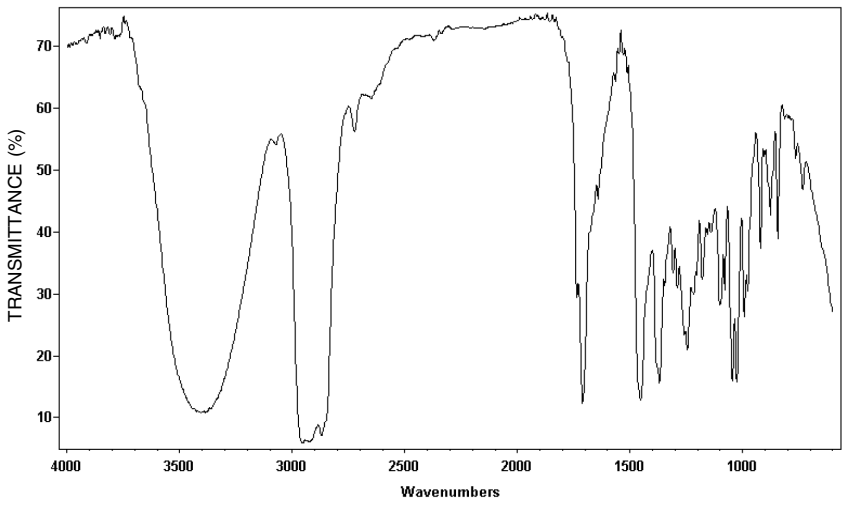
2,3-Pentanedione View Flavor Chemical



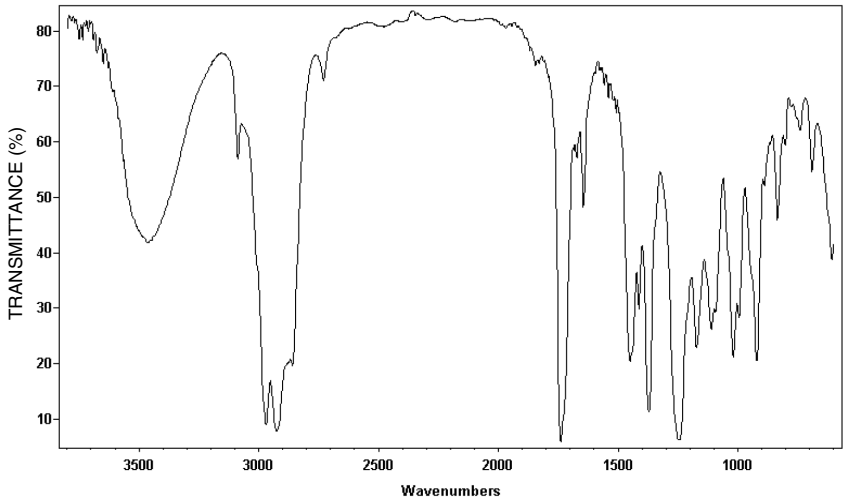
2-Pentanone View Flavor Chemical



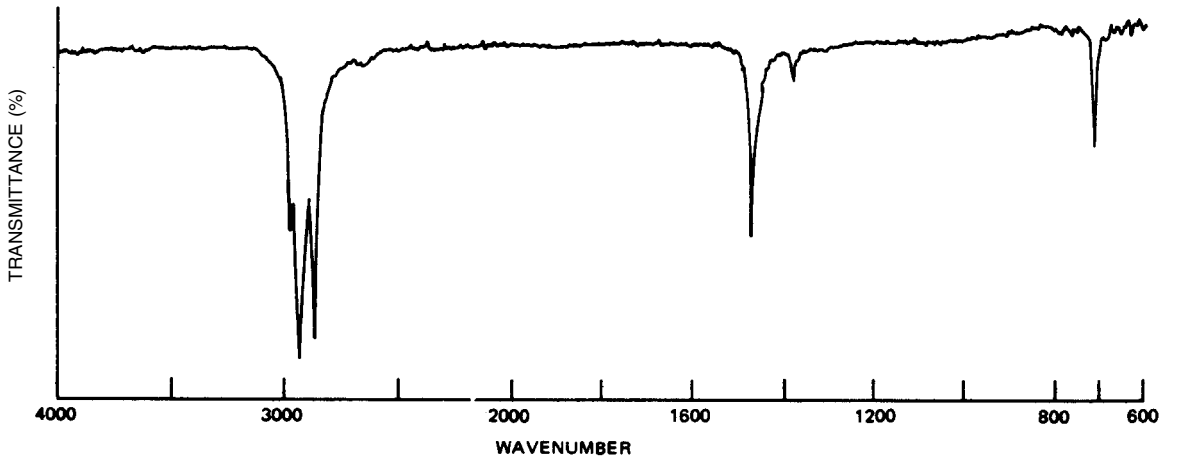
Peppermint Oil View Monograph



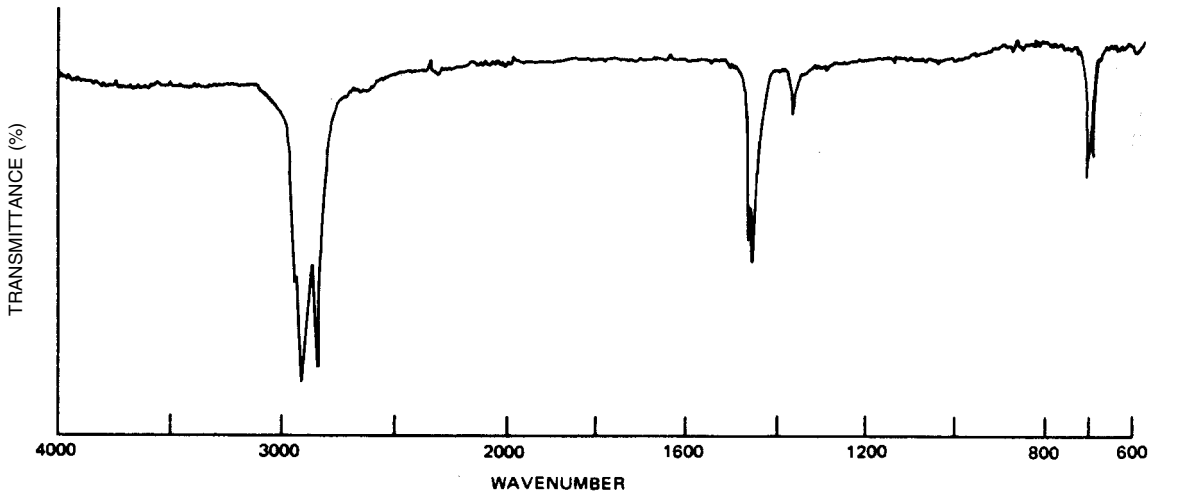
Petitgrain Oil, Paraguay Type View Monograph



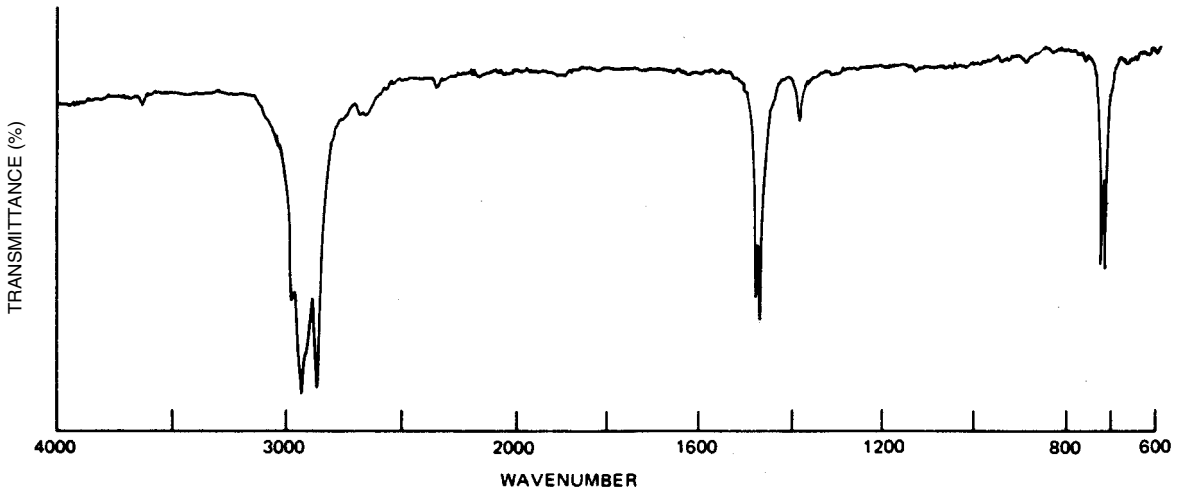
Petroleum Wax (Refined) View Monograph



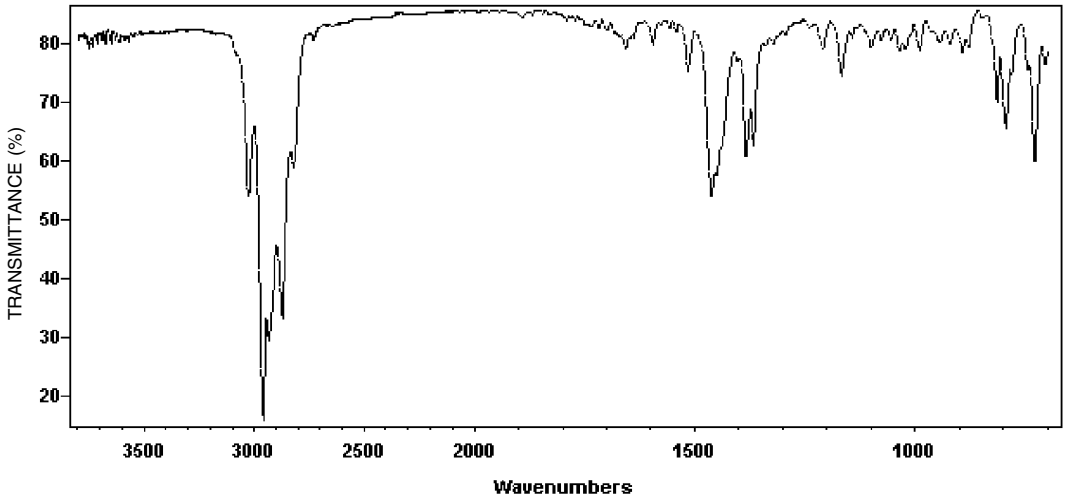
Petroleum Wax (Microcrystalline) View Monograph



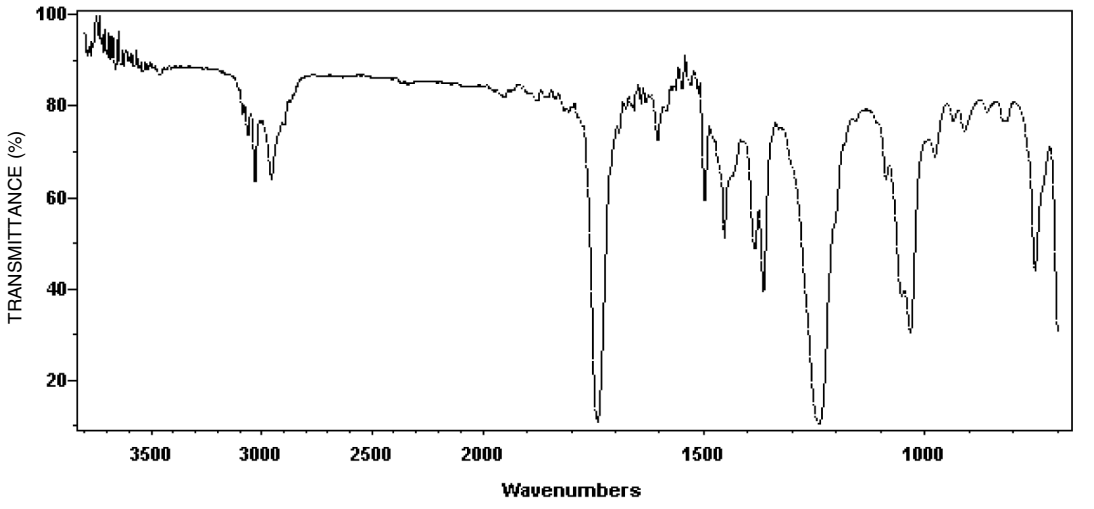
Petroleum Wax, Synthetic View Monograph



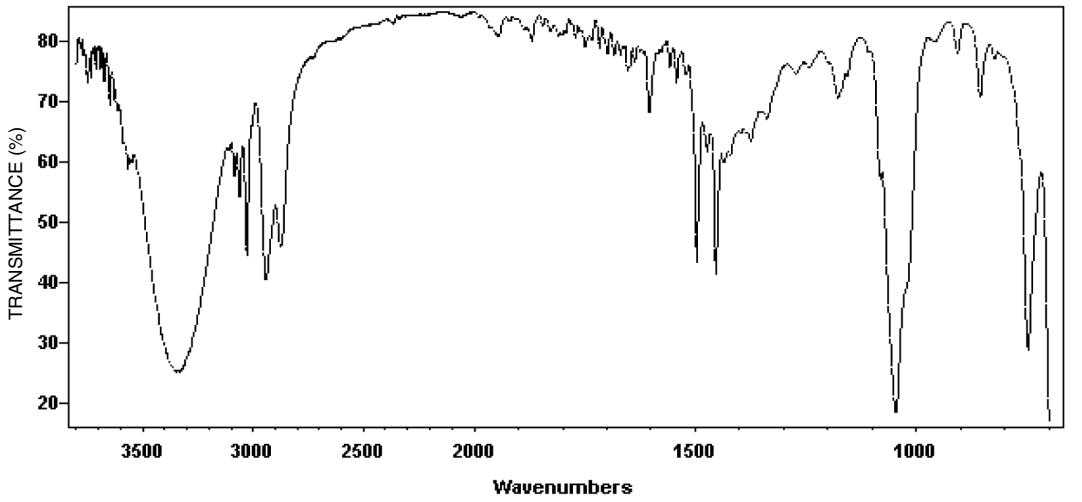
α-Phellandrene View Flavor Chemical



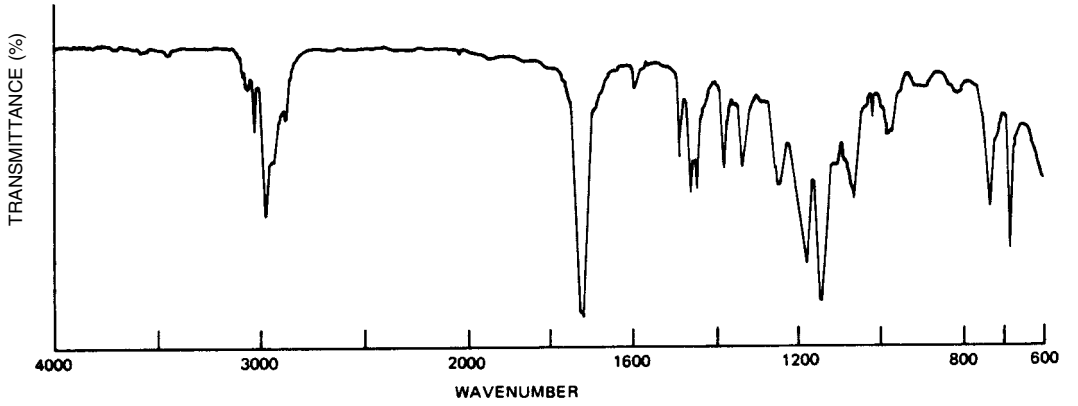
Phenethyl Acetate View Flavor Chemical



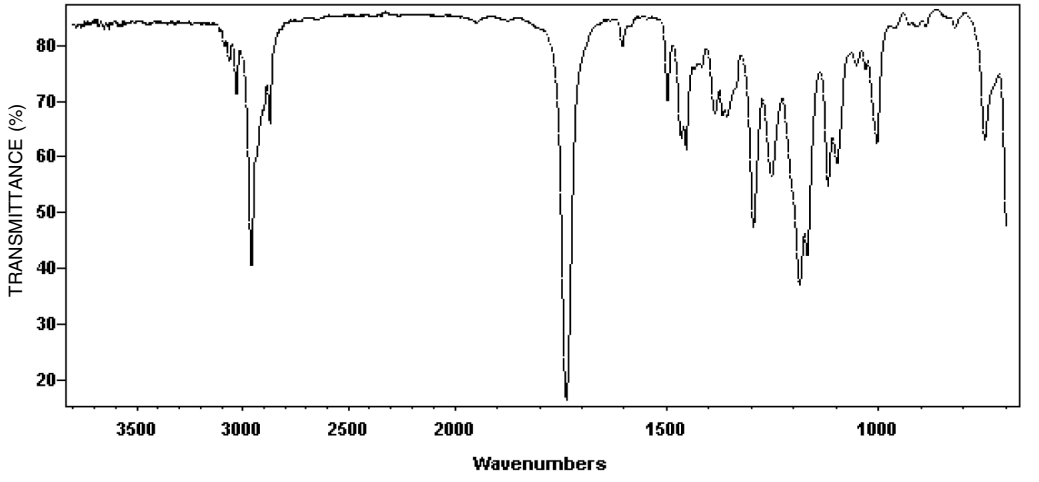
Phenethyl Alcohol View Flavor Chemical



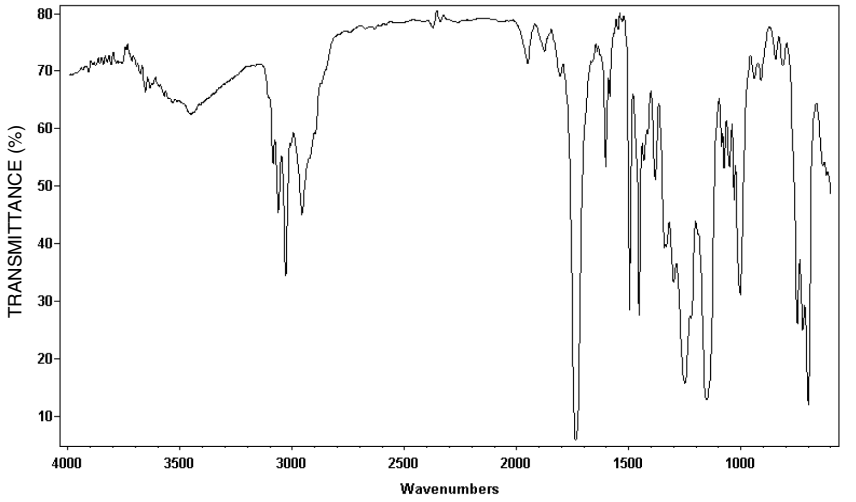
Phenethyl Isobutyrate View Flavor Chemical



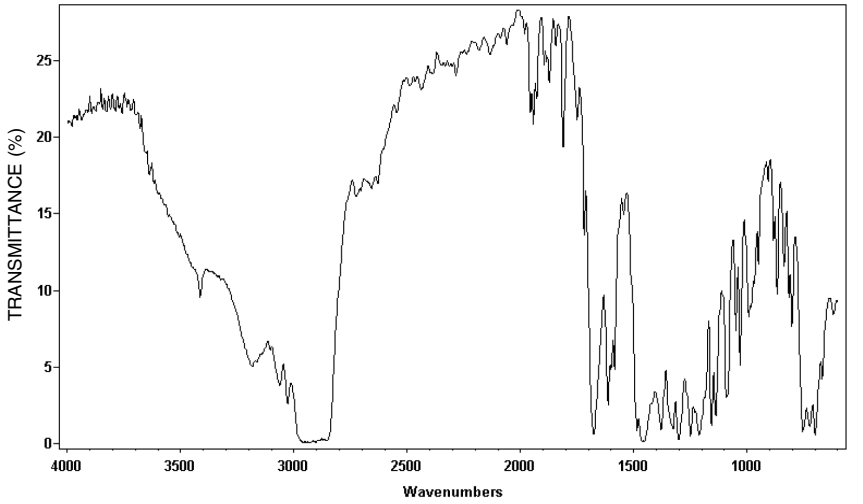
Phenethyl Isovalerate View Flavor Chemical



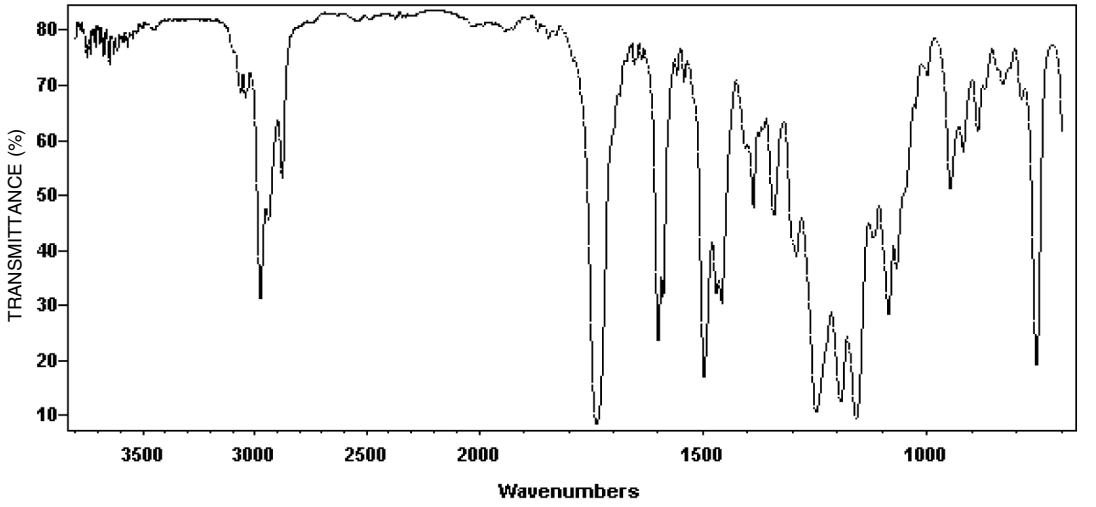
Phenethyl Phenylacetate View Flavor Chemical



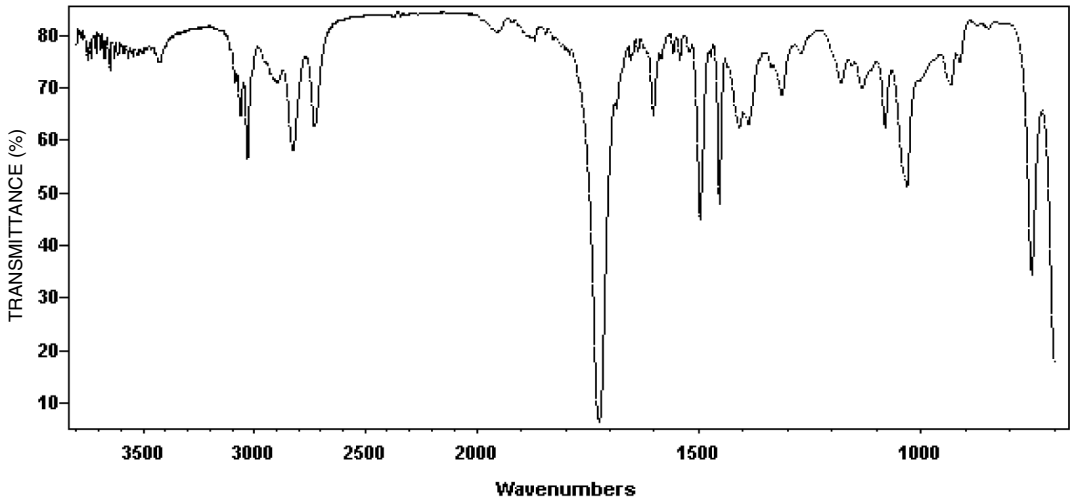
Phenethyl Salicylate View Flavor Chemical



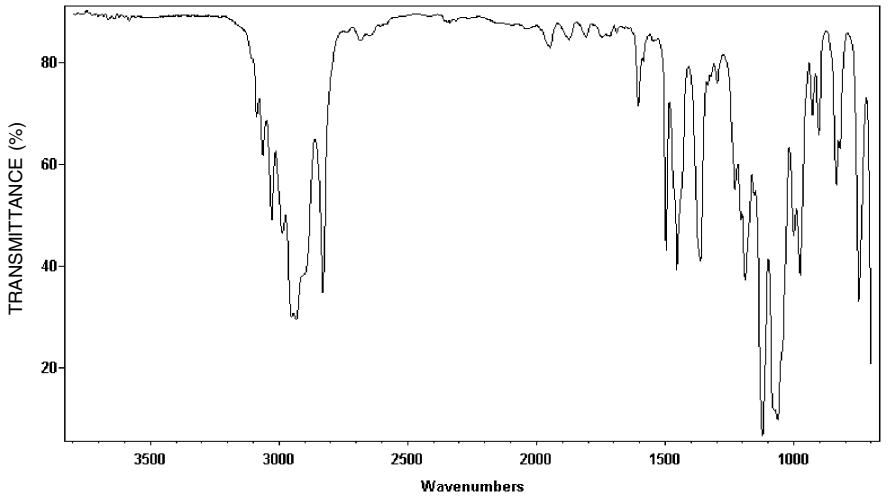
Phenoxyethyl Isobutyrate View Flavor Chemical



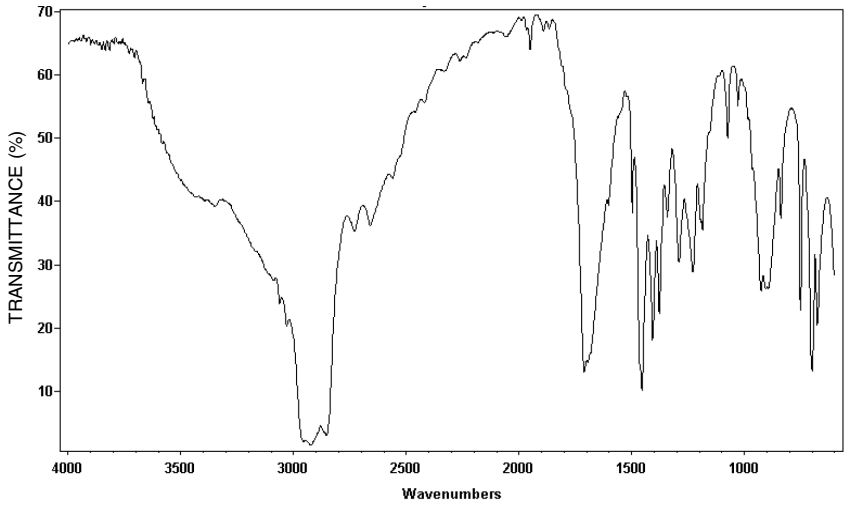
Phenylacetaldehyde View Flavor Chemical



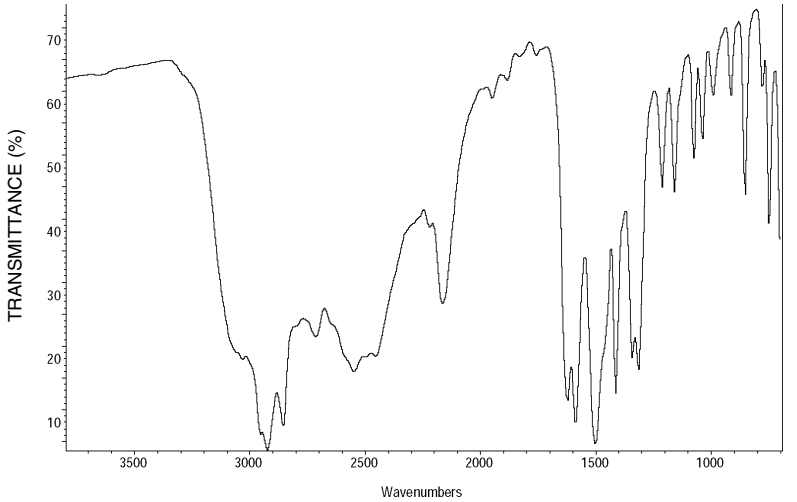
Phenylacetaldehyde Dimethyl Acetal View Flavor Chemical



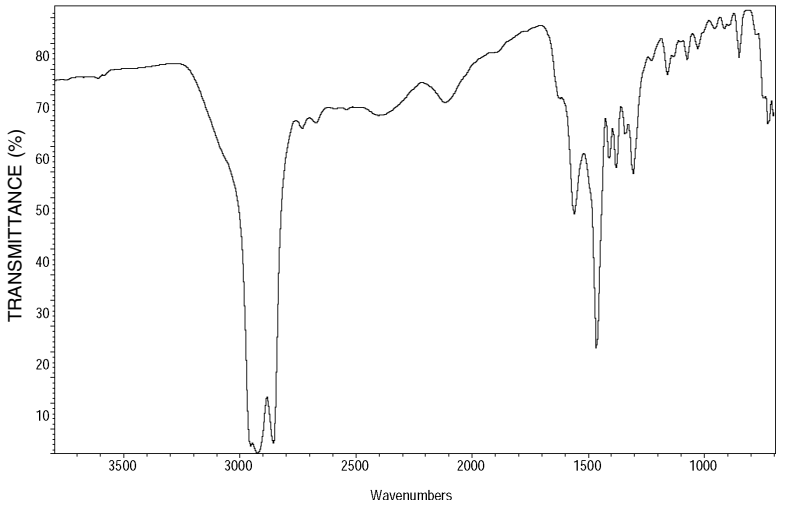
Phenylacetic Acid View Flavor Chemical



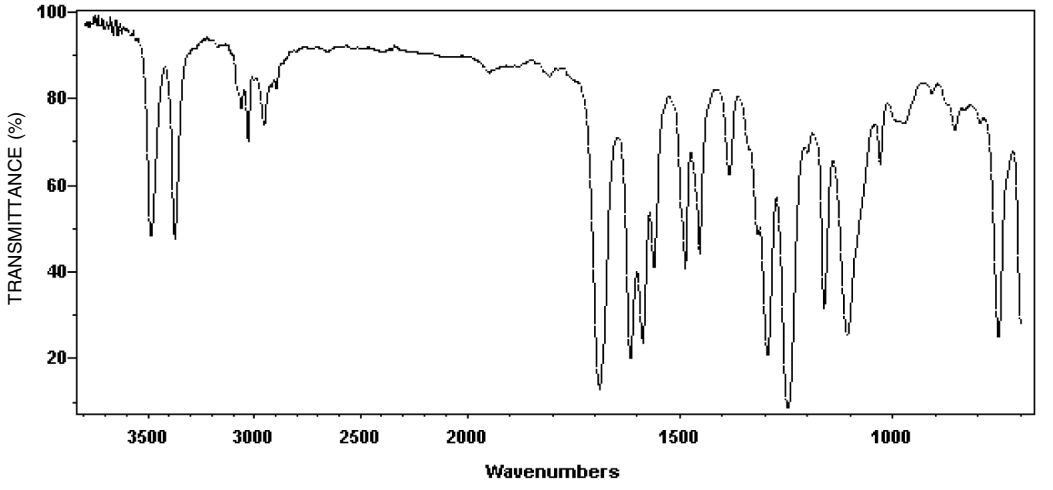
DL-Phenylalanine (Mineral Oil Mull) View Monograph



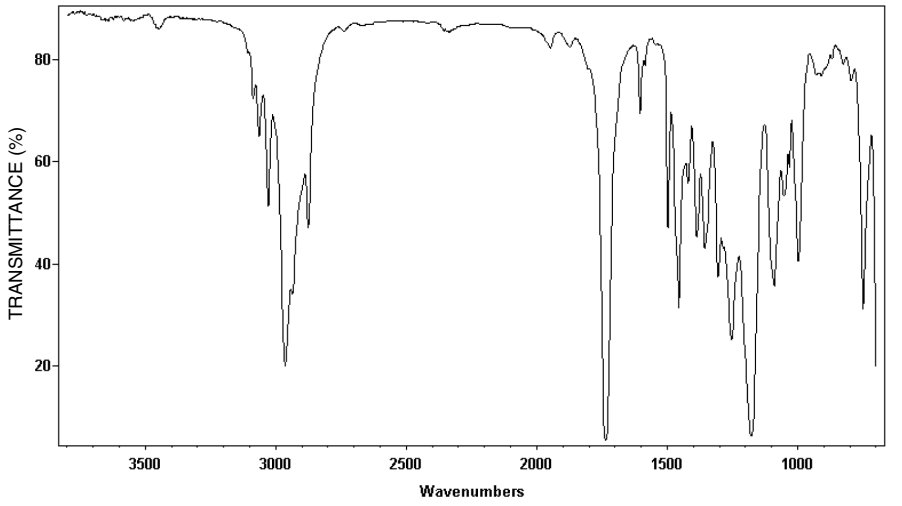
L-Phenylalanine (Mineral Oil Mull) View Monograph



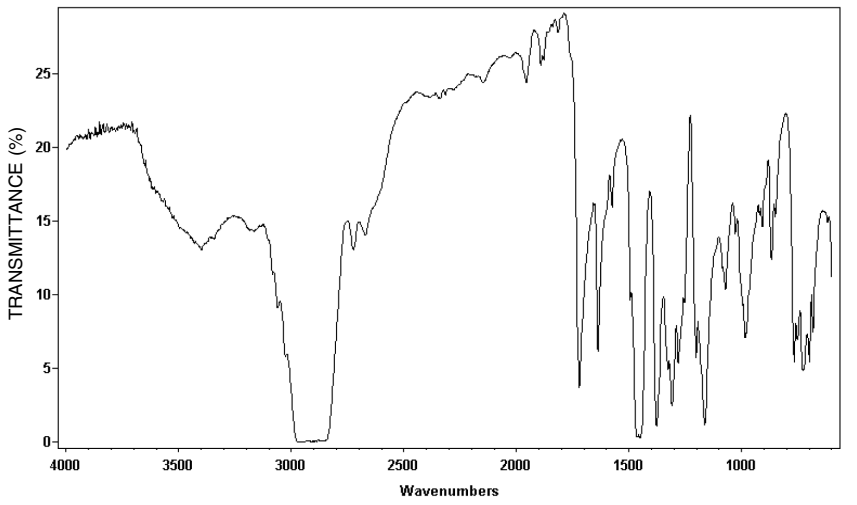
Phenylethyl Anthranilate View Flavor Chemical



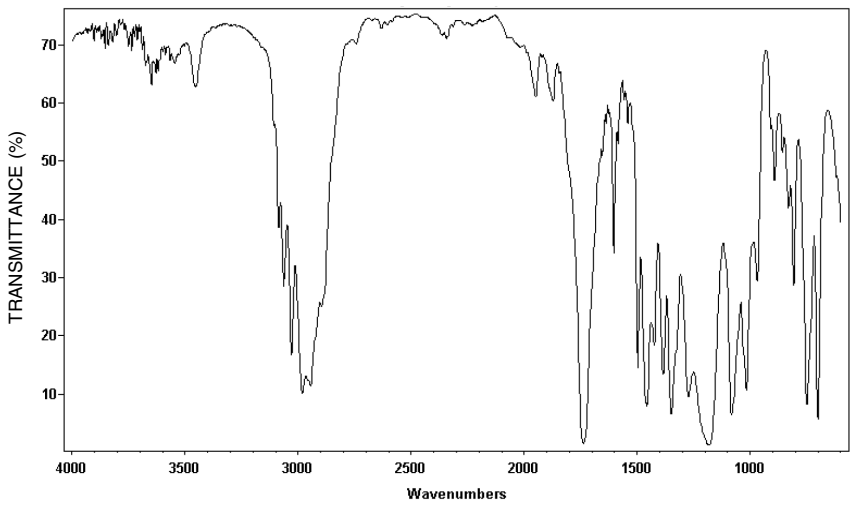
Phenylethyl Butyrate **View Flavor Chemical**



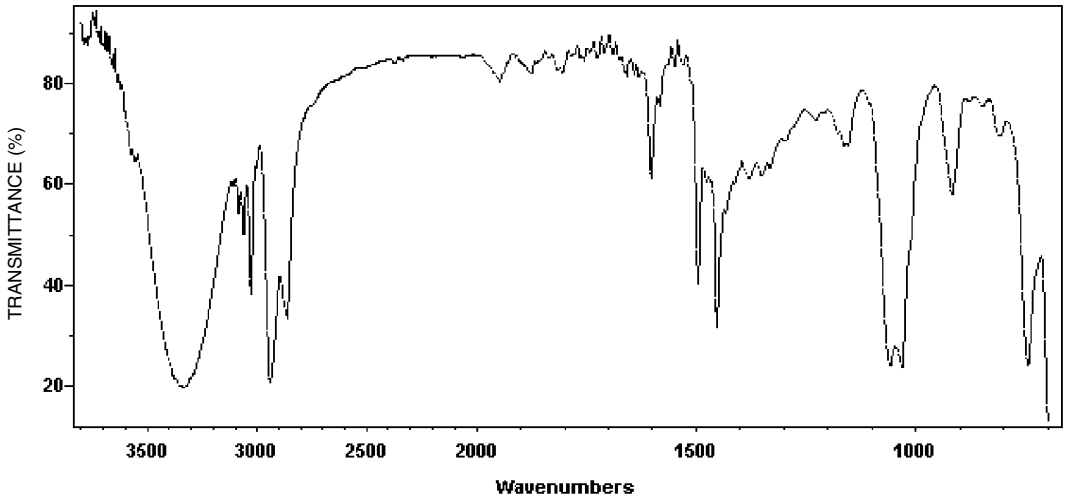
Phenyl Ethyl Cinnamate **View Flavor Chemical**



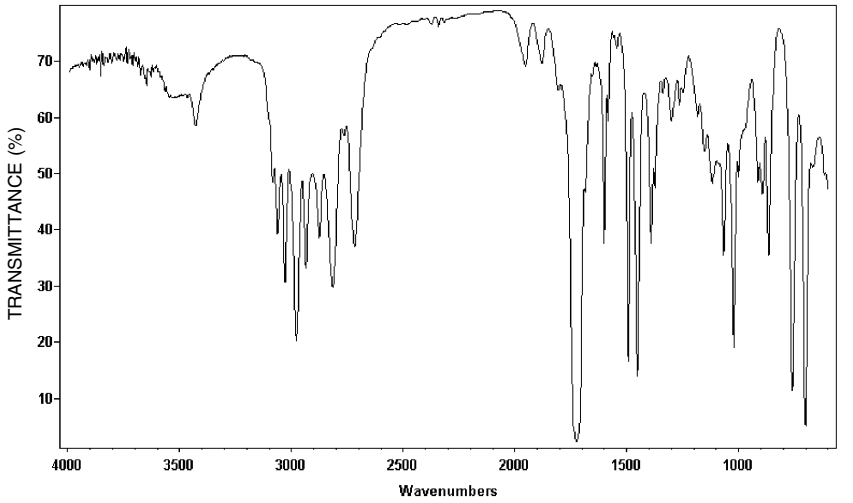
Phenyl Ethyl Propionate **View Flavor Chemical**



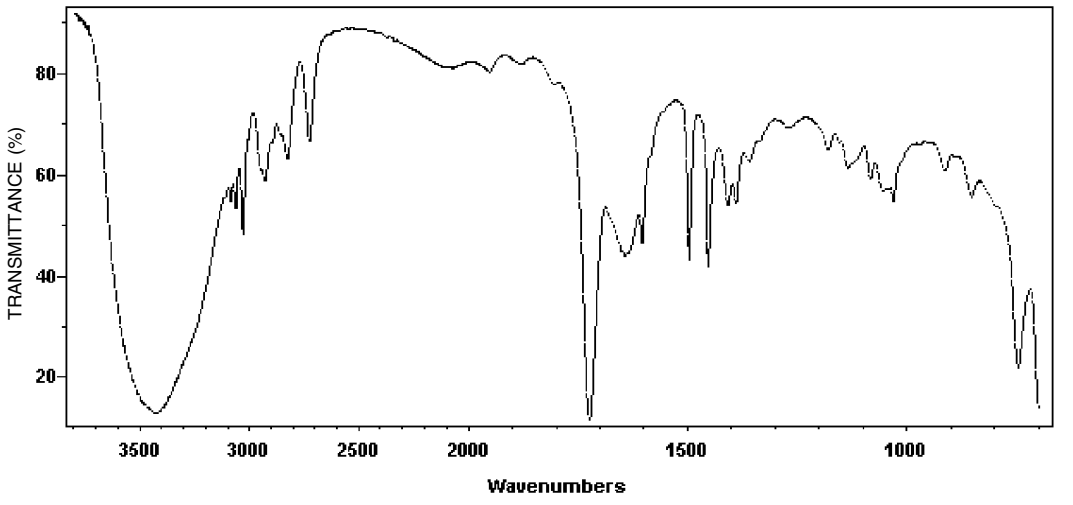
3-Phenyl-1-propanol View Flavor Chemical



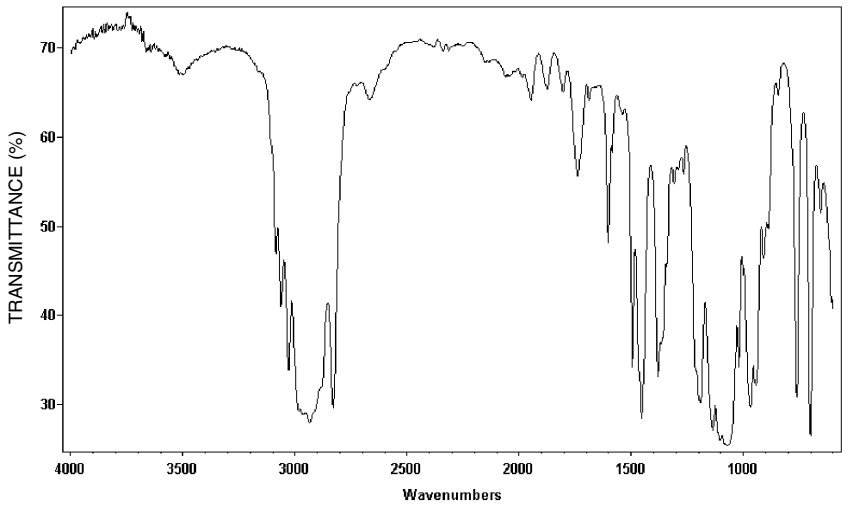
2-Phenylpropionaldehyde View Flavor Chemical



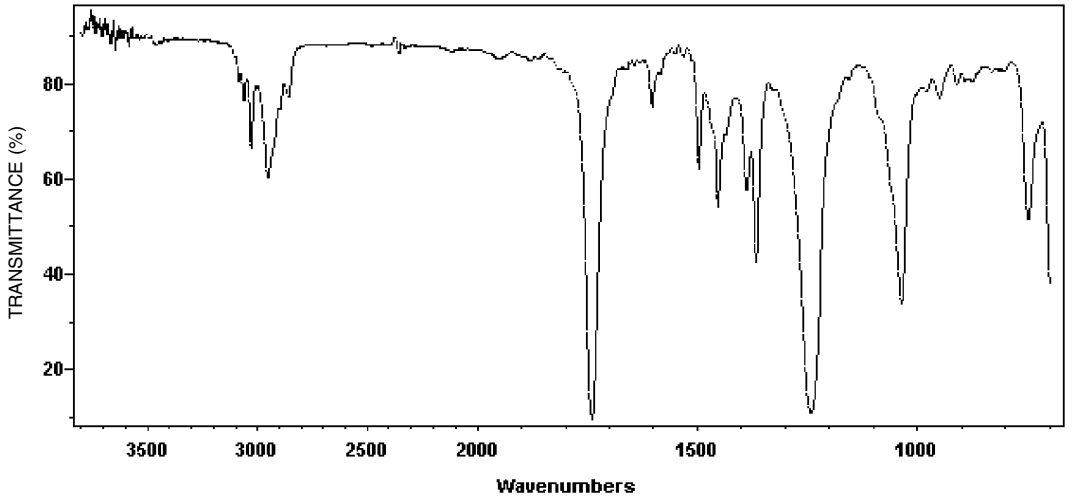
3-Phenylpropionaldehyde View Flavor Chemical



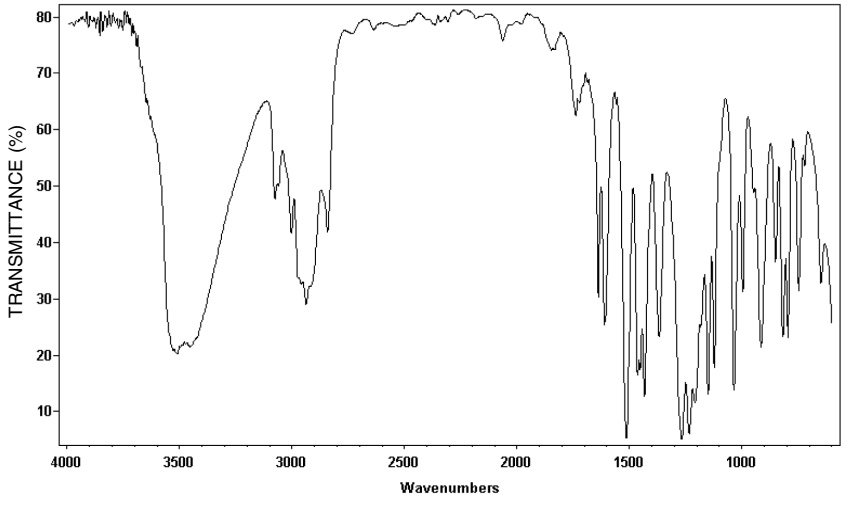
2-Phenylpropionaldehyde Dimethyl Acetal View Flavor Chemical



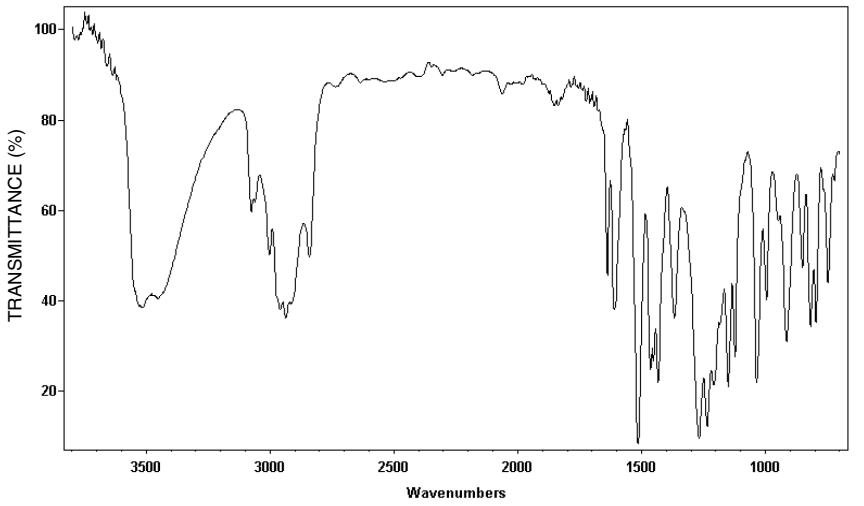
3-Phenylpropyl Acetate View Flavor Chemical



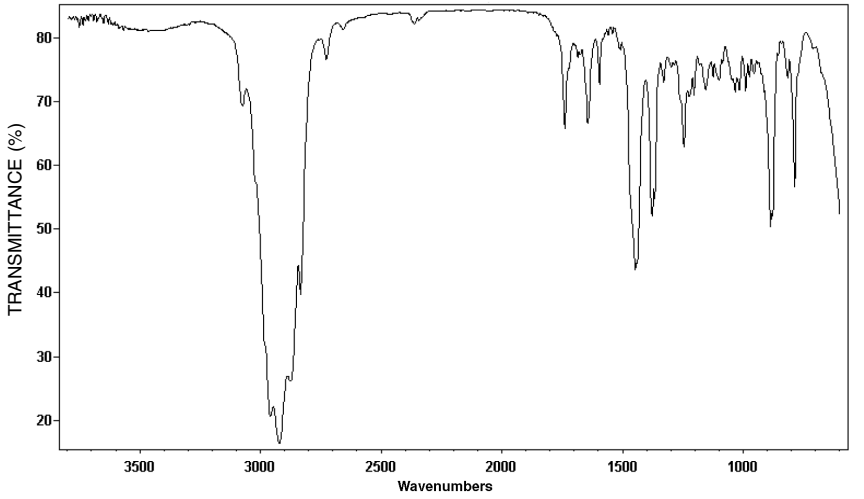
Pimenta Leaf Oil View Monograph



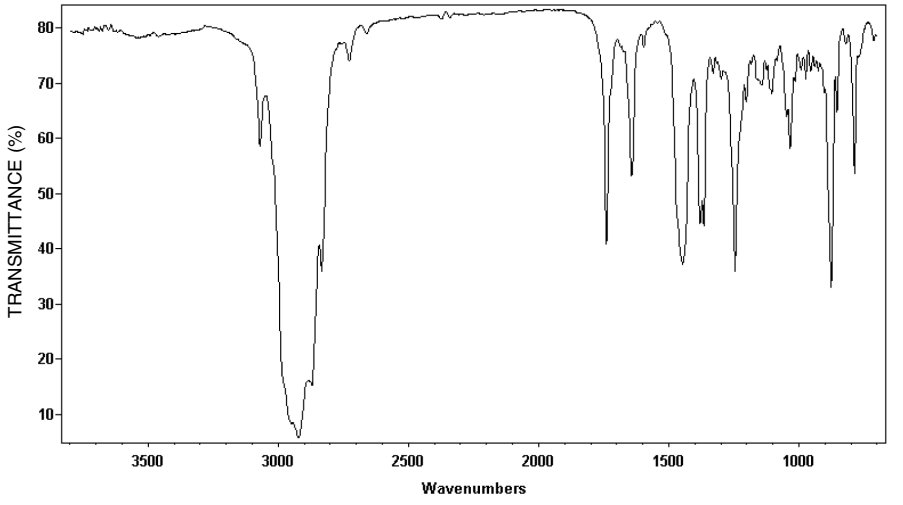
Pimenta Oil View Monograph



Pine Needle Oil, Dwarf View Monograph

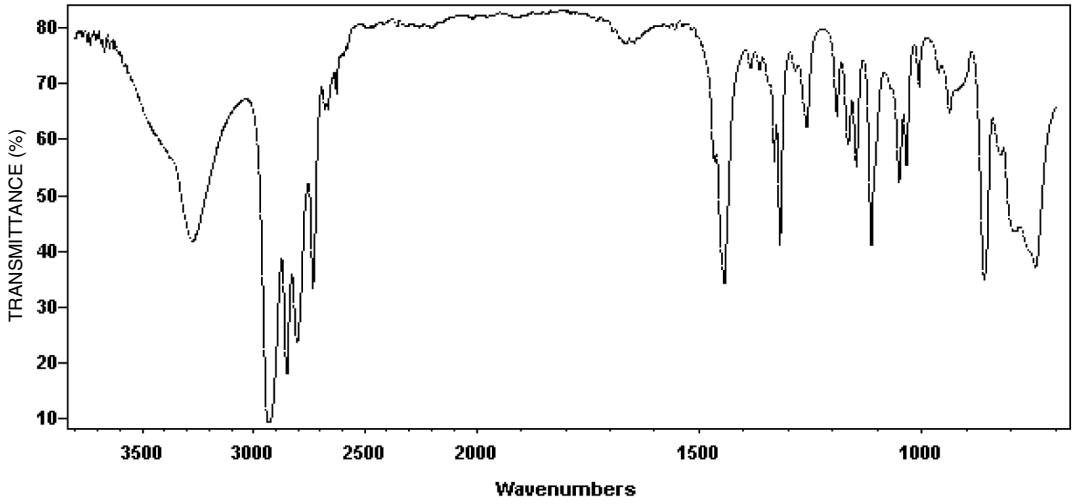


Pine Needle Oil, Scotch Type View Monograph



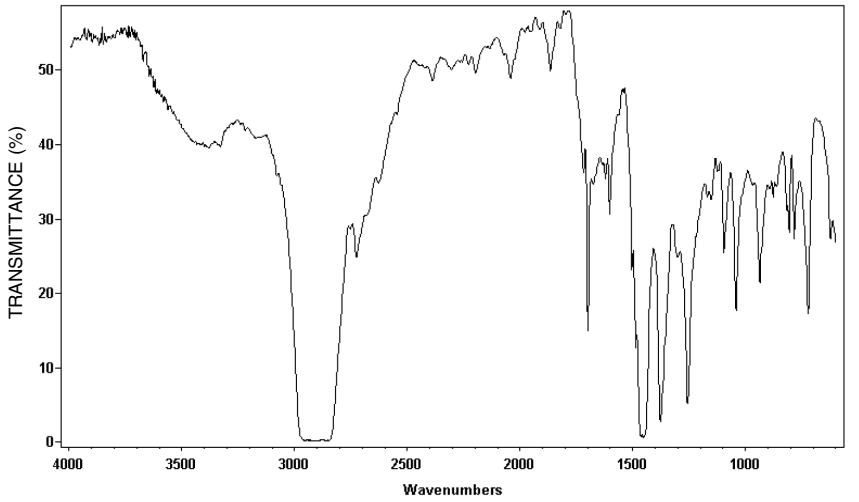
Piperidine

View Flavor Chemical



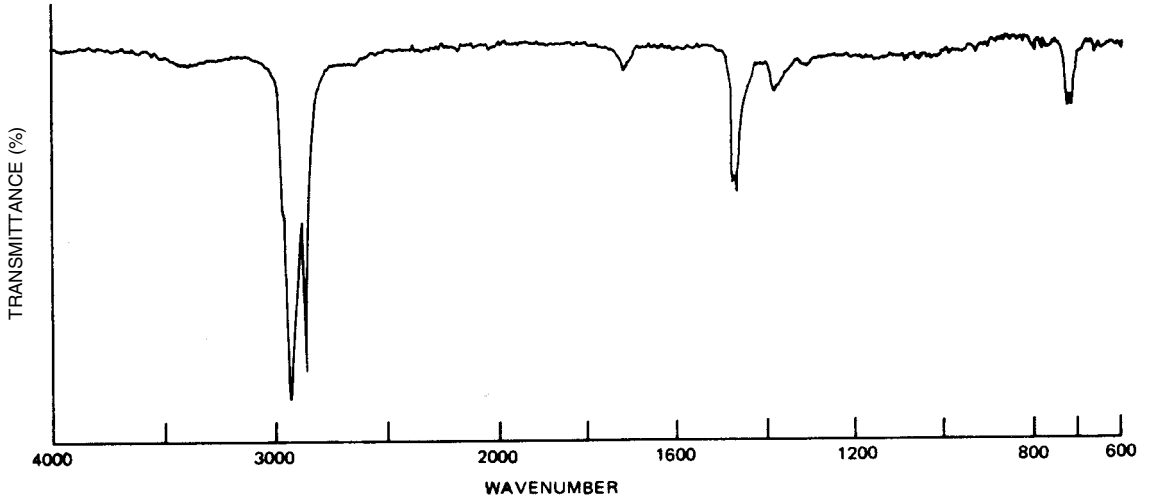
Piperonal

View Flavor Chemical

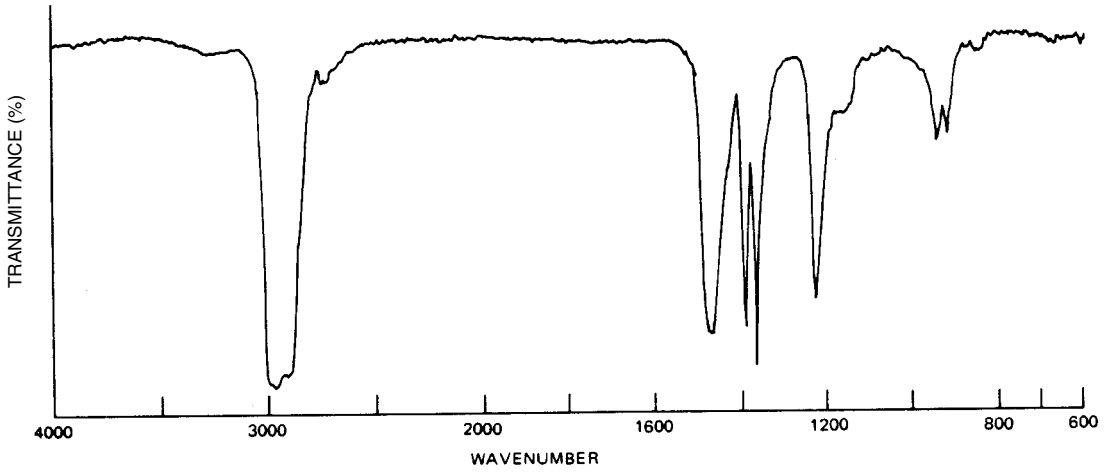


Polyethylene

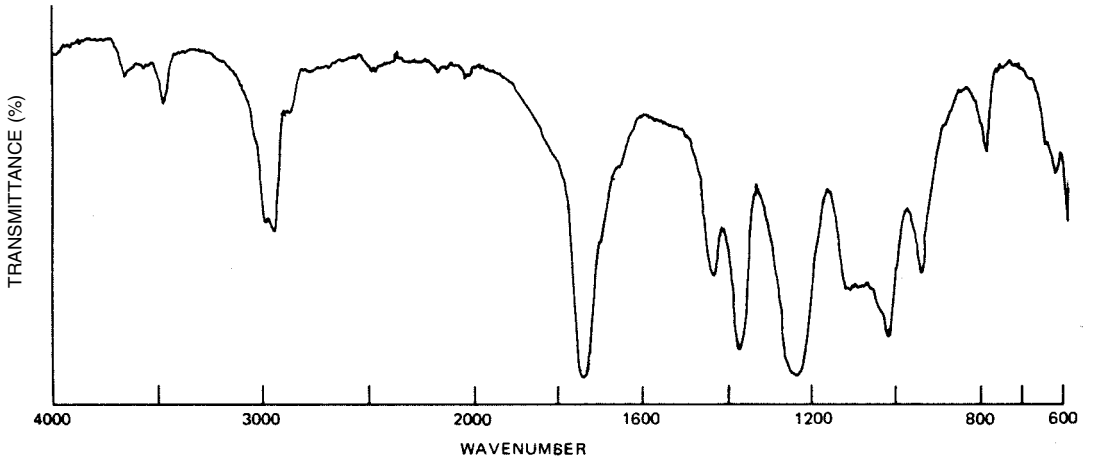
View Monograph



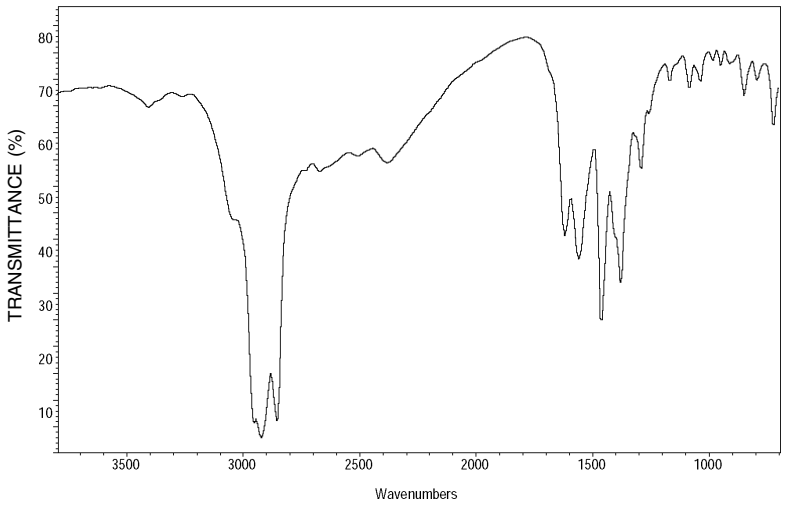
Polyisobutylene View Monograph



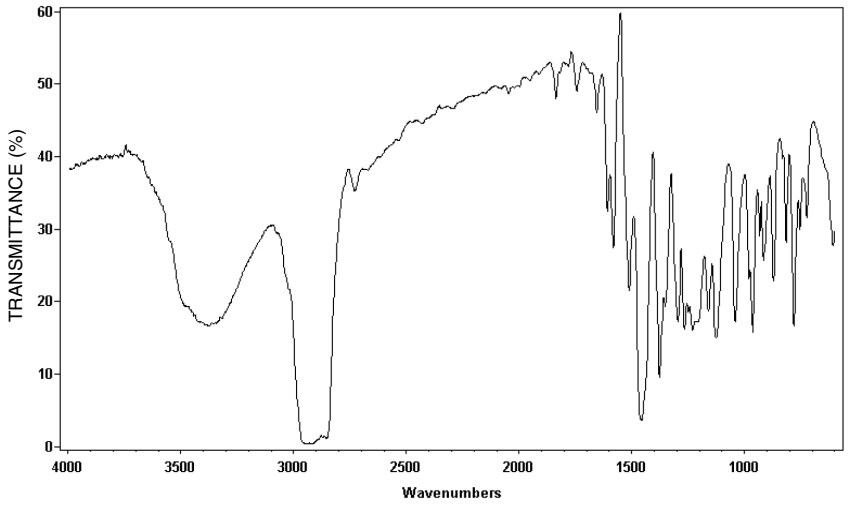
Polyvinyl Acetate View Monograph



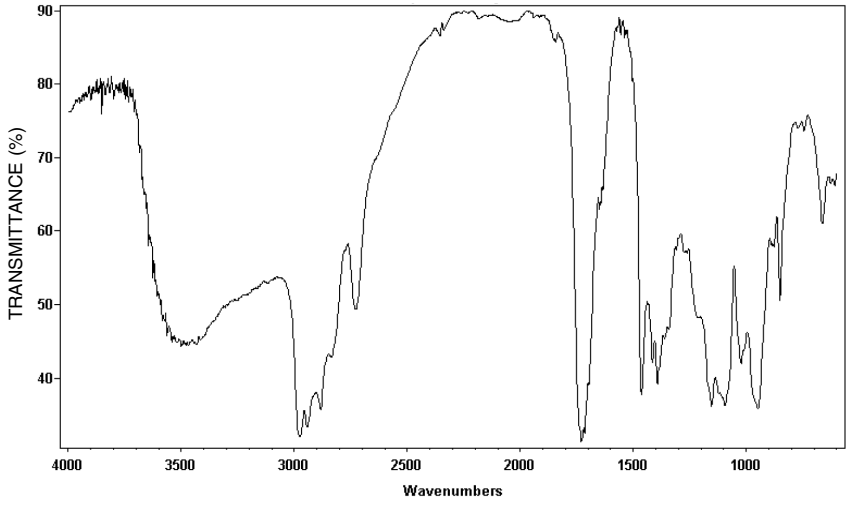
L-Proline (Mineral Oil Mull) View Monograph



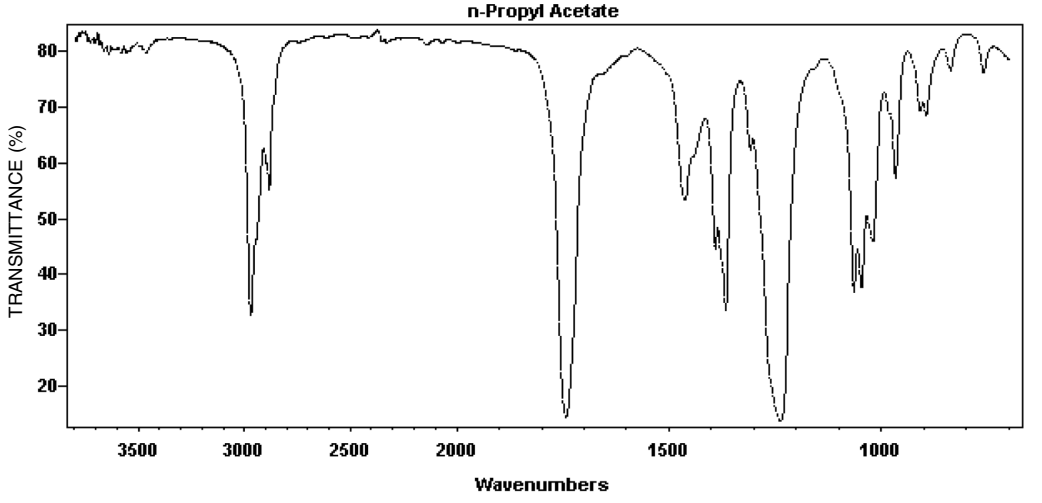
Propenylguaethol View Flavor Chemical



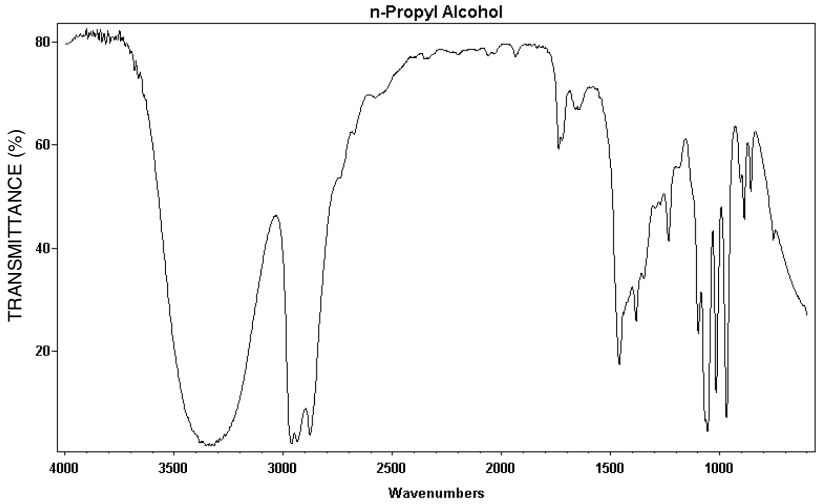
Propionaldehyde View Flavor Chemical



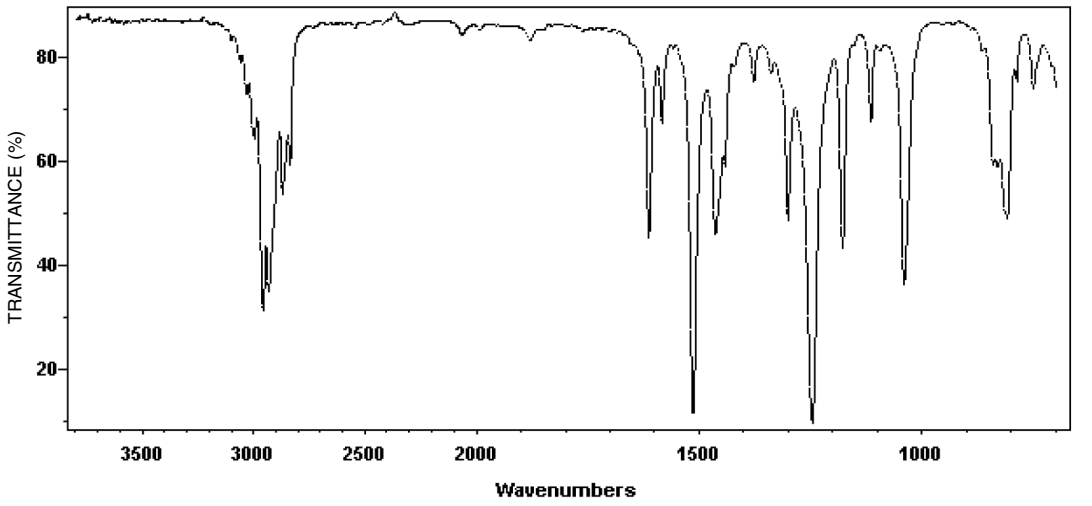
Propyl Acetate View Flavor Chemical



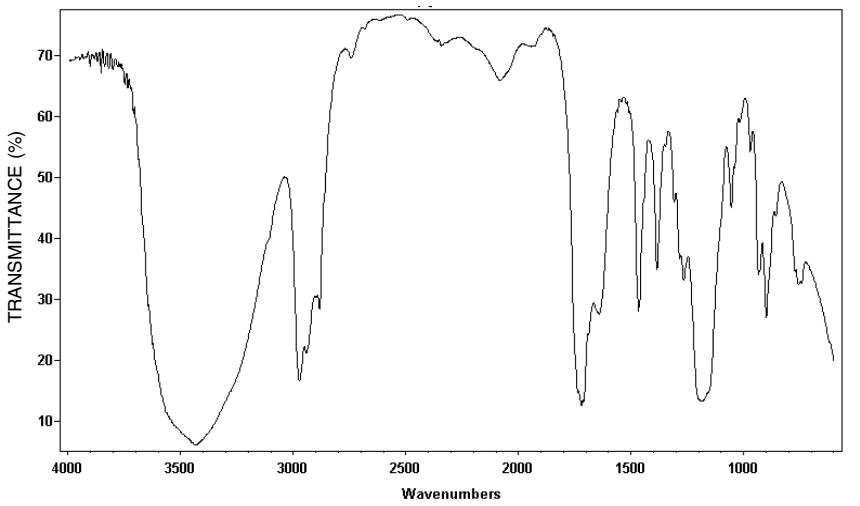
Propyl Alcohol View Flavor Chemical



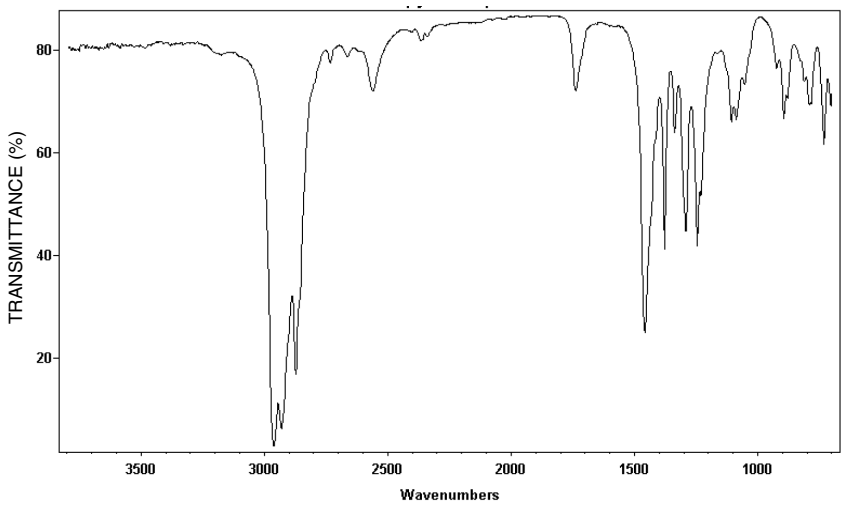
***p*-Propyl Anisole** View Flavor Chemical



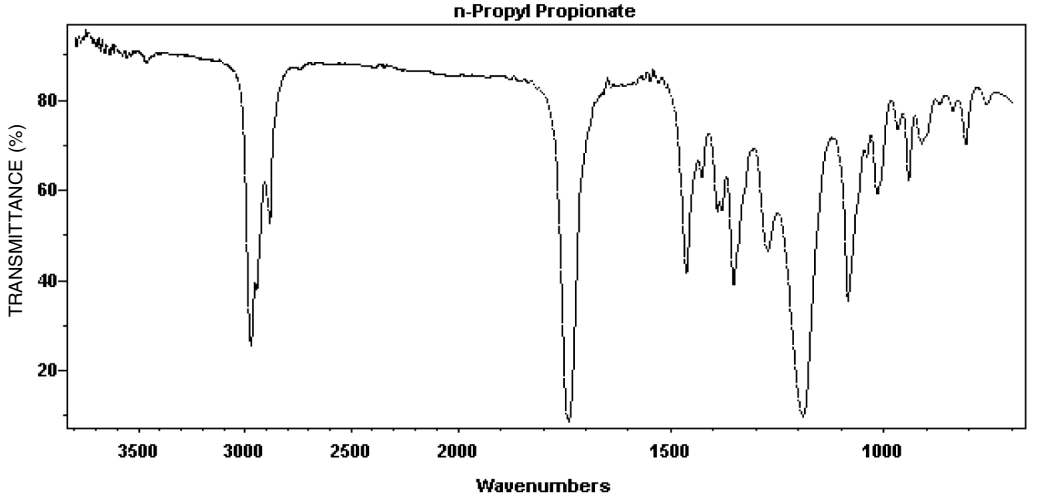
Propyl Formate View Flavor Chemical



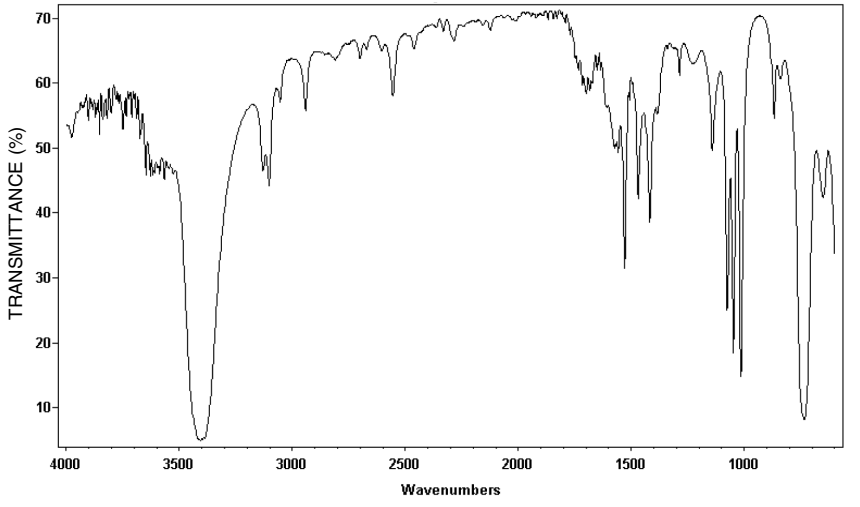
Propyl Mercaptan View Flavor Chemical



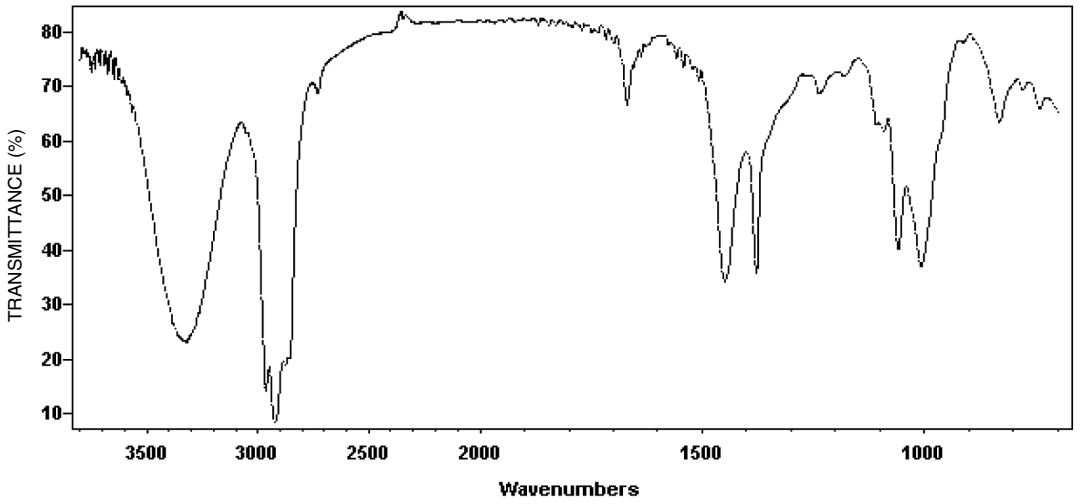
Propyl Propionate View Flavor Chemical



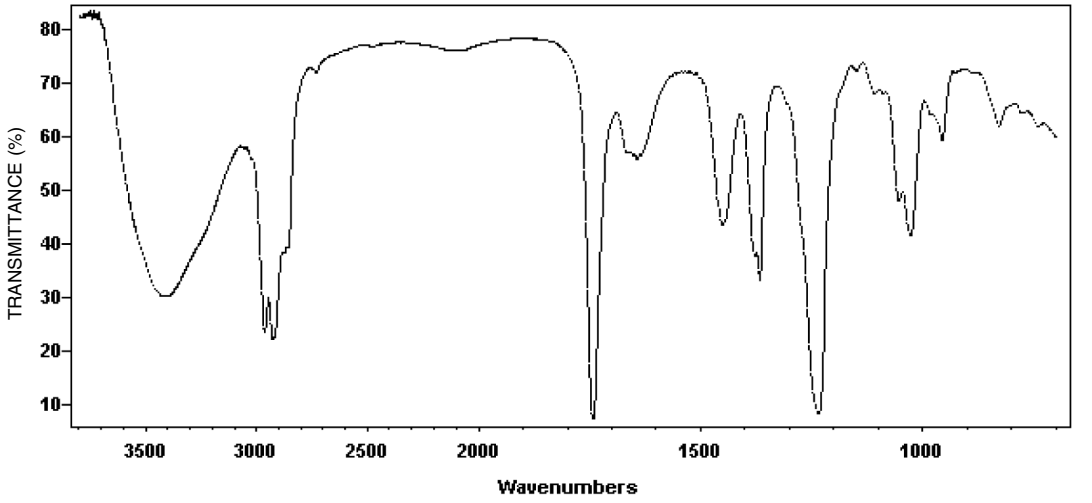
Pyrrole View Flavor Chemical



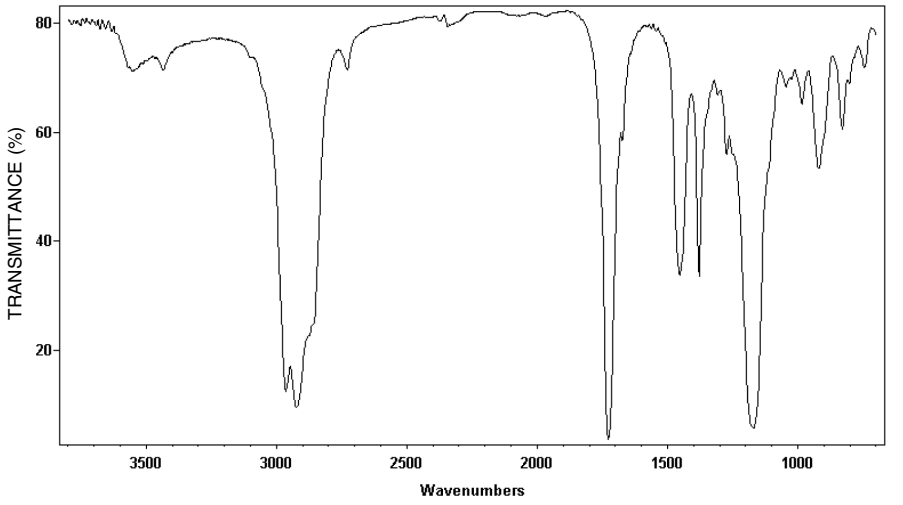
Rhodinol View Flavor Chemical



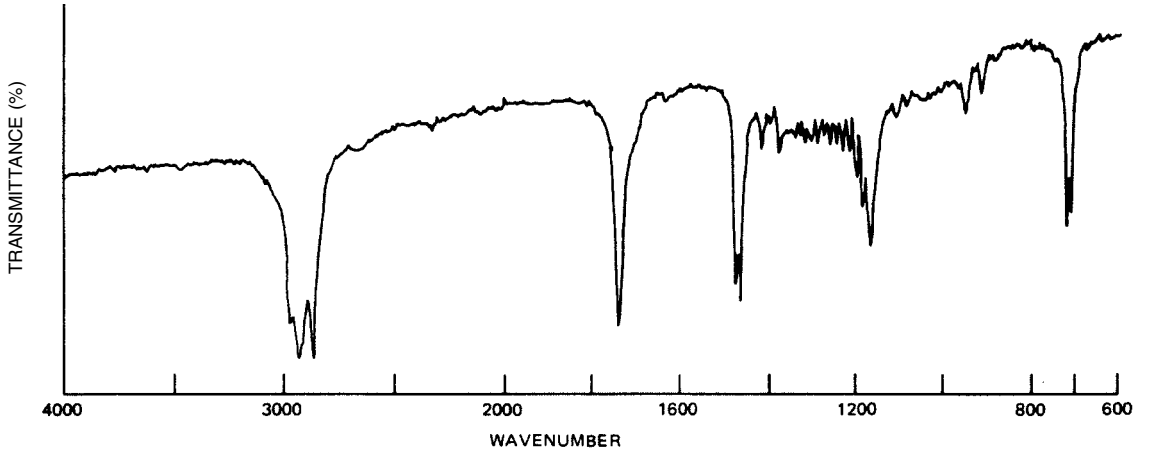
Rhodinyl Acetate View Flavor Chemical



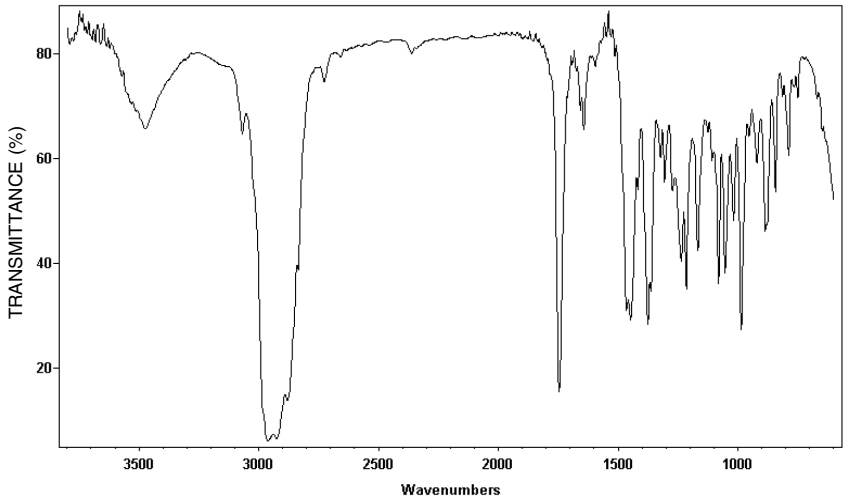
Rhodinyl Formate View Flavor Chemical



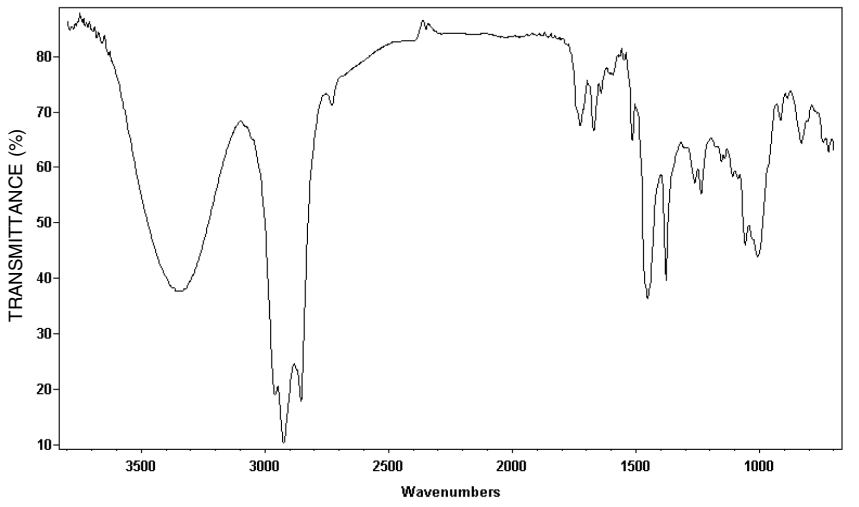
Rice Bran Wax View Monograph



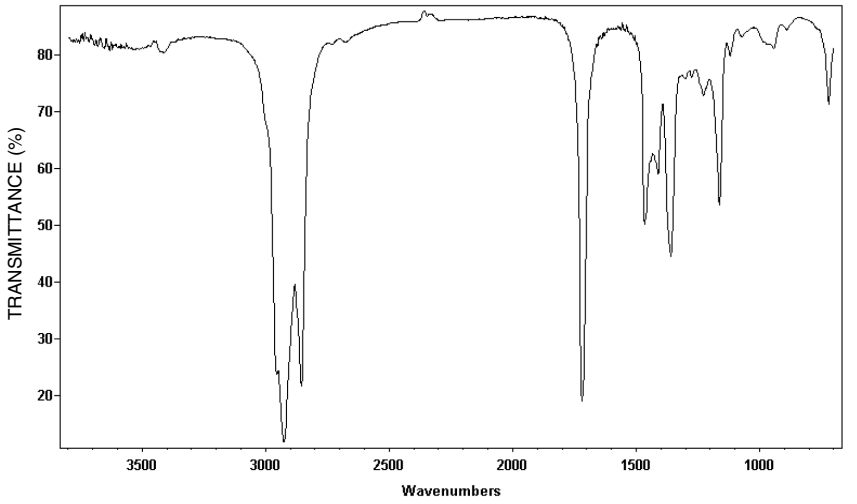
Rosemary Oil View Monograph



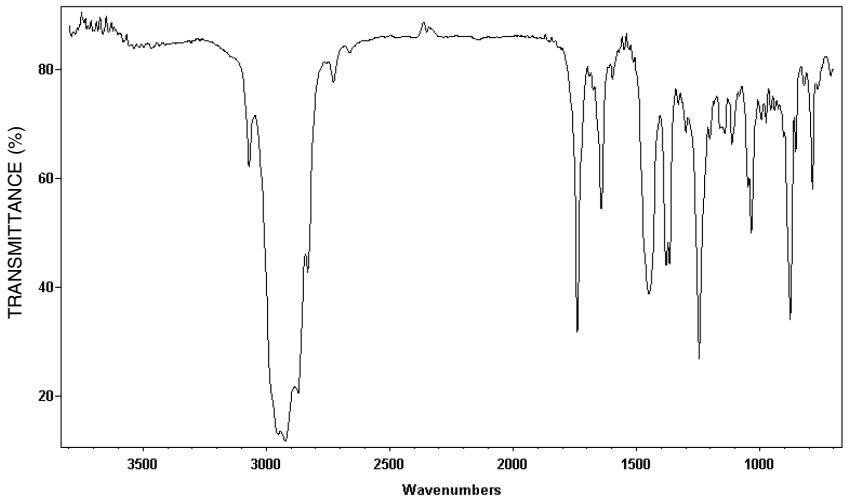
Rose Oil View Monograph



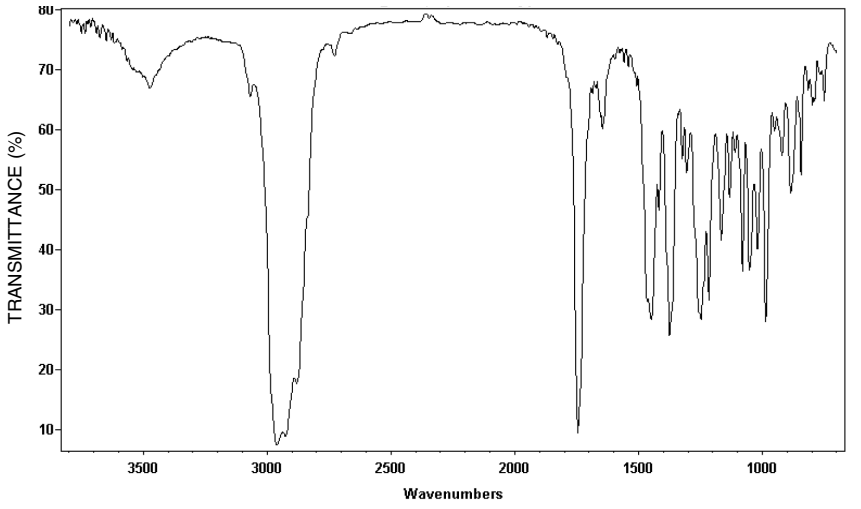
Rue Oil View Monograph



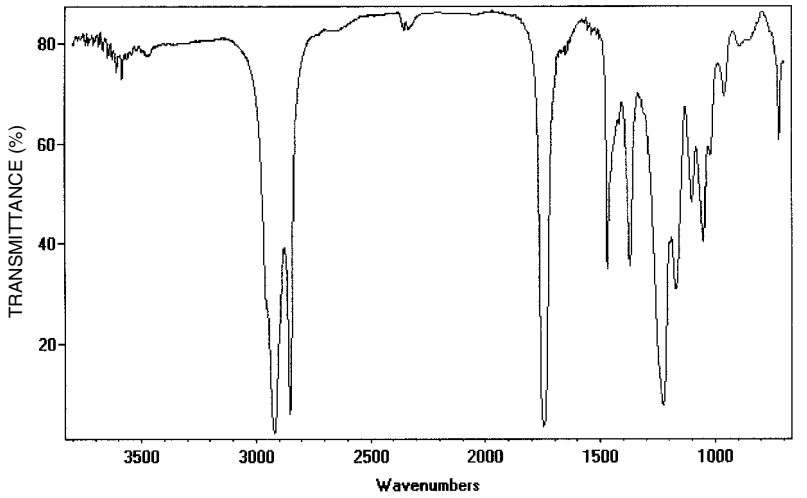
Sage Oil, Dalmatian Type View Monograph



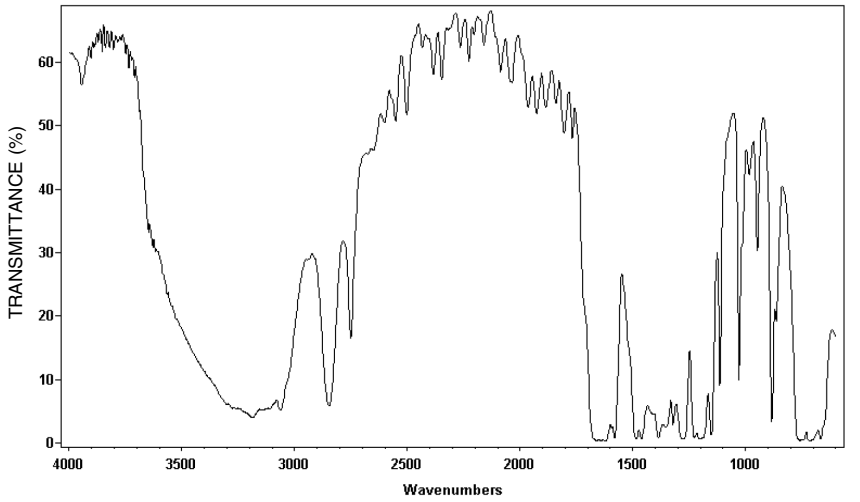
Sage Oil, Spanish Type View Monograph



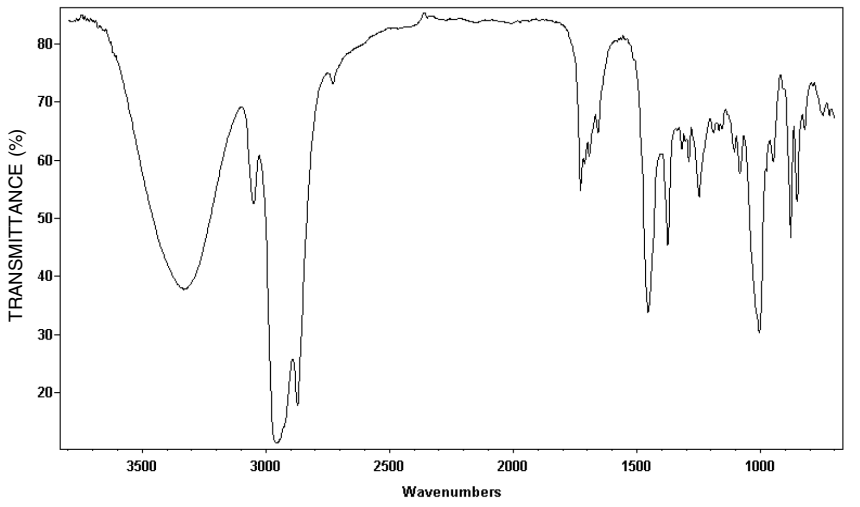
Salatrim View Monograph



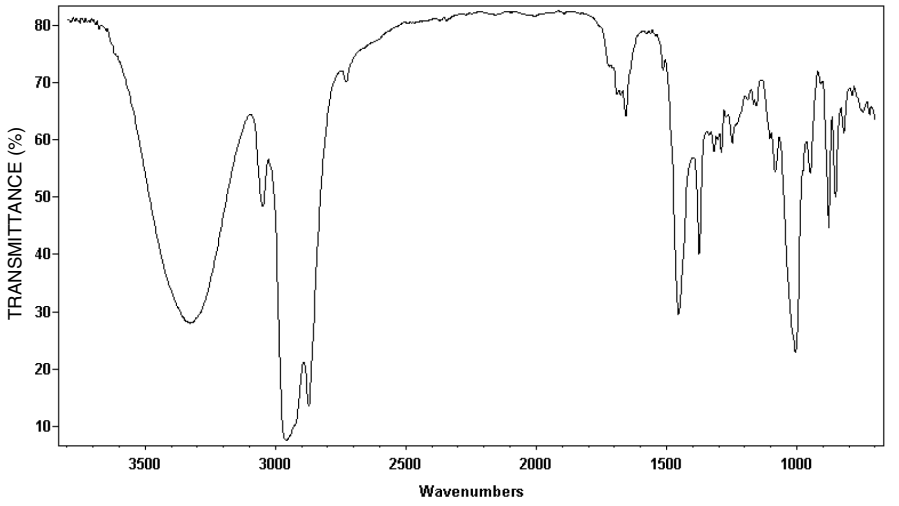
Salicylaldehyde View Flavor Chemical



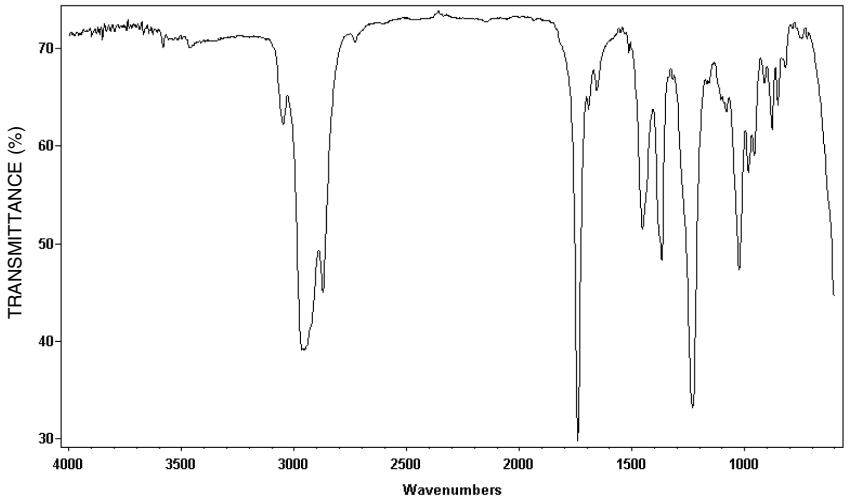
Sandalwood Oil, East Indian Type View Monograph



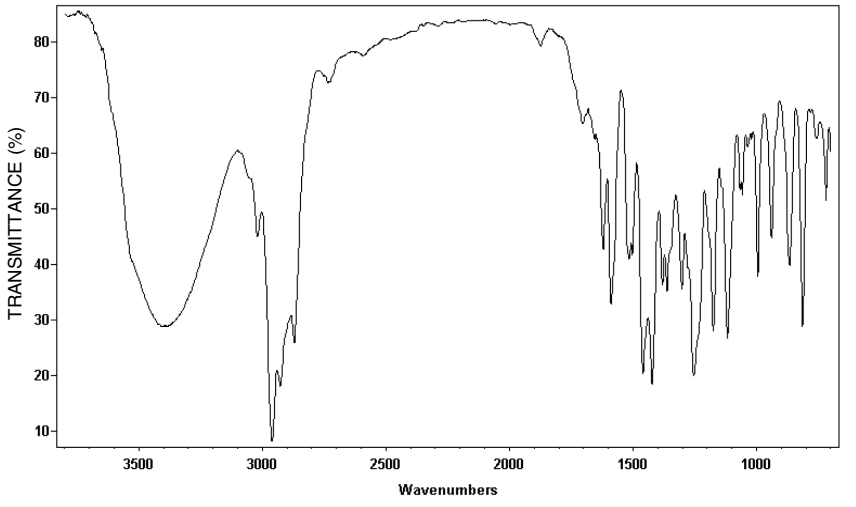
Santalol View Flavor Chemical



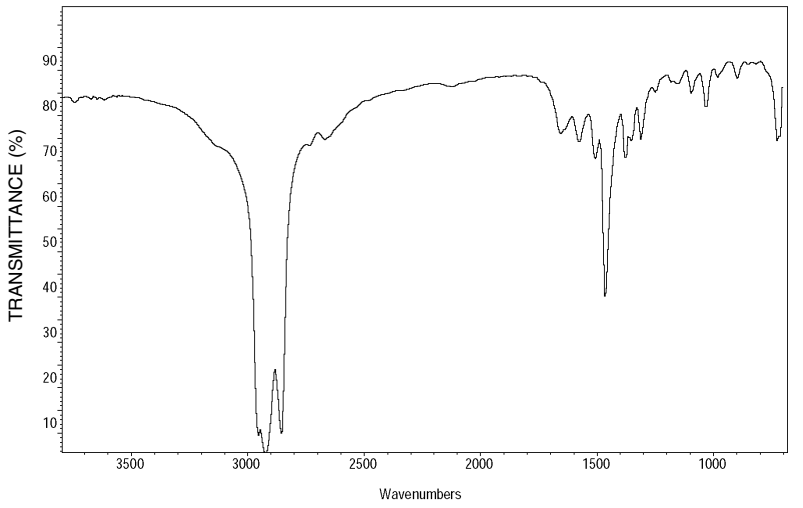
Santalyl Acetate View Flavor Chemical



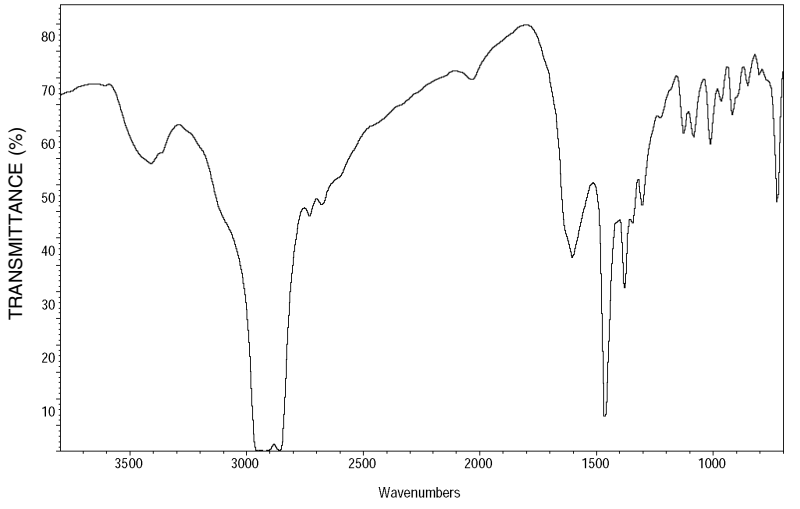
Savory Oil (Summer Variety) View Monograph



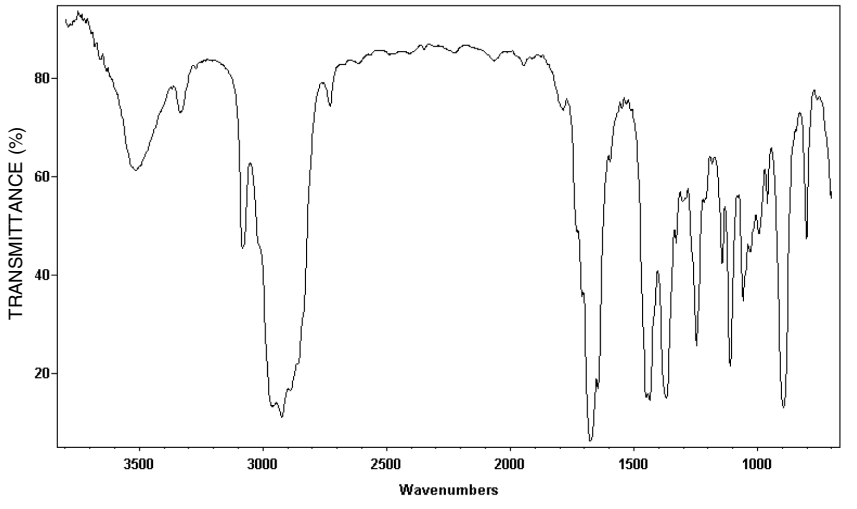
DL-Serine (Mineral Oil Mull) View Monograph



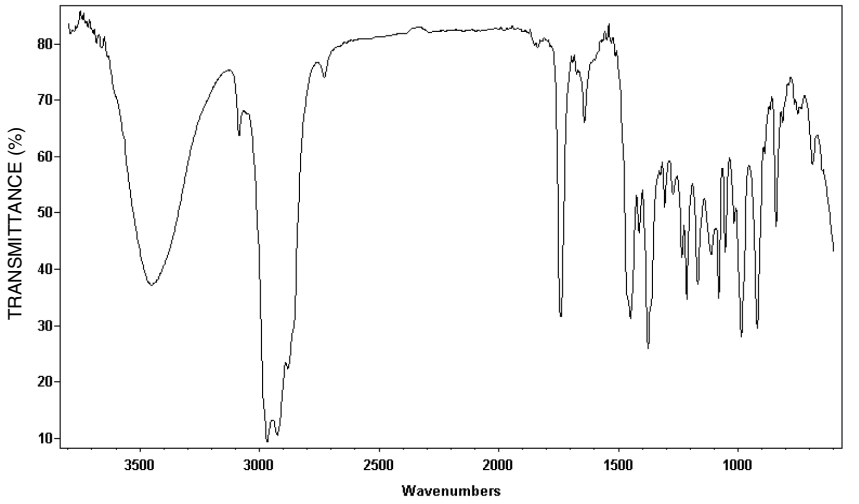
L-Serine (Mineral Oil Mull) View Monograph



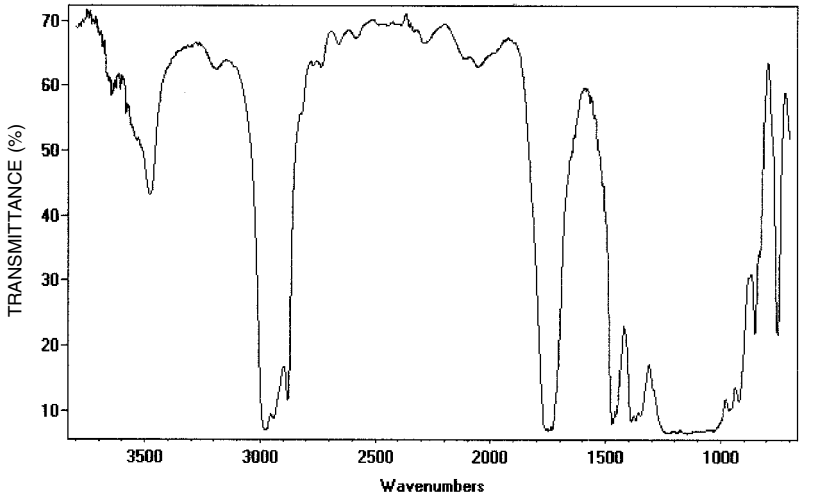
Spearmint Oil View Monograph



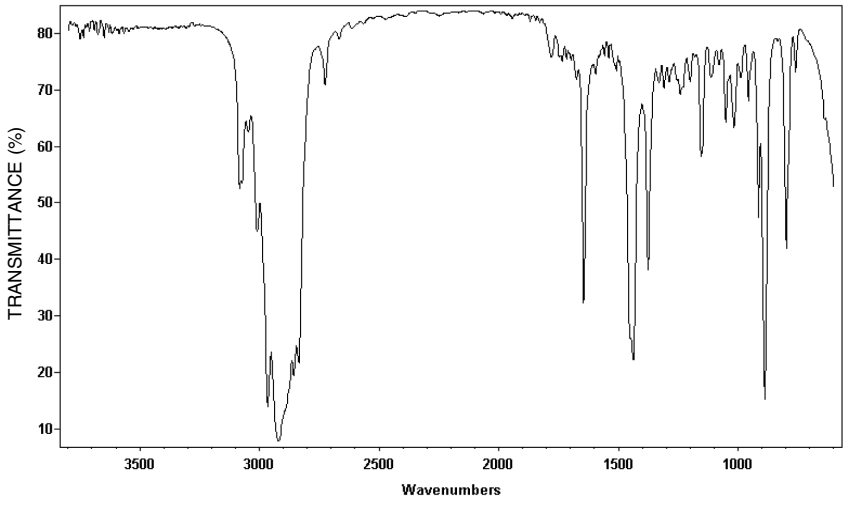
Spike Lavender Oil View Monograph



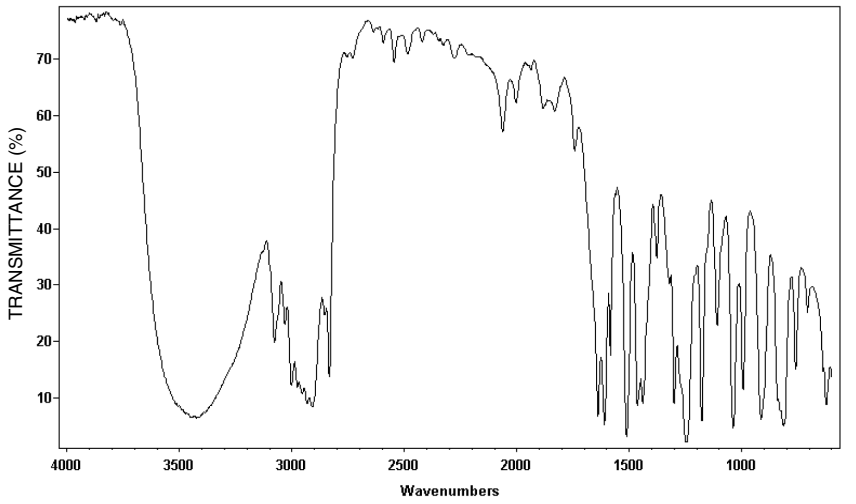
Sucrose Acetate Isobutyrate View Monograph



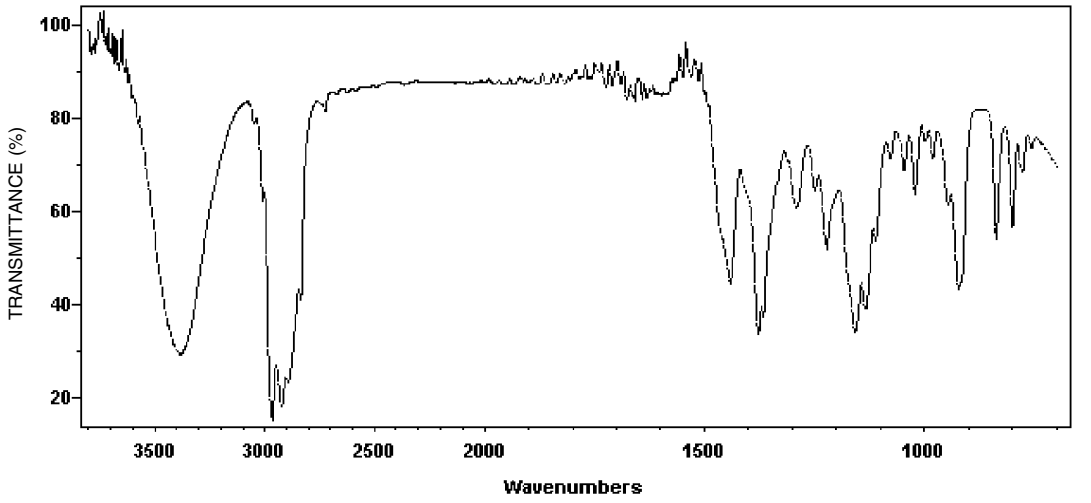
Tangerine Oil, Coldpressed View Monograph



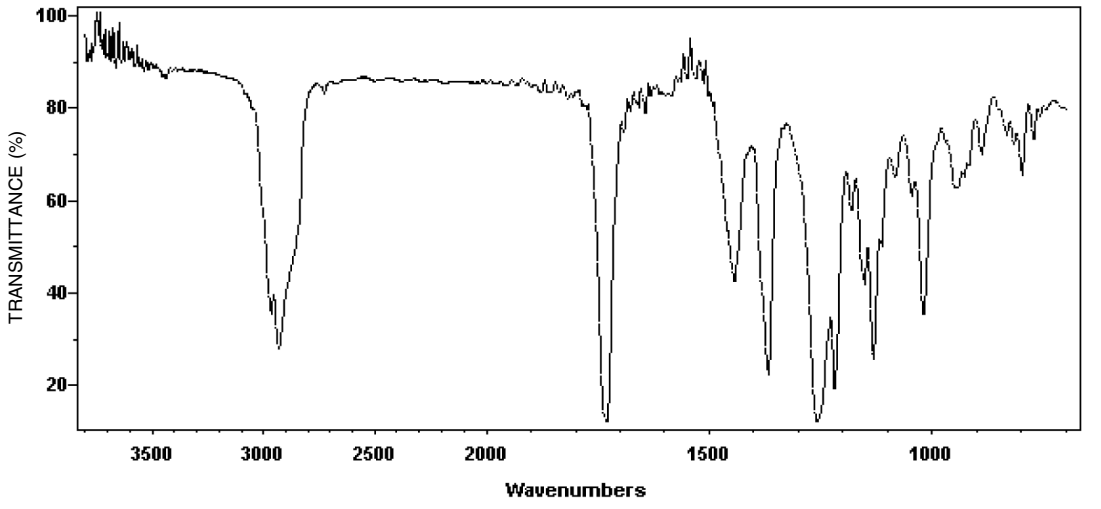
Tarragon Oil View Monograph



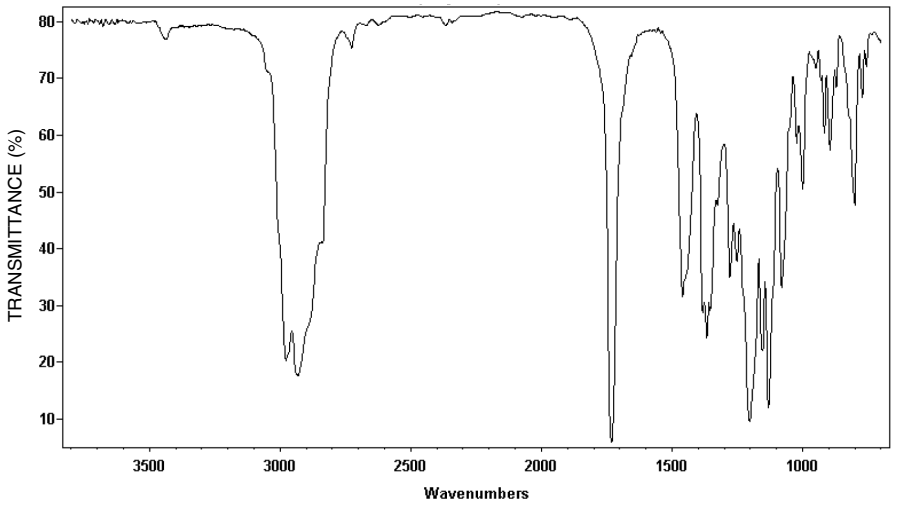
α-Terpineol View Flavor Chemical



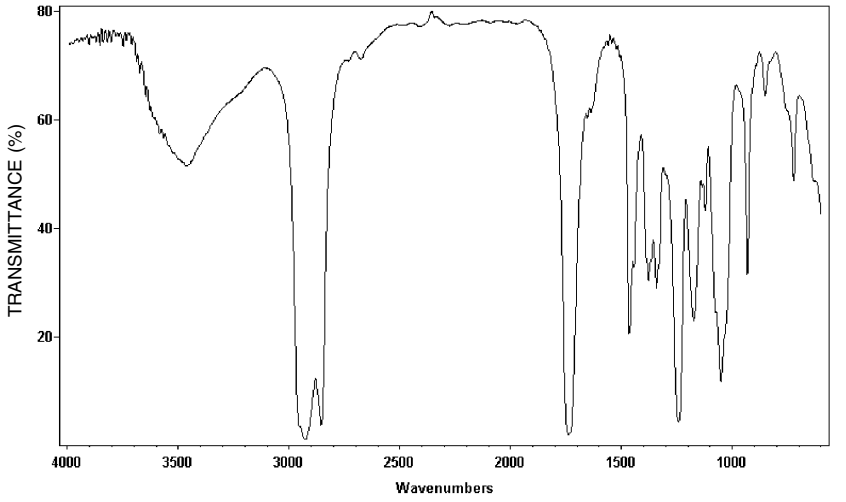
Terpinyl Acetate View Flavor Chemical



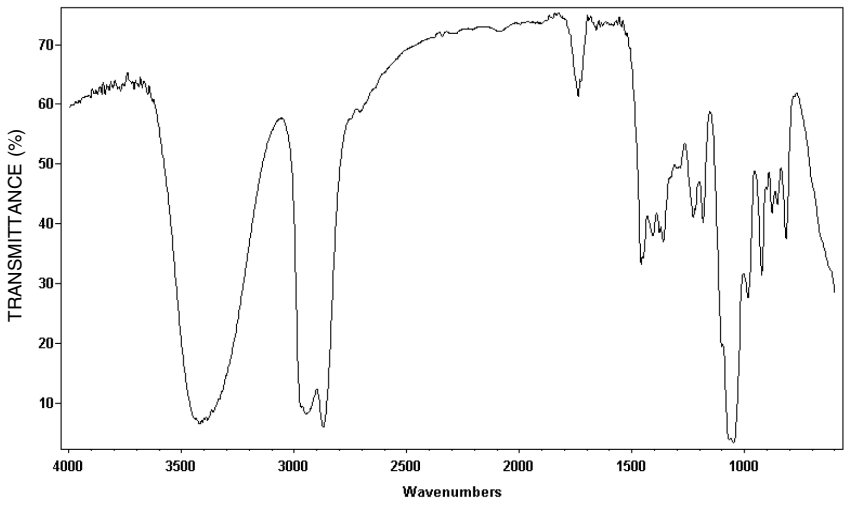
Terpinyl Propionate View Flavor Chemical



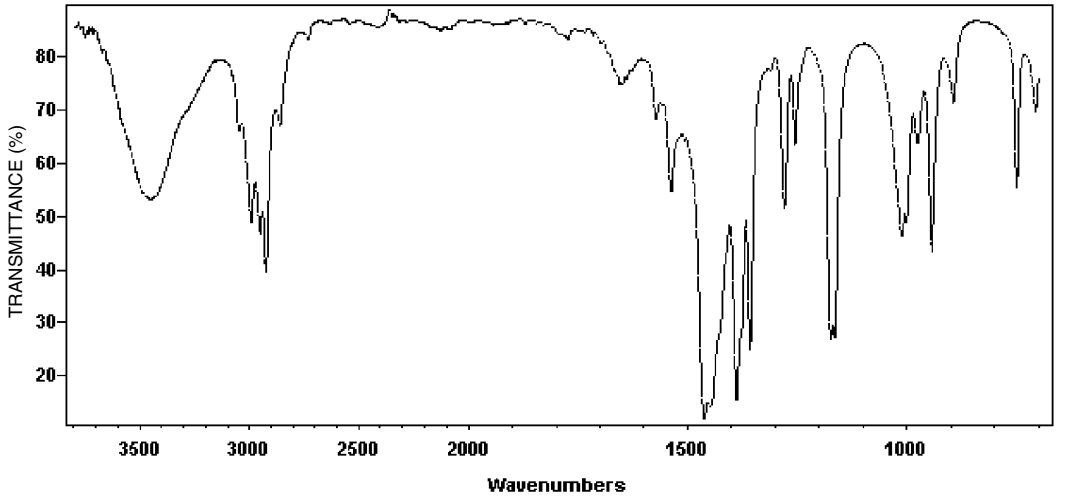
δ-Tetradecalactone View Flavor Chemical



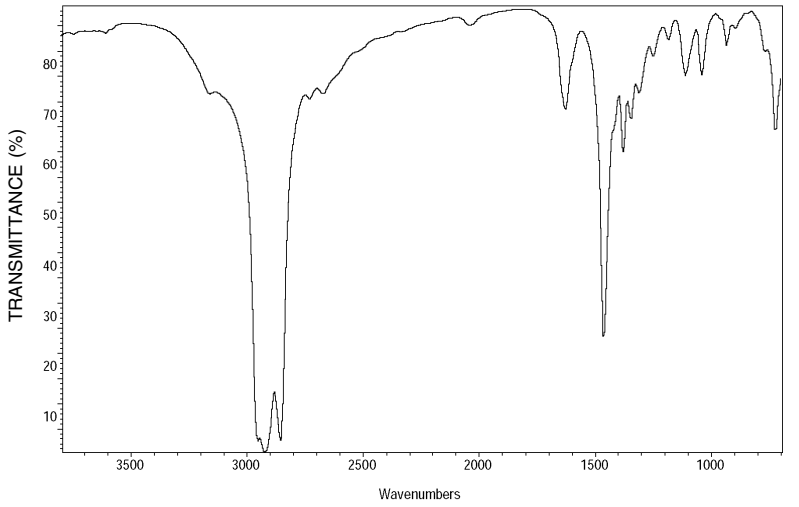
Tetrahydrofurfuryl Alcohol View Flavor Chemical



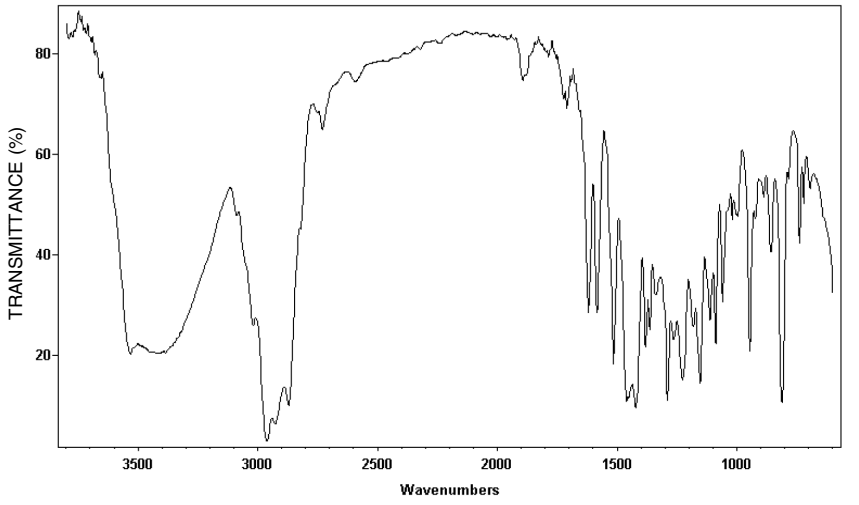
2,3,5,6-Tetramethylpyrazine **View Flavor Chemical**



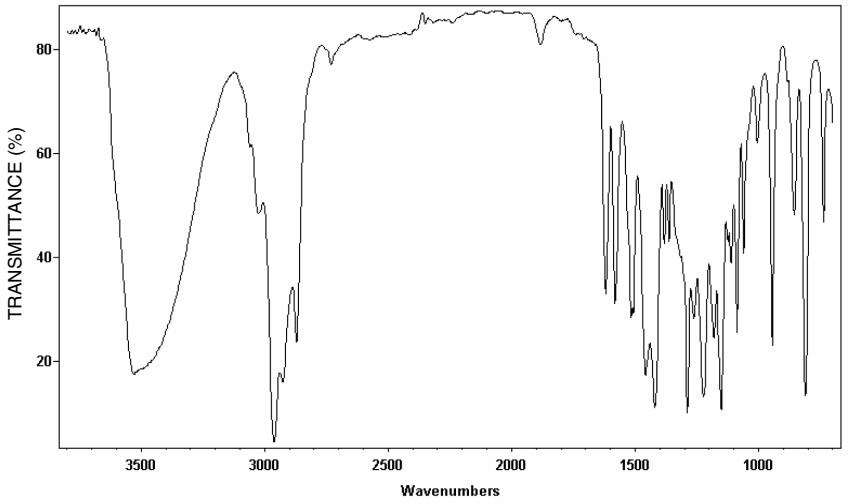
L-Threonine (Mineral Oil Mull) **View Monograph**



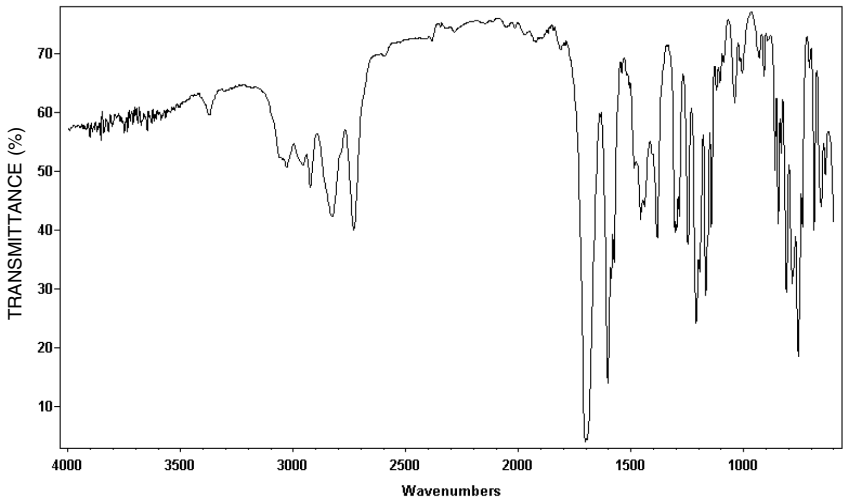
Thyme Oil **View Monograph**



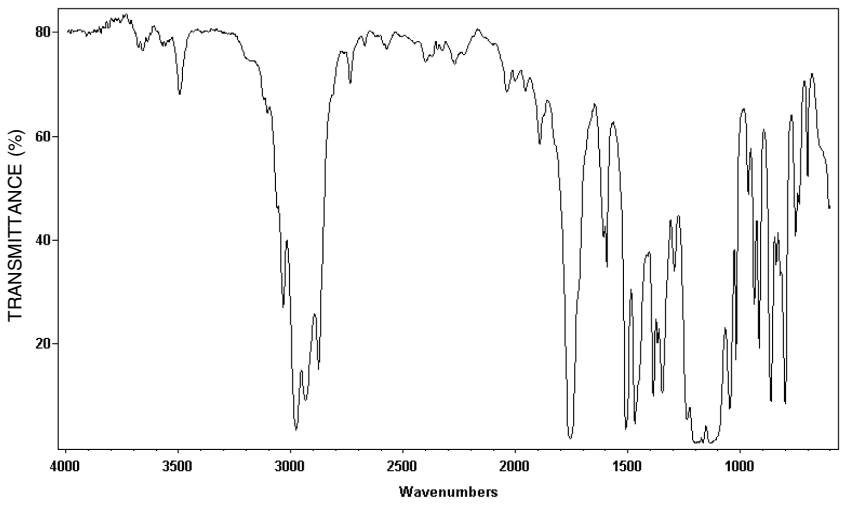
Thymol View Flavor Chemical



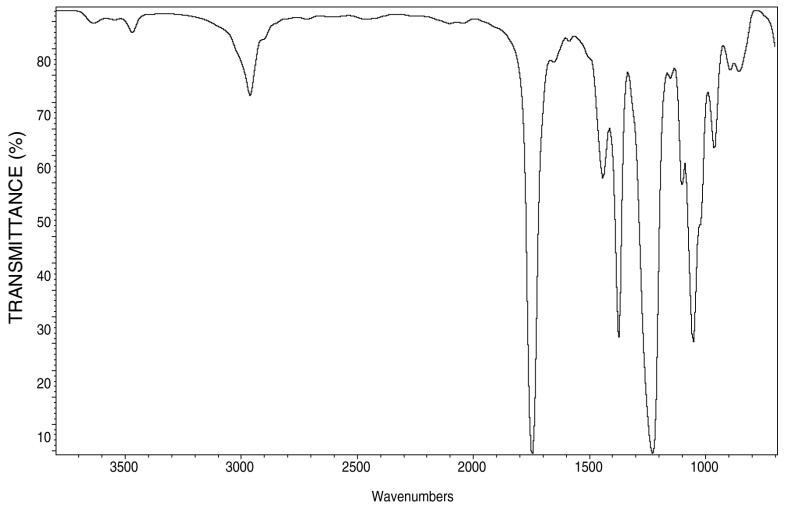
Tolualdehyde (Mixed Isomers) View Flavor Chemical



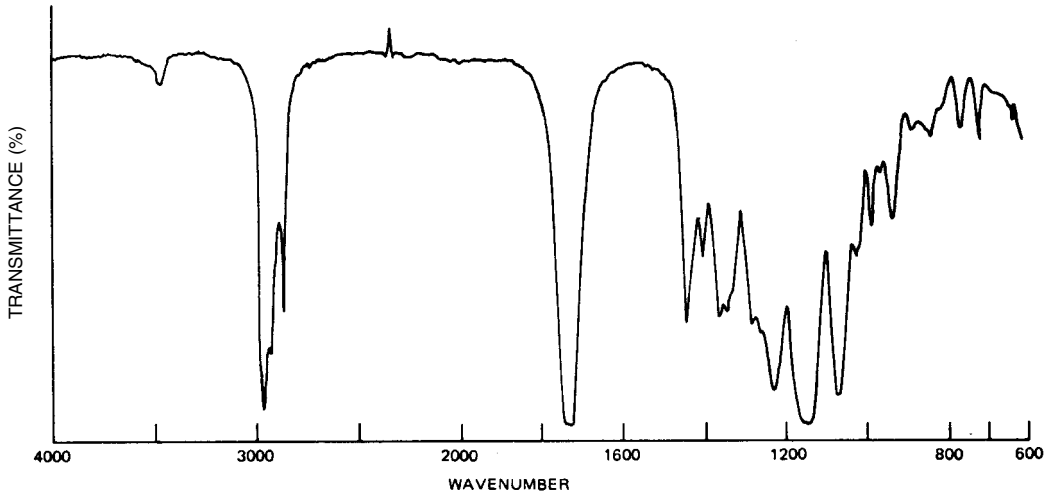
***p*-Tolyl Isobutyrate** View Flavor Chemical



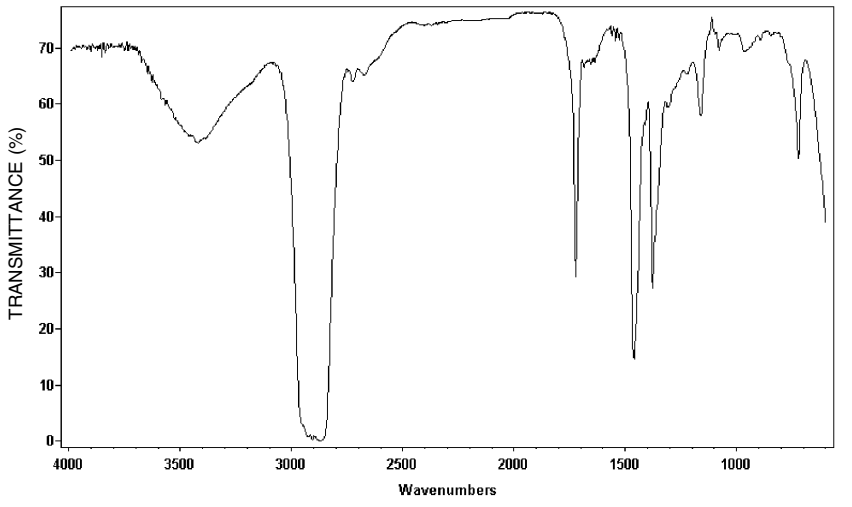
Triacetin View Monograph



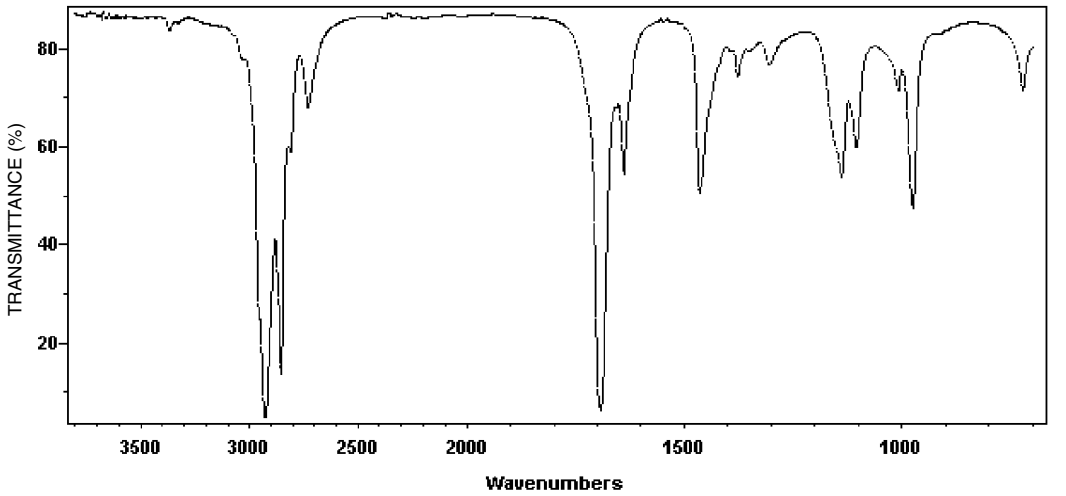
Tributyrin View Flavor Chemical



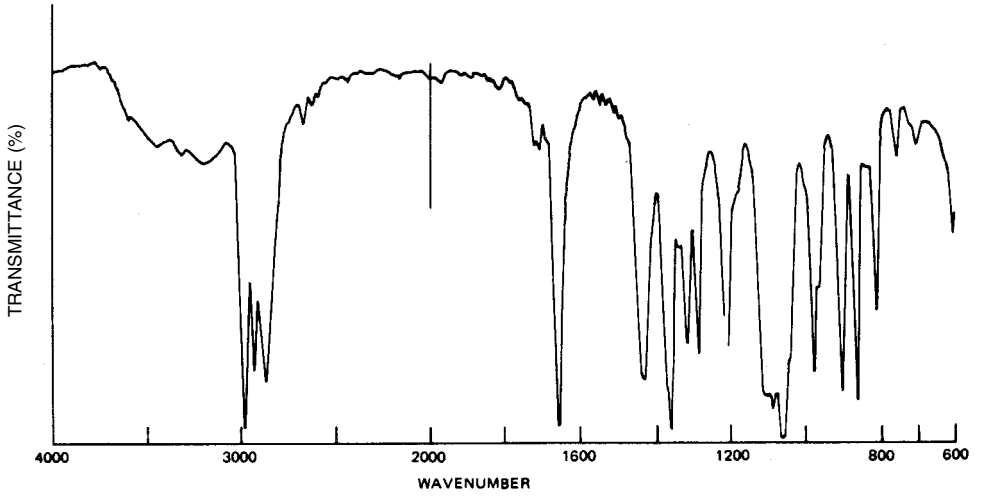
2-Tridecanone View Flavor Chemical



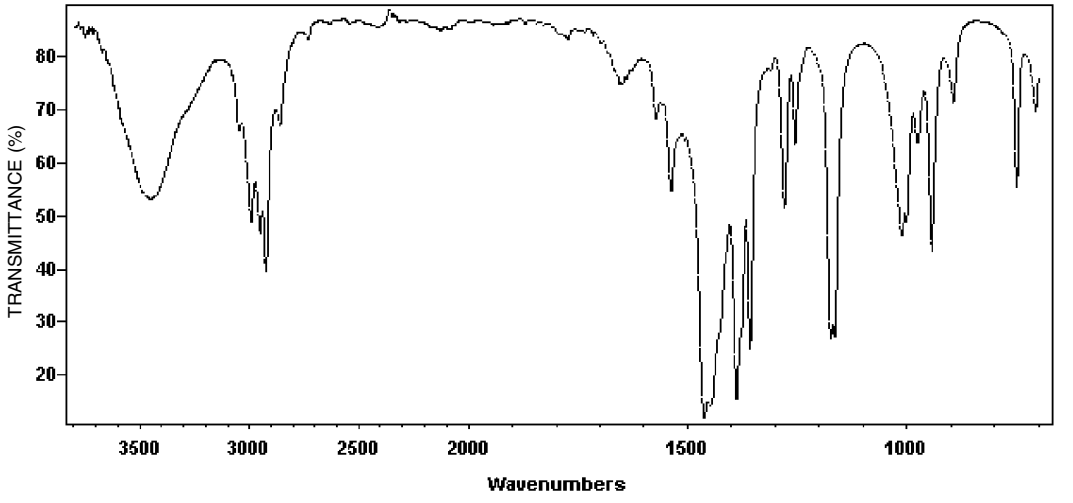
2-Tridecenal View Flavor Chemical



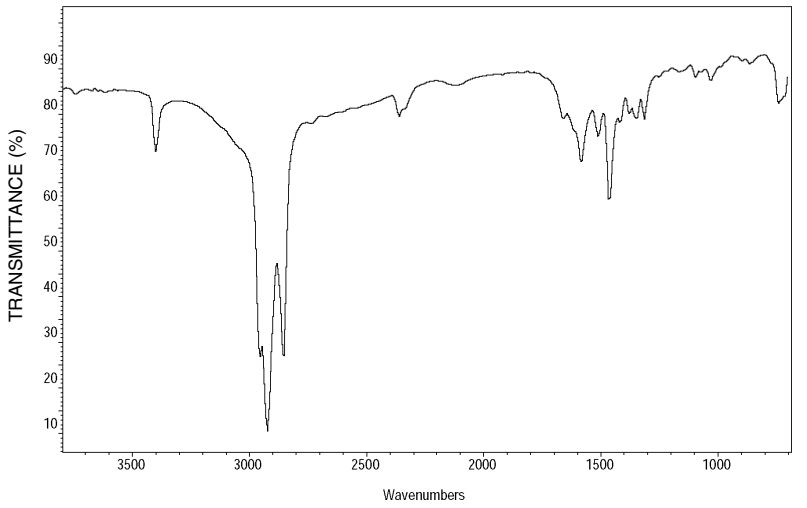
2,4,5-Trimethyl δ-3-Oxazoline View Flavor Chemical



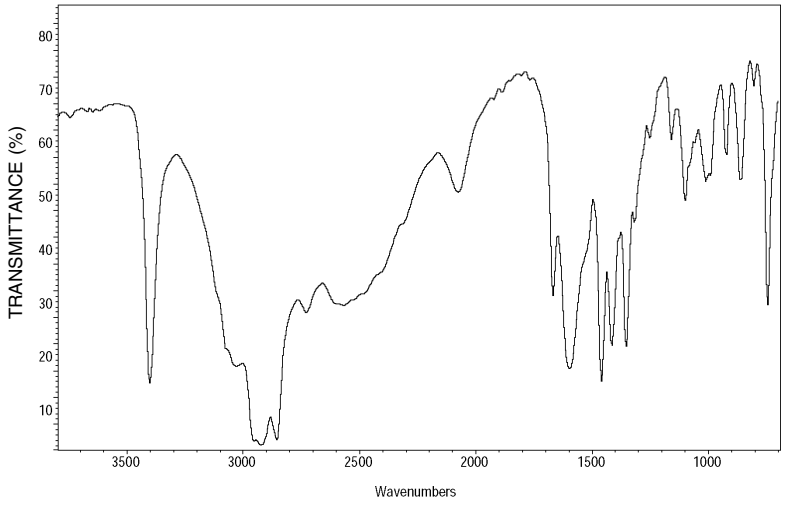
2,3,5-Trimethylpyrazine View Flavor Chemical



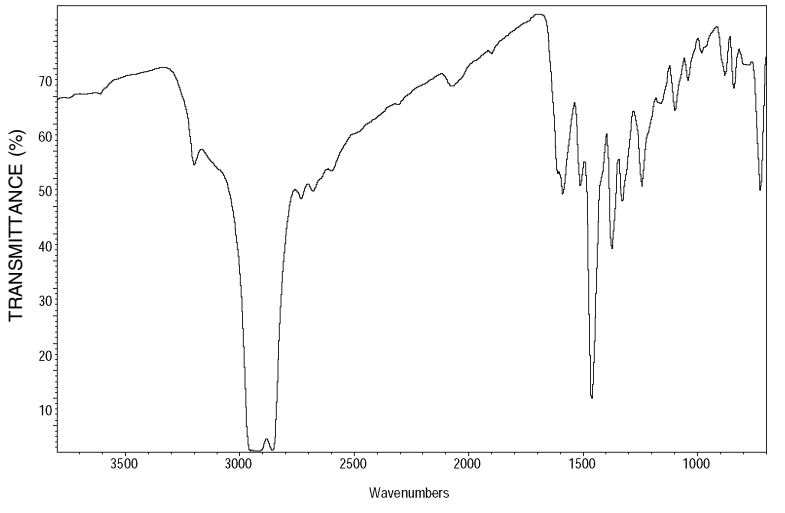
DL-Tryptophan (Mineral Oil Mull) View Monograph



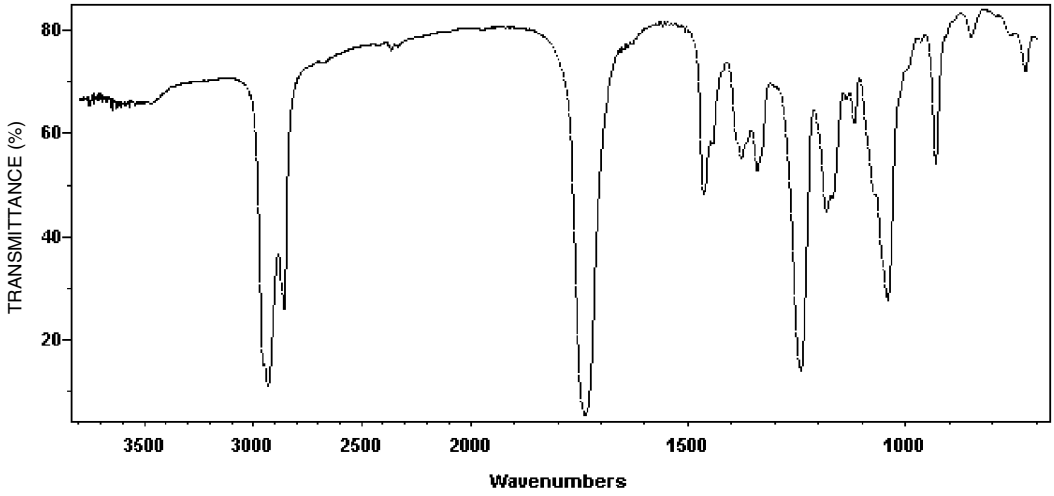
L-Tryptophan (Mineral Oil Mull) View Monograph



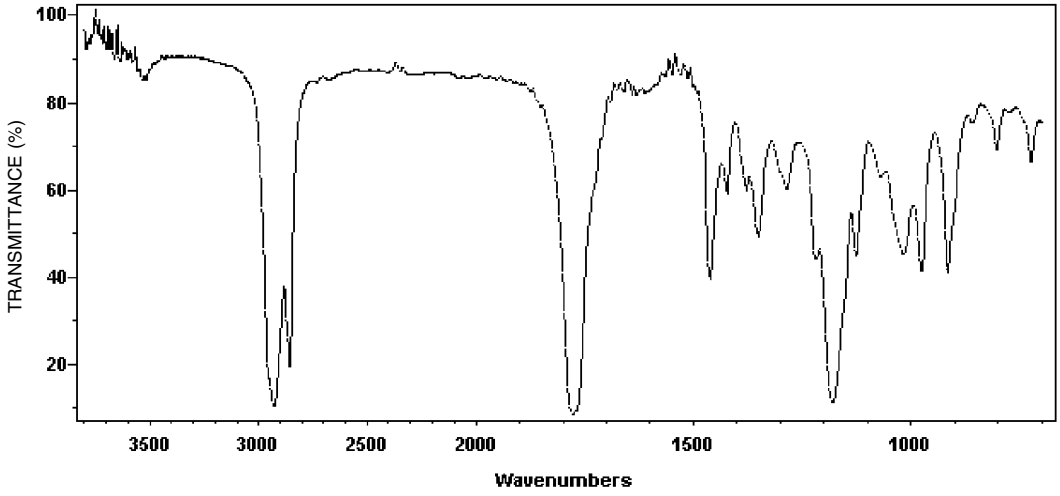
L-Tyrosine (Mineral Oil Mull) View Monograph



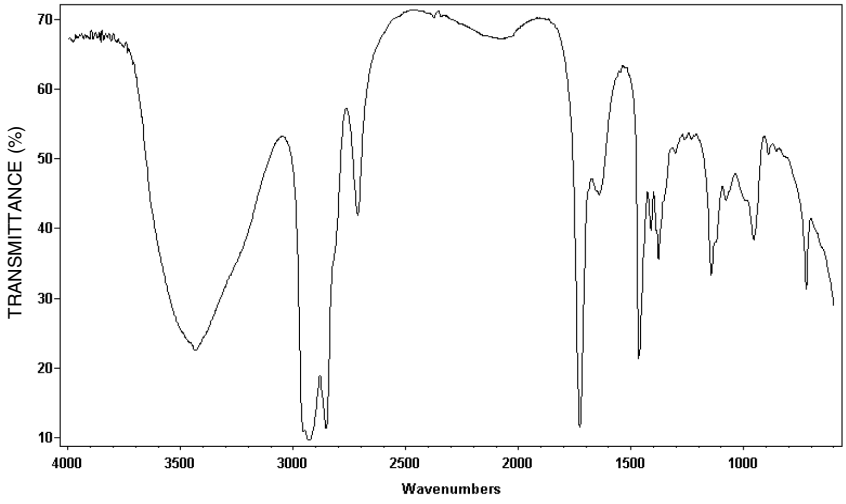
δ-Undecalactone View Flavor Chemical



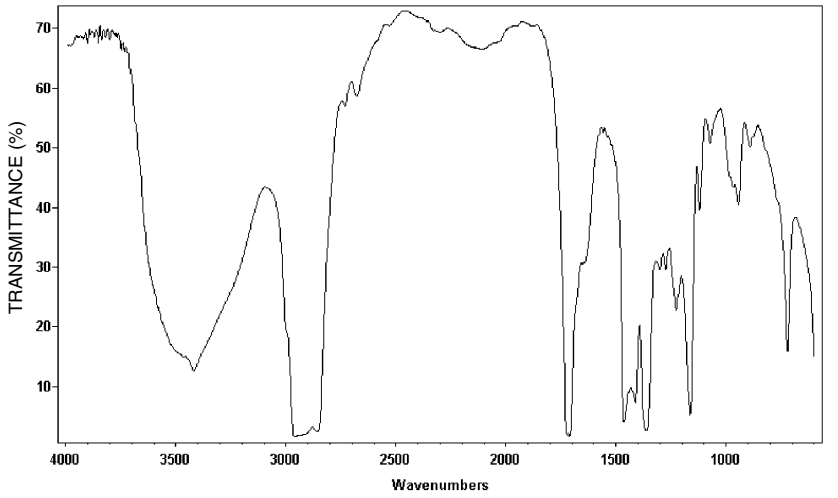
γ-Undecalactone View Flavor Chemical



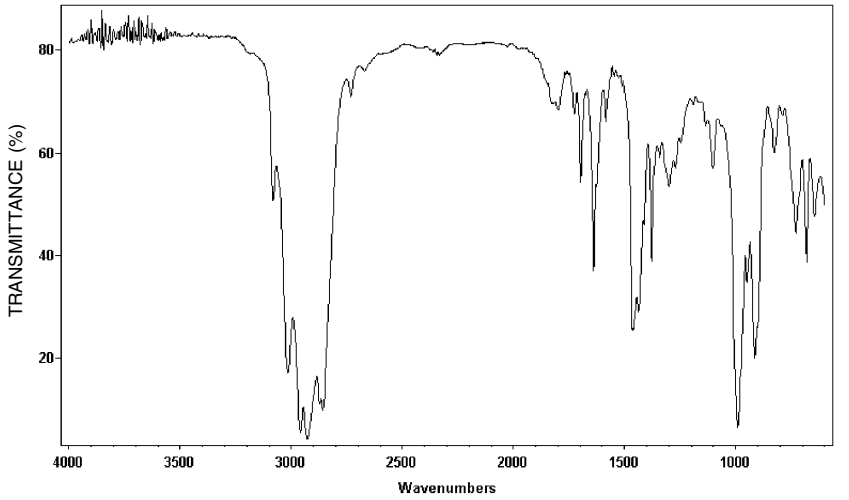
Undecanal View Flavor Chemical



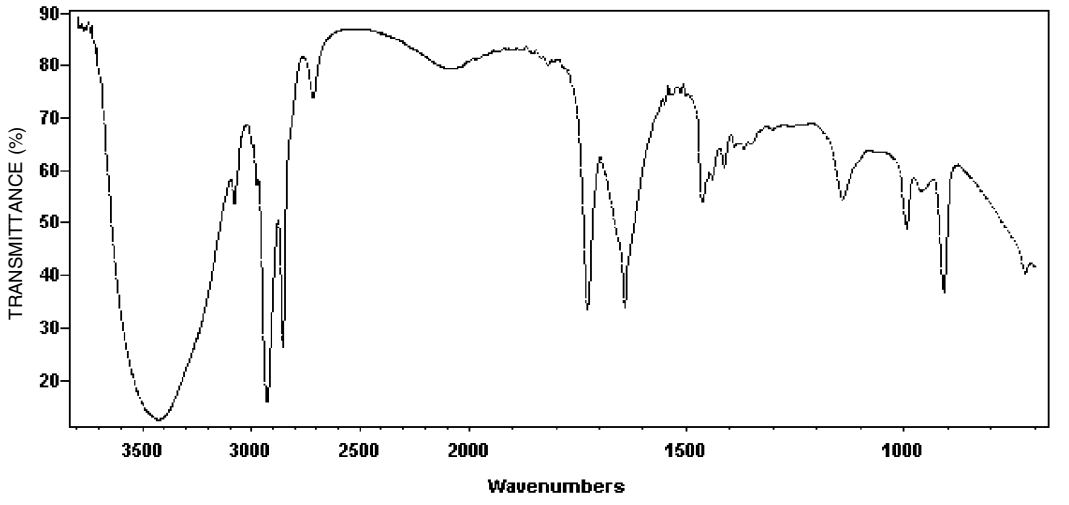
2-Undecanone View Flavor Chemical



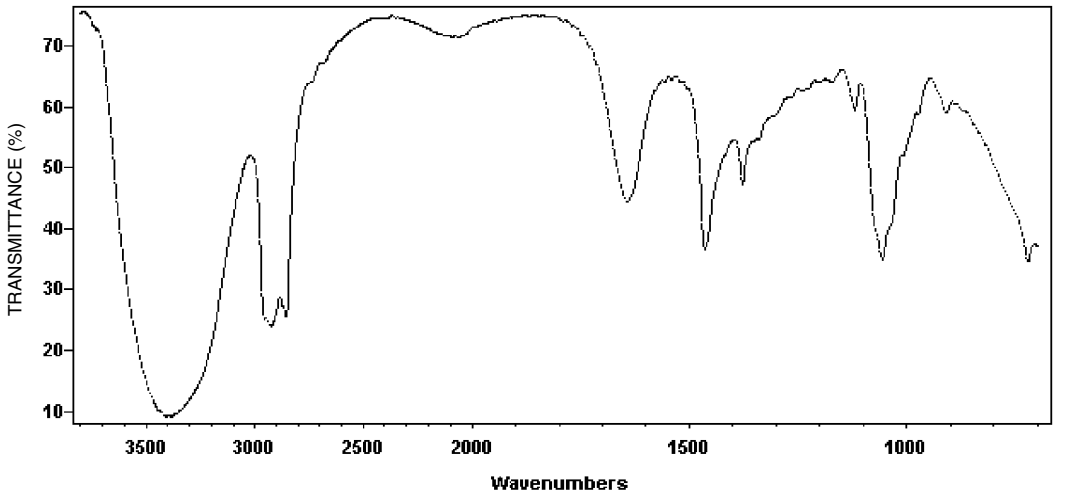
1,3,5-Undecatriene View Flavor Chemical



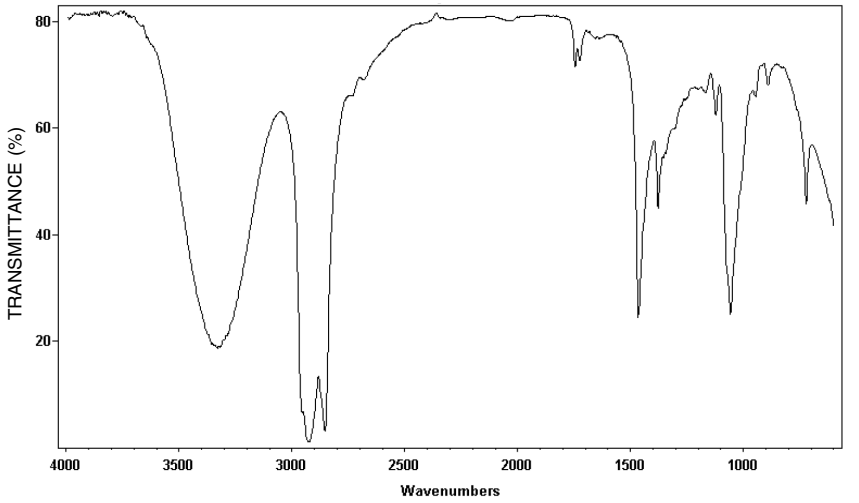
10-Undecenal View Flavor Chemical



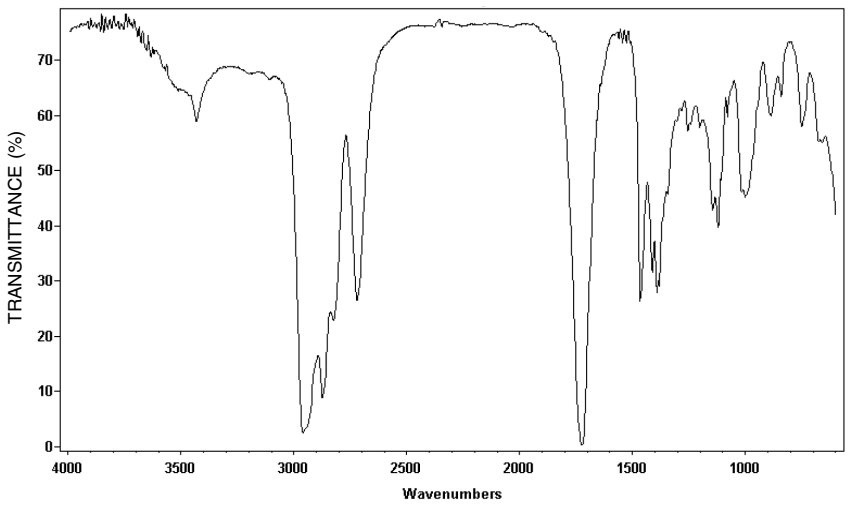
(*E*)-2-Undecenol View Flavor Chemical



Undecyl Alcohol View Flavor Chemical

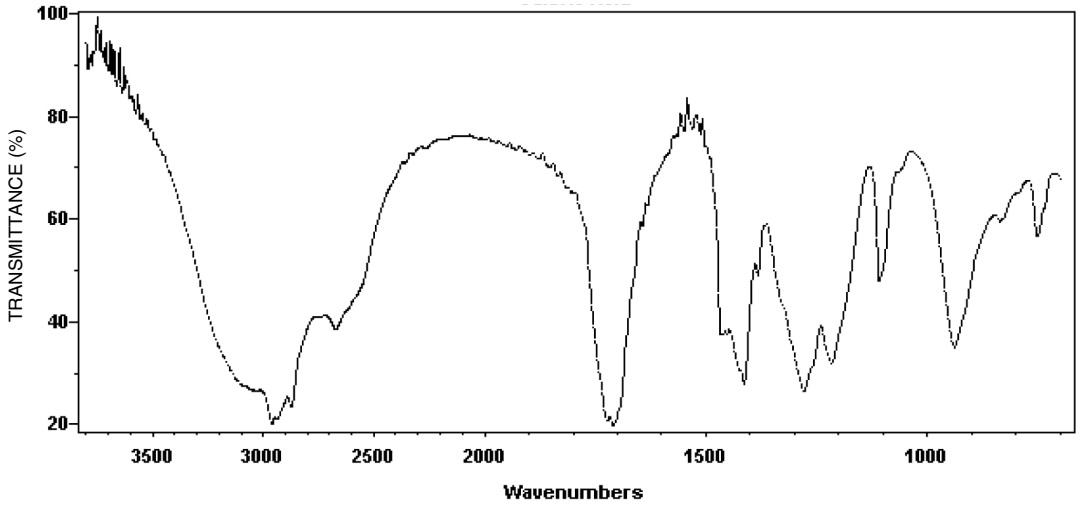


Valeraldehyde View Flavor Chemical



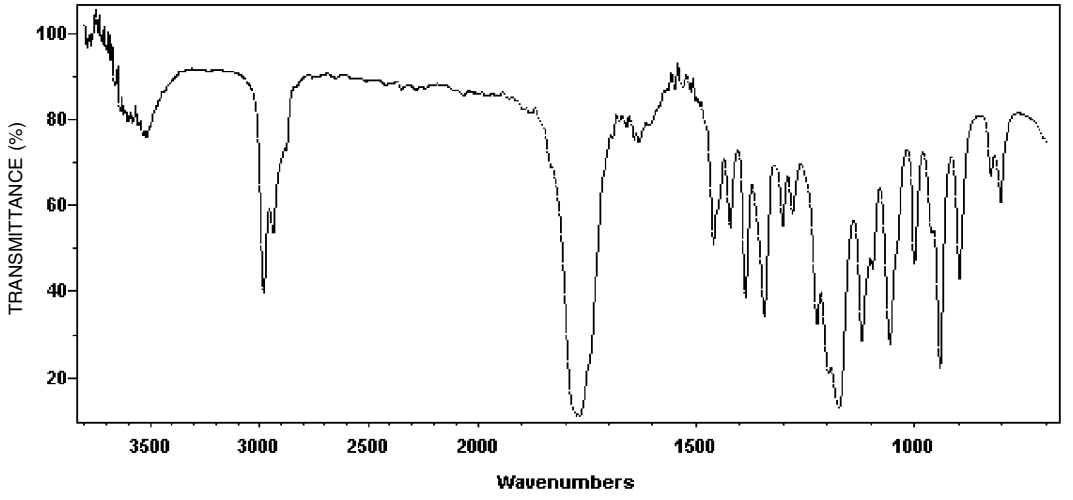
Valeric Acid

View Flavor Chemical



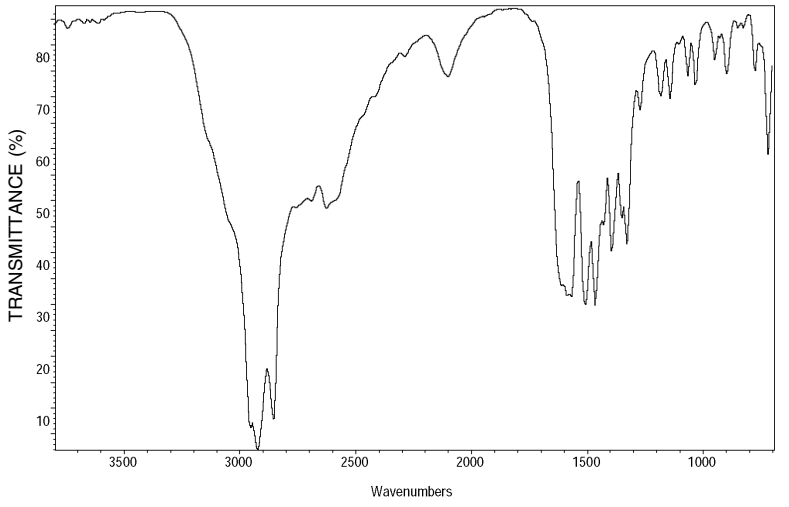
γ-Valerolactone

View Flavor Chemical

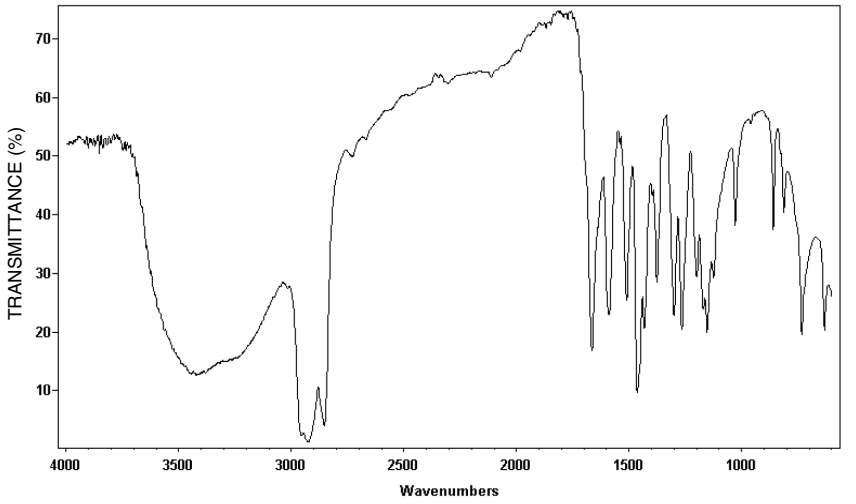


L-Valine (Mineral Oil Mull)

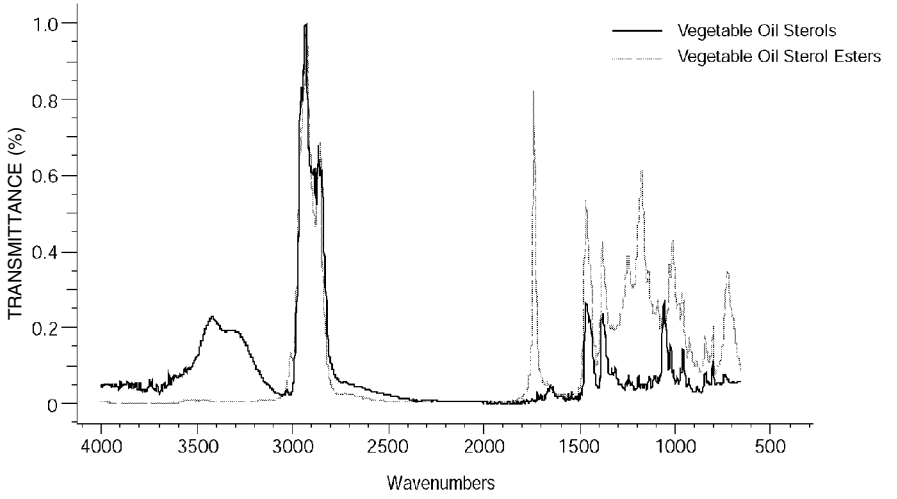
View Monograph



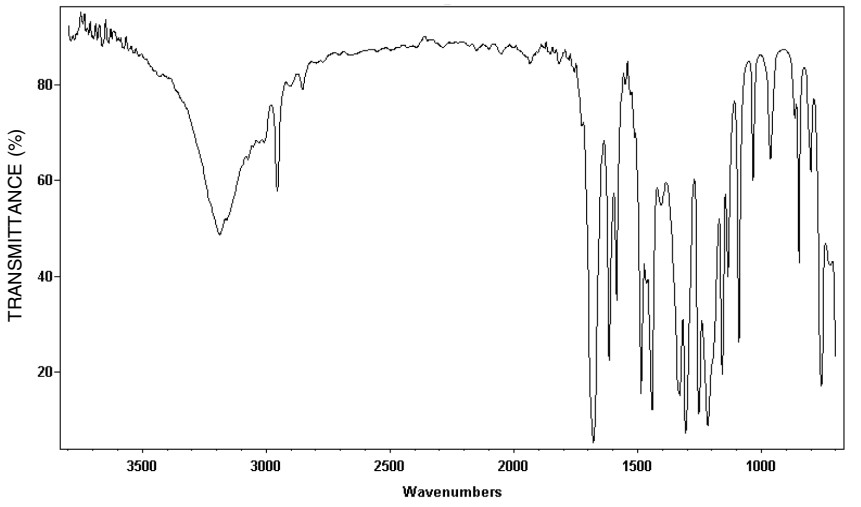
Vanillin View Flavor Chemical



Vegetable Oil Phytosterol Esters View Monograph

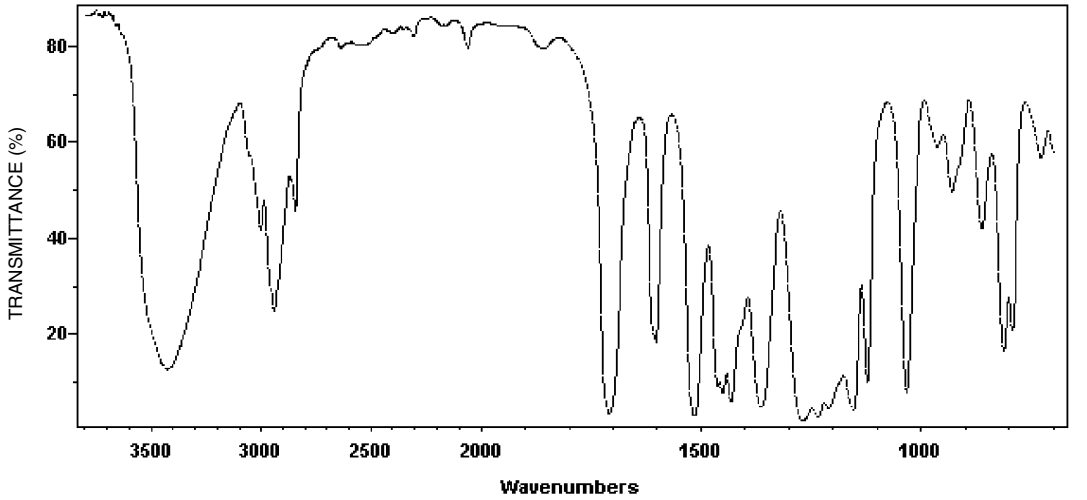


Wintergreen Oil View Monograph



Zingerone

View Flavor Chemical



5 / *General Tests and Assays*

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APPENDIX I: APPARATUS FOR TESTS AND ASSAYS

OXYGEN FLASK COMBUSTION

Apparatus The apparatus consists of a heavy-walled, deeply lipped or cupped, conical flask of a volume suitable for the complete combustion of the sample in which the particular element is being determined (e.g., see *Selenium Limit Test*, Appendix IIIB). The flask is fitted with a ground-glass stopper to which is fused a sample carrier consisting of heavy-gauge platinum wire and a piece of welded platinum gauze measuring about 1.5 × 2 cm. A suitable apparatus may be obtained as Catalog Nos. 6513-C20 (500-mL capacity) and 6513-C30 (1000-mL capacity) from the Arthur H. Thomas Co., P.O. Box 779, Philadelphia, PA 19105. Equivalent apparatus available from other sources, or other suitable apparatus embodying the principles described herein, may also be used.

Procedure (**Caution**: Analysts should wear safety glasses and should use a suitable safety shield between themselves and the apparatus. Further safety measures should be observed as necessary to ensure maximum protection of the analysts. Furthermore, the flask must be scrupulously clean and free from even traces of organic solvents. Samples containing water of hydration or more than 1% of moisture should be dried at 140° for 2 h before combustion, unless otherwise directed.) Accurately weigh the amount of sample specified in the monograph or general test. Solids should be weighed on a 4-cm square piece of halide-free paper, which should be folded around the sample. Liquid samples not exceeding 0.2 mL in volume should be weighed in tared cellulose acetate capsules [available as Catalog Nos. 6513-C80 (100 capsules) and 6513-C82 (1000 capsules) from the Arthur H. Thomas Co.]; gelatin capsules are satisfactory for liquid samples exceeding 0.2 mL in volume.

> **Note**: Gelatin capsules may contain significant amounts of combined halide or sulfur, in which case a blank determination should be made as necessary.

Place the sample, together with a filter paper fuse-strip, in the platinum gauze sample holder. Place the absorbing liquid, as specified in the individual monograph or general test, into the flask, moisten the joint of the stopper with water, and flush the air from the flask with a stream of rapidly flowing oxygen, swirling the liquid to facilitate its taking up oxygen.

> **Note**: Saturation of the liquid with oxygen is essential for successful performance of this procedure.

Ignite the fuse-strip by suitable means. If the strip is ignited outside the flask, immediately plunge the sample holder into the flask, invert the flask so that the absorption solution makes a seal around the stopper, and hold the stopper firmly in place. If the ignition is carried out in a closed system, the inversion of the flask may be omitted. After combustion is complete, shake the flask vigorously, and allow it to stand for not less than 10 min with intermittent shaking. Continue as directed in the individual monograph or general test chapter.

THERMOMETERS

Thermometers suitable for *Food Chemicals Codex* use conform to the specifications of the American Society for Testing and Materials, ASTM Standards E 1, and are standardized in accordance with ASTM Method E 77.

The thermometers are of the mercury in glass type, and the column above the liquid is filled with nitrogen. They may be standardized for ''total immersion'' or for ''partial immersion'' and should be used as near as practicable under the same condition of immersion.

Total immersion means standardization with the thermometer immersed to the top of the mercury column, with the remainder of the stem and the upper expansion chamber exposed to the ambient temperature. Partial immersion means standardization with the thermometer immersed to the indicated immersion line etched on the front of the thermometer, with the remainder of the stem exposed to the ambient temperature. If used under any other condition of immersion, an emergent-stem correction is necessary to obtain correct temperature readings.

Thermometer Specifications

ASTM No. E1	Range °C	Range °F	Subdivisions °C	Subdivisions °F	Immersion (mm)
For General Use					
1 C	–20 to +150	—	1	—	76
1 F	—	0 to 302	—	2	76
2 C	–5 to +300	—	1	—	76
2 F	—	20 to 580	—	2	76
3 C	–5 to +400	—	1	—	76
3 F	—	20 to 760	—	2	76
For Determination of Softening Point					
15 C	–2 to +80	—	0.2	—	total
15 F	—	30 to 180	—	0.5	total
16 C	30 to 200	—	0.5	—	total
16 F	—	85 to 392	—	1	total
For Determination of Kinematic Viscosity					
44 F	—	66.5 to 71.5	—	0.1	total
45 F	—	74.5 to 79.5	—	0.1	total
28 F	—	97.5 to 102.5	—	0.1	total
46 F	—	119.5 to 124.5	—	0.1	total
29 F	—	127.5 to 132.5	—	0.1	total
47 F	—	137.5 to 142.5	—	0.1	total
48 F	—	177.5 to 182.5	—	0.1	total
30 F	—	207.5 to 212.5	—	0.1	total

Thermometer Specifications (*continued*)

ASTM No. E1	Range °C	Range °F	Subdivisions °C	Subdivisions °F	Immersion (mm)
For Determination of Distillation Range					
37 C	–2 to +52	—	0.2	—	100
38 C	24 to 78	—	0.2	—	100
39 C	48 to 102	—	0.2	—	100
40 C	72 to 126	—	0.2	—	100
41 C	98 to 152	—	0.2	—	100
102 C	123 to 177	—	0.2	—	100
103 C	148 to 202	—	0.2	—	100
104 C	173 to 227	—	0.2	—	100
105 C	198 to 252	—	0.2	—	100
106 C	223 to 277	—	0.2	—	100
107 C	248 to 302	—	0.2	—	100
For Determination of Solidification Point					
89 C	–20 to +10	—	0.1	—	76
90 C	0 to 30	—	0.1	—	76
91 C	20 to 50	—	0.1	—	76
92 C	40 to 70	—	0.1	—	76
93 C	60 to 90	—	0.1	—	76
94 C	80 to 110	—	0.1	—	76
95 C	100 to 130	—	0.1	—	76
96 C	120 to 150	—	0.1	—	76
100 C	145 to 205	—	0.2	—	76
101 C	195 to 305	—	0.5	—	76
For Special Use					
14 C[a]	38 to 82	—	0.1	—	79
38 C[b]	–2 to +68	—	0.2	—	45
18 C[c]	34 to 42	—	0.1	—	total
18 F[c]	—	94 to 108	—	0.2	total
22 C[c]	95 to 103	—	0.1	—	total
22 F[c]	—	204 to 218	—	0.2	total
23 C[d]	18 to 28	—	0.2	—	90
24 C[d]	30 to 54	—	0.2	—	90
54 F[e]	—	68 to 213	—	0.5	total
71 F[f]	—	–35 to +70	—	1	76

[a]For determination of melting range of Class III solids.
[b]For determination of the titer of fatty acids.
[c]For determination of Saybolt viscosity.
[d]For determination of Engler viscosity.
[e]For determination of congealing point.
[f]For determination of oil in wax.

In selecting a thermometer, careful consideration should be given to the conditions under which it is to be used. The preceding table lists several ASTM thermometers, together with their usual conditions of use, which may be required in *Food Chemicals Codex* tests. Complete specifications for these thermometers are given in ''ASTM Standards on Thermometers.''

VOLUMETRIC APPARATUS

Most of the volumetric apparatus available in the United States is calibrated at 20°, although the temperatures generally prevailing in laboratories more nearly approach 25°, which is the temperature specified generally for tests and assays. This discrepancy is inconsequential provided the room temperature is reasonably constant and the apparatus has been calibrated accurately prior to and under the conditions of its intended use.

Before use, all volumetric ware must be cleaned in such a manner that when rinsed with water, no droplet of water can be seen on the inside walls. Many kinds of ''degreasing'' solutions are available, and the user should consult the manufacturer's literature for the system of choice.

Use To attain the degree of precision required in many assays involving volumetric measurements and directing that a quantity be ''accurately measured,'' the apparatus must be chosen and used with exceptional care. Where less than 10 mL of titrant is to be measured, a 10-mL buret or microburet generally is required.

The design of volumetric apparatus is an important factor in ensuring accuracy. For example, the length of the graduated portions of graduated cylinders should be not less than five times the inside diameter, and the tips of burets should permit an outflow of not more than 0.5 mL/s.

Pipets and burets must be allowed to drain properly in use. Usually, transfer pipets for dilute aqueous solutions should drain for the time specified by the manufacturer before the tip is touched to the wall of the vessel. Buret volumes should not be read immediately upon delivery of the titrant. A suitable length of time should elapse to allow the titrant retained on the walls to drain down. A time interval of 5 to 10 s is usually sufficient.

Standards of Accuracy The capacity tolerances for volumetric flasks, transfer pipets, and burets are those accepted by the National Institute of Standards and Technology (Class A),[1] as indicated in the accompanying tables. Use Class A volumetric apparatus unless otherwise specified in the individual monograph. For plastic volumetric apparatus, the accepted capacity tolerances are Class B.[2]

Volumetric Flasks

	Designated Volume (mL)						
	10	25	50	100	250	500	1000
Limit of error (mL)	0.02	0.03	0.05	0.08	0.12	0.15	0.30
Limit of error (%)	0.20	0.12	0.10	0.08	0.05	0.03	0.03

Transfer Pipets

	Designated Volume (mL)						
	1	2	5	10	25	50	100
Limit of error (mL)	0.006	0.006	0.01	0.02	0.03	0.05	0.08
Limit of error (%)	0.6	0.30	0.20	0.20	0.12	0.10	0.08

[1] See ''Testing of Glass Volumetric Apparatus,'' NBS Circ. 602, April 1, 1959. Apparatus meeting the specifications of NB SIR 74–461 (''The Calibration of Small Volumetric Laboratory Glassware''), as well as of ANSI/ASTM E 694–79 (''Specifications for Volumetric Ware''), is also acceptable.

[2] See ASTM E 288, Fed. Spec. NNN-F-289, and ISO Standard 284.

Burets

	Designated Volume (mL)		
	10 (''micro'' type)	25	50
Subdivisions (mL)	0.02	0.10	0.10
Limit of error (mL)	0.02	0.03	0.05

The capacity tolerances for measuring (i.e., ''graduated'') pipets of up to and including 10-mL capacity are somewhat larger than those for the corresponding sizes of transfer pipets, namely, 0.01, 0.02, and 0.03 mL for the 2-, 5-, and 10-mL sizes, respectively.

Transfer and measuring pipets calibrated ''to deliver'' should be drained in a vertical position and then touched against the wall of the receiving vessel to drain the tips. Volume readings on burets should be estimated to the nearest 0.01 mL for 25- and 50-mL burets, and to the nearest 0.005 mL for 5- and 10-mL burets. Pipets calibrated ''to contain'' may be called for in special cases, generally for measuring viscous fluids. In such cases, the pipet should be washed clean, after draining, and the washings added to the measured portion.

WEIGHTS AND BALANCES

Food Chemicals Codex tests and assays are designed for use with three types of analytical balances, known as micro-, semimicro-, and macro-.

By custom, microbalances weigh objects with a sensitivity down to the microgram range (or lower); semimicrobalances down to the 0.01-mg range; and analytical macrobalances down to the 0.1-mg range.

Tolerances The analytical weights meet the tolerances of the American National Standard ANSI/ASTM E617, ''Laboratory Weights and Precision Mass Standards.'' This standard is incorporated by reference and should be consulted for full descriptions and information on the tolerances and construction of weights.[3] Where quantities of 25 mg or less are to be ''accurately weighed,'' any applicable corrections for weights should be used.

Class 1.1 weights are used for calibration of low-capacity, high-sensitivity balances. They are available in various denominations from 1 to 500 mg. The tolerance for any denomination in this class is 5 μg. They are recommended for calibration of balances using optical or electrical methods for accurately weighing quantities below 20 mg.

Class 1 weights are designated as high-precision standards for calibration. They may be used for accurately weighing quantities below 20 mg.

Class 2 weights are used as working standards for calibration, built-in weights for analytical balances, and laboratory weights for routine analytical work.

Class 3 and Class 4 weights are used with moderate-precision laboratory balances.

Use Where substances are to be ''accurately weighed'' in an assay or a test, the weighing is to be performed in such manner as to limit the error to 0.1% or less. For example, a quantity of 50 mg is to be weighed to the nearest 0.05 mg; a quantity of 0.1 g is to be weighed to the nearest 0.1 mg; and a quantity of 10 g is to be weighed to the nearest 10 mg.

Calibration All precision balances and weights should be calibrated periodically (preferably at least once a year) and a record kept of the calibration date and results. The user may have a set of weights calibrated by the nearest Department of Weights and Measurements (or its equivalent). This is usually done for little or no charge. Alternatively, an independent, outside company may be retained for the purpose of performing such calibrations.

Buoyancy Effect When a weighing is to be performed with an accuracy of 0.1% or better, the buoyancy effect should not be neglected. The equation to be used in correcting for this effect is

$$M_V = M_A[1 + 0.0012(1/D_O + D_W)],$$

in which M_V is the mass in vacuum; M_A is the mass in air; 0.0012 is the density of air; D_O is the density of the weighed object; and D_W is the density of the calibrated weights.

[3] Copies of ASTM Standard E 617-81 (Reapproved 1985) may be obtained from the American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

APPENDIX II: PHYSICAL TESTS AND DETERMINATIONS

A. CHROMATOGRAPHY

Note: Chromatographic separations may also be characterized according to the type of instrumentations or apparatus used. The types of chromatography that may be used in the *Food Chemicals Codex* are column, thin-layer, gas, and high-pressure or high-performance liquid chromatography.

The Committee on Food Chemicals Codex recognizes that the field of chromatography continues to advance. Accordingly, the use of equivalent or improved systems is acceptable with appropriate validation.

For the purposes of the *Food Chemicals Codex*, chromatography is defined as an analytical technique whereby a mixture of chemicals may be separated by virtue of their differential affinities for two immiscible phases. One of these, the stationary phase, consists of a fixed bed of small particles with a large surface area, while the other, the mobile phase, is a gas or liquid that moves constantly through, or over the surface of, the fixed phase. Chromatographic systems achieve their ability to separate mixtures by selectively retarding the passage of some compounds through the stationary phase while permitting others to move more freely. Therefore, the chromatogram may be evaluated qualitatively by determining the R_f, or retardation factor, for each of the eluted substances. The R_f is a measure of that fraction of its total elution time that any compound spends in the mobile phase. Because this fraction is directly related to the fraction of the total amount of the solute that is in the mobile phase, the R_f can be expressed as

$$R_f = V_m C_m/(V_m C_m + V_s C_s),$$

in which V_m and V_s are the volumes of the mobile and stationary phase, respectively, and C_m and C_s are the concentrations of the solute in either phase at any time. This can be simplified to

$$R_f = V_m/(V_m + KV_s),$$

in which $K = C_s/C_m$ and is an equilibrium constant that indicates this differential affinity of the solute for the phases. Alternatively, a new constant, k, the capacity factor, may be introduced, giving another form of the expression:

$$R_f = 1/(1 + k),$$

in which $k = KV_s/V_m$. The capacity factor, k, which is normally constant for small samples, is a parameter that expresses the ability of a particular chromatographic system to interact with a solute. The larger the k value, the more the sample is retarded.

Both the retardation factor and the capacity factor may be used for qualitative identification of a solute or for developing strategies for improving separation. In terms of parameters easily obtainable from the chromatogram, the R_f is defined as the ratio of the distance traveled by the solute band to the distance traveled by the mobile solvent in a particular time. The capacity factor, k, can be evaluated by the expression

$$k = (t_r - t_o)/t_o,$$

in which t_r, the retention time, is the elapsed time from the start of the chromatogram to the elution maximum of the solute, and t_o is the retention time of a solute that is not retained by the chromatographic system.

Retardation of the solutes by the stationary phase may be achieved by one or a combination of mechanisms. Certain substances, such as alumina or silica gel, interact with the solutes primarily by *adsorption*, either *physical adsorption*, in which the binding forces are weak and easily reversible, or *chemisorption*, in which strong bonding to the surface can occur. Another important mechanism of retardation is *partition*, which occurs when the solute dissolves in the stationary phase, usually a liquid coated as a thin layer on the surface of an inert particle or chemically bonded to it. If the liquid phase is a polar substance (e.g., polyethylene glycol) and the mobile phase is nonpolar, the process is termed *normal-phase chromatography*. When the stationary phase is nonpolar (e.g., octadecylsilane) and the mobile phase is polar, the process is *reversed-phase chromatography*. For the separation of mixtures of ionic species, insoluble polymers called *ion exchangers* are used as the stationary phase. Ions of the solutes contained in the mobile phase are adsorbed onto the surface of the ion exchanger while at the same time displacing an electrically equivalent amount of less strongly bound ions to maintain the electroneutrality of both phases. The chromatographic separation of mixtures of large molecules such as proteins may be accomplished by a mechanism called *size exclusion chromatography*. The stationary phases used are highly cross-linked polymers that have imbibed a sufficient amount of solvent to form a gel. The separation is based on the physical size of the solvated solutes; those that are too large to fit within the interstices of the gel are eluted rapidly, while the smaller molecules permeate into the pores of the gel and are eluted later. In any chromatographic separation, more than one of the above mechanisms may be occurring simultaneously.

Chromatographic separations may also be characterized according to the type of instrumentation or apparatus used. The types of chromatography that may be used in the FCC are column, thin-layer, gas, and high-performance liquid chromatography.

COLUMN CHROMATOGRAPHY

Apparatus The equipment needed for column chromatography is not elaborate, consisting only of a cylindrical glass or

Teflon tube that has a restricted outflow orifice. The dimensions of the tube are not critical and may vary from 10 to 40 mm in inside diameter and from 100 to 600 mm in length. For a given separation, greater efficiency may be obtained with a long narrow column, but the resultant flow rate will be lower. A fritted-glass disk may be seated in the end of the tube to act as a support for the packing material. The column is fitted at the end with a stopcock or other flow-restriction device to control the rate of delivery of the eluant.

Procedure The stationary phase is introduced into the column either as a dry powder or as a slurry in the mobile phase. Because a homogeneous bed free of void spaces is necessary to achieve maximum separation efficiency, the packing material is introduced in small portions and allowed to settle before further additions are made. Settling may be accomplished by allowing the mobile phase to flow through the bed, by tapping or vibrating the column if a dry powder is used, or by compressing each added portion using a tamping rod. The rod can be a solid glass, plastic, or metal cylinder whose diameter is slightly smaller than that of the column, or it can be a thinner rod onto the end of which has been attached a disk of suitable diameter. Ion-exchange resins and exclusion polymers are never packed as dry powders because after introduction of the mobile phase, they will swell and create sufficient pressure to shatter the column. When the packing has been completed, the sample is introduced onto the top of the column. If the sample is soluble, it is dissolved in a minimum amount of the mobile phase, pipetted onto the column, and allowed to percolate into the top of the bed. If it is not soluble or if the volume of solution is too large, it may be mixed with a small amount of the column packing. This material is then transferred to the chromatographic tube to form the top of the bed.

The chromatogram is then developed by adding the mobile phase to the column in small portions and allowing it to percolate through the packed bed either by gravity or under the influence of pressure or vacuum. Development of the chromatogram takes place by selective retardation of the components of the mixture as a result of their interaction with the stationary phase. In column chromatography, the stationary phase may act by adsorption, partition, ion exchange, exclusion of the solutes, or a combination of these effects.

When the development is complete, the components of the sample mixture may be detected and isolated by either of two procedures. The entire column may be extruded carefully from the tube, and if the compounds are colored or fluorescent under ultraviolet light, the appropriate segments may be cut from the column using a razor blade. If the components are colorless, they may be visualized by painting or spraying a thin longitudinal section of the surface of the chromatogram with color-developing reagents. The chemical may then be separated from the stationary phase by extraction with a strong solvent such as methanol and subsequently quantitated by suitable methods.

In the second procedure, the mobile phase may be allowed to flow through the column until the components of the mixture successively appear in the effluent. This eluate may be collected in fractions and the mobile phase evaporated if desired. The chemicals present in each fraction may then be determined by suitable analytical techniques.

THIN-LAYER CHROMATOGRAPHY

In thin-layer chromatography (TLC), the stationary phase is a uniform layer of a finely divided powder that has been coated on the surface of a glass or plastic sheet and that is held in place by a binder. The capacity of the system is dependent on the thickness of the layer, which may range from 0.1 to 2.0 mm. The thinner layers are used primarily for analytical separations, while the thicker layers, because of their greater sample-handling ability, are useful for preparative work.

Substances that are used as coatings in TLC include silica gel, alumina, cellulose, and reversed-phase packings. Separations occur because of adsorption of the solutes from the mobile phase onto the surface of the thin layer. However, adsorption of water from the air or solvent components from the mobile phase can give rise to partition or liquid–liquid chromatography. Specially coated plates are available that permit ion-exchange or reversed-phase separations.

Apparatus Acceptable apparatus and materials for thin-layer chromatography consist of the following:

Glass Plates Flat glass plates of uniform thickness throughout their areas. The most common sizes are 20, 10, and 5 cm × 20 cm. (Aluminum plates also are commonly used.)

Aligning Tray An aligning tray or other suitable flat surface is used to align and hold plates during application of the adsorbent.

Adsorbent The adsorbent may consist of finely divided adsorbent materials for chromatography. It can be applied directly to the glass plate, or it can be bonded to the plate by means of plaster of Paris or with starch paste. Pretreated chromatographic plates are available commercially.

Spreader A suitable spreading device that, when moved over the glass plate, applies a uniform layer of adsorbent of the desired thickness over the entire surface of the plate.

Storage Rack A rack of convenient size to hold the prepared plates during drying and transportation.

Developing Chamber A glass chamber that can accommodate one or more plates and can be properly closed and sealed. It is fitted with a plate-support rack that can support the plates when the lid of the chamber is in place.

> **Note**: Preformed TLC plates available commercially may also be used.

Procedure Clean the plates scrupulously, as by immersion in a chromic acid cleansing mixture, rinse them with copious quantities of water until the water runs off the plates without leaving any visible water or oily spots, and dry.

Arrange the plate or plates on the aligning tray, and secure them so that they will not slip during the application of the adsorbent. Mix an appropriate quantity of adsorbent and liq-

uid, usually water, which when shaken for 30 s gives a smooth slurry that will spread evenly with the aid of a spreader. Transfer the slurry to the spreader, and apply the coating at once before the binder begins to harden. Move the spreader smoothly over the plates from one end of the tray to the other. Remove the spreader, and wipe away excess slurry. Allow the plates to set for 10 min, and then place them in the storage rack and dry at 105° for 30 min or as directed in the individual monograph. Store the finished plates in a desiccator.

Equilibrate the atmosphere in the *Developing Chamber* by placing in it a volume of the mobile phase in excess of that required for complete development of the chromatogram, cover the chamber with its lid, and allow it to stand for at least 30 min.

Apply the *Sample Solution* and the *Standard Solution* at points about 1.5 cm apart and about 2 cm from the lower edge of the plate (the lower edge is the first part over which the spreader moves in the application of the adsorbent layer), and allow to dry. A template will aid in determining the spot points and the 10- to 15-cm distance through which the solvent front should move.

Arrange the plate on the supporting rack (sample spots on the bottom), and introduce the rack into the developing chamber. The solvent in the chamber must be deep enough to reach the lower edge of the adsorbent, but must not touch the spot points. Seal the cover in place, and maintain the system until the solvent ascends to a point 10 to 15 cm above the initial spots; this usually requires 15 min to 1 h. Remove the plates, and dry them in air. Measure and record the distance of each spot from the point of origin. If so directed, spray the spots with the reagent specified, observe, and compare the sample with the standard chromatogram.

Detection and Identification Detection and identification of solute bands is done by methods essentially the same as those described in *Column Chromatography*. However, in TLC an additional method called *fluorescence quenching* is also used. In this procedure, an inorganic phosphor is mixed with the adsorbent before it is coated on the plate. When the developed chromatogram is irradiated with ultraviolet light, the surface of the plate fluoresces with a characteristic color, except in those places where ultraviolet-absorbing solutes are situated. These quench the fluorescence and are detectable as dark spots.

Detection with an ultraviolet light source suitable for observations with short (254-nm) and long (360-nm) ultraviolet wavelengths may be called for in some cases.

Quantitative Analysis Two methods are available if quantitation of the solute is necessary. In the first, the bands are detected and their positions marked. Those areas of adsorbent containing the compounds of interest are scraped from the surface of the plate into a centrifuge tube. The chemicals are extracted from the adsorbent with the aid of a suitable strong solvent, the suspension is centrifuged, and the supernatant layer is subjected to appropriate methods of quantitative analysis.

The second method involves the use of a scanning densitometer. This is a spectrophotometric device that directs a beam of monochromatic radiation across the surface of the plate. After interaction with the solutes in the adsorbent layer, the radiation is detected as transmitted or reflected light and a recording of light intensity versus distance traveled is produced. The concentration of a particular species is proportional to the area under its peak and can be determined accurately by comparison with standards.

GAS CHROMATOGRAPHY

The distinguishing features of gas chromatography are a gaseous mobile phase and a solid or immobilized liquid stationary phase. Liquid stationary phases are available in packed or capillary columns. In the packed columns, the liquid phase is deposited on a finely divided, inert solid support, such as diatomaceous earth or porous polymer, which is packed into a column that typically has a 2- to 4-mm id and is 1 to 3 m long. In capillary columns, which contain no particles, the liquid phase is deposited on the inner surface of the fused silica column and may be chemically bonded to it. In gas–solid chromatography, the solid phase is an active adsorbent, such as alumina, silica, or carbon, packed into a column. Polyaromatic porous resins, which are sometimes used in packed columns, are not coated with a liquid phase.

When a volatile compound is introduced into the carrier gas and carried into the column, it is partitioned between the gas and stationary phases by a dynamic countercurrent distribution process. The compound is carried down the column by the carrier gas, retarded to a greater or lesser extent by sorption and desorption in the stationary phase. The elution of the compound is characterized by the partition ratio, k, a dimensionless quantity also called the capacity factor. It is equivalent to the ratio of the time required for the compound to flow through the column (the retention time) to the retention time of a nonretarded compound. The value of the capacity factor depends on the chemical nature of the compound; the nature, amount, and surface area of the liquid phase; and the column temperature. Under a specified set of experimental conditions, a characteristic capacity factor exists for every compound. Separation by gas chromatography occurs only if the compounds concerned have different capacity factors.

Apparatus A gas chromatograph consists of a carrier gas source, an injection port, column, detector, and recording device. The injection port, column, and detector are carefully temperature controlled. The typical carrier gas is helium or nitrogen, depending on the column and detector in use. The gas is supplied from a high-pressure cylinder and passes through suitable pressure-reducing valves to the injection port and column. Compounds to be chromatographed, either in solution or as gases, are injected into the gas stream at the injection port. Depending on the configuration of the apparatus, the test mixture may be injected directly into the column or be vaporized in the injection port and mixed into the flowing carrier gas before entering the column.

Once in the column, compounds in the test mixture are separated by virtue of differences in their capacity factors, which in turn depend on their vapor pressure and degree of interaction with the stationary phase. The capacity factor, which governs resolution and retention times of components of the test mixture, is also temperature dependent. The use of temperature-programmable column ovens takes advantage of this dependence to achieve efficient separation of compounds differing widely in vapor pressure.

As resolved compounds emerge from the column, they pass through a detector, which responds to the amount of each compound present. The type of detector to be used depends on the nature of the compounds to be analyzed, and is specified in the individual monograph. Detectors are heated above the maximum column operating temperature to prevent condensation of the eluting compounds.

Detector output is recorded as a function of time, producing a chromatogram, which consists of a series of peaks on a time axis. Each peak represents a compound in the vaporized test mixture, although some peaks may overlap. The elution time is characteristic of the individual compounds (qualitative analysis), and the peak area is a function of the amount present (quantitative analysis).

Injectors Sample injection devices range from simple syringes to fully programmable automatic injectors. The amount of sample that can be injected into a capillary column without overloading is small compared with the amount that can be injected into a packed column, and may be less than the smallest amount that can be manipulated satisfactorily by syringe. Capillary columns are therefore used with injectors able to split samples into two fractions, a small one that enters the column and a large one that goes to waste (split injector). Such injectors may also be used in a splitless mode for analyses of trace or minor components.

Purge and trap injectors are equipped with a sparging device by which volatile compounds in solution are carried into a low-temperature trap. When sparging is complete, trapped compounds are thermally desorbed into the carrier gas by rapid heating of the temperature-programmable trap.

Headspace injectors are equipped with a thermostatically controlled sample-heating chamber. Solid or liquid samples in tightly closed containers are heated in the chamber for a fixed period of time, allowing the volatile components in the sample to reach an equilibrium between the nongaseous phase and the gaseous or headspace phase.

After this equilibrium has been established, the injector automatically introduces a fixed amount of the headspace in the sample container into the gas chromatograph.

Columns Capillary columns, which are usually made of fused silica, have a 0.2- to 0.53-mm id and are 5 to 30 m long. The liquid or stationary phase is 0.1 to 1.0 μm thick, although nonpolar stationary phases may be up to 5 μm thick.

Packed columns, made of glass or metal, are 1 to 3 m long, with a 2- to 4-mm id. Those used for analysis typically have liquid phase loadings of about 5% (w/w) on a solid support.

Supports for analysis of polar compounds on low-capacity, low-polarity liquid phase columns must be inert to avoid peak tailing. The reactivity of support materials can be reduced by silanizing before coating with liquid phase. Acid-washed, flux-calcined diatomaceous earth is often used for drug analysis. Support materials are available in various mesh sizes, with 80- to 100-mesh and 100- to 120-mesh being more commonly used with 2- to 4-mm columns. Because of the absence of a solid support, capillary compounds are much more inert than packed columns.

Retention time and the peak efficiency depend on the carrier gas flow rate; retention time is also directly proportional to column length, while resolution is proportional to the square root of the column length. For packed columns, the carrier gas flow rate is usually expressed in milliliters per minute at atmospheric pressure and room temperature. It is measured at the detector outlet with a soap film flow meter while the column is at operating temperature. Unless otherwise specified in the individual monograph, flow rates for packed columns are 60 to 75 mL/min for 4-mm id columns and ~30 mL/min for 2-mm id columns.

For capillary columns, linear flow velocity is often used instead of flow rate. This is conveniently determined from the length of the column and the retention time of a dilute methane sample, provided a flame-ionization detector is in use. Typical linear velocities are 20 to 60 cm/s for helium. At high operating temperatures there is sufficient vapor pressure to result in a gradual loss of liquid phase, a process called ''bleeding.''

Detectors Flame-ionization detectors are used for most analyses, with lesser use made of thermal conductivity, electron-capture, nitrogen–phosphorus, and mass spectrometric detectors. For quantitative analyses, detectors must have a wide linear dynamic range: the response must be directly proportional to the amount of compound present in the detector over a wide range of concentrations. Flame-ionization detectors have a wide linear range ($\sim 10^6$) and are sensitive to organic compounds. Unless otherwise specified in individual monographs, flame-ionization detectors with either helium or nitrogen carrier gas are to be used for packed columns, and helium is used for capillary columns.

The thermal conductivity detector detects changes in the thermal conductivity of the gas stream as solutes are eluted. Although its linear dynamic range is smaller than that of the flame-ionization detector, it is quite rugged and occasionally used with packed columns, especially for compounds that do not respond to flame-ionization detectors.

The alkali flame-ionization detector, sometimes called an NP or nitrogen–phosphorus detector, contains a thermionic source, such as an alkali-metal salt or a glass element containing rubidium or other metal, that results in the efficient ionization of organic nitrogen and phosphorus compounds. It is a selective detector that shows little response to hydrocarbons.

The electron-capture detector contains a radioactive source (usually ^{63}Ni) of ionizing radiation. It exhibits an extremely high response to compounds containing halogens and nitro groups but little response to hydrocarbons. The sensitivity increases with the number and atomic weight of the halogen atoms.

Data Collection Devices Modern data stations receive the detector output, calculate peak areas, and print chromato-

grams, complete with run parameters and peak data. Chromatographic data may be stored and reprocessed, with integration and other calculation variables being changed as required. Data stations are used also to program the chromatograph, controlling most operational variables and providing for long periods of unattended operation.

Data can also be collected for manual measurement on simple recorders or on integrators whose capabilities range from those providing a printout of peak areas to those providing chromatograms with peak areas and peak heights calculated and data stored for possible reprocessing.

Procedure Capillary columns must be tested to ensure that they comply with the manufacturers' specifications before they are used. These tests consist of the following injections: a dilute methane sample to determine the linear flow velocity; a mixture of alkanes (e.g., C_{14}, C_{15}, and C_{16}) to determine resolution; and a polarity test mixture to check for active sites on the column. The latter mixture may include a methyl ester, an unsaturated compound, a phenol, an aromatic amine, a diol, a free carboxylic acid, and a polycyclic aromatic compound, depending on the samples to be analyzed.

Packed columns must be conditioned before use until the baseline and other characteristics are stable. This may be done by operation at a temperature above that called for by the method or by repeated injections of the compound or mixture to be chromatographed. A suitable test for support inertness should be done. Very polar molecules (like free fatty acids) may require a derivatization step.

Before any column is used for assay purposes, a calibration curve should be constructed to verify that the instrumental response is linear over the required range and that the curve passes through the origin. If the compound to be analyzed is adsorbed within the system, the calibration curve will intersect the abscissa at a nonzero value. This may result in error, particularly for compounds at low concentrations determined by a procedure based on a single reference point. At high concentrations, the liquid phase may be overloaded, leading to loss of peak height and symmetry.

Assays require quantitative comparison of one chromatogram with another. A major source of error is irreproducibility in the amount of sample injected, notably when manual injections are made with a syringe. The effects of variability can be minimized by addition of an internal standard, a noninterfering compound present at the same concentration as in the sample and standard solutions. The ratio of peak response of the analyte to that of the internal standard is compared from one chromatogram to another. Where the internal standard is chemically similar to the substance being determined, there is also compensation for minor variations in column and detector characteristics. In some cases, the internal standard may be carried through the sample preparation procedure before gas chromatography to control other quantitative aspects of the assay. Automatic injectors greatly improve the reproducibility of sample injections and reduce the need for internal standards.

Many monographs require that system suitability requirements be met before samples are analyzed, see *System Suitability* below.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

High-performance liquid chromatography (HPLC) is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, exclusion, or ion-exchange processes, depending on the type of stationary phase used. HPLC has distinct advantages over gas chromatography for the analysis of nonvolatile organic compounds. Compounds to be analyzed are dissolved in a liquid, and most separations take place at room temperature.

As in gas chromatography, the elution time of a compound can be described by the capacity factor, k, which depends on the chemical nature of the composition and flow rate of the mobile phase, and the composition and surface area of the stationary phase. Column length is an important determinant of resolution. Only compounds having different capacity factors can be separated by HPLC.

Apparatus A liquid chromatograph consists of one, two, or more reservoirs containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data collection device such as a computer, integrator, or recorder. Short, 3-, 5-, 10-, and 25-cm, small-bore columns containing densely packed particles of stationary phase provide for the rapid exchange of compounds between the mobile and stationary phases. In addition to receiving and reporting detector output, computers are used to control chromatographic settings and operations, thus providing for long periods of unattended operation.

Pumping Systems HPLC pumping systems deliver metered amounts of mobile phase from the solvent reservoirs to the column through high-pressure tubing and fittings. Modern systems consist of one or more computer-controlled metering pumps that can be programmed to vary the ratio of mobile phase components, as is required for gradient chromatography, or to mix isocratic mobile phases (i.e., mobile phases having a fixed ratio of solvents). However, the proportion of ingredients in premixed isocratic mobile phases can be more accurately controlled than in those delivered by most pumping systems. Operating pressures up to 5000 psi with delivery rates up to about 10 mL/min are typical. Pumps used for quantitative analysis should be constructed of materials inert to corrosive mobile phase components and be capable of delivering the mobile phase at a constant rate with minimal fluctuations over extended periods of time.

Injectors After dissolution in mobile phase or other suitable solution, compounds to be chromatographed are injected into the mobile phase, either manually by syringe or loop injectors, or automatically by autosamplers. The latter consist of a carousel or rack to hold sample vials with tops that have a pierceable septum or stopper and an injection device to transfer sample from the vials to a calibrated, fixed-volume loop from which it is loaded into the chromatograph. Some autosamplers can be programmed to control sample volume, the number of injections and loop rinse cycles, the interval between injections, and other operating variables.

Some valve systems incorporate a calibrated sample loop that is filled with test solution for transfer to the column in the mobile phase. In other systems, test solution is transferred to a cavity by syringe and then switched into the mobile phase.

Columns For most analyses, separation is achieved by partition of compounds in the test solution between the mobile and stationary phases. Systems consisting of polar stationary phases and nonpolar mobile phases are described as normal phase, while the opposite arrangement, polar mobile phases and nonpolar stationary phases, is called reversed-phase chromatography. Partition chromatography is almost always used for hydrocarbon-soluble compounds of a molecular weight that is less than 1000. The affinity of a compound for the stationary phase, and thus its retention time on the column, is controlled by making the mobile phase more or less polar. Mobile phase polarity can be varied by the addition of a second, and sometimes a third or even a fourth, component.

Stationary phases for modern, reversed-phase liquid chromatography typically consist of an organic phase chemically bound to silica or other materials. Particles are usually 3, 5, or 10 μm in diameter, but sizes may range up to 50 μm for preparative columns. Small particles thinly coated with organic phase allow fast mass transfer and, hence, rapid transfer of compounds between the stationary and mobile phases. Column polarity depends on the polarity of the bound functional groups, which range from relatively nonpolar octadecyl silane to very polar nitrile groups.

Columns used for analytical separations usually have internal diameters of 2 to 4.6 mm; larger diameter columns are used for preparative chromatography. Columns may be heated to give more efficient separations, but only rarely are they used at temperatures above 60° because of potential stationary phase degradation or mobile phase volatility. Unless otherwise specified in the individual monograph, columns are used at an ambient temperature.

Ion-exchange chromatography is used to separate water-soluble, ionizable compounds of molecular weights that are less than 2000. The stationary phases are usually synthetic organic resins; cation-exchange resins contain negatively charged active sites and are used to separate basic substances such as amines; while anion-exchange resins have positively charged active sites for separation of compounds with negatively charged groups such as phosphate, sulfonate, or carboxylate groups. Water-soluble ionic or ionizable compounds are attracted to the resins, and differences in affinity bring about the chromatographic separation. The pH of the mobile phase, temperature, ion type, ionic concentration, and organic modifiers affect the equilibrium, and these variables can be adjusted to obtain the desired degree of separation.

In size-exclusion chromatography, columns are packed with a porous stationary phase. Molecules of the compounds being chromatographed are filtered according to size. Those too large to enter the pores pass unretained through the column (total exclusion). Smaller molecules enter the pores and are increasingly retained as molecular size decreases. These columns are typically used to remove high molecular weight matrices or to characterize the molecular weight distribution of a polymer.

Detectors Many compendial HPLC methods require the use of spectrophotometric detectors. Such a detector consists of a flow-through cell mounted at the end of the column. A beam of ultraviolet radiation passes through the flow cell and into the detector. As compounds elute from the column, they pass through the cell and absorb the radiation, resulting in measurable energy level changes.

Fixed, variable, and photodiode array (PDA) detectors are widely available. Fixed wavelength detectors operate at a single wavelength, typically 254 nm, emitted by a low-pressure mercury lamp. Variable wavelength detectors contain a continuous source, such as a deuterium or high-pressure xenon lamp, and a monochromator or an interference filter to generate monochromatic radiation at a wavelength selected by the operator. Modern variable wavelength detectors can be programmed to change wavelength while an analysis is in progress. Multi-wavelength detectors measure absorbance at two or more wavelengths simultaneously. In diode array multi-wavelength detectors, continuous radiation is passed through the sample cell, then resolved into its constituent wavelengths, which are individually detected by the photodiode array. These detectors acquire absorbance data over the entire UV-visible range, thus providing the analyst with chromatograms at multiple, selectable wavelengths and spectra of the eluting peaks. Diode array detectors usually have lower signal-to-noise ratios than fixed or variable wavelength detectors, and thus are less suitable for analysis of compounds present at low concentrations.

Differential refractometer detectors measure the difference between the refractive index of the mobile phase alone and that of the mobile phase containing chromatographed compounds as it emerges from the column. Refractive index detectors are used to detect non-UV absorbing compounds, but they are less sensitive than UV detectors. They are sensitive to small changes in solvent composition, flow rate, and temperature, so that a reference column may be required to obtain a satisfactory baseline.

Fluorometric detectors are sensitive to compounds that are inherently fluorescent or that can be converted to fluorescent derivatives either by chemical transformation of the compound or by coupling with fluorescent reagents at specific functional groups. If derivatization is required, it can be done before chromatographic separation or, alternatively, the reagent can be introduced into the mobile phase just before its entering the detector.

Potentiometric, voltammetric, or polarographic electrochemical detectors are useful for the quantitation of species that can be oxidized or reduced at a working electrode. These detectors are selective, sensitive, and reliable, but require conducting mobile phases free of dissolved oxygen and reducible metal ions. A pulseless pump must be used, and care must be taken to ensure that the pH, ionic strength, and temperature of the mobile phase remain constant. Working electrodes are prone to contamination by reaction products with consequent variable responses.

Electrochemical detectors with carbon-paste electrodes may be used advantageously to measure nanogram quantities of easily oxidized compounds, notably phenols and catechols.

Data Collection Devices Modern data stations receive and store detector output and print out chromatograms complete with peak heights, peak areas, sample identification, and method variables. They are also used to program the liquid chromatograph, controlling most variables and providing for long periods of unattended operation.

Data also may be collected on simple recorders for manual measurement or on stand-alone integrators, which range in complexity, from those providing a printout of peak areas to those providing a printout of peak areas and peak heights calculated and data stored for possible subsequent reprocessing.

Procedure The mobile phase composition significantly influences chromatographic performance and the resolution of compounds in the mixture being chromatographed. Composition has a much greater effect than temperature on the capacity factor, *k*.

In partition chromatography, the partition coefficient, and hence the separation, can be changed by addition of another component to the mobile phase. In ion-exchange chromatography, pH and ionic strength as well as changes in the composition of the mobile phase affect capacity factors. The technique of continuously increasing mobile phase strength during the chromatographic run is called gradient elution or solvent programming. It is sometimes used to chromatograph complex mixtures of components differing greatly in their capacity factors. Detectors that are sensitive to change in solvent composition, such as the differential refractometer, are more difficult to use with the gradient elution technique.

For accurate quantitative work, high-purity, ''HPLC-grade'' solvents and reagents must be used. The detector must have a broad linear dynamic range, and compounds to be measured must be resolved from any interfering substances. The linear dynamic range of a compound is the range over which the detector signal response is directly proportional to the amount of the compound. For maximum flexibility in quantitative work, this range should be about three orders of magnitude. HPLC systems are calibrated by plotting peak responses in comparison with known concentrations of a reference standard, using either an external or an internal standardization procedure.

Reliable quantitative results are obtained by external calibration if automatic injectors or autosamplers are used. This method involves direct comparison of the peak responses obtained by separately chromatographing the test and reference standard solutions. If syringe injection, which is irreproducible at the high pressures involved, must be used, better quantitative results are obtained by the internal calibration procedure where a known amount of a noninterfering compound, the internal standard, is added to the test and reference standard solutions, and the ratios of peak responses of the analyte and internal standard are compared.

Because of normal variations in equipment, supplies, and techniques, a system suitability test is required to ensure that a given operating system may be generally applicable. The main features of *System Suitability* tests are described below.

For information on the interpretation of results, see the section *Interpretation of Chromatograms*.

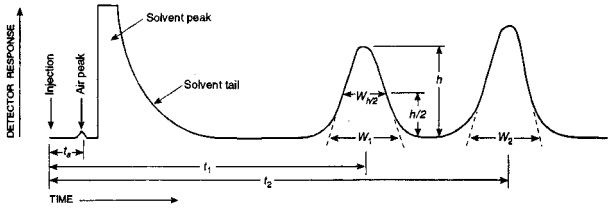


FIGURE 1 Chromatographic Separation of Two Substances.

Interpretation of Chromatograms Fig. 1 represents a typical chromatographic separation of two substances, 1 and 2, in which $t_{R(1)}$ and $t_{R(2)}$ are the respective retention times; h, $h/2$, and $W_{h/2}$ are the height, the half-height, and the width at half-height, respectively, for peak 1; and W_1 and W_2 are the respective widths of peaks 1 and 2 at the baseline. Air peaks are a feature of gas chromatograms and correspond to the solvent front in liquid chromatography.

Chromatographic retention times are characteristic of the compounds they represent but are not unique. Coincidence of retention times of a test and a reference substance can be used as a feature in construction of an identity profile but is insufficient on its own to establish identity. Absolute retention times of a given compound vary from one chromatogram to the next. Comparisons are normally made in terms of relative retention, which is calculated by the equation

$$\alpha = (t_{R(2)} - t_{R(0)})/(t_{R(1)} - t_O),$$

in which $t_{R(2)}$ and $t_{R(1)}$ are the retention times, measured from the point of injection, of the test and reference substances, respectively, determined under identical experimental conditions on the same column, and t_O is the retention time of a nonretained substance, such as methane in this case, of gas chromatography.

In this and the following expressions, the corresponding retention volumes or linear separations on the chromatogram, both of which are directly proportional to retention time, may be substituted in the equations. Where the value of t_O is small, R_r may be estimated from the retention times measured from the point of injection $(t_{R(2)}/t_{R(1)})$.

The number of theoretical plates, N, is a measure of column efficiency. For Gaussian peaks, it is calculated by the equations

$$N = 16(t_R/W)^2 \text{ or } N = 5.54(t_R/W_{½})^2,$$

in which t_R is the retention time of the substance and W is the width of the peak at its base, obtained by extrapolating the relatively straight sides of the peak to the baseline. $W_{1/2}$ is the peak width at half-height, obtained directly by electronic integrators. The value of N depends on the substance being chromatographed as well as the operating conditions such as mobile phase or carrier gas flow rates and temperature, the quality of the packing, the uniformity of the packing within the column, and for capillary columns, the thickness of the stationary phase film and the internal diameter and length of the column.

The separation of two components in a mixture, the resolution, R, is determined by the equation

$$R = 2(t_{R(2)} - t_{R(1)})/(W_2 + W_1),$$

in which $t_{R(2)}$ and $t_{R(1)}$ are the retention times of the two components, and W_2 and W_1 are the corresponding widths at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

Peak areas and peak heights are usually proportional to the quantity of compound eluting. These are commonly measured by electronic integrators but may be determined by more classical approaches. Peak areas are generally used but may be less accurate if peak interference occurs. For manual measurements, the chart should be run faster than usual, or a comparator should be used to measure the width at half-height and the width at the base of the peak, to minimize error in these measurements. For accurate quantitative work, the components to be measured should be separated from any interfering components. Peak tailing and fronting and the measurement of peaks on solvent tails are to be avoided (see Fig. 2). The relative standard deviation is expressed by the equation

$$S_R\ (\%) = (100/\overline{X})\ \{[\sum_{i-1}^{N} (X_i - \overline{X})^2]/(N-1)\}^{1/2},$$

in which S_R is the relative standard deviation in percent, $\overline{X}$ is the mean of the set of N measurements, and X_i is an individual measurement. When an internal standard is used, the measurement X_i usually refers to the measurement of relative area, A_s,

$$X_i = A_s = a_r/a_i,$$

in which a_r is the area of the peak corresponding to the standard substance and a_i is the area of the peak corresponding to the internal standard. When peak heights are used, the measurement X_i refers to the measurement of relative heights, H_s,

$$X_i = H_s = h_r/h_i,$$

in which h_r is the height of the peak corresponding to the standard substance and h_i is the height of the peak corresponding to the internal standard.

System Suitability Such tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such.

The resolution, R, is a function of column efficiency, N, and is specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the analyte. Column efficiency may be specified also as a system suitability requirement, especially if there is only one peak of interest in the chromatogram; however, it is a less reliable means to ensure resolution than direct measurement. Column efficiency is a measure of peak sharpness, which is important for the detection of trace components.

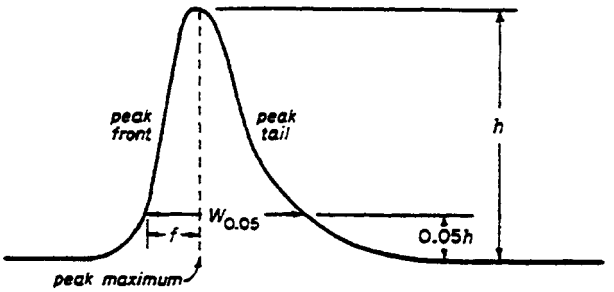


FIGURE 2 Asymmetrical Chromatographic Peak.

Replicate injections of a standard preparation used in the assay or other standard solution are compared to ascertain whether requirements for precision are met. Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate the relative standard deviation if the requirement is 2.0% or less; data from six replicate injections are used if the relative standard deviation requirement is more than 2.0%.

The tailing factor, T, a measure of peak symmetry, is unity for perfectly symmetrical peaks, and its value increases as tailing becomes more pronounced. In some cases, values less than unity may be observed. As peak asymmetry increases, integration, and hence precision, becomes less reliable. The calculation is expressed by the equation

$$\text{tailing factor} = T = W_{0.05}/2f.$$

These tests are performed by collecting data from replicate injections of standard or other solutions as specified in the individual monograph. The specification of definitive parameters in a monograph does not preclude the use of other suitable operating conditions (see *Procedures* under *Tests and Assays* in *General Provisions*). Adjustments of operating conditions to meet system suitability requirements may be necessary.

Unless otherwise directed in the monograph, system suitability parameters are determined from the analyte peak.

To ascertain the effectiveness of the final operating system, it should be subjected to a suitability test before use and during testing whenever there is a significant change in equipment or in a critical reagent or when a malfunction is suspected.

B. PHYSICOCHEMICAL PROPERTIES

DISTILLATION RANGE

Scope This method is to be used for determining the distillation range of pure or nearly pure compounds or mixtures having a relatively narrow distillation range of about 40° or less. The result so determined is an indication of purity, not necessarily of identity. Products having a distillation range of greater than 40° may be determined by this method if a wide-range thermometer, such as ASTM E1, 1C, 2C, or 3C, is specified in the individual monograph.

Definitions

Distillation Range The difference between the temperature observed at the start of a distillation and that observed at which a specified volume has distilled, or at which the dry point is reached.

Initial Boiling Point The temperature indicated by the distillation thermometer at the instant the first drop of condensate leaves the end of the condenser tube.

Dry Point The temperature indicated at the instant the last drop of liquid evaporates from the lowest point in the distillation flask, disregarding any liquid on the side of the flask.

Apparatus

Distillation Flask A 200-mL round-bottom distilling flask of heat-resistant glass is preferred when sufficient sample (in excess of 100 mL) is available for the test. If a sample of less than 100 mL must be used, a smaller flask having a capacity of at least double the volume of the liquid taken may be employed. The 200-mL flask has a total length of 17 to 19 cm, and the inside diameter of the neck is 20 to 22 mm. Attached about midway on the neck, approximately 12 cm from the bottom of the flask, is a side arm 10 to 12.7 cm long and 5 mm in internal diameter, which forms an angle of 70° to 75° with the lower portion of the neck.

Condenser Use a straight glass condenser of heat-resistant tubing, 56 to 60 cm long and equipped with a water jacket so that about 40 cm of the tubing is in contact with the cooling medium. The lower end of the condenser may be bent to provide a delivery tube or it may be connected to a bent adapter that serves as the delivery tube.

> **Note**: All-glass apparatus with standard-taper ground joints may be used alternatively if the assembly employed provides results equal to those obtained with the flask and condenser described above.

Receiver The receiver is a 100-mL cylinder that is graduated in 1-mL subdivisions and calibrated ''to contain.'' It is used for measuring the sample as well as for receiving the distillate.

Thermometer An accurately standardized partial-immersion thermometer having the smallest practical subdivisions (not greater than 0.2°) is recommended to avoid the necessity for an emergent stem correction. Suitable thermometers are available as the ASTM E1 Series 37C through 41C, and 102C through 107C, or as the MCA types R-1 through R-4 (see *Thermometers*, Appendix I).

Source of Heat A Bunsen burner is the preferred source of heat. An electric heater may be used, however, if it is shown to give results comparable to those obtained with the gas burner.

Shield The entire burner and flask assembly should be protected from external air currents. Any efficient shield may be employed for this purpose.

Flask Support A heat-resistant board, 5 to 7 mm in thickness and having a 10-cm circular hole, is placed on a suitable ring or platform support and fitted loosely inside the shield to ensure that hot gases from the source of heat do not come in contact with the sides or neck of the flask. A second 5- to 7-mm thick heat-resistant board, 14- to 16-cm square and provided with a 30- to 40-mm circular hole, is placed on top of the first board. This board is used to hold the 200-mL distillation flask, which should be fitted firmly on the board so that direct heat is applied to the flask only through the opening in the board.

Procedure (**Note**: For materials boiling below 50°, cool the liquid to below 10° before sampling, receive the distillate in a water bath cooled to below 10°, and use water cooled to below 10° in the condenser.) Measure 100 ± 0.5 mL of the liquid in the 100-mL graduate, and transfer the sample, together with an efficient antibumping device, into the distilling flask. Do not use a funnel in the transfer or allow any of the sample to enter the side arm of the flask. Place the flask on the heat-resistant boards, which are supported on a ring or platform, and position the shield for the flask and burner. Connect the flask and condenser, place the graduate under the outlet of the condenser tube, and insert the thermometer. The thermometer should be located in the center of the neck so that the top of the contraction chamber (or bulb, if 37C or 38C is used) is level with the bottom of the outlet to the side arm. Regulate the heating so that the first drop of liquid is collected within 5 to 10 min. Read the thermometer at the instant the first drop of distillate falls from the end of the condenser tube, and record as the initial boiling point. Continue the distillation at the rate of 4 or 5 mL of distillate per minute, noting the temperature as soon as the last drop of liquid evaporates from the bottom of the flask (dry point) or when the specified percentage has distilled over. Correct the observed temperature readings for any variation in the barometric pressure from the normal (760 mm) by allowing 0.1° for each 2.7 mm of variation, adding the correction if the pressure is lower, or subtracting if higher, than 760 mm.

When a total-immersion thermometer is used, correct for the temperature of the emergent stem by the formula

$$0.00015 \times N(T - t),$$

in which N represents the number of degrees of emergent stem from the bottom of the stopper, T represents the observed temperatures of the distillation, and t represents the temperature registered by an auxiliary thermometer, the bulb of which is placed midway of the emergent stem, adding the correction to the observed readings of the main thermometer.

MELTING RANGE OR TEMPERATURE

For purposes of the FCC, the melting range or temperature of a solid is defined as those points of temperature within which or the point at which the solid coalesces and is completely melted when determined as directed below. Any apparatus or method capable of equal accuracy may be used. The accuracy should be checked frequently by the use of one or more of the six USP Melting Point Reference Standards, preferably the one that melts nearest the melting temperature of the compound to be tested.

Five procedures for the determination of melting range or temperature are given herein, varying in accordance with the nature of the substance. When no class is designated in the monograph, use the procedure for *Class I*.

The procedure known as the mixed melting point determination, whereby the melting range of a solid under test is compared with that of an intimate mixture of equal parts of the solid and an authentic specimen of it, may be used as a confirmatory identification test. Agreement of the observations on the original and the mixture usually constitutes reliable evidence of chemical identity.

Apparatus The melting range apparatus consists of a glass container for a bath of colorless fluid, a suitable stirring device, an accurate thermometer (see Appendix I), and a controlled source of heat. The bath fluid is selected consistent with the temperature required, but light paraffin is used generally, and certain liquid silicones are well adapted to the higher temperature ranges. The fluid is deep enough to permit immersion of the thermometer to its specified immersion depth so that the bulb is still about 2 cm above the bottom of the bath. The heat may be supplied electrically or by an open flame. The capillary tube is about 10 cm long, with an internal diameter of 0.8 to 1.2 mm, and with walls 0.2 to 0.3 mm thick.

The thermometer is preferably one that conforms to the specifications provided under *Thermometers*, Appendix I, selected for the desired accuracy and range of temperature.

Procedure for Class I Reduce the sample to a very fine powder, and unless otherwise directed, render it anhydrous when it contains water of hydration by drying it at the temperature specified in the monograph, or when the substance contains no water of hydration, dry it over a suitable desiccant for 16 to 24 h.

Charge a capillary glass tube, one end of which is sealed, with a sufficient amount of the dry powder to form a column in the bottom of the tube 2.5 to 3.5 mm high when packed down as closely as possible by moderate tapping on a solid surface.

Heat the bath until a temperature approximately 30° below the expected melting point is reached, attach the capillary tube to the thermometer, and adjust its height so that the material in the capillary is level with the thermometer bulb. Return the thermometer to the bath, continue the heating, with constant stirring, at a rate of rise of approximately 3°/min until a temperature 3° below the expected melting point is attained, then carefully regulate the rate to about 1° to 2°/min until melting is complete.

The temperature at which the column of the sample is observed to collapse definitely against the side of the tube at any point is defined as the beginning of melting, and the temperature at which the sample becomes liquid throughout is defined as the end of melting. The two temperatures fall within the limits of the melting range.

Procedure for Class Ia Prepare the sample and charge the capillary glass tube as directed for *Class I*. Heat the bath until a temperature 10° ± 1° below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of 3° ± 0.5°/min until melting is complete. Record the melting range as for *Class I*.

Procedure for Class Ib Place the sample in a closed container, and cool to 10° or lower for at least 2 h. Without previous powdering, charge the cooled material into the capillary tube as directed for *Class I*, immediately place the charged tube in a vacuum desiccator, and dry at a pressure not exceeding 20 mm Hg for 3 h. Immediately upon removal from the desiccator, fire-seal the open end of the tube. As soon as is practicable, proceed with the determination of the melting range as follows: Heat the bath until a temperature of 10° ± 1° below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of 3° ± 0.5°/min until melting is complete. Record the melting range as directed in *Class I*.

If the particle size of the material is too large for the capillary, precool the sample as directed above, then with as little pressure as possible, gently crush the particles to fit the capillary, and immediately charge the tube.

Procedure for Class II Carefully melt the material to be tested at as low a temperature as possible, and draw it into a capillary tube that is left open at both ends to a depth of about 10 mm. Cool the charged tube at 10°, or lower, for 24 h, or in contact with ice for at least 2 h. Then attach the tube to the thermometer by means of a rubber band, adjust it in a water bath so that the upper edge of the material is 10 mm below the water level, and heat as directed for *Class I*, except within 5° of the expected melting temperature, regulate the rate of rise of temperature to 0.5° to 1.0°/min. The temperature at which the material is observed to rise in the capillary tube is the melting temperature.

Procedure for Class III Melt a quantity of the substance slowly, while stirring, until it reaches a temperature of 90° to 92°. Remove the source of heat, and allow the molten substance to cool to a temperature of 8° to 10° above the expected melting point. Chill the bulb of an ASTM 14C thermometer (see Appendix I) to 5°, wipe it dry, and while it is still cold, dip it into the molten substance so that approximately the lower half of the bulb is submerged. Withdraw it immediately, and hold it vertically away from the heat until the wax surface dulls, then dip it for 5 min into a water bath having a temperature not higher than 16°.

Fix the thermometer securely in a test tube so that the lower point is 15 mm above the bottom of the test tube. Suspend the test tube in a water bath adjusted to about 16°, and raise the temperature of the bath at the rate of 2°/min to 30°, then change to a rate of 1°/min, and note the temperature at which the first drop of melted substance leaves the thermometer. Repeat the determination twice on a freshly melted portion of the sample. If the variation of three determinations is less than 1°, take the average of the three as the melting point. If the variation of three determinations is greater than 1°, make two additional determinations and take the average of the five.

OPTICAL (SPECIFIC) ROTATION

Many chemicals in a pure state or in solution are optically active in the sense that they cause incident polarized light to emerge in a plane forming a measurable angle with the plane of the incident light. When this effect is large enough for precise measurement, it may serve as the basis for an assay or an identity test. In this connection, the optical rotation is expressed in degrees, as either *angular rotation* (observed) or *specific rotation* (calculated with reference to the specific concentration of 1 g of solute in 1 mL of solution, measured under stated conditions).

Specific rotation of a liquid substance usually is expressed by the equation $[\alpha]_x^t = a/ld$, and for solutions of solid substances, expressed by the equation $[\alpha]_x^t = 100a/lpd = 100a/lc$, in which a is the corrected observed rotation, in degrees, at temperature t; x is the wavelength of the light used; l is the length of the polarimeter cell, in dm; d is the specific gravity of the liquid or solution at the temperature of observation; p is the concentration of the solution expressed as the number of grams of substance in 100 g of solution; and c is the concentration of the solution expressed as the number of grams of substance in 100 mL of solution. The concentrations p and c should be calculated on the dried or anhydrous basis, unless otherwise specified. Spectral lines most frequently employed are the D line of sodium (doublet at 589.0 and 589.6 nm) and the yellow-green line of mercury at 546.1 nm. The specific gravity and the rotatory power vary appreciably with the temperature.

The accuracy and precision of optical rotatory measurements will be increased if they are carried out with due regard for the following general considerations.

Supplement the source of illumination with a filtering system capable of transmitting light of a sufficiently monochromatic nature. Precision polarimeters generally are designed to accommodate interchangeable disks to isolate the D line from sodium light or the 546.1-nm line from the mercury spectrum. With polarimeters not thus designed, cells containing suitably colored liquids may be employed as filters (see also A. Weissberger and B. W. Rossiter, *Techniques of Chemistry*, Vol. I: *Physical Methods of Chemistry*, Part 3, Wiley-Interscience, New York, 1972).

Pay special attention to temperature control of the solution and of the polarimeter. Make accurate and reproducible observations to the extent that differences between replicates, or between observed and true values of rotation (the latter value having been established by calibration of the polarimeter scale with suitable standards), calculated in terms of either specific rotation or angular rotation, whichever is appropriate, do not exceed one-fourth of the range given in the individual monograph for the rotation of the article being tested. Generally, a polarimeter accurate to 0.05° of angular rotation, and capable of being read with the same precision, suffices for FCC purposes; in some cases, a polarimeter accurate to 0.01°, or less, of angular rotation, and read with comparable precision, may be required.

Fill polarimeter tubes in such a way as to avoid creating or leaving air bubbles, which interfere with the passage of the beam of light. Interference from bubbles is minimized with tubes in which the bore is expanded at one end. However, tubes of uniform bore, such as semimicro- or micro-tubes, require care for proper filling. At the time of filling, the tubes and the liquid or solution should be at a temperature not higher than that specified for the determination to guard against the formation of a bubble upon cooling and contraction of the contents.

In closing tubes having removable end plates fitted with gaskets and caps, the latter should be tightened only enough to ensure a leak-proof seal between the end plate and the body of the tube. Excessive pressure on the end plate may set up strains that result in interference with the measurements. In determining the specific rotation of a substance of low rotatory power, loosen the caps and tighten them again between successive readings in the measurement of both the rotation and the zero point. Differences arising from end plate strain thus generally will be revealed and appropriate adjustments to eliminate the cause may be made.

Procedure In the case of a solid, dissolve the substance in a suitable solvent, reserving a separate portion of the latter for a blank determination. Make at least five readings of the rotation of the solution, or of the substance itself if liquid, at 25° or the temperature specified in the individual monograph. Replace the solution with the reserved portion of the solvent (or, in the case of a liquid, use the empty tube), make the same number of readings, and use the average as the zero point value. Subtract the zero point value from the average observed rotation if the two figures are of the same sign, or add if opposite in sign, to obtain the corrected observed rotation.

Calculation Calculate the specific rotation of a liquid substance, or of a solid in solution, by application of one of the following formulas:

(I) for liquid substances,

$$[\alpha]_x^t = a/ld,$$

(II) for solutions of solids,

$$[\alpha]_x^t = 100a/lpd = 100a/lc,$$

in which a is the corrected observed rotation, in degrees, at temperature t; x is the wavelength of the light used; l is the length, in dm, of the polarimeter cell; d is the specific gravity of the liquid or solution at the temperature of observation; p is the concentration of the solution expressed as the number of grams of substance in 100 g of solution; and c is the concentration of the solution expressed as the number of grams of substance in 100 mL of solution. The concentrations p and c should be calculated on the dried or anhydrous basis, unless otherwise specified.

pH DETERMINATION

Principle The definition of pH is the negative log of the hydrogen ion concentration in moles per liter of aqueous

solutions. Measure pH potentiometrically by using a pH meter or colorimetrically by using pH indicator paper.

Scope This method is suitable to determine the pH of aqueous solutions. While pH meters, calibrated with aqueous solutions, are sometimes used to make measurements in semiaqueous solutions or in nonaqueous polar solutions, the value obtained is the apparent pH value only and should not be compared with the pH of aqueous solutions. For nonpolar solutions, pH has no meaning, and pH electrodes may be damaged by direct contact with these solutions. References to the pH of nonpolar solutions or liquids usually indicate the pH of a water extract of the nonpolar liquid or the apparent pH of a mixture of the nonpolar liquid in a polar liquid such as alcohol or alcohol–water mixtures.

Procedure [Potentiometric Method (pH Meter)]

Calibration Select two standard buffers to bracket, if possible, the anticipated pH of the unknown substances. These commercially available standards and the sample should be at the same temperature, within two degrees. Set the temperature compensator of the pH meter to the temperature of the samples and standards. Follow the manufacturer's instructions for setting temperature compensation and for adjusting the output during calibration. Rinse the electrodes with distilled or deionized water, and blot them dry with clean, absorbent laboratory tissue. Place the electrode(s) in the first standard buffer solution, and adjust the standardization control so that the pH reading matches the stated pH of the standard buffer. Repeat this procedure with fresh portions of the first buffer solution until two successive readings are within ± 0.02 pH units with no further adjustment. Rinse the electrodes, blot them dry, and place them in a portion of the second standard buffer solution. Following the manufacturer's instructions, adjust the slope control (not the standardization control) until the output displays the pH of the second standard buffer.

Repeat the sequence of standardization with both buffers until pH readings are within ± 0.02 pH units for both buffers without adjustments to either the slope or standardization controls. The pH of the unknown may then be measured, using either a pH electrode in combination with a reference electrode or a single combination electrode. Select electrodes made of chemically resistant glass when measuring samples of either low or high pH.

pH Indicator Paper Test papers impregnated with acid–base indicators, although less accurate than pH meters, offer a convenient way to determine the pH of an aqueous solution. They may be purchased in rolls or strips covering all or part of the pH range; papers covering a narrow part of the pH range can be sensitive to differences of 0.2 pH units. Some test papers comprise a plastic strip with small squares of test paper attached. The different squares are sensitive to different pH ranges. When using this type of test paper, wet all of the squares with the test sample to ensure a correct pH reading.

Test paper can contaminate the sample being tested; therefore, do not dip it into the sample. Either use a clean glass rod to remove a drop of the test solution and place it on the test paper, or transfer a small amount of the sample to a small container, dip the test paper into this portion, and compare the developed color with the color comparison chart provided with the test paper to determine the pH of the sample.

READILY CARBONIZABLE SUBSTANCES

Reagents

Sulfuric Acid, 95% Add a quantity of sulfuric acid of known concentration to sufficient water to adjust the final concentration to between 94.5% and 95.5% of H_2SO_4. Because the acid concentration may change upon standing or upon intermittent use, check the concentration frequently and either adjust solutions assaying more than 95.5% or less than 94.5% by adding either diluted or fuming sulfuric acid, as required, or discard them.

Cobaltous Chloride CS Dissolve about 65 g of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 5 mL of this solution into a 250-mL iodine flask, add 5 mL hydrogen peroxide TS (3%) and 15 mL of a 1:5 solution of sodium hydroxide, boil for 10 min, cool, and add 2 g of potassium iodide and 20 mL of 1:4 sulfuric acid. When the precipitate has dissolved, titrate the liberated iodine with 0.1 *N* sodium thiosulfate. The titration is sensitive to air oxidation and should be blanketed with carbon dioxide. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 23.79 mg of $CoCl_2 \cdot 6H_2O$. Adjust the final volume of the solution by adding enough of the mixture of hydrochloric acid and water so that each milliliter contains 59.5 mg of $CoCl_2 \cdot 6H_2O$.

Cupric Sulfate CS Dissolve about 65 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 10 mL of this solution into a 250-mL iodine flask; add 40 mL of water, 4 mL of acetic acid, and 3 g of potassium iodide; and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 24.97 mg of $CuSO_4 \cdot 5H_2O$. Adjust the final volume of the solution by adding enough of the mixture of hydrochloric acid and water so that each milliliter contains 62.4 mg of $CuSO_4 \cdot 5H_2O$.

Ferric Chloride CS Dissolve about 55 g of ferric chloride ($FeCl_3 \cdot 6H_2O$) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 10 mL of this solution into a 250-mL iodine flask; add 15 mL of water, 5 mL of hydrochloric acid, and 3 g of potassium iodide; and allow the mixture to stand for 15 min. Dilute with 100 mL of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents and in the same manner, and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 27.03 mg of $FeCl_3 \cdot 6H_2O$. Adjust the final volume of the solution by adding the mixture of hydrochloric acid and water so that each milliliter contains 45.0 mg of $FeCl_3 \cdot 6H_2O$.

Platinum–Cobalt CS Transfer 1.246 g of potassium chloroplatinate (K_2PtCl_6) and 1.00 g of crystallized cobaltous chloride ($CoCl_2 \cdot 6H_2O$) into a 1000-mL volumetric flask, dissolve in about 200 mL of water and 100 mL of hydrochloric acid, dilute to volume with water, and mix. This solution has a color of 500 APHA units.

Note: Use this solution only when specified in an individual monograph.

Procedure Unless otherwise directed, add the specified quantity of the substance, finely powdered if in solid form, in small portions to the comparison container, which is made of colorless glass resistant to the action of sulfuric acid and contains the specified volume of *95% Sulfuric Acid*.

Stir the mixture with a glass rod until solution is complete, allow the solution to stand for 15 min, unless otherwise directed, and compare the color of the solution with that of the specified matching fluid in a comparison container that also is of colorless glass and has the same internal and cross-section dimensions, viewing the fluids transversely against a background of white porcelain or white glass.

When heat is directed to effect solution of the substance in the *95% Sulfuric Acid*, mix the sample and the acid in a test tube, heat as directed, cool, and transfer the solution to the comparison container for matching.

Matching Fluids For purposes of comparison, a series of 20 matching fluids, each designated by a letter of the alphabet, is provided, the composition of each being as indicated in the accompanying table. To prepare the matching fluid specified, pipet the prescribed volumes of the colorimetric test solutions (CS) and water into one of the matching containers, and mix the solutions in the container.

Matching Fluids[a]

Matching Fluid	Parts of Cobaltous Chloride CS	Parts of Ferric Chloride CS	Parts of Cupric Sulfate CS	Parts of Water
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	8.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	0.0	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	0.0	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	0.0	0.0
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	0.0	4.9	0.1	0.0
O	0.1	4.8	0.1	0.0
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	0.0	4.7
T	0.5	0.5	0.4	3.6

[a]Solutions A–D, very light brown-yellow.
Solutions E–L, yellow through red-yellow.
Solutions M–O, green-yellow.
Solutions P–T, light pink.

REFRACTIVE INDEX

The refractive index of a transparent substance is the ratio of the velocity of light in air to its velocity in that material under like conditions. It is equal to the ratio of the sine of the angle of incidence made by a ray in air to the sine of the angle of refraction made by the ray in the material being tested. The refractive index values specified in this Codex are for the D line of sodium (589 nm) unless otherwise specified. The determination should be made at the temperature specified in the individual monograph, or at 25° if no temperature is specified. This physical constant is used as a means for identification of, and detection of impurities in, volatile oils and other liquid substances. The Abbé refractometer, or other refractometers of equal or greater accuracy, may be employed at the discretion of the operator.

SOLIDIFICATION POINT

Scope This method is designed to determine the solidification point of food-grade chemicals having appreciable heats of fusion. It is applicable to chemicals having solidification points between −20° and +150°. Necessary modifications will be noted in individual monographs.

Definition Solidification Point is an empirical constant defined as the temperature at which the liquid phase of a substance is in approximate equilibrium with a relatively small portion of the solid phase. It is measured by noting the maximum temperature reached during a controlled cooling cycle after the appearance of a solid phase.

The solidification point is distinguished from the freezing point in that the latter term applies to the temperature of equilibrium between the solid and liquid state of pure compounds.

Some chemical compounds have more than one temperature at which there may be an equilibrium between the solid and

liquid state depending on the crystal form of the solid that is present.

Apparatus The apparatus illustrated in Figs. 3 and 4 consists of the components described in the following paragraphs.

Thermometer A thermometer having a range not exceeding 30°, graduated in 0.1° divisions, and calibrated for 76-mm immersion should be employed. A satisfactory series of thermometers, covering a range from −20° to +150°, is available as ASTM-E1 89C through 96C (see *Thermometers*, Appendix I). A thermometer should be chosen such that the solidification point is not obscured by the cork stopper of the sample container.

Sample Container Use a standard glass 25- × 150-mm test tube with a lip, fitted with a two-hole cork stopper to hold the thermometer in place and to allow adequate stirring with a stirrer.

Air Jacket For the air jacket, use a standard glass 38- × 200-mm test tube with a lip and fitted with a cork or rubber stopper bored with a hole into which the sample container can easily be inserted up to the lip.

Cooling Bath Use a 2000-mL beaker or a similar, suitable container as a cooling bath. Fill it with an appropriate cooling medium such as glycerin, mineral oil, water, water and ice, or alcohol–dry ice.

Stirrer The stirrer (Fig. 4) consists of a 1-mm in diameter (B & S gauge 18), corrosion-resistant wire bent into a series of three loops about 25 mm apart. It should be made so that it will move freely in the space between the thermometer and the inner wall of the sample container. The shaft of the stirrer should be of a convenient length designed to pass loosely through a hole in the cork holding the thermometer. Stirring may be hand operated or mechanically activated at 20 to 30 strokes/min.

Assembly Assemble the apparatus in such a way that the cooling bath can be heated or cooled to control the desired temperature ranges. Clamp the air jacket so that it is held rigidly just below the lip, and immerse it in the cooling bath to a depth of 160 mm.

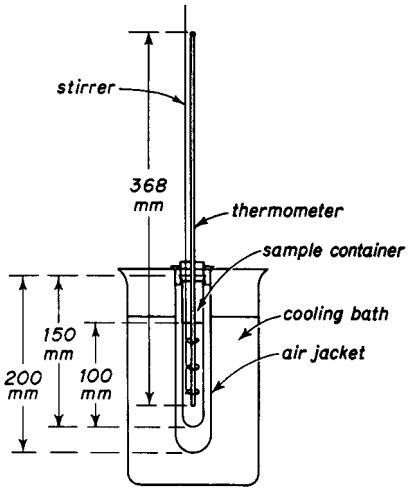


FIGURE 3 Apparatus for Determination of Solidification Point.

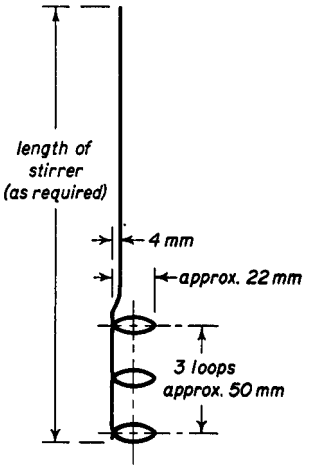


FIGURE 4 Stirrer for Solidification Point Determination.

Sample Preparation The solidification point of chemicals is usually determined as they are received. Some may be hygroscopic, however, and will require special drying. If this is necessary, it will be noted in the individual monographs.

Products that are normally solid at room temperature must be carefully melted at a temperature about 10° above the expected solidification point. Care should be observed to avoid heating in such a way as to decompose or distill any portion of a sample.

Procedure Adjust the temperature of the cooling bath to about 5° below the expected solidification point. Fit the thermometer and stirrer with a cork stopper so that the thermometer is centered and the bulb is about 20 mm from the bottom of the sample container. Transfer a sufficient amount of the sample, previously melted if necessary, into the sample container to fill it to a depth of about 90 mm when in the molten state. Place the thermometer and stirrer in the sample container, and adjust the thermometer so that the immersion line will be at the surface of the liquid and so that the end of the bulb is 20 ± 4 mm from the bottom of the sample container. When the temperature of the sample is about 5° above the expected solidification point, place the assembled sample tube in the air jacket.

Allow the sample to cool while stirring, at the rate of 20 to 30 strokes/min, in such a manner that the stirrer does not touch the thermometer. Stir the sample continuously during the remainder of the test.

The temperature at first will gradually fall, then will become constant as crystallization starts and continues under equilibrium conditions, and finally will start to drop again. Some chemicals may supercool slightly below (0.5°) the solidification point; as crystallization begins, the temperature will rise and remain constant as equilibrium conditions are established. Other products may cool more than 0.5° and cause deviation from the normal pattern of temperature change. If the temperature rise exceeds 0.5° after the initial crystallization begins, repeat the test, and seed the melted compound with small crystals of the sample at 0.5° intervals as the temperature approaches the expected solidification point. Crystals for seed-

ing may be obtained by freezing a small sample in a test tube directly in the cooling bath. It is preferable that seed of the stable phase be used from a previous determination.

Observe and record the temperature readings at regular intervals until the temperature rises from a minimum, due to supercooling, to a maximum and then finally drops. The maximum temperature reading is the solidification point. Readings 10 s apart should be taken to establish that the temperature is at the maximum level and should continue until the drop in temperature is established.

VISCOSITY

Viscosity is a fluid's measured internal resistance to flow. Thick, slow-moving fluids have higher viscosities than thin, free-flowing fluids. The basic unit of measure for viscosity is the poise or Pascal second, Pa·s, in SI units. The relationship between poise and Pa·s is 1 poise = 0.1 Pa·s. Since commonly encountered viscosities are often fractions of 1 poise, viscosities are commonly expressed as centipoises (one centipoise = 0.01 poise). Poise or centipoise is the unit of measure for absolute viscosity. Kinematic viscosity also is commonly used and is determined by dividing the absolute viscosity of the test liquid by the density of the test liquid at the same temperature as the viscosity measurement and is expressed as stokes or centistokes (poise/density = stokes). The specified temperature is important: viscosity varies greatly with temperature, generally decreasing with increasing temperature.

Absolute viscosity can be determined directly if accurate dimensions of the measuring instruments are known. It is common practice to calibrate an instrument with a fluid of known viscosity and to determine the unknown viscosity of another fluid by comparison with that of the known viscosity.

Many substances, such as gums, have a variable viscosity, and most of them are less resistant to flow at higher flow (more correctly, shear) rates. In such cases, select a given set of conditions for measurement, and consider the measurement obtained to be an apparent viscosity. Since a change in the conditions of measurement would yield a different value for the apparent viscosity of such substances, the operator must closely adhere to the instrument dimensions and conditions for measurement.

Measuring Viscosity Several common methods are available for measuring viscosity. Two very common ones are the use of capillary tubes such as Ubbelohde, Ostwald, or Cannon-Fenske viscometer tubes and the use of a rotating spindle such as the Brookfield viscometer.

Determine the viscosity in capillary tubes by measuring the amount of time it takes for a given volume of liquid to flow through a calibrated capillary tube. Calibrate the capillary tube by using liquids of known viscosity. The calibration may be supplied with the viscometer tube when purchased along with specific instructions for its use. Many types of capillary viscometer tubes are available, and exact procedures will vary with the type of tube chosen. Examples of procedures are in the following sections: *Viscosity of Dimethylpolysiloxane* and *Viscosity of Methylcellulose*. In general, calibrate capillary viscometers by filling the viscometers per the manufacturer's instructions and allowing the filled tube to equilibrate to the given temperature in a constant-temperature bath. Draw the liquid to the top graduation line, and measure the time, in seconds, it takes for the liquid to flow from the upper mark to the lower mark in the capillary tube. Calculate the viscometer constant, k, by the equation

$$k = v/dt,$$

in which v is the known viscosity, in centipoises, of the standard liquid; d is the density, at the specified temperature, of the liquid; and t is the time, in seconds, for the liquid to pass from the upper mark to the lower mark. It is not necessary to recalibrate the tube unless changes or repairs are made to it. To measure viscosity, introduce the unknown liquid into the viscometer tube in the same way as the calibration standard was introduced, and measure the time, in seconds, it takes for the liquid to flow from the upper mark to the lower mark. Calculate viscosity by the equation

$$v = kdt,$$

in which v is the viscosity to be determined, k is the viscometer constant, and d is the density of the liquid being measured.

Using rotational viscometers provides a particularly rapid and convenient method for determining viscosity. They employ a rotating spindle or cup immersed in the liquid, and they measure the resistance of the liquid to the rotation of the spindle or cup. A wide range of viscosities can be measured with one instrument by using spindles or cups of different sizes and by rotating them at different speeds. The manufacturer supplies the calibration of viscosity versus the spindle size and speed, which can be checked by using fluids of known viscosity. Take a measurement by allowing the sample to come to the desired temperature in a constant-temperature bath and immersing the spindle or cup to the depth specified by the manufacturer. Allow the spindle or cup to rotate until a constant reading is obtained. Multiply the reading by a factor supplied by the manufacturer for a given spindle or cup and given rotational speed to obtain the viscosity. The exact procedures will vary with the particular instrument. An example is given in the section on *Viscosity of Cellulose Gum*.

Another method to determine viscosity uses the falling-ball viscometer. Determine viscosity by noting the time it takes for a ball to fall through the distance between two marks on a tube filled with the unknown liquid (the tube is generally in a constant-temperature bath). Use balls of different weights to measure a wide range of viscosities. Calculate the viscosity by using manufacturer-supplied constants for the ball used. These instruments can be quite precise for Newtonian liquids, that is, liquids that do not have viscosities that vary with flow (more correctly, shear) rate.

Three specific methods are described below:

Viscosity of Dimethylpolysiloxane

Apparatus The Ubbelohde suspended level viscometer, shown in Fig. 5, is preferred to determine the viscosity of

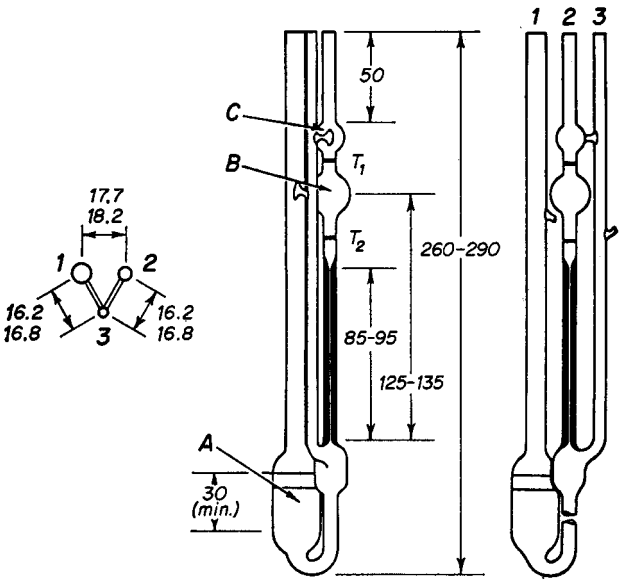


FIGURE 5 Ubbelohde Viscometer for Dimethylpolysiloxane (all dimensions are in mm).

dimethylpolysiloxane. Alternatively, a Cannon-Ubbelohde viscometer may be used.

Select a viscometer having a minimum flow time of at least 200 s. Use a No. 3 size Ubbelohde, or a No. 400 size Cannon-Ubbelohde, viscometer for the range of 300 to 600 centistokes. The viscometer should be fitted with holders that satisfy the dimensional positions of the separate tubes as shown in the diagram and that hold the viscometer vertically. Filling lines in bulb *A* indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb *B* is approximately 5 mL.

Calibration of the Viscometer Determine the viscosity constant, *C*, for each viscometer by using an oil of known viscosity.[1] Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb *A* below the capillary, and then introduce enough of the sample into tube *l* to bring the level up to the lower filling line. The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube *l*. Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air.

After the viscometer has been in a constant-temperature bath (25° ± 0.2°) long enough for the sample to reach temperature equilibrium, place a finger over tube *3*, and apply suction to tube *2* until the liquid reaches the center of bulb *C*. Remove suction from tube *2*, then remove the finger from tube *3*, and place it over tube *2* until the sample drops away from the lower end of the capillary. Remove the finger from tube *2*, and measure the time, to the nearest 0.1 s, required for the meniscus to pass from the first timing mark (T_1) to the second (T_2).

Calculate the viscometer constant, *C*, by the equation

$$C = cs/t_1,$$

in which *cs* is the viscosity, in centistokes, and t_1 is the efflux time, in seconds, for the standard liquid.

Determination of the Viscosity of Dimethylpolysiloxane Charge the viscometer with the sample in the same manner as described for the calibration procedure; determine the efflux time, t_2; and calculate the viscosity of the dimethylpolysiloxane by the formula

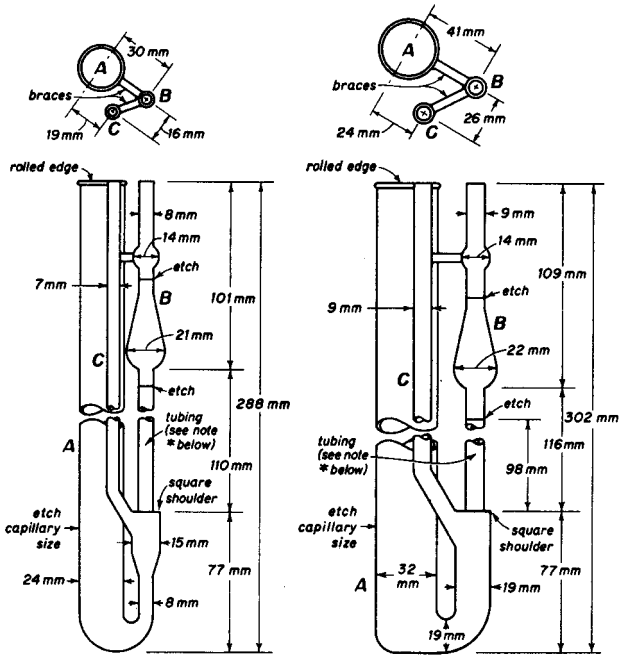
$$V = C \times t_2.$$

Viscosity of Methylcellulose

Apparatus Viscometers used to determine the viscosity of methylcellulose and some related compounds are illustrated in Fig. 6 and consist of three parts: a large filling tube, *A*; an orifice tube, *B*; and an air vent to the reservoir, *C*.

There are two basic types of methylcellulose viscometers—one for cellulose derivatives of a range between 1500 and 4000 centipoises, and the other for less viscous ones. Each type of viscometer is modified slightly for the different viscosities.

Calibration of the Viscometer Determine the viscometer constant, *K*, for each viscometer by using an oil of known



Viscometer for Low Viscosity
*Precision bore capillary tubing 1.5 mm id for 15 cps, 1.8 mm id for 25 cps, 2.4 mm id for 100 cps, and 3.2 mm id for 400 cps viscosities.

Viscometer for High Viscosity
*Precision bore capillary tubing 5.0 mm id for 1500 cps and 6.0 mm id for 4000 cps viscosities.

FIGURE 6 Methylcellulose Viscometers.

[1]Oils of known viscosities may be obtained from the Cannon Instrument Co., P.O. Box 812, State College, PA 16801. For determining the viscosity of dimethylpolysiloxane, choose an oil with a viscosity as close as possible to that of the type of sample to be tested.

viscosity.[2] Place an excess of the liquid that is to be tested (adjusted to 20° ± 0.1°) in the filling tube, *A*, and transfer it to the orifice tube, *B*, by gentle suction, taking care to keep the liquid free from air bubbles by closing the air vent tube, *C*. Adjust the column of liquid in tube *B* so it is even with the top graduation line. Open both tubes *B* and *C* to permit the liquid to flow into the reservoir against atmospheric pressure.

Note: Failure to open air vent tube *C* before determining the viscosity will yield false values.

Record the time, in seconds, for the liquid to flow from the upper mark to the lower mark in tube *B*.

Calculate the viscometer constant, *K*, from the equation

$$K = V/dt,$$

in which *V* is the viscosity, in centipoises, of the liquid; *K* is the viscometer constant; *d* is the specific gravity of the liquid tested at 20°/20°; and *t* is the time, in seconds, for the liquid to pass from the upper to the lower mark.

For the calibration, all values in the equation are known or can be determined except *K*, which must be solved. If a tube is repaired, it must be recalibrated to avoid obtaining significant changes in the value of *K*.

Determination of the Viscosity of Methylcellulose Prepare a 2% solution of methylcellulose or other cellulose derivative, by weight, as directed in the monograph. Place the solution in the proper viscometer and determine the time, *t*, required for the solution to flow from the upper mark to the lower mark in orifice tube *B*. Separately determine the specific gravity, *d*, at 20°/20°. Viscosity, $V = Kdt$.

Viscosity of Cellulose Gum

Apparatus Use a Brookfield Model LV series viscometer, analog or digital, or equivalent type viscometer for the determination of viscosity of aqueous solutions of cellulose gum within the range of 25 to 10,000 centipoises at 25°. Rotational viscometers of this type have spindles for use in determining the viscosity of different viscosity types of cellulose gum. The spindles and speeds for determining viscosity within different ranges are tabulated below.

Viscometer Spindles Required for Given Speeds

Viscosity Range (centipoises)	Spindle No.	Speed (rpm)	Scale	Factor
10–100	1	60	100	1
100–200	1	30	100	2
200–1000	2	30	100	10
1000–4000	3	30	100	40
4000–10,000	4	30	100	200

[2]Oils of known viscosities may be obtained from the Cannon Instrument Co., P.O. Box 812, State College, PA 16801. For determining the viscosity of methylcellulose, choose an oil that has a viscosity as close as possible to that of the type of sample to be tested.

Mechanical Stirrer Use an agitator essentially as shown in Fig. 7 that can be attached to a variable-speed motor capable of operating at 900 ± 100 rpm under varying load conditions.

Note: The agitator may be fabricated from stainless steel or glass as shown in Fig. 7. Where this procedure is specified for viscosity measurements by reference in other monographs, equivalent three-blade agitators may be used. Agitators are commercially available from Divtech Equipment Company, Cincinnati, Ohio, or from Hercules, Inc., Wilmington, Delaware.

Sample Container Use a glass jar about 152 mm deep having an od of approximately 64 mm and a capacity of about 340 g.

Water Bath Use a water bath capable of maintaining a constant temperature. Set the temperature to 25°, and maintain it within ±0.2°.

Thermometer Use an ASTM Saybolt Viscosity Thermometer having a range from 19° to 27° and conforming to the requirements for Thermometer 17C as described in ASTM Specification E1.

Sample Preparation Accurately weigh an amount of sample equivalent to 4.8 g of cellulose gum on the dried basis, and record the actual quantity required, in grams, as *S*. Transfer an accurately measured volume of water equivalent to 240 – *S* g into the sample container. Position the stirrer in the sample container, allowing minimal clearance between the stirrer and the bottom of the container. Begin stirring, and slowly add the sample. Adjust the stirring speed to approximately 900 ± 100 rpm. Mix for exactly 2 h. Do not allow

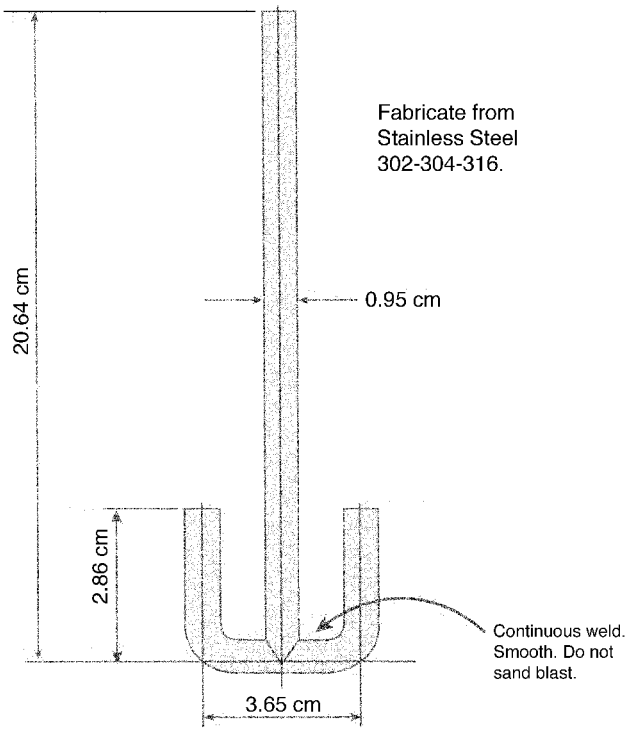


FIGURE 7 Agitator for Viscosity of Cellulose Gum.

the stirring speed to exceed 1200 rpm. Remove the stirrer, cap the sample container, and transfer the sample container into a constant-temperature water bath, maintained at 25° ± 0.2°, for 1 h. Check the sample temperature with a thermometer at the end of 1 h to ensure that the test temperature has been reached.

Procedure Remove the sample container from the water bath, shake vigorously for 10 s, and measure the viscosity with the Brookfield viscometer, using the proper spindle and speed indicated in the accompanying table. Be sure to use the viscometer guard, and allow the spindle to rotate for 3 min before taking the reading. Calculate the viscosity, in centipoises, by multiplying the reading observed by the appropriate factor from the table.

WATER DETERMINATION

Method I (Karl Fischer Titrimetric Method)

Determine the water by *Method Ia*, unless otherwise specified in the individual monograph.

Method Ia (Direct Titration)

Principle The titrimetric determination of water is based on the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as *Karl Fischer Reagent*, the sulfur dioxide and iodine are dissolved in pyridine and methanol. Pyridine-free reagents are more commonly used now. The test specimen may be titrated with the *Karl Fischer Reagent* directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of the determination depends on such factors as the relative concentrations of the *Karl Fischer Reagent* ingredients, the nature of the inert solvent used to dissolve the test specimen, the apparent pH of the final mixture, and the technique used in the particular determination. Therefore, an empirically standardized technique is used to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however, other suitable solvents may be used for special or unusual test specimens.

Substances that may interfere with the test results are ferric ion, chlorine, and similar oxidizing agents, as well as significant amounts of strong acids or bases, phosgene, or anything that will reduce iodide to iodine, poison the reagent, and show the sample to be bone dry when water may be present (false negative). 8-Hydroxyquinoline may be added to the vessel to eliminate interference from ferric ion. Chlorine interference can be eliminated with SO_2 or unsaturated hydrocarbon. Excess pyridine or other amines may be added to the vessel to eliminate the interference of strong acids. Excess acetic acid or other carboxylic acid can be added to reduce the interference of strong bases. Aldehydes and ketones may react with the solution, showing the sample to be wet while the detector never reaches an endpoint (false positive).

Apparatus Any apparatus may be used that provides for adequate exclusion of atmospheric moisture and for determination of the endpoint. In the case of a colorless solution that is titrated directly, the endpoint may be observed visually as a change in color from canary yellow to amber. The reverse is observed in the case of a test specimen that is titrated residually. More commonly, however, the endpoint is determined electrometrically with an apparatus employing a simple electrical circuit that serves to impress about 200 mV of applied potential between a pair of platinum electrodes (about 5 mm^2 in area and about 2.5 cm apart) immersed in the solution to be titrated. At the endpoint of the titration, a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 s to 30 min, depending on the solution being titrated. The time is shortest for substances that dissolve in the reagent. The longer times are required for solid materials that do not readily go into solution in the *Karl Fischer Reagent*. With some automatic titrators, the abrupt change in current or potential at the endpoint serves to close a solenoid-operated valve that controls the buret delivering the titrant. A commercially available apparatus generally comprises a closed system consisting of one or two automatic burets and a tightly covered titration vessel fitted with the necessary electrodes and a magnetic stirrer. The air in the system is kept dry with a suitable desiccant such as phosphorus pentoxide, and the titration vessel may be purged by means of a stream of dry nitrogen or a current of dry air.

Reagent The *Karl Fischer Reagent* may be prepared as follows: Add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine, and cool. Place 100 mL of pyridine in a 250-mL graduated cylinder, and keeping the pyridine cold in an ice bath, pass in dry sulfur dioxide until the volume reaches 200 mL. Slowly add this solution, with shaking, to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus, and allow the solution to stand overnight before standardizing. One milliliter of this solution, when freshly prepared, is equivalent to approximately 5 mg of water, but it deteriorates gradually; therefore, standardize it within 1 h before use, or daily in continual use. Protect the solution from light while in use. Store any bulk stock of the solution in a suitably sealed, glass-stoppered container, fully protected from light and under refrigeration.

A commercially available, stabilized solution of a Karl Fischer-type reagent may be used. Commercially available reagents containing solvents or bases other than pyridine and/or alcohols other than methanol also may be used. These may be single solutions or reagents formed in situ by combining the components of the reagents present in two discrete solutions. The diluted *Karl Fischer Reagent* called for in some monographs should be diluted as directed by the manufacturer. Either methanol, or another suitable solvent such as ethylene glycol monomethyl ether, may be used as the diluent.

Test Preparation Unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 10 to 250 mg of water.

Where the monograph specifies that the specimen under test is hygroscopic, accurately weigh a sample of the specimen into a suitable container. Use a dry syringe to inject an appropriate volume of methanol, or other suitable solvent, accurately measured, into the container and shake to dissolve the specimen. Dry the syringe, and use it to remove the solution from the container and transfer it to a titration vessel prepared as directed under *Procedure*. Repeat the procedure with a second portion of methanol, or other suitable solvent, accurately measured; add this washing to the titration vessel; and immediately titrate. Determine the water content, in milligrams, of a portion of solvent of the same total volume as that used to dissolve the specimen and to wash the container and syringe, as directed under *Standardization of Water Solution for Residual Titrations*, and subtract this value from the water content, in milligrams, obtained in the titration of the specimen under test.

Standardization of the Reagent Place enough methanol or other suitable solvent in the titration vessel to cover the electrodes, and add sufficient *Karl Fischer Reagent* to give the characteristic color or 100 ± 50 microamperes of direct current at about 200 mV of applied potential. Pure methanol can make the detector overly sensitive, particularly at low ppm levels of water, causing it to deflect to dryness and slowly recover with each addition of reagent. This slows down the titration and may allow the system to actually pick up ambient moisture during the resulting long titration. Adding chloroform or a similar nonconducting solvent will retard this sensitivity and can improve the analysis.

For determination of trace amounts of water (less than 1%), quickly add 25 µL (25 mg) of pure water, using a 25- or 50-µL syringe, and titrate to the endpoint. The water equivalence factor *F*, in milligrams of water per milliliter of reagent, is given by the formula

$$25/V,$$

in which *V* is the volume, in milliliters, of the *Karl Fischer Reagent* consumed in the second titration.

For the precise determination of significant amounts of water (more than 1%), quickly add between 25 and 250 mg (25 to 250 µL) of pure water, accurately weighed by difference from a weighing pipet or from a precalibrated syringe or micropipet, the amount of water used being governed by the reagent strength and the buret size, as referred to under *Volumetric Apparatus*. Titrate to the endpoint. Calculate the water equivalence factor, *F*, in milligrams of water per milliliter of reagent by the formula

$$W/V,$$

in which *W* is the weight, in milligrams, of the water, and *V* is the volume, in milliliters, of the *Karl Fischer Reagent* required.

Procedure Unless otherwise specified, transfer 35 to 40 mL of methanol or other suitable solvent to the titration vessel, and titrate with the *Karl Fischer Reagent* to the electrometric or visual endpoint to consume any moisture that may be present. (Disregard the volume consumed because it does not enter into the calculations.) Quickly add the *Test Preparation*, mix, and again titrate with the *Karl Fischer Reagent* to the electrometric or visual endpoint. Calculate the water content of the specimen, in milligrams, by the formula

$$SF,$$

in which *S* is the volume, in milliliters, of the *Karl Fischer Reagent* consumed in the second titration, and *F* is the water equivalence factor of the *Karl Fischer Reagent*.

Method 1b (Residual Titration)

Principle See the information in the section entitled *Principle* under *Method Ia*. In the residual titration, add excess *Karl Fischer Reagent* to the test specimen, allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Karl Fischer Reagent* with a standard solution of water in a solvent such as methanol. The residual titration procedure is generally applicable and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

Apparatus, **Reagent**, and **Test Preparation** Use those in *Method Ia*.

Standardization of Water Solution for Residual Titration Prepare a *Water Solution* by diluting 2 mL of pure water to 1000 mL with methanol or another suitable solvent. Standardize this solution by titrating 25.0 mL with the *Karl Fischer Reagent*, previously standardized as directed under *Standardization of the Reagent*. Calculate the water content, in milligrams per milliliter, of the *Water Solution* with the formula

$$VF/25,$$

in which *V* is the volume of the *Karl Fischer Reagent* consumed, and *F* is the water equivalence factor of the *Karl Fischer Reagent*. Determine the water content of the *Water Solution* weekly, and standardize the *Karl Fischer Reagent* against it periodically as needed. Store the *Water Solution* in a tightly capped container.

Procedure Where the individual monograph specifies the water content is to be determined by *Method Ib*, transfer 35 to 40 mL of methanol or other suitable solvent into the titration vessel, and titrate with the *Karl Fischer Reagent* to the electrometric or visual endpoint. Quickly add the *Test Preparation*, mix, and add an accurately measured excess of the *Karl Fischer Reagent*. Allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Karl Fischer Reagent* with standardized *Water Solution* to the electrometric or visual endpoint. Calculate the water content of the specimen, in milligrams, with the formula

$$F(X' - XR),$$

in which *F* is the water equivalence factor of the *Karl Fischer Reagent*; *X′* is the volume, in milliliters, of the *Karl Fischer*

Reagent added after introduction of the specimen; *X* is the volume, in milliliters, of standardized *Water Solution* required to neutralize the unconsumed *Karl Fischer Reagent*; and *R* is the ratio *V*/25 (milliliters of *Karl Fischer Reagent*/milliliters of *Water Solution*), determined from the *Standardization of Water Solution for Residual Titration*.

Method Ic (Coulometric Titration)

Principle Use the Karl Fischer reaction in the coulometric determination of water. In this determination, iodine is not added in the form of a volumetric solution, but is produced in an iodide-containing solution by anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with the water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which can be detected potentiometrically, thus indicating the endpoint. Preelectrolysis, which can take several hours, eliminates moisture from the system. Therefore, changing the *Karl Fischer Reagent* after each determination is not practical. Individual determinations may be carried out in succession in the same reagent solution. A requirement for this method is that each component of the test specimen be compatible with the other components and that no side reactions take place. Samples may be transferred into the vessel as solids or as solutions by means of injection through a septum. Gases can be introduced into the cell by means of a suitable gas inlet tube. For the water determination of solids, another common technique is to dissolve the solid in a suitable solvent and then inject a portion of this solution into the cell. In the case of insoluble solids, water may be extracted using suitable solvents, and then the extracts injected into the coulometric cell. Alternatively, an evaporation technique may be used in which the sample is heated in a tube and the water is evaporated and carried into the cell by means of a stream of dry, inert gas. Precision in the method is predominantly governed by the extent to which atmospheric moisture is excluded from the system. Control of the system may be monitored by measuring the amount of baseline drift. The titration of water in solid test specimens is usually carried out with the use of anhydrous methanol as the solvent. Other suitable solvents may be used for special or unusual test specimens. This method is particularly suited to chemically inert substances such as hydrocarbons, alcohols, and ethers. In comparison with the volumetric Karl Fischer titration, coulometry is a micro-method. The method uses extremely small amounts of current. It is predominantly used for substances with a very low water content (0.1% to 0.0001%).

Apparatus Any commercially available apparatus consisting of an absolutely tight system fitted with the necessary electrodes and a magnetic stirrer is appropriate. The instrument's microprocessor controls the analytical procedure and displays the results. Calibration of the instrument is not necessary as the current consumed can be measured absolutely. Proper operation of the instrument can be confirmed by injecting 1 μL of water into the vessel. The instrument should read 1000 μg of water on reaching the endpoint.

Reagent See *Reagent* under *Method Ia*.

Test Preparation Using a dry syringe, inject an appropriate volume of test specimen estimated to contain 0.5 to 5 mg of water, accurately measured, into the anolyte solution. The sample may also be introduced as a solid, accurately weighed, into the anolyte solution. Perform coulometric titration, and determine the water content of the specimen under test.

Alternatively, when the specimen is a suitable solid, dissolve an appropriate quantity, accurately weighed, in anhydrous methanol or another suitable solvent, and inject a suitable portion into the anolyte solution.

When the specimen is an insoluble solid, extract the water by using a suitable anhydrous solvent from which an appropriate quantity, accurately weighed, may be injected into the anolyte solution. Alternatively use an evaporation technique.

Procedure Quickly inject the *Test Preparation*, or transfer the solid sample, into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the *Test Preparation* directly from the instrument's display, and calculate the percent that is present in the substance.

Method II (Toluene Distillation Method)

Principle This method determines water by distillation of a sample with an immiscible solvent, usually toluene.

Apparatus Use a glass distillation apparatus (see Fig. 8) provided with 24/40 ground-glass connections. The components consist of a 500-mL short-neck, round-bottom flask connected by means of a trap to a 400-mm water-cooled condenser. The lower tip of the condenser should be about 7 mm above the surface of the liquid in the trap after distillation conditions have been established (see *Procedure*).

The trap should be constructed of well-annealed glass, the receiving end of which is graduated to contain 5 mL and subdivided into 0.1-mL divisions, with each 1-mL line numbered from 5 mL beginning at the top. Calibrate the receiver by adding 1 mL of water, accurately measured, to 100 mL of toluene contained in the distillation flask. Conduct the distillation, and calculate the volume of water obtained as directed in the *Procedure*. Add another milliliter of water to the cooled apparatus, and repeat the distillation. Continue in this manner until five 1-mL portions of water have been added. The error at any indicated capacity should not exceed 0.05 mL. The source of heat is either an oil bath or an electric heater provided with a suitable means of temperature control. The distillation may be better controlled by insulating the tube leading from the flask to the receiver. It is also advantageous to protect the flask from drafts. Clean the entire apparatus with potassium dichromate–sulfuric acid cleaning solution, rinse thoroughly, and dry completely before using.

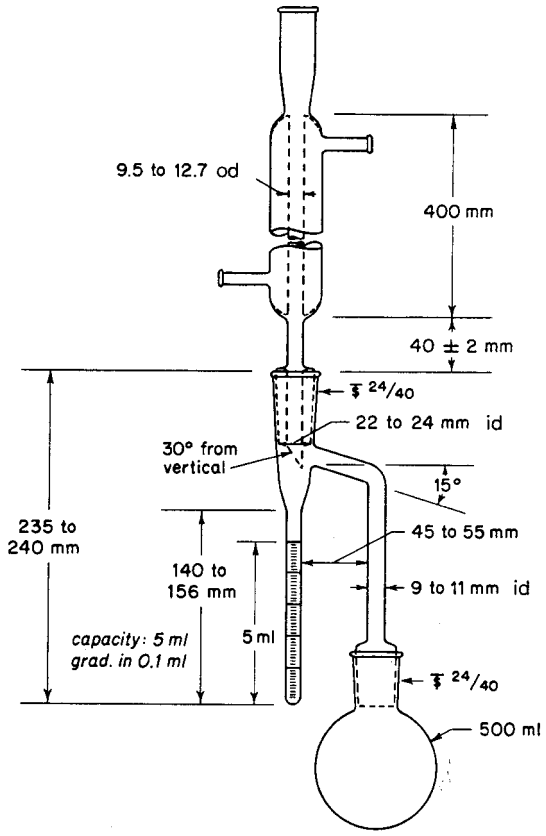


FIGURE 8 Moisture Distillation Apparatus.

Procedure Place in the previously cleaned and dried flask a quantity of the substance, weighed accurately to the nearest 0.01 g, that is expected to yield from 1.5 to 4 mL of water. If the substance is of a pastelike consistency, weigh it in a boat of metal foil that will pass through the neck of the flask. If the substance is likely to cause bumping, take suitable precautions to prevent it. Transfer about 200 mL of ACS reagent-grade toluene into the flask, and swirl to mix it with the sample. Assemble the apparatus, fill the receiver with toluene by pouring it through the condenser until it begins to overflow into the flask, and insert a loose cotton plug in the top of the condenser. Heat the flask so that the distillation rate will be about 200 drops/min, and continue distilling until the volume of water in the trap remains constant for 5 min. Discontinue the heating, use a copper or nichrome wire spiral to dislodge any drops of water that may be adhering to the inside of the condenser tube or receiver, and wash down with about 5 mL of toluene. Disconnect the receiver, immerse it in water at 25° for at least 15 min or until the toluene layer is clear, and then read the volume of water. Conduct a blank determination using the same volume of toluene as used when distilling the sample mixture, and make any necessary correction (see *General Provisions*).

C. OTHERS

ASH (Acid-Insoluble)

Boil the ash obtained as directed under *Ash (Total)*, below, with 25 mL of 2.7 *N* hydrochloric acid for 5 min, collect the insoluble matter on a tared, porous-bottom porcelain filter crucible or ashless filter, wash it with hot water, ignite to constant weight at 675° ± 25°, and weigh. Calculate the percent acid-insoluble ash from the weight of the sample taken.

Note: Avoid exposing the crucible to sudden temperature changes.

ASH (Total)

Unless otherwise directed, accurately weigh about 3 g of the sample in a tared crucible, ignite it at a low temperature (about 550°), not to exceed a very dull redness, until it is free from carbon, cool it in a desiccator, and weigh. If a carbon-free ash is not obtained, wet the charred mass with hot water, collect the insoluble residue on an ashless filter paper, and ignite the residue and filter paper until the ash is white or nearly so. Finally, add the filtrate, evaporate it to dryness, and heat the whole to a dull redness. If a carbon-free ash is still not obtained, cool the crucible, add 15 mL of ethanol, break up the ash with a glass rod, then burn off the ethanol, again heat the whole to a dull redness, cool it in a desiccator, and weigh.

HYDROCHLORIC ACID TABLE

°Bé	Sp. Gr.	Percent HCl	°Bé	Sp. Gr.	Percent HCl
1.00	1.0069	1.40	7.25	1.0526	10.55
2.00	1.0140	2.82	7.50	1.0545	10.94
3.00	1.0211	4.25	7.75	1.0564	11.32
4.00	1.0284	5.69	8.00	1.0584	11.71
5.00	1.0357	7.15	8.25	1.0603	12.09
5.25	1.0375	7.52	8.50	1.0623	12.48
5.50	1.0394	7.89	8.75	1.0642	12.87
5.75	1.0413	8.26	9.00	1.0662	13.26
6.00	1.0432	8.64	9.25	1.0681	13.65
6.25	1.0450	9.02	9.50	1.0701	14.04
6.50	1.0469	9.40	9.75	1.0721	14.43
6.75	1.0488	9.78	10.00	1.0741	14.83
7.00	1.0507	10.17	10.25	1.0761	15.22

°Bé	Sp. Gr.	Percent HCl	°Bé	Sp. Gr.	Percent HCl
10.50	1.0781	15.62	21.7	1.1760	34.64
10.75	1.0801	16.01	21.8	1.1770	34.83
11.00	1.0821	16.41	21.9	1.1779	35.02
11.25	1.0841	16.81	22.0	1.1789	35.21
11.50	1.0861	17.21	22.1	1.1798	35.40
11.75	1.0881	17.61	22.2	1.1808	35.59
12.00	1.0902	18.01	22.3	1.1817	35.78
12.25	1.0922	18.41	22.4	1.1827	35.97
12.50	1.0943	18.82	22.5	1.1836	36.16
12.75	1.0964	19.22	22.6	1.1846	36.35
13.00	1.0985	19.63	22.7	1.1856	36.54
13.25	1.1006	20.04	22.8	1.1866	36.73
13.50	1.1027	20.44	22.9	1.1875	36.93
13.75	1.1048	20.86	23.0	1.1885	37.14
19.2	1.1526	30.00	23.1	1.1895	37.36
19.3	1.1535	30.18	23.2	1.1904	37.58
19.4	1.1544	30.35	23.3	1.1914	37.80
19.5	1.1554	30.53	23.4	1.1924	38.03
19.6	1.1563	30.71	23.5	1.1934	38.26
19.7	1.1572	30.90	23.6	1.1944	38.49
19.8	1.1581	31.08	23.7	1.1953	38.72
19.9	1.1590	31.27	23.8	1.1963	38.95
20.0	1.1600	31.45	23.9	1.1973	39.18
20.1	1.1609	31.64	24.0	1.1983	39.41
20.2	1.1619	31.82	24.1	1.1993	39.64
20.3	1.1628	32.01	24.2	1.2003	39.86
20.4	1.1637	32.19	24.3	1.2013	40.09
20.5	1.1647	32.38	24.4	1.2023	40.32
20.6	1.1656	32.56	24.5	1.2033	40.55
20.7	1.1666	32.75	24.6	1.2043	40.78
20.8	1.1675	32.93	24.7	1.2053	41.01
20.9	1.1684	33.12	24.8	1.2063	41.24
21.0	1.1694	33.31	24.9	1.2073	41.48
21.1	1.1703	33.50	25.0	1.2083	41.72
21.2	1.1713	33.69	25.1	1.2093	41.99
21.3	1.1722	33.88	25.2	1.2103	42.30
21.4	1.1732	34.07	25.3	1.2114	42.64
21.5	1.1741	34.26	25.4	1.2124	43.01
21.6	1.1751	34.45	25.5	1.2134	43.40

Source: Courtesy of the Manufacturing Chemists' Association of the United States.

Specific gravity determinations were made at 60°F, compared with water at 60°F.

From the specific gravities, the corresponding degrees Baumé were calculated by the following formula:

$$\text{degrees Baumé} = 145 - (145/\text{sp. gr.}).$$

Baumé hydrometers for use with this table must be graduated by the above formula, which should always be printed on the scale.

Allowance for Temperature

10° to 15°Bé: 1/40 °Bé or 0.0002 sp. gr. for 1°F
15° to 22°Bé: 1/30 °Bé or 0.0003 sp. gr. for 1°F
22° to 25°Bé: 1/28 °Bé or 0.00035 sp. gr. for 1°F

LOSS ON DRYING

This procedure is used to determine the amount of volatile matter expelled under the conditions specified in the monograph. Because the volatile matter may include material other than adsorbed moisture, this test is designed for compounds in which the loss on drying may not definitely be attributable to water alone. For substances appearing to contain water as the only volatile constituent, the *Direct (Karl Fischer) Titration Method*, provided under *Water*, Appendix IIB, is usually appropriate.

Procedure Unless otherwise directed in the monograph, conduct the determination on 1 to 2 g of the substance, previously mixed and accurately weighed. If the sample is in the form of large crystals, reduce the particle size to about 2 mm, quickly crushing the sample to avoid absorption or loss of moisture. Tare a glass-stoppered, shallow weighing bottle that has been dried for 30 min under the same conditions to be used in the determination. Transfer the sample to the bottle, replace the cover, and weigh the bottle and its contents. By gentle sideways shaking, distribute the sample as evenly as possible to a depth of about 5 mm for most substances and not over 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber, and dry at the temperature and for the length of time specified in the monograph. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature, preferably in a desiccator, before weighing.

Where drying in vacuum is specified in the monograph, use a pressure as low as that obtainable by an aspirating water pump (not higher than 20 mm Hg).

If the test substance melts at a temperature lower than that specified for the determination, preheat the bottle and its contents for 1 to 2 h at a temperature 5° to 10° below the melting range, then continue drying at the specified temperature for the determination. When drying the sample in a desiccator, ensure that the desiccant is kept fully effective by replacing it frequently.

OIL CONTENT OF SYNTHETIC PARAFFIN

Apparatus

Filter Stick Use either a 10-mm diameter sintered-glass filter stick of 10- to 15-μm maximum pore diameter, or a filter stick made of stainless steel and having a 0.5-in. disk of 10- to 15-μm maximum pore diameter. Determine conformance with the pore diameter specified as follows: Clean sintered-glass filter sticks by soaking in hydrochloric acid, or stainless steel sticks by soaking in nitric acid, wash with water, rinse with acetone, and dry in air followed by drying in an oven at 105° for 30 min.

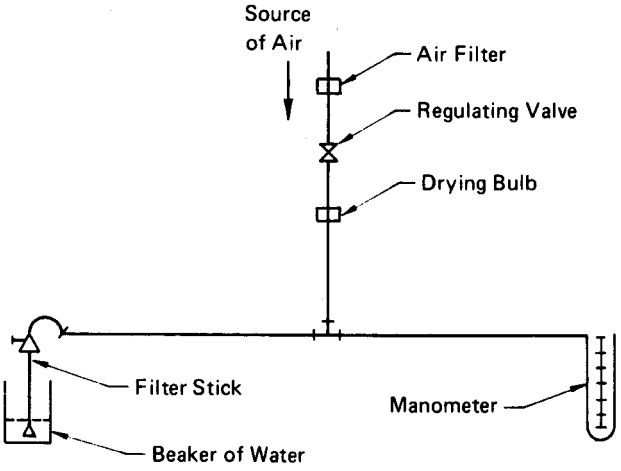


FIGURE 9 Assembly for Checking Pore Diameter of Filter Sticks.

Thoroughly wet the clean filter stick by soaking in water, and then connect it with an apparatus (see Fig. 9) consisting of a mercury-filled manometer, readable to 0.5 mm; a clean and filtered air supply; a drying bulb filled with silica gel; and a needle-valve type air pressure regulator. Apply pressure slowly from the air source, and immerse the filter just below the surface of water contained in a beaker.

Note: If a head of liquid is noted above the surface of the filter after it is inserted into the water, the back pressure thus produced should be subtracted from the observed pressure when the pore diameter is calculated as directed below.

Increase the air pressure to 10 mm below the acceptable pressure limit, and then increase the pressure at a slow, uniform rate of about 3 mm Hg per minute until the first bubble passes through the filter. This can be conveniently observed by placing the beaker over a mirror. Read the manometer when the first bubble passes off the underside of the filter. Calculate the pore diameter, in micrometers, by the formula

$$2180/p,$$

in which p is the observed pressure, in millimeters, corrected for any back pressure as mentioned above.

Filtration Assembly Connect the *Filter Stick* with an air pressure inlet tube and delivery nozzle and ground-glass joint to fit a 25- × 170-mm test tube as shown in Fig. 10. If a stainless steel *Filter Stick* is used, make the connection to the test tube by means of a cork.

Cooling Bath Use a suitable insulated box having 1-in. holes in the center to accommodate any desired number of test tubes. The bath may be filled with a suitable medium such as kerosene and may be cooled by circulating a refrigerant through coils, or by using solid carbon dioxide, to produce a temperature of 30° ± 2°F.

Air Pressure Regulator Use a suitable pressure-reduction valve, or other suitable regulator, that will supply air to the *Filtration Assembly* at the volume and pressure required to give an even flow of filtrate (see *Procedure*). Connect the regulator with rubber tubing to the end of the *Filter Stick* in the *Filtration Assembly*.

Thermometer Use an ASTM Oil in Wax Thermometer having the range of −35° to +70°F and conforming to the requirements for an ASTM 71F thermometer (see *Thermometers*, Appendix I).

Weighing Bottles Use glass-stoppered conical bottles having a capacity of 15 mL. The bottles are used as evaporating flasks in the *Procedure*.

Evaporation Assembly The assembly consists of an evaporating cabinet capable of maintaining a temperature of 95° ± 2°F around the evaporation flasks, and air jets (4 ± 0.2 mm id) for delivering a stream of clean, dry air vertically downward into the flasks. In the *Procedure* below, support each jet so that the tip is 15 ± 5 mm above the surface of the liquid at the start of the evaporation. Supply the air (purified by passage through a tube of 1-cm bore packed loosely to a height of 20 cm with absorbent cotton) at the rate of 2 to 3 L/min per jet. The cleanliness of the air should be checked periodically to ensure that not more than 0.1 mg of residue is obtained when 4 mL of methyl ethyl ketone is evaporated as directed in the *Procedure*.

Wire Stirrer Use a 250-mm length of stiff iron or nichrome wire of about No. 20 B & S gauge. Form a 10-mm diameter loop at each end, and bend the loop at the bottom end so that the plane of the loop is perpendicular to the length of the wire.

Sample Selection If the sample weighs about 1 kg or less, obtain a representative portion by melting the entire sample

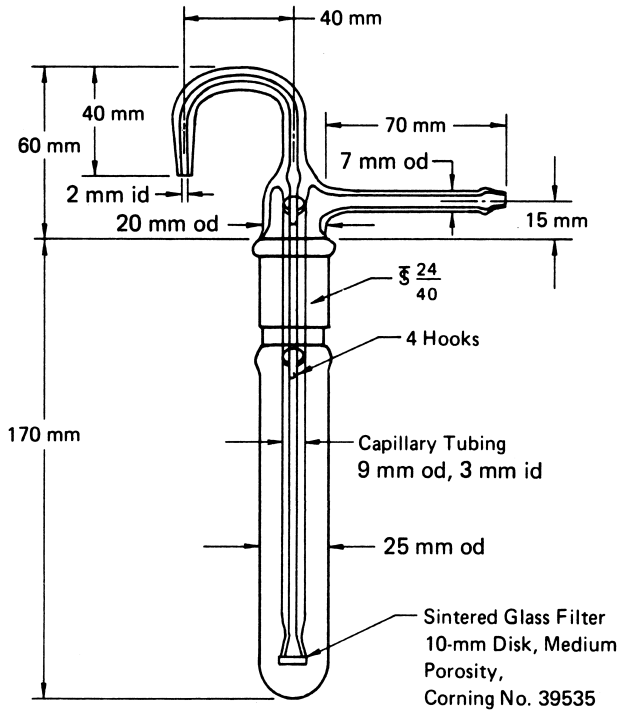


FIGURE 10 Filtration Assembly for Determination of Oil Content.

and stirring thoroughly. For samples heavier than about 1 kg, exercise special care to ensure that a truly representative portion is obtained, noting that the oil may not be distributed uniformly throughout the sample and that mechanical operations may have expressed some of the oil.

Procedure Melt a representative portion of the sample in a beaker, using a water bath or oven maintained at 160° to 210°F. As soon as the sample is completely melted, thoroughly mix it by stirring. Preheat a dropper pipet, provided with a rubber bulb and calibrated to deliver 1 ± 0.05 g of molten sample, and withdraw a 1-g portion of the sample as soon as possible after it has melted. Hold the pipet in a vertical position, and carefully transfer its contents into a clean, dry test tube previously weighed to the nearest milligram. Evenly coat the bottom of the tube by swirling, allow the tube to cool, and weigh to the nearest milligram. Calculate the sample weight, in grams, and record it as *B* (see *Calculation*). Pipet 15 mL of methyl ethyl ketone (ASTM Specification D 740, or equivalent) into the tube, and immerse the tube up to the top of the liquid in a hot water or steam bath. Stir with an up-and-down motion with the wire stirrer, and continue heating and stirring until a homogeneous solution is obtained, exercising care to avoid loss of solvent by prolonged boiling.

> **Note**: If it appears that a clear solution will not be obtained, stir until any undissolved material is well dispersed so as to produce a slightly cloudy solution.

After the sample solution is prepared, plunge the test tube into an 800-mL beaker of ice water, and continue to stir until the contents are cold. Remove the stirrer, then remove the test tube from the bath, dry the outside of the tube with a cloth, and weigh to the nearest 100 mg. Calculate the weight, in grams, of solvent in the test tube, and record it as *C* (see *Calculation*). Place the tube in the cooling bath, maintained at −30° ± 2°F, and stir continuously with the thermometer until the temperature reaches −25° ± 0.5°F, maintaining the slurry at a uniform consistency and taking precautions to prevent the sample from setting up on the walls of the tube or forming crystals.

Place the filter stick in a test tube and cool at −30° ± 2°F in the cooling bath for a minimum of 10 min. Immerse the cooled filter stick in the sample, then connect the filtration assembly, seating the ground-glass joint of the filter so as to make an airtight seal. Placed an unstoppered weighing bottle, previously weighed together with the glass stopper to the nearest 0.1 mg, under the delivery nozzle of the filtration assembly.

> **Note**: Suitable precautions and proper analytical technique should be applied to ensure the accuracy of the weight of the bottle. Before determining its weight, the bottle and its stopper should have been cleaned and dried, then rinsed with methyl ethyl ketone, wiped dry on the outside, dried in the evaporation assembly for about 5 min, and cooled. Then allow it to stand for about 10 min near the balance before weighing.

Apply air pressure to the filtration assembly, immediately collect about 4 mL of filtrate in the weighing bottle, and release the air pressure to permit the liquid to drain back slowly from the delivery nozzle. Stopper the bottle, and weigh it to the nearest 10 mg without waiting for it to come to room temperature. Remove the stopper, transfer the bottle to the evaporation assembly maintained at 95° ± 2°F, and place it under an air jet centered inside the neck, with the tip 15 ± 5 mm above the surface of the liquid. After the solvent has evaporated (usually less than 30 min), stopper the bottle, and allow it to stand near the balance for about 10 min before it is weighed to the nearest 0.1 mg. Repeat the evaporation procedure for 5-min periods until the loss between successive weighings is not more than 0.2 mg. Determine the weight of the oil residue, in grams, by subtracting the weight of the empty stoppered bottle from the weight of the stoppered bottle plus the oil residue after the evaporation procedure, and record the results as *A* (see *Calculation*). Determine the weight of solvent evaporated, in grams, by subtracting the weight of the bottle plus oil residue from the weight of the bottle plus filtrate, and record the result as *D* (see *Calculation*).

Calculation Calculate the percent, by weight, of oil in the sample by the formula

$$(100\ AC/BD) - 0.15,$$

in which 0.15 is a factor to correct for solubility of the sample in the solvent at −25°F.

RESIDUE ON IGNITION (Sulfated Ash)

Method I (for Solids)
Transfer the quantity of the sample directed in the individual monograph onto a tared 50- to 100-mL platinum dish or other suitable container, and add sufficient 2 *N* sulfuric acid to moisten the entire sample. Heat gently, using a hot plate, an Argand burner, or an infrared heat lamp, until the sample is dry and thoroughly charred, then continue heating until all of the sample has been volatilized or nearly all of the carbon has been oxidized, and cool. Moisten the residue with 0.1 mL of sulfuric acid, and heat in the same manner until the remainder of the sample and any excess sulfuric acid have been volatilized. To promote volitilization of sulfuric acid, add a few pieces of ammonium carbonate just before completing ignition. Finally, ignite to constant weight in a muffle furnace at 800° ± 25° for 15 min, or longer if necessary to complete ignition, cool in a desiccator, and weigh.

Method II (for Liquids)
Unless otherwise directed, transfer the required weight of the sample onto a tared 75- to 100-mL platinum dish. Heat gently, using an Argand or Meker burner, until the sample ignites, then allow the sample to burn until it self-extinguishes. Cool, then wet the residue with 2 mL of concentrated sulfuric acid, and heat the sample over a low flame until dry. Ignite to

constant weight in a muffle furnace at 800° ± 25° for 30 min, or longer if necessary for complete ignition, cool in a desiccator, and weigh.

SIEVE ANALYSIS OF GRANULAR METAL POWDERS

(Based on ASTM Designation: B 214)

Apparatus

Sieves Use a set of standard sieves, ranging from 80-mesh to 325-mesh, conforming to the specifications in ASTM Designation: E 11 (Sieves for Testing Purposes).

Sieve Shaker Use a mechanically operated sieve shaker that imparts to the set of sieves a horizontal rotary motion of between 270 and 300 rotations/min and a tapping action of between 140 and 160 taps/min. The sieve shaker is fitted with a plug to receive the impact of the tapping device. The entire apparatus is rigidly mounted—bolted to a solid foundation, preferably of concrete. Preferably a time switch is provided to ensure the accuracy of test duration.

Procedure Assemble the sieves in consecutive order by opening size, with the coarsest sieve (80-mesh) at the top, and place a solid-collecting pan below the bottom sieve (325-mesh). Place 100.0 g of the test sample, *W*, on the top sieve, and close the sieve with a solid cover. Securely fasten the assembly to the sieve shaker, and operate the shaker for 15 min. Remove the most coarse sieve from the nest, gently tap its contents to one side, and pour the contents onto a tared, glazed paper. Using a soft brush, transfer onto the next finer sieve any material adhering to the bottom of the sieve and frame. Place the sieve just removed upside down on the paper containing the retained portion, and tap the sieve. Accurately weigh the paper and its contents, and record the net weight of the fraction, *F*, obtained. Repeat this process for each sieve in the nest and for the portion of the sample that has been collected in the bottom pan. Record the total of the fractions retained on the sieves as *T* and that portion collected in the pan as *t*. The combined total, *S*, of $T + t$ is the amount of the sample, *W*, recovered in the test. Calculate the percent recovery by the formula

$$S/W \times 100.$$

If the percent recovery is less than 99.0%, check the condition of the sieves and for possible errors in weighing, and repeat the test. If the percent recovery is not less than 99.0%, calculate the percent retained on each sieve by the formula

$$F/W \times 100.$$

Calculate the percent through the smallest mesh sieve from the portion collected in the pan by the formula

$$[(100 - t)/W] \times 100.$$

SULFURIC ACID TABLE

°Bé	Sp. Gr.	Percent H_2SO_4	°Bé	Sp. Gr.	Percent H_2SO_4
0	1.0000	0.00	36	1.3303	42.63
1	1.0069	1.02	37	1.3426	43.99
2	1.0140	2.08	38	1.3551	45.35
3	1.0211	3.13	39	1.3679	46.72
4	1.0284	4.21	40	1.3810	48.10
5	1.0357	5.28	41	1.3942	49.47
6	1.0432	6.37	42	1.4078	50.87
7	1.0507	7.45	43	1.4216	52.26
8	1.0584	8.55	44	1.4356	53.66
9	1.0662	9.66	45	1.4500	55.07
10	1.0741	10.77	46	1.4646	56.48
11	1.0821	11.89	47	1.4796	57.90
12	1.0902	13.01	48	1.4948	59.32
13	1.0985	14.13	49	1.5104	60.75
14	1.1069	15.25	50	1.5263	62.18
15	1.1154	16.38	51	1.5426	63.66
16	1.1240	17.53	52	1.5591	65.13
17	1.1328	18.71	53	1.5761	66.63
18	1.1417	19.89	54	1.5934	68.13
19	1.1508	21.07	55	1.6111	69.65
20	1.1600	22.25	56	1.6292	71.17
21	1.1694	23.43	57	1.6477	72.75
22	1.1789	24.61	58	1.6667	74.36
23	1.1885	25.81	59	1.6860	75.99
24	1.1983	27.03	60	1.7059	77.67
25	1.2083	28.28	61	1.7262	79.43
26	1.2185	29.53	62	1.7470	81.30
27	1.2288	30.79	63	1.7683	83.34
28	1.2393	32.05	64	1.7901	85.66
29	1.2500	33.33	64.25	1.7957	86.33
30	1.2609	34.63	64.50	1.8012	87.04
31	1.2719	35.93	64.75	1.8068	87.81
32	1.2832	37.26	65	1.8125	88.65
33	1.2946	38.58	65.25	1.8182	89.55
34	1.3063	39.92	65.50	1.8239	90.60
35	1.3182	41.27	66	1.8354	93.19

Source: Courtesy of the Manufacturing Chemists' Association of the United States.

Specific gravity determinations were made at 60°F, compared with water at 60°F. The values given above for aqueous sulfuric acid solutions were adopted as standard in 1904 by the Manufacturing Chemists' Association of the United States.

From the specific gravities, the corresponding degrees Baumé were calculated by the following equation:

$$°\text{Baumé} = 145 - (145/\text{sp. gr.}).$$

Baumé hydrometers for use with this table must be graduated by the above formula, which should always be printed on the scale. Acids stronger than 66°Bé should have their percentage compositions determined by chemical analysis.

APPENDIX III: CHEMICAL TESTS AND DETERMINATIONS

A. IDENTIFICATION TESTS

The identification tests described in *Section A* of this Appendix are frequently referred to in the *Food Chemicals Codex* for the presumptive identification of FCC-grade chemicals taken from labeled containers. These tests are not intended to be applied to mixtures unless so specified.

Acetate
Acetic acid or acetates, when warmed with sulfuric acid and alcohol, form ethyl acetate, recognizable by its characteristic odor. With neutral solutions of acetates, ferric chloride TS produces a deep red color that is destroyed by the addition of a mineral acid.

Aluminum
Solutions of aluminum salts yield with 6 *N* ammonia a white, gelatinous precipitate that is insoluble in an excess of the 6 *N* ammonia. The same precipitate is produced by 1 *N* sodium hydroxide, but it dissolves in an excess of this reagent.

Ammonium
Ammonium salts are decomposed by 1 *N* sodium hydroxide with the evolution of ammonia, recognizable by its alkaline effect on moistened red litmus paper. The decomposition is accelerated by warming.

Benzoate
Neutral solutions of benzoates yield a salmon colored precipitate with ferric chloride TS. From moderately concentrated solutions of benzoate, 2 *N* sulfuric acid precipitates free benzoic acid, which is readily soluble in ether.

Bicarbonate
See *Carbonate*.

Bisulfite
See *Sulfite*.

Bromide
Free bromine is liberated from solutions of bromides upon the dropwise addition of chlorine TS. When shaken with chloroform, the bromine dissolves, coloring the chloroform red to red-brown. A yellow-white precipitate, which is insoluble in nitric acid and slightly soluble in 6 *N* ammonia, is produced when solutions of bromides are treated with silver nitrate TS.

Calcium
Insoluble oxalate salts are formed when solutions of calcium salts are treated in the following manner: Using 2 drops of methyl red TS as the indicator, neutralize a 1:20 solution of a calcium salt with 6 *N* ammonia, then add 2.7 *N* hydrochloric acid, dropwise, until the solution is acid. A white precipitate of calcium oxalate forms upon the addition of ammonium oxalate TS. This precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

Calcium salts moistened with hydrochloric acid impart a transient yellow-red color to a nonluminous flame.

Carbonate
Carbonates and bicarbonates effervesce with acids, yielding a colorless gas that produces a white precipitate immediately when passed into calcium hydroxide TS. Cold solutions of soluble carbonates are colored red by phenolphthalein TS, whereas solutions of bicarbonates remain unchanged or are slightly changed.

Chloride
Solutions of chlorides yield with silver nitrate TS a white, curdy precipitate that is insoluble in nitric acid but soluble in a slight excess of 6 *N* ammonia.

Citrate
To 15 mL of pyridine add a few milligrams of a citrate salt, dissolved or suspended in 1 mL of water, and shake. Add 5 mL of acetic anhydride to this mixture, and shake. A light red color appears.

Cobalt
Solutions of cobalt salts (1:20) in 2.7 *N* hydrochloric acid yield a red precipitate when heated on a steam bath with an equal volume of a hot, freshly prepared 1:10 solution of 1-nitroso-2-naphthol in 9 *N* acetic acid. Solutions of cobalt salts yield a yellow precipitate when saturated with potassium chloride and treated with potassium nitrite and acetic acid.

Copper
When solutions of cupric compounds are acidified with hydrochloric acid, a red film of metallic copper is deposited on a bright untarnished surface of metallic iron. An excess of 6 *N* ammonia, added to a solution of a cupric salt, produces first a blue precipitate and then a deep blue colored solution. Solutions of cupric salts yield with potassium ferrocyanide TS a red-brown precipitate, insoluble in diluted acids.

Hypophosphite
Hypophosphites evolve spontaneously flammable phosphine when strongly heated. Solutions of hypophosphites yield a white precipitate with mercuric chloride TS. This precipitate becomes gray when an excess of hypophosphite is present. Hypophosphite solutions, acidified with sulfuric acid and warmed with copper sulfate TS, yield a red precipitate.

Iodide
Solutions of iodides, upon the dropwise addition of chlorine TS, liberate iodine, which colors the solution yellow to red. Chloroform is colored violet when shaken with this solution.

The iodine thus liberated gives a blue color with starch TS. In solutions of iodides, silver nitrate TS produces a yellow, curdy precipitate that is insoluble in nitric acid and in 6 *N* ammonia.

Iron
Solutions of ferrous and ferric compounds yield a black precipitate with ammonium sulfide TS. This precipitate is dissolved by cold 2.7 *N* hydrochloric acid with the evolution of hydrogen sulfide.

Ferric Salts Potassium ferrocyanide TS (10%) produces a dark blue precipitate in acid solutions of ferric salts. With an excess of 1 *N* sodium hydroxide, a red-brown precipitate is formed. Solutions of ferric salts produce with ammonium thiocyanate TS (1.0 *N*) a deep red color that is not destroyed by diluted mineral acids.

Ferrous Salts Potassium ferricyanide TS (10%) produces a dark blue precipitate in solutions of ferrous salts. This precipitate, which is insoluble in dilute hydrochloric acid, is decomposed by 1 *N* sodium hydroxide. Solutions of ferrous salts yield with 1 *N* sodium hydroxide a green-white precipitate, the color rapidly changing to green and then to brown when shaken.

Lactate
When solutions of lactates are acidified with sulfuric acid, potassium permanganate TS (0.1 *N*) is added, and the mixture is heated, acetaldehyde is evolved. This can be detected by allowing the vapor to come into contact with a filter paper that has been moistened with a freshly prepared mixture of equal volumes of 20% aqueous morpholine and sodium nitroferricyanide TS. A blue color is produced.

Magnesium
Solutions of magnesium salts in the presence of ammonium chloride yield no precipitate with ammonium carbonate TS, but a white crystalline precipitate, which is insoluble in 6 *N* ammonium hydroxide, is formed on the subsequent addition of sodium phosphate TS (6%).

Manganese
Solutions of manganous salts yield with ammonium sulfide TS a salmon colored precipitate that dissolves in acetic acid.

Nitrate
When a solution of a nitrate is mixed with an equal volume of sulfuric acid, the mixture cooled, and a solution of ferrous sulfate superimposed, a brown color is produced at the junction of the two liquids. Brown-red fumes are evolved when a nitrate is heated with sulfuric acid and metallic copper. Nitrates do not decolorize acidified potassium permanganate TS (0.1 *N*) (distinction from nitrites).

Nitrite
Nitrites yield brown-red fumes when treated with diluted mineral acids or acetic acid. A few drops of potassium iodide TS (15%) and a few drops of 2 *N* sulfuric acid added to a solution of nitrite liberate iodine, which colors starch TS blue.

Peroxide
Solutions of peroxides slightly acidified with sulfuric acid yield a deep blue color on the addition of potassium dichromate TS. On shaking the mixture with an equal volume of diethyl ether and allowing the liquids to separate, the blue color is transferred to the ether layer.

Phosphate
Neutral solutions of orthophosphates yield with silver nitrate TS (0.1 *N*) a yellow precipitate, which is soluble in 1.7 *N* nitric acid or in 6 *N* ammonium hydroxide. With ammonium molybdate TS, a yellow precipitate, which is soluble in 6 *N* ammonium hydroxide, is formed.

Potassium
Potassium compounds impart a violet color to a nonluminous flame if not masked by the presence of small quantities of sodium. In neutral, concentrated or moderately concentrated solutions of potassium salts, sodium bitartrate TS (10%) slowly produces a white, crystalline precipitate that is soluble in 6 *N* ammonium hydroxide and in solutions of alkali hydroxides or carbonates. The precipitation may be accelerated by stirring or rubbing the inside of the test tube with a glass rod or by the addition of a small amount of glacial acetic acid or alcohol.

Sodium
Dissolve 0.1 g of the sodium compound in 2 mL of water. Add 2 mL of 15% potassium carbonate, and heat to boiling. No precipitate is formed. Add 4 mL of potassium pyroantimonate TS, and heat to boiling. Allow to cool in ice water, and if necessary, rub the inside of the test tube with a glass rod. A dense precipitate is formed. Sodium compounds impart an intense yellow color to a nonluminous flame.

Sulfate
Solutions of sulfates yield with barium chloride TS (10%) a white precipitate that is insoluble in hydrochloric and nitric acids. Sulfates yield with lead acetate TS (8%) a white precipitate that is soluble in ammonium acetate solution. Hydrochloric acid produces no precipitate when added to solutions of sulfates (distinction from thiosulfates).

Sulfite
When treated with 2.7 *N* hydrochloric acid, sulfites and bisulfites yield sulfur dioxide, recognizable by its characteristic odor. This gas blackens filter paper moistened with mercurous nitrate TS.

Tartrate
When a few milligrams of a tartrate are added to a mixture of 15 mL of pyridine and 5 mL of acetic anhydride, an emerald green color is produced.

Thiosulfate
With hydrochloric acid, solutions of thiosulfates yield a white precipitate that soon turns yellow, liberating sulfur dioxide, recognizable by its odor. The addition of ferric chloride TS

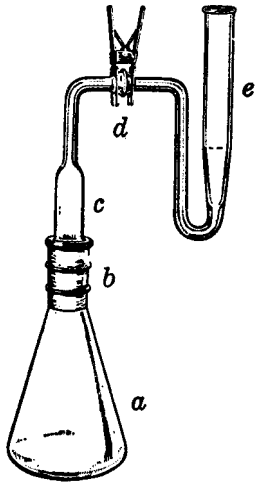


FIGURE 11 General Apparatus for Arsenic Limit Test. (Courtesy of the Fisher Scientific Co., Pittsburgh, PA.)

to solutions of thiosulfates produces a dark violet color that quickly disappears.

Zinc

Zinc salts, in the presence of sodium acetate, yield a white precipitate with hydrogen sulfide. This precipitate, which is insoluble in acetic acid, is dissolved by 2.7 *N* hydrochloric acid. A similar precipitate is produced by ammonium sulfide TS in neutral or alkaline solutions. Solutions of zinc salts yield with potassium ferrocyanide TS (10%) a white precipitate that is insoluble in 2.7 *N* hydrochloric acid.

B. LIMIT TESTS

ARSENIC LIMIT TEST

Silver Diethyldithiocarbamate Colorimetric Method

Note: All reagents used in this test should be very low in arsenic content.

Apparatus Use the general apparatus shown in Fig. 11 unless otherwise specified in an individual monograph. It consists of a 125-mL arsine generator flask (*a*) fitted with a scrubber unit (*c*) and an absorber tube (*e*), with a 24/40 standard-taper joint (*b*) and a ball-and-socket joint (*d*), secured with a No. 12 clamp, connecting the units. The tubing between *d* and *e* and between *d* and *c* is a capillary having an id of 2 mm and an od of 8 mm. Alternatively, an apparatus embodying the principle of the general assembly described and illustrated may be used.

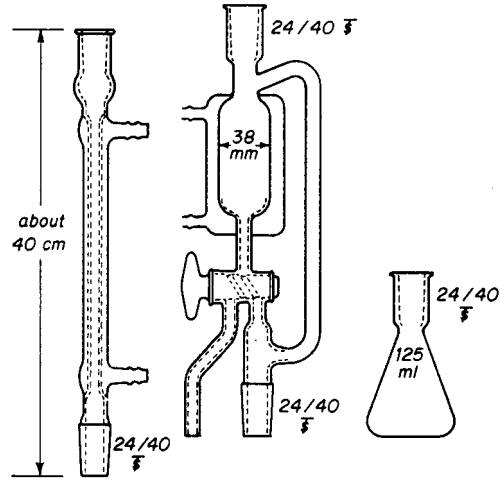


FIGURE 12 Modified Bethge Apparatus for the Distillation of Arsenic Tribromide.

Note: The special assemblies shown in Figs. 12, 13, and 14 are to be used only when specified in certain monographs.

Standard Arsenic Solution Accurately weigh 132.0 mg of arsenic trioxide that has been previously dried at 105° for 1 h, and dissolve it in 5 mL of a 1:5 sodium hydroxide solution. Neutralize the solution with 2 *N* sulfuric acid, add 10 mL in excess, and dilute to 1000.0 mL with recently boiled water. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 2 *N* sulfuric acid, dilute to volume with recently boiled water, and mix. Use this final solution, which contains 1 μg of arsenic in each milliliter, within 3 days.

Silver Diethyldithiocarbamate Solution Dissolve 1 g of ACS reagent-grade silver diethyldithiocarbamate in 200 mL of recently distilled pyridine. Store this solution in a light-resistant container and use within 1 month.

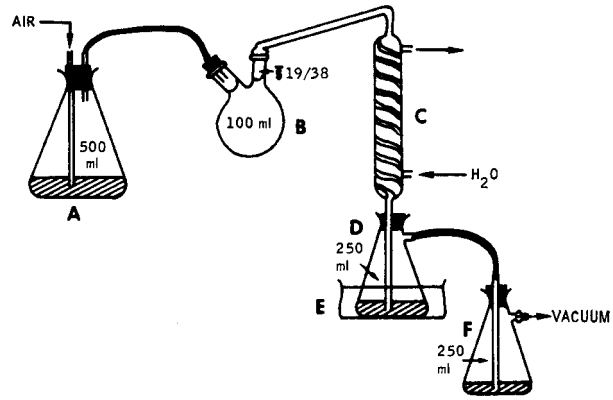


FIGURE 13 Special Apparatus for the Distillation of Arsenic Trichloride. (Flask *A* contains 150 mL of hydrochloric acid; flasks *D* and *F* contain 20 mL of water. Flask *D* is placed in an ice water bath, *E*.)

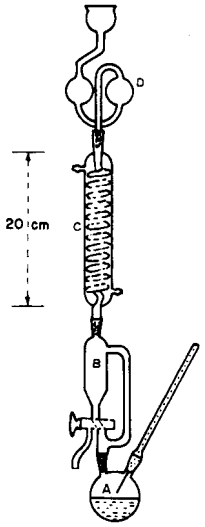


FIGURE 14 Special Apparatus for the Determination of Inorganic Arsenic. (*A*, 250-mL distillation flask; *B*, receiver chamber, approximately 50-mL capacity; *C*, reflux condenser; *D*, splash head.)

Stannous Chloride Solution Dissolve 40 g of stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) in 100 mL of hydrochloric acid. Store the solution in glass containers and use within 3 months.

Lead Acetate-Impregnated Cotton Soak cotton in a saturated solution of lead acetate trihydrate, squeeze out the excess solution, and dry in a vacuum at room temperature.

Sample Solution Use directly as the *Sample Solution* in the *Procedure* the solution obtained by treating the sample as directed in an individual monograph. Prepare sample solutions of organic compounds in the generator flask (*a*), unless otherwise directed, according to the following general procedure:

> **Caution**: Some substances may react unexpectedly with explosive violence when digested with hydrogen peroxide. Use appropriate safety precautions at all times.
>
> **Note**: If halogen-containing compounds are present, use a lower temperature while heating the sample with sulfuric acid; do not boil the mixture; and add the peroxide, with caution, before charring begins to prevent loss of trivalent arsenic.

Transfer 1.0 g of sample into the generator flask, add 5 mL of sulfuric acid and a few glass beads, and digest at a temperature not exceeding 120° until charring begins, preferably using a hot plate in a fume hood. (Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 mL.) After the acid has initially decomposed the sample, cautiously add, dropwise, hydrogen peroxide (30%), allowing the reaction to subside and reheating the sample between drops. Add the first few drops very slowly with sufficient mixing to prevent a rapid reaction, and discontinue heating if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls or bottom of the flask during digestion.

> **Note**: Maintain oxidizing conditions at all times during the digestion by adding small quantities of the peroxide whenever the mixture turns brown or darkens.

Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250° to 300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, cautiously add 10 mL of water, heat again to strong fuming, and cool. Cautiously add 10 mL of water, mix, wash the sides of the flask with a few milliliters of water, and dilute to 35 mL.

Procedure If the *Sample Solution* was not prepared in the generator flask, transfer to the flask a volume of the solution, prepared as directed, equivalent to 1.0 g of the substance being tested, and add water to make 35 mL. Add 20 mL of 1:5 sulfuric acid, 2 mL of potassium iodide TS, 0.5 mL of *Stannous Chloride Solution*, and 1 mL of isopropyl alcohol, and mix. Allow the mixture to stand for 30 min at room temperature. Pack the scrubber unit (*c*) with two plugs of *Lead Acetate-Impregnated Cotton*, leaving a small air space between the two plugs, lubricate joints *b* and *d* with stopcock grease, if necessary, and connect the scrubber unit with the absorber tube (*e*). Transfer 3.0 mL of *Silver Diethyldithiocarbamate Solution* to the absorber tube, add 3.0 g of granular zinc (20-mesh) to the mixture in the flask, and immediately insert the standard-taper joint (*b*) into the flask. Allow the evolution of hydrogen and color development to proceed at room temperature (25° ± 3°) for 45 min, swirling the flask gently at 10-min intervals. Disconnect the absorber tube from the generator and scrubber units, and transfer the *Silver Diethyldithiocarbamate Solution* to a 1-cm absorption cell. Determine the absorbance at the wavelength of maximum absorption between 535 nm and 540 nm, with a suitable spectrophotometer or colorimeter, using *Silver Diethyldithiocarbamate Solution* as the blank. The absorbance due to any red color from the solution of the sample does not exceed that produced by 3.0 mL of *Standard Arsenic Solution* (3 μg As) when treated in the same manner and under the same conditions as the sample. The room temperature during the generation of arsine from the standard should be held to within ±2° of that observed during the determination of the sample.

Interferences Metals or salts of metals such as chromium, cobalt, copper, mercury, molybdenum, nickel, palladium, and silver may interfere with the evolution of arsine. Antimony, which forms stibine, is the only metal likely to produce a positive interference in the color development with the silver diethyldithiocarbamate. Stibine forms a red color with silver diethyldithiocarbamate that has a maximum absorbance at 510 nm, but at 535 to 540 nm, the absorbance of the antimony complex is so diminished that the results of the determination would not be altered significantly.

CADMIUM LIMIT TEST

Spectrophotometer Use any suitable atomic absorption spectrophotometer equipped with a Boling-type burner, an air–acetylene flame, and a hollow-cathode cadmium lamp. The instrument should be capable of operating within the sensitivity necessary for the determination.

Standard Solution Transfer 100 mg of cadmium chloride crystals ($CdCl_2 \cdot 2\frac{1}{2}H_2O$), accurately weighed, into a 1000-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, add 1 mL of hydrochloric acid, dilute to volume with water, and mix. Each milliliter contains 12.5 μg of cadmium.

Sample Solution Transfer 10 g of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

Test Solutions Transfer 5.0 mL of *Sample Solution* into each of five separate 25-mL volumetric flasks. Dilute the contents of *Flask 1* to volume with water, and mix. Add 1.00, 2.00, 3.00, and 4.00 mL of *Standard Solution*, to *Flasks 2*, *3*, *4*, and *5*, respectively, then dilute each flask to volume with water, and mix. The *Test Solutions* contain, respectively, 0, 0.5, 1.0, 1.5, and 2.0 μg/mL of cadmium.

Procedure Determine the absorbance of each *Test Solution* at 228.8 nm, setting the instrument to previously established optimum conditions, using water as a blank. Plot the absorbance of the *Test Solutions* versus their contents of cadmium, in micrograms per milliliter. Draw the straight line best fitting the five points, and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the amount, in micrograms, of cadmium in each milliliter of the *Test Solution* containing 0 mL of the *Standard Preparation.* Calculate the quantity, in milligrams per kilogram, of cadmium in the sample by multiplying this value by 25.

CHLORIDE AND SULFATE LIMIT TESTS

Where limits for chloride and sulfate are specified in the individual monograph, compare the *Sample Solution* and control in appropriate glass cylinders of the same dimensions and matched as closely as practicable with respect to their optical characteristics.

If the solution is not perfectly clear after acidification, filter it through filter paper that has been washed free of chloride and sulfate. Add identical quantities of the precipitant (silver nitrate TS or barium chloride TS) in rapid succession to both the *Sample Solution* and the control solution.

Experience has shown that visual turbidimetric comparisons are best made between solutions containing from 10 to 20 μg of chloride (Cl) ion or from 200 to 400 μg of sulfate (SO_4) ion in 50 mL. Weights of samples are specified on this basis in the individual monographs in which these limits are included.

Chloride Limit Test

Standard Chloride Solution Dissolve 165 mg of sodium chloride in water and dilute to 100.0 mL. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. Each milliliter of the final solution contains 10 μg of chloride (Cl) ion.

Procedure Unless otherwise directed, dissolve the specified amount of the test substance in 30 to 40 mL of water; neutralize to litmus external indicator with nitric acid, if necessary; and add 1 mL in excess. Add 1 mL of silver nitrate TS to the clear solution or filtrate, dilute to 50 mL with water, mix, and allow to stand for 5 min protected from direct sunlight. Compare the turbidity, if any, with that produced similarly in a control solution containing the required volume of *Standard Chloride Solution* and the quantities of the reagents used for the sample.

Sulfate Limit Test

Standard Sulfate Solution Dissolve 148 mg of anhydrous sodium sulfate in water, and dilute to 100.0 mL. Transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute to volume with water, and mix. Each milliliter of the final solution contains 10 μg of sulfate (SO_4).

Procedure Unless otherwise directed, dissolve the specified amount of the test substance in 30 to 40 mL of water; neutralize to litmus external indicator with hydrochloric acid, if necessary; then add 1 mL of 2.7 *N* hydrochloric acid. Add 3 mL of barium chloride TS to the clear solution or filtrate, dilute to 50 mL with water, and mix. After 10 min compare the turbidity, if any, with that produced in a solution containing the required volume of *Standard Sulfate Solution* and the quantities of the reagents used for the sample.

1,4-DIOXANE LIMIT TEST

Vacuum Distillation Apparatus Assemble a closed-system vacuum distillation apparatus employing glass vacuum stopcocks (*A*, *B*, and *C*), as shown in Fig. 15. The concentrator tube (*D*) is made of borosilicate or quartz (not flint) glass, graduated precisely enough to measure the 0.9 mL or more of distillate and marked so that the analyst can accurately dilute to 2.0 mL (available as Chromaflex concentrator tube, Kontes Glass Co., Vineland, NJ, Catalog No. K42560-0000).

Standard Preparation Prepare a solution of 1,4-dioxane in water containing 100 μg/mL. Keep the solution refrigerated, and prepare fresh weekly.

Sample Preparation Transfer 20 g of the sample, accurately weighed, into a 50-mL round-bottom flask (*E*) having

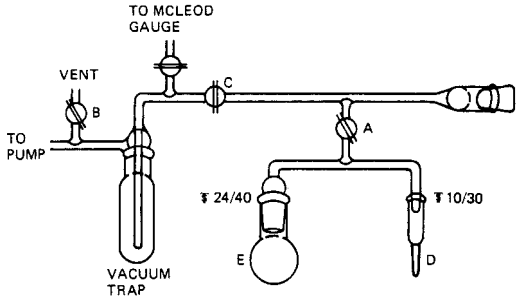


FIGURE 15 Closed-System Vacuum Distillation Apparatus for 1,4-Dioxane.

a 24/40 ground-glass neck. Semisolid or waxy samples should be liquefied by heating on a steam bath before making the transfer. Add 2.0 mL of water to the flask for crystalline samples, and 1.0 mL for liquid, semisolid, or waxy samples. Place a small Teflon-covered stirring bar in the flask, stopper, and stir to mix. Immerse the flask in an ice bath, and chill for about 1 min.

Wrap heating tape around the tube connecting the Chromaflex tube (*D*) and the round-bottom flask (*E*), and apply about 10 V to the tape. Apply a light coating of high-vacuum silicone grease to the ground-glass joints, and connect the Chromaflex tube to the 10/30 joint and the round-bottom flask to the 24/40 joint. Immerse the vacuum trap in a Dewar flask filled with liquid nitrogen, close stopcocks *A* and *B*, open stopcock *C*, and begin evacuating the system with a vacuum pump. Prepare a slush bath from powdered dry ice and methanol, and raise the bath to the neck of the round-bottom flask. After freezing the contents of the flask for about 10 min, and when the vacuum system is operating at 0.05 mm pressure or lower, open stopcock *A* for 20 s, and then close it. Remove the slush bath, and allow the flask to warm in air for about 1 min. Immerse the flask in a water bath at 20° to 25°, and after about 5 min warm the water in the bath to 35° to 40° (sufficient to liquefy most samples) while stirring slowly but constantly with the magnetic bar. Cool the water in the bath by adding ice, and chill for about 2 min. Replace the water bath with the slush bath, freeze the contents of the flask for about 10 min, then open stopcock *A* for 20 s, and close it. Remove the slush bath, and repeat the heating steps as before, this time reaching a final temperature of 45° to 50° or a temperature necessary to melt the sample completely. If there is any condensation in the tube connecting the round-bottom flask to the Chromaflex tube, slowly increase the voltage to the heating tape and heat until condensation disappears.

Stir with the magnetic stirrer throughout the following steps: Very slowly immerse the Chromaflex tube in the Dewar flask containing liquid nitrogen.

> **Caution**: When there is liquid distillate in the Chromaflex tube, the tube must be immersed in the nitrogen very slowly, or the tube will break.

Water will begin to distill into the tube. As ice forms in the tube, raise the Dewar flask to keep the liquid nitrogen level only slightly below the level of ice in the tube. When water begins to freeze in the neck of the 10/30 joint, or when liquid nitrogen reaches the 2.0-mL graduation mark on the Chromaflex tube, remove the Dewar flask and let the ice melt without heating. After the ice has melted, check the volume of water that has distilled, and repeat the sequence of chilling and thawing until at least 0.9 mL of water has been collected. Freeze the tube once again for about 2 min, and release the vacuum first by opening stopcock *B*, followed by stopcock *A*. Remove the Chromaflex tube from the apparatus, close it with a greased stopper, and let the ice melt without heating. Mix the contents of the tube by swirling, note the volume of distillate, and dilute to 2.0 mL with water, if necessary. Use this *Sample Preparation* as directed under *Chromatography* (below).

Chromatography (See *Chromatography*, Appendix IIA.) Use a gas chromatograph equipped with a flame-ionization detector. Under typical conditions, the instrument contains a 4-mm (id) × 6-ft glass column, or equivalent, packed with 80-/100- or 100-/120-mesh Chromosorb 104, or equivalent. The column is maintained isothermally at about 140°, the injection port at 200°, and the detector at 250°. Nitrogen is the carrier gas, flowing at a rate of about 35 mL/min. Install an oxygen scrubber between the carrier gas line and the column. The column should be conditioned for about 72 h at 250° with 30 to 40 mL/min carrier flow.

> **Note**: Chromosorb 104 is oxygen sensitive. Both new and used columns should be flushed with carrier gas for 30 to 60 min before heating each time they are installed in the gas chromatograph.

Inject a volume of the *Standard Preparation*, accurately measured, to give about 20% of maximum recorder response. Where possible, keep the injection volume in the range of 2 to 4 µL, and use the solvent-flush technique to minimize errors associated with injection volumes. In the same manner, inject an identical volume of the *Sample Preparation*. The height of the peak produced by the *Sample Preparation* does not exceed that produced by the *Standard Preparation*.[1]

FLUORIDE LIMIT TEST

Method I (Thorium Nitrate Colorimetric Method)

Use this method unless otherwise directed in the individual monograph.

> **Caution**: When applying this test to organic compounds, rigidly control at all times the temperature at which the distillation is conducted to the recommended range of 135° to 140° to avoid the possibility of explosion.

[1] If the sample fails the test because of known or suspected interference, another aliquot may be run on a 6-ft × 2-mm (id) column, or equivalent, of 0.2% Carbowax 1500 on Carbopak C, operating at 100° isothermal, with 20 mL/min of helium carrier flow. Under these conditions, the 1,4-dioxane elutes in about 4 min.

Note: To minimize the distillation blank resulting from fluoride leached from the glassware, treat the distillation apparatus as follows: Treat the glassware with hot 10% sodium hydroxide solution, followed by flushing with tap water and rinsing with distilled water. At least once daily, treat in addition by boiling down 15 to 20 mL of 1:2 sulfuric acid until the still is filled with fumes; cool, pour off the acid, treat again with 10% sodium hydroxide solution, and rinse thoroughly. For further details, see AOAC method 944.08.

Unless otherwise directed, place a 5.0-g sample and 30 mL of water in a 125-mL Pyrex distillation flask having a side arm and trap. The flask is connected with a condenser and carries a thermometer and a capillary tube, both of which must extend into the liquid. Slowly add, with continuous stirring, 10 mL of 70% perchloric acid, and then add 2 or 3 drops of a 1:2 solution of silver nitrate and a few glass beads. Connect a small dropping funnel or a steam generator to the capillary tube. Support the flask on a flame-resistant mat or shielding board, with a hole that exposes about one-third of the flask to the low, ''clean'' flame of a Bunsen burner.

Note: The shielding is essential to prevent the walls of the flask from overheating above the level of its liquid contents.

Distill until the temperature reaches 135°. Add water from the funnel or introduce steam through the capillary, maintaining the temperature between 135° and 140° at all times. Continue the distillation until 100 mL of distillate has been collected. After the 100-mL portion (*Distillate A*) is collected, collect an additional 50-mL portion of distillate (*Distillate B*) to ensure that all of the fluorine has been volatilized.

Place 50 mL of *Distillate A* in a 50-mL Nessler tube. In another, similar Nessler tube, place 50 mL of water distilled through the apparatus as a control. Add to each tube 0.1 mL of a filtered 1:1000 solution of sodium alizarinsulfonate and 1 mL of a freshly prepared 1:4000 solution of hydroxylamine hydrochloride, and mix well. Add, dropwise and with stirring, either 1 *N* or 0.05 *N* sodium hydroxide, depending on the expected volume of volatile acid distilling over, to the tube containing the distillate until its color just matches that of the control, which is faintly pink. Then add to each tube 1.0 mL of 0.1 *N* hydrochloric acid, and mix well. From a buret, graduated in 0.05 mL, add slowly to the tube containing the distillate enough of a 1:4000 solution of thorium nitrate so that, after mixing, the color of the liquid just changes to a faint pink. Note the volume of the solution added, then add exactly the same volume to the control, and mix. Now add to the control solution sodium fluoride TS (10 μg F per milliliter) from a buret to make the colors of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final color comparison. Check the endpoint by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in color should take place. Note the volume of sodium fluoride TS added.

Dilute *Distillate B* to 100 mL, and mix well. Place 50 mL of this solution in a 50-mL Nessler tube, and follow the procedure used for *Distillate A*. The total volume of sodium fluoride TS required for the solutions from both *Distillate A* and *Distillate B* should not exceed 2.5 mL.

Method II (Ion-Selective Electrode Method A)

Buffer Solution Dissolve 36 g of cyclohexylenedinitrilotetraacetic acid (CDTA) in sufficient 1 *N* sodium hydroxide to make 200 mL. Transfer 20 mL of this solution (equivalent to 4 g of disodium CDTA) into a 1000-mL beaker containing 500 mL of water, 57 mL of glacial acetic acid, and 58 g of sodium chloride, and stir to dissolve. Adjust the pH of the solution to between 5.0 and 5.5 by the addition of 5 *N* sodium hydroxide, then cool to room temperature, dilute to 1000 mL with water, and mix.

Procedure Unless otherwise directed in the individual monograph, transfer 8.0 g of sample and 20 mL of water into a 250-mL distilling flask, cautiously add 20 mL of perchloric acid, and then add 2 or 3 drops of a 1:2 solution of silver nitrate and a few glass beads.

Caution: Handle perchloric acid in an appropriate fume hood.

Following the directions, and observing the *Caution* and *Notes*, as given under *Method I*, distill the solution until 200 mL of distillate has been collected.

Transfer a 25.0-mL aliquot of the distillate into a 250-mL plastic beaker, and dilute to 100 mL with the *Buffer Solution*. Place the fluoride ion and reference electrodes (or a combination fluoride electrode) of a suitable ion-selective electrode apparatus in the solution. Adjust the calibration control until the indicator needle points to the center on the logarithmic concentration scale, allowing sufficient time for equilibration (about 20 min), and stirring constantly during the equilibration period and throughout the remainder of the procedure. Pipet 1.0 mL of a solution containing 100 μg of fluoride (F) ion per milliliter (prepared by dissolving 22.2 mg of sodium fluoride, previously dried at 200° for 4 h, in sufficient water to make 100.0 mL) into the beaker, allow the electrode to come to equilibrium, and record the final reading on the logarithmic concentration scale.

Note: Follow the instrument manufacturer's instructions regarding precautions and interferences, electrode filling and check, temperature compensation, and calibration.

Calculations Calculate the fluoride content, in milligrams per kilogram, of the sample taken by the formula

$$[IA/(R - I)] \times 100 \times (200/25W),$$

in which I is the initial scale reading before the addition of the sodium fluoride solution; A is the concentration, in micrograms per milliliter, of fluoride in the sodium fluoride solution added to the sample solution; R is the final scale reading after addition of the sodium fluoride solution; and W is the original weight, in grams, of the sample.

Method III (Ion-Selective Electrode Method B)

Sodium Fluoride Solution (5 μg F per milliliter) Transfer 2.210 g of sodium fluoride, previously dried at 200° for 4 h

and accurately weighed, into a 400-mL plastic beaker, add 200 mL of water, and stir until dissolved. Quantitatively transfer this solution into a 1000-mL volumetric flask with the aid of water, dilute to volume with water, and mix. Store this stock solution in a plastic bottle. On the day of use, transfer 5.0 mL of the stock solution into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Calibration Curve Transfer 1.0, 2.0, 3.0, 5.0, 10.0, and 15.0 mL of the *Sodium Fluoride Solution* into separate 250-mL plastic beakers; add 50 mL of water, 5 mL of 1 *N* hydrochloric acid, 10 mL of 1 *M* sodium citrate, and 10 mL of 0.2 *M* disodium EDTA to each beaker; and mix. Transfer each solution into separate 100-mL volumetric flasks, dilute to volume with water, and mix. Transfer a 50-mL portion of each solution into separate 125-mL plastic beakers, and measure the potential of each solution with a suitable ion-selective electrode apparatus (such as the Orion Model No. 94-09, with solid-state membrane), using a suitable reference electrode (such as the Orion Model No. 90-01, with single junction). Plot the calibration curve on two-cycle semilogarithmic paper (such as K & E No. 465130), with micrograms of F per 100 mL solution on the logarithmic scale.

Procedure Transfer 1.00 g of sample into a 150-mL glass beaker, add 10 mL of water, and, while stirring continuously, slowly add 20 mL of 1 *N* hydrochloric acid to dissolve the sample. Boil rapidly for 1 min, then transfer into a 250-mL plastic beaker, and cool rapidly in ice water. Add 15 mL of 1 *M* sodium citrate and 10 mL of 0.2 *M* disodium EDTA, and mix. Adjust the pH to 5.5 ± 0.1 with 1 *N* hydrochloric acid or 1 *N* sodium hydroxide, if necessary; transfer into a 100-mL volumetric flask; dilute to volume with water; and mix. Transfer a 50-mL portion of this solution into a 125-mL plastic beaker, and measure the potential of the solution with the apparatus described under *Calibration Curve*. Determine the fluoride content, in micrograms, of the sample from the *Calibration Curve*.

Method IV (Ion-Selective Electrode Method C)

Buffer Solution Dissolve 150 g of sodium citrate dihydrate and 10.3 g of disodium EDTA dihydrate in 800 mL of water, adjust the pH to 8.0 with 50% sodium hydroxide solution, and dilute to 1000 mL with water.

Fluoride Standard Solutions

1000 mg/kg Fluoride Standard Transfer 2.2108 g of sodium fluoride, previously dried at 200° for 4 h, into a 1000-mL volumetric flask and dissolve in and dilute to volume with water. The resulting solution contains 1000 μg of fluoride per milliliter.

50 mg/kg Fluoride Standard Pipet 50 mL of the *1000 mg/kg Fluoride Standard* into a 1000-mL volumetric flask. Dilute to volume with water.

10 mg/kg Fluoride Standard Pipet 100 mL of the *50 mg/kg Fluoride Standard* into a 500-mL volumetric flask. Dilute to volume with water.

Fluoride Limit Solutions (for a 1-g sample)

50 mg/kg Fluoride Limit Solution (1 mg/kg fluoride standard) Pipet 50 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute to volume with water.

10 mg/kg Fluoride Limit Solution (0.2 mg/kg fluoride standard) Pipet 10 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute to volume with water.

Fluoride Limit Solutions (for a 2-g sample)

50 mg/kg Fluoride Limit Solution (2 mg/kg fluoride standard) Pipet 100 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute to volume with water.

10 mg/kg Fluoride Limit Solution (0.4 mg/kg fluoride standard) Pipet 20 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute to volume with water.

Note: Store all standard and limit solutions in plastic containers.

Sample Preparation Accurately weigh the amount of sample specified in the monograph, transfer it into a 100-mL volumetric flask, and dissolve it in a minimal amount of water. Add 50.0 mL of the *Buffer Solution,* dilute to volume with water, and mix.

Electrode Calibration Pipet 50 mL of the *Buffer Solution* into a plastic beaker. Place the fluoride ion and reference electrodes (or a combination fluoride electrode) into the plastic beaker and stir. At 5-min intervals, add 100 μL and 1000 μL of the *1000 mg/kg Fluoride Standard* and read the potential, in millivolts, after each addition. The difference between the two readings is the slope of the fluoride electrode and should typically be in the range of 54 to 60 mV at 25°. If the difference in potential is not within this range, check, and, if necessary, replace the electrode, instrument, or solutions.

Procedure Transfer the entire sample into a plastic beaker. Place the electrode into the beaker, allow the solution to equilibrate for 5 min with stirring, and read the potential, in millivolts. Remove and rinse the electrode(s) with water. In another beaker, using a pipet, add 50 mL of the *Buffer Solution* followed by 50 mL of the *Fluoride Limit Solution* that best reflects the fluoride limit of the sample. Place the electrode in the beaker, equilibrate for 3 min, and read the potential in millivolts. If the potential of the *Fluoride Limit Solution* is less than that of the sample, the sample passes the test criterion for maximum acceptable fluoride level limit.

Method V

Lime Suspension Carefully shake about 56 g of low-fluorine calcium oxide (about 2 mg/kg of F) with 250 mL of water, and while stirring, slowly add 250 mL of 60% perchloric acid. Add a few glass beads, and boil until copious fumes of perchloric acid evolve, then cool, add 200 mL of water, and boil again.

Caution: Handle perchloric acid in an appropriate fume hood.

Repeat the dilution and boiling once more, cool, dilute considerably, and if precipitated silicon dioxide forms, filter through

a fritted-glass filter. While stirring, pour the clear solution into 1000 mL of a 1:10 solution of sodium hydroxide, allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing five times with water in large centrifuge bottles, shaking the mass thoroughly each time. Finally, shake the precipitate into a suspension and dilute with water to 2000 mL. Store in paraffin-lined bottles, and shake well before use.

Note: 100 mL of this suspension should give no appreciable fluoride blank when evaporated, distilled, and titrated as directed under *Method I*.

Procedure Assemble the distilling apparatus as described under *Method I*, and add 1.67 g of sample, accurately weighed, and 25 mL of 1:2 sulfuric acid to the distilling flask. Distill until the temperature reaches 160°, then maintain at 160° to 165° by adding water from the funnel, collecting 300 mL of distillate. Oxidize the distillate by cautiously adding 2 or 3 mL of fluorine-free 30% hydrogen peroxide (to remove sulfates), allow to stand for a few minutes, and evaporate in a platinum dish with an excess of *Lime Suspension*. Ignite briefly at 600°, then cool and wet the ash with about 10 mL of water. Cover the dish with a watch glass, and cautiously introduce under the watch glass just sufficient 60% perchloric acid to dissolve the ash. Add the contents of the dish through the dropping funnel of a freshly prepared distilling apparatus (the distilling flask should contain a few glass beads), using a total of 20 mL of the 60% perchloric acid to dissolve the ash and transfer the solution. Add 10 mL of water and a few drops of a 1:2 solution of silver perchlorate through the dropping funnel, and continue as directed under *Method I*, beginning with ''Distill until the temperature reaches 135°. . . .''

LEAD LIMIT TEST

Note: Unless otherwise specified in the monograph, use the *Dithizone Method* to determine lead levels.

Dithizone Method

Special Reagents Select reagents having as low a lead content as practicable, and store all solutions in containers of borosilicate glass. Rinse all glassware thoroughly with warm, 1:2 nitric acid followed by water.

Ammonia–Cyanide Solution Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute to 100 mL with water.

Ammonium Citrate Solution Dissolve 40 g of citric acid in 90 mL of water, add 2 or 3 drops of phenol red TS, then cautiously add ammonium hydroxide until the solution acquires a red color. Extract it with 20-mL portions of *Dithizone Extraction Solution* until the dithizone solution retains its green color or remains unchanged.

Diluted Standard Lead Solution (1 μg Pb in 1 mL)

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL with water, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* to 100.0 mL with water. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 μg of lead (Pb) ion.

Diluted Standard Lead Solution Immediately before use, transfer 10.0 mL of *Standard Lead Solution* into a 100-mL volumetric flask, dilute to volume with 1:100 nitric acid, and mix.

Dithizone Extraction Solution Dissolve 30 mg of dithizone in 1000 mL of chloroform, add 5 mL of alcohol, and mix. Store in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1:100 nitric acid, discarding the nitric acid. Do not use if more than 1 month old.

Hydroxylamine Hydrochloride Solution Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 mL, transfer the solution into a separator, add a few drops of thymol blue TS, then add ammonium hydroxide until the solution assumes a yellow color. Add 10 mL of a 1:25 solution of sodium diethyldithiocarbamate, mix, and allow to stand for 5 min. Extract the solution with successive 10- to 15-mL portions of chloroform until a 5-mL test portion of the chloroform extract does not assume a yellow color when shaken with cupric sulfate TS. Add 2.7 *N* hydrochloric acid until the extracted solution is pink, adding 1 or 2 drops more of thymol blue TS if necessary, then dilute to 100 mL with water, and mix.

Potassium Cyanide Solution Dissolve 50 g of potassium cyanide in sufficient water to make 100 mL. Remove the lead from the solution by extraction with successive portions of *Dithizone Extraction Solution* as described under *Ammonium Citrate Solution*, then extract any dithizone remaining in the cyanide solution by shaking with chloroform. Finally, dilute the cyanide solution with sufficient water so that each 100 mL contains 10 g of potassium cyanide.

Standard Dithizone Solution Dissolve 10 mg of dithizone in 1000 mL of chloroform, keeping the solution in a glass-stoppered, lead-free bottle suitably wrapped to protect it from light and stored in a refrigerator.

Sample Solution Use the solution obtained by treating the sample as directed in an individual monograph as the *Sample Solution* in the *Procedure*. Sample solutions of organic compounds are prepared, unless otherwise directed, according to the following general method:

Caution: Some substances may react unexpectedly with explosive violence when digested with hydrogen peroxide. Use appropriate safety precautions at all times.

Transfer 1.0 g of sample into a suitable flask, add 5 mL of sulfuric acid and a few glass beads, and digest at a temperature not exceeding 120° until charring begins, using preferably, a hot plate in a fume hood. (Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 mL.) After the sample has initially been decomposed by the acid, add with caution, dropwise, hydrogen peroxide (30%), allowing the reaction to subside and reheating between drops. The first few drops must be added very slowly with sufficient mixing to prevent a rapid reaction, and heating should be discontinued if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls or bottom of the flask during the digestion.

Note: Add small quantities of the peroxide when the solution begins to darken.

Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250° to 300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, cautiously add 10 mL of water, again evaporate to strong fuming, and cool. Quantitatively transfer the solution into a separator with the aid of small quantities of water.

Procedure Transfer the *Sample Solution*, prepared as directed in the individual monograph, into a separator, and unless otherwise directed, add 6 mL of *Ammonium Citrate Solution* and 2 mL of *Hydroxylamine Hydrochloride Solution*. (Use 10 mL of the citrate solution when determining lead in iron salts.) Add 2 drops of phenol red TS to the separator, and make the solution just alkaline (red in color) by the addition of ammonium hydroxide. Cool the solution, if necessary, under a stream of tap water, then add 2 mL of *Potassium Cyanide Solution*. Immediately extract the solution with 5-mL portions of *Dithizone Extraction Solution*, draining each extract into another separator, until the dithizone solution retains its green color. Shake the combined dithizone solutions for 30 s with 20 mL of 1:100 nitric acid; discard the chloroform layer; add 5.0 mL of *Standard Dithizone Solution* and 4 mL of *Ammonia–Cyanide Solution* to the acid solution; and shake for 30 s. The purple hue in the chloroform solution of the sample caused by any lead dithizonate present does not exceed that in a control, containing the volume of *Diluted Standard Lead Solution* equivalent to the amount of lead specified in the monograph, when treated in the same manner as the sample.

Flame Atomic Absorption Spectrophotometric Method

Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 1:2 nitric acid followed by water.

Lead Nitrate Stock Solution (100 μg/mL) Dissolve 159.8 mg of reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid in a 1000-mL volumetric flask, and dilute to volume with water.

Standard Lead Solution (10 μg/mL) On the day of use, transfer 10 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute to volume with water.

Diluted Standard Lead Solutions On the day of use, prepare a set of standard lead solutions that corresponds to the lead limit specified in the monograph:

1 mg/kg Lead Limit (0.5, 1.0, and 1.5 μg/mL standards) On the day of use, transfer 5.0, 10.0, and 15.0 mL of *Standard Lead Solution* into three separate 100-mL volumetric flasks, add 10 mL of 3 *N* hydrochloric acid to each, and dilute to volume with water.

5 mg/kg Lead Limit (1.0, 5.0, and 10.0 μg/mL standards) On the day of use, transfer 10.0 and 50.0 mL of *Standard Lead Solution* into two separate 100-mL volumetric flasks, add 10 mL of 3 *N* hydrochloric acid to each, and dilute to volume with water. The final standard, 10.0 μg/mL, is taken directly from the *Standard Lead Solution*.

10 mg/kg Lead Limit (5.0, 10.0, and 15.0 μg/mL standards) On the day of use, transfer 5.0, 10.0, and 15.0 mL of *Lead Nitrate Stock Solution* into three separate 100-mL volumetric flasks, add 10 mL of 3 *N* hydrochloric acid to each, and dilute to volume with water.

25% Sulfuric Acid Solution (by volume) Cautiously add 100 mL of sulfuric acid to 300 mL of water with constant stirring while cooling in an ice bath.

Sample Preparation Transfer the sample weight as specified in the monograph, weighed to the nearest 0.1 mg, into an evaporating dish. Add a sufficient amount of *25% Sulfuric Acid Solution*, and distribute the sulfuric acid uniformly through the sample. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the sample by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the sample until the residue appears free from carbon. Prepare a *Sample Blank* by ashing 5 mL of 25% sulfuric acid. Cool and cautiously wash down the inside of each evaporation dish with water.

Add 5 mL of 1 *N* hydrochloric acid. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 *N* hydrochloric acid and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer each solution quantitatively to a 10-mL volumetric flask, dilute to volume, and mix.

Procedure Concomitantly determine the absorbances of the *Sample Blank*, the *Diluted Standard Lead Solutions*, and the *Sample Preparation* at the lead emission line of 283.3 nm, using a slit-width of 0.7 nm. Use a suitable atomic absorption spectrophotometer equipped with a lead electrodeless discharge lamp (EDL), an air–acetylene flame, and a 4-in. burner head. Use water as the blank.

Calculations Determine the corrected absorbance values by subtracting the *Sample Blank* absorbance from each of the *Diluted Standard Lead Solutions* and from the *Sample Preparation* absorbances. Prepare a standard curve by plotting the corrected *Diluted Standard Lead Solutions* absorbance values versus their corresponding concentrations expressed as micrograms per milliliter. Determine the lead concentration in the *Sample Preparation* by reference to the calibration curve. Calculate the quantity of lead, in milligrams per kilogram, in the sample taken by the formula

$$10C/W_S,$$

in which C is the concentration, in micrograms per milliliter, of lead from the standard curve; and W_S is the weight, in grams, of the sample taken.

Atomic Absorption Spectrophotometric Graphite Furnace Method

The following methods are primarily intended for the analysis of applicable substances containing less than 1 mg/kg of lead.

Method I

This method is intended for the quantitation of lead in substances that are soluble in water, such as sugars and sugar syrups, at levels as low as 0.03 mg/kg. The method detection limit is approximately 5 ng/kg.

Apparatus Use a suitable graphite furnace atomic absorption spectrophotometer set at 283.3 nm and equipped with an autosampler, pyrolytically coated graphite tubes, solid pyrolytic graphite platforms, and an adequate means of background correction. Zeeman effect or Smith-Hieftje background correction is preferred, but deuterium arc background correction should be acceptable. (This method was developed on a Perkin-Elmer Model Z5100, 0.7-nm slit, HGA-600 furnace, AS-60 autosampler with Zeeman background correction.) If the instrument does not have a well-defined calibration function, a separate calculator or computer is required for linear least squares, nonlinear, or quadratic calibrations. Use either a hollow cathode lamp or an electrode-less discharge lamp as the source, and use argon as the purge gas and breathing-quality air (for oxygen ashing to avoid residue build up during the char step) as the alternate gas. Set up the instrument according to the manufacturer's specifications with consideration of current good GFAAS practices—addressing such factors as line voltage, cooling water temperature, graphite part specifications, and furnace temperature. If an optical pyrometer or thermocouple is not available to check the furnace controller temperature calibration, dim the room lights, and observe the furnace emission through the sample introduction port while increasing the furnace temperature. A characteristic cherry red glow should begin to appear at 800°. If it glows at a lower temperature, then the furnace is hotter, and temperatures must be adjusted downward accordingly.

Use acid-cleaned [in a mixture of 5% sub-boiling, distilled nitric acid and 5% sub-boiling, distilled hydrochloric acid made up in deionized, distilled water (18 megohm), and thoroughly rinsed with deionized, distilled water (18 megohm)] autosampler cups (PE B008-7600 Teflon, or equivalent) to avoid contamination. Use micropipets with disposable tips free of lead contamination for dilution. Ensure accuracy and precision of micropipets and tips by dispensing and weighing 5 to 10 replicate portions of water onto a microbalance. Use acid-cleaned volumetric glassware to prepare standards and dilute samples to a final volume. For digestion, use acid-cleaned, high-density polyethylene tubes, polypropylene tubes, Teflon tubes, or quartz tubes. Store final diluted samples in plastic tubes.

Standard Solutions Prepare all lead solutions in 5% sub-boiling distilled nitric acid. Use a single-element 1000- or 10,000-μg/mL lead stock to prepare (weekly) an intermediate 10-μg/mL standard in 5% nitric acid. Prepare (daily) a *Lead Standard Solution* (1 μg/mL) by diluting the intermediate 10-μg/mL stock solution 1:10. Prepare *Working Calibration Standards* of 100.0, 50.0, 25.0, and 10.0 ng/mL from this, using appropriate dilutions. Store standards in acid-cleaned polyethylene test tubes or bottles. If the GFAAS autosampler is used to automatically dilute standards, ensure calibration accuracy by pipetting volumes of 3 μL or greater.

Modifier Stock Solution Weigh 20 g of ultrapure magnesium nitrate hexahydrate and dilute to 100 mL. Just before use, prepare a *Modifier Working Solution* by diluting stock solution 1:10. A volume of 5 μL will provide 0.06 mg of magnesium nitrate.

Sample Digestion (**Caution**: Perform the procedure in a fume hood, and wear safety glasses.) Obtain a representative subsample to be analyzed. For liquid samples such as sugar syrups, ultrasonicate and/or vortex mix before weighing. For solid samples such as crystalline sucrose, make a sugar solution using equal weights of sample (5-g minimum) and deionized, distilled (18 megohm) water. Mix samples until completely dissolved. Transfer approximately 1.5 g (record to nearest mg) of sample (or 3.0 g of sugar solution), accurately weighed, into a digestion tube. Run a *Sample Preparation Blank* of 1.5 g of deionized, distilled (18 megohm) water through the entire procedure with each batch of samples. Add 0.75 mL of sub-boiling, distilled nitric acid. Heat plastic tubes in a water bath, quartz tubes in a water bath or heating block, warming slowly to between 90° and 95° to avoid spattering. Monitor the temperature by using a ''dummy'' sample. Heat until all brown vapors have dissipated and any rust-colored tint is gone (20 to 30 min). Cool. Add 0.5 mL of 50% hydrogen peroxide dropwise, heat at 90° to 95° for 5 min, and cool. Add a second 0.5-mL portion of 50% hydrogen peroxide, dropwise, and heat at 90° to 100° for 5 to 10 min until clear. Cool, and dilute to a final volume of 10 mL.

Procedure The furnace program is as follows: (1) dry at 200°, using a 20-s ramp and a 30-s hold and a 300-mL/min argon flow; (2) char the sample at 750°, using a 40-s ramp and a 40-s hold and a 300-mL/min air flow; (3) cool down, and purge the air from the furnace for 60 s, using a 20° set temperature and a 300-mL/min argon flow; (4) atomize at 1800°, using a 0-s ramp and a 10-s hold with the argon flow

stopped; (5) clean out at 2600°, with a 1-s ramp and a 7-s hold; (6) cool down the furnace (if necessary) at 20°, with a 1-s ramp and a 5-s hold with a 300-mL/min argon flow.

Use the autosampler to inject 20 µL of blanks, calibration standards, and sample solutions and 5 µL of *Modifier Working Solution*. Inject each respective solution in triplicate, and average results. Use peak area measurements for all quantitation. After ensuring that the furnace is clean by running a 5% nitric acid blank, check the instrument sensitivity by running the 25-ng/mL calibration standard. If the integrated absorbance is less than 0.14 abs-sec for a standard, 28-mm × 6-mm, end-heated furnace tube, correct the cause of insufficient sensitivity before proceeding. If the integrated absorbance is greater than 0.25 abs-sec, contamination is likely, and the source should be investigated. Calculate the characteristic mass (m_o) (mass of Pb pg necessary to produce an integrated absorbance of 0.0044 abs-sec) as follows:

$$m_o = (0.0044 \text{ abs-sec})(25 \text{ pg/µL})(20 \text{ µL})/(\text{measured } 25 \text{ pg/µL abs-sec}).$$

Record and track the integrated absorbance and m_o for reference and quality assurance.

Standard Curve Inject each calibration standard in triplicate. Normal instrument linearity extends to 25 ng/mL. If nonlinear calibration capability is not available, limit the working calibration curve to ≤25 ng/mL. Use the calibration algorithms provided in the instrument software. Recheck calibration periodically (≤15 samples) by running a 25- or 50-ng/mL calibration standard interspersed with samples. If recheck differs from calibration by >10%, recalibrate the instrument. The instrumental detection limit (DL) and quantitation limit (QL), in picograms, may be based on 7 to 10 replicates of the *Sample Preparation Blank* and calculated as follows:

$$DL = (3)(\text{s.d. blank abs-sec})(10 \text{ pg/µL})(20 \text{ µL})/(\text{abs-sec } 10 \text{ ng/mL std}),$$

$$QL = (10)(\text{s.d. blank abs-sec})(10 \text{ pg/µL})(20 \text{ µL})/(\text{abs-sec } 10 \text{ ng/mL std}).$$

During method development, detection limits were typically 10 to 14 pg, corresponding to 0.5 to 0.7 ng/mL for 20 µL. This corresponds to a method detection limit of 3.3 to 4.7 ng/g of sugar.

Sample Analyses Inject each sample digest in triplicate, and record the integrated absorbance. If instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample response into working range, and note the dilution factor (DF). Sample solutions having a final concentration of >25 ng/mL should be diluted 1:10 to facilitate analysis in the linear range for systems not equipped with nonlinear calibration. All sample analyses should be blank corrected using the sample preparation blank. This can typically be done automatically by the software after identifying and running a representative sample preparation blank. Use the calibration algorithm provided in the instrument software to calculate a blank-corrected, digest lead concentration (in nanograms per milliliter).

Calculation of Lead Content Calculate the lead level in the original sample as follows:

$$\text{Pb(ng/g)} = (\text{blank-corrected Pb ng/mL})(\text{DF})[\text{sample vol (10 mL)}][\text{sample wt (approx. 1.5 g)}].[1]$$

Quality Assurance To ensure analytical accuracy, NIST SRM 1643c acidified water or a similar material should be analyzed before the unknown samples are. The certified content of SRM 1643c is 35.3 ± 0.9 ng/mL. If the concentration determined is not within 10% of the mean reference value (31.8 to 38.8 ng/mL), the reason for inaccuracy should be evaluated, and unknown samples should not be analyzed until acceptable accuracy is achieved. Also prepare an in-house control solution made from uncontaminated table sugar or reagent-grade sucrose (or other appropriate substance with a Pb content <5 ng/g as received) mixed with an equal volume of water. Spike this solution with Pb to produce a concentration of 100 ng/g. Analyze with each batch of samples. Recoveries should be 100% ± 20%, and the precision for complete replicate digestions should be <5% RSD. Periodically, a sample digest should be checked using the method of standard additions to ensure that there are no multiplicative or chemical interferences. Spiking samples and checking recoveries is always a good practice.

Method II

This method is primarily intended for the determination of lead at levels of less than 1 mg/kg in substances immiscible with water, such as edible oils.

Apparatus Use a suitable atomic absorption spectrophotometer (Perkin-Elmer Model 3100 or equivalent) fitted with a graphite furnace (Perkin-Elmer HGA 600 or equivalent). Use a lead hollow-cathode lamp (Perkin-Elmer or equivalent) with argon as the carrier gas. Follow the manufacturers' directions for setting the appropriate instrument parameters for lead determination.

> **Note**: For this test, use reagent-grade chemicals with as low a lead content as is practicable, as well as high-purity water and gases. Before use in this analysis, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse them thoroughly with high-purity water, preferably obtained from a mixed-bed strong-acid, strong-base ion-exchange cartridge capable of producing water with an electrical resistivity of 12 to 15 megohms.

Hydrogen Peroxide–Nitric Acid Solution Dissolve equal volumes of 10% hydrogen peroxide and 10% nitric acid.

> **Note**: Use caution.

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate (alternatively, use NIST Standard Reference Material, containing 10 mg of lead per kilogram, or equivalent) in 100 mL of *Hydrogen Peroxide–Nitric Acid Solution*. Dilute to 1000.0 mL with *Hydrogen Peroxide–Nitric*

[1] If a sample solution was prepared initially to ensure sample homogeneity, this is the weight of the original sugar digested (not the weight of the solution).

Acid Solution, and mix. Prepare and store this solution in glass containers that are free from lead salts. Each milliliter of this solution contains the equivalent of 100 μg of lead (Pb) ion.

Standard Lead Solution On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* to 100.0 mL with *Hydrogen Peroxide–Nitric Acid Solution*, and mix. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 μg of lead (Pb) ion.

Butanol–Nitric Acid Solution Slowly add 50 mL of nitric acid to approximately 500 mL of butanol contained in a 1000-mL volumetric flask. Dilute to volume with butanol, and mix.

Standard Solutions Prepare a series of lead standard solutions serially diluted from the *Standard Lead Solution* in *Butanol–Nitric Acid Solution*. Pipet into separate 100-mL volumetric flasks 0.2, 0.5, 1, and 2 mL, respectively, of *Standard Lead Solution*, dilute to volume with *Butanol–Nitric Acid Solution*, and mix. The *Standard Solutions* contain, respectively, 0.02, 0.05, 0.1, and 0.2 μg of lead per milliliter. (For lead limits greater than 1 mg/kg, prepare a series of standard solutions in a range encompassing the expected lead concentration in the sample.)

Sample Solution (**Caution**: Perform this procedure in a fume hood, and wear safety glasses.) Transfer 1 g of sample, accurately weighed, into a large test tube. Add 1 mL of nitric acid. Place the test tube in a rack in a boiling water bath. As soon as the rusty tint is gone, add 1 mL of 30% hydrogen peroxide dropwise to avoid a vigorous reaction, and wait for bubbles to form. Stir with an acid-washed plastic spatula if necessary. Remove the test tube from the water bath, and let it cool. Transfer the solution into a 10-mL volumetric flask, and dilute to volume with *Butanol–Nitric Acid Solution*, and mix. Use this solution for analysis.

Procedure

Tungsten Solution Transfer 0.1 g of tungstic acid (H_2WO_4) and 5 g of sodium hydroxide pellets into a 50-mL plastic bottle. Add 5.0 mL of high-purity water, and mix. Heat the mixture in a hot water bath until complete solution is achieved. Cool, and store at room temperature.

Procedure Place the graphite tube in the furnace. Inject a 20-μL aliquot of the *Tungsten Solution* into the graphite tube, using a 300-mL/min argon flow and the following sequence of conditions: Dry at 110° for 20 s, char at 700° to 900° for 20 s, and with the argon flow stopped, atomize at 2700° for 10 s; repeat this procedure once more using a second 20-μL aliquot of the *Tungsten Solution*. Clean the quartz windows.

Standard Curve [**Note**: The sample injection technique is the most crucial step in controlling the precision of the analysis; the volume of the sample must remain constant. Rinse the μL pipet tip (Eppendorf or equivalent) three times with either the *Standard Solutions* or *Sample Solution* before injection. Use a fresh pipet tip for each injection, and start the atomization process immediately after injecting the sample. Between injections, flush the graphite tube of any residual lead by purging at a high temperature as recommended by the manufacturer.] With the hollow cathode lamp properly aligned for maximum absorbance and the wavelength set at 283.3 nm, atomize 20-μL aliquots of the four *Standard Solutions*, using a 300-mL/min argon flow and the following sequence of conditions: Dry at 110° for 30 s, with a 20-s ramp period and a 10-s hold time; then char at 700° for 42 s, with a 20-s ramp period and a 22-s hold time; and then, with the argon flow stopped, atomize at 2300° for 7 s.

Plot a standard curve using the concentration, in micrograms per milliliter, of each *Standard Solution* versus its maximum absorbance value compensated for background correction as directed for the particular instrument, and draw the best straight line.

Atomize 20 μL of the *Sample Solution* under identical conditions, and measure its corrected maximum absorbance. From the *Standard Curve*, determine the concentration, *C*, in micrograms per milliliter, of the *Sample Solution*. Calculate the quantity, in milligrams per kilogram, of lead in the sample by the formula

$$10C/W,$$

in which *W* is the weight, in grams, of the sample taken.

APDC Extraction Method

Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 1:2 nitric acid followed by water.

2% APDC Solution Dissolve 2.0 g of ammonium pyrrolidinedithiocarbamate (APDC) in 100 mL of water. Filter any slight residue of insoluble APDC from the solution before use.

Lead Nitrate Stock Solution (100 μg/mL) Dissolve 159.8 mg of reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid in a 1000-mL volumetric flask, and dilute to volume with water.

Standard Lead Solution (2 μg/mL) On the day of use, transfer 2.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute to volume with water.

Sample Preparation Transfer a 10.0-g sample to a clean 150-mL beaker, and 10 mL of water to a second 150-mL beaker to serve as the blank. Add to each 30 mL of water and the minimum amount of hydrochloric acid needed to dissolve the sample, plus an additional 1 mL of hydrochloric acid to ensure the dissolution of any lead present. Heat to boiling, and boil for several minutes. Allow to cool, and dilute to about 100 mL with deionized water. Adjust the pH of the resulting solution to between 1.0 and 1.5 with 25% NaOH. Quantitatively transfer the pH-adjusted solution to a clean 250-mL separatory funnel, and dilute to about 200 mL with water. Add 2 mL of *2% APDC Solution*, and mix. Extract with two 20-mL portions of chloroform, collecting the extracts in a clean 50-mL beaker. Evaporate to dryness on a steam bath. Add 3 mL of nitric acid to the residue, and heat to near

dryness. Then add 0.5 mL of nitric acid and 10 mL of deionized water to the beaker, and heat until the volume is reduced to about 3 to 5 mL. Transfer the digested extract to a clean 10-mL volumetric flask, and dilute to volume with water.

Procedure Concomitantly determine the absorbances of the *Standard Lead Solution* and the *Sample Preparation* against the blank at the lead emission line of 283.3 nm, using a slit-width of 0.7 nm. Use a suitable atomic absorption spectrophotometer equipped with a lead electrode-less discharge lamp (EDL), or equivalent; an air–acetylene flame; and a 4-in. burner head. Use water as the blank. The absorbance of the *Sample Preparation* is not greater than that of the *Standard Lead Solution*.

MANGANESE LIMIT TEST

Manganese Detection Instrument Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder or other readout device and capable of measuring the radiation absorbed by manganese atoms at the manganese resonance line of 279.5 nm.

Standard Preparations Transfer 1000 mg, accurately weighed, of manganese metal powder into a 1000-mL volumetric flask, dissolve by warming in a mixture of 10 mL of water and 10 mL of 0.5 *N* hydrochloric acid, cool, dilute to volume with water, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute to volume with water, and mix. Finally, pipet 5.0, 10.0, 15.0, and 25.0 mL of this solution into separate 1000-mL volumetric flasks, dilute each flask to volume with water, and mix. The final solutions contain 0.5, 1.0, 1.5, and 2.5 mg/kg of Mn, respectively.

Sample Preparation Transfer 10.000 g of the sample into a 200-mL Kohlrausch volumetric flask, previously rinsed with 0.5 *N* hydrochloric acid, add 140 mL of 0.5 *N* hydrochloric acid, and shake vigorously for 15 min, preferably with a mechanical shaker. Dilute to volume with 0.5 *N* hydrochloric acid, and shake. Centrifuge approximately 100 mL of the sample mixture in a heavy-walled centrifuge tube at 2000 rpm for 5 min, and use the clear supernatant liquid in the following *Procedure*.

Procedure Aspirate 0.5 *N* hydrochloric acid through the air–acetylene burner for 5 min, and obtain a baseline reading at 279.5 nm, following the manufacturer's instructions for operating the atomic absorption spectrophotometer being used for the analysis. Aspirate a portion of each *Standard Preparation* in the same manner, note the readings, then aspirate a portion of the *Sample Preparation*, and note the reading. Prepare a standard curve by plotting the mg/kg of Mn in each *Standard Preparation* against the respective readings. From the graph determine the mg/kg of Mn in the *Sample Preparation*, and multiply this value by 20 to obtain the mg/kg of Mn in the original sample taken for analysis.

MERCURY LIMIT TEST

Method I

Mercury Detection Instrument Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder and capable of measuring the radiation absorbed by mercury vapors at the mercury resonance line of 253.6 nm. A simple mercury vapor meter or detector equipped with a variable span recorder also is satisfactory.

> **Note**: Wash all glassware associated with the test with nitric acid, and rinse thoroughly with water before use.

Aeration Apparatus The apparatus, shown in Fig. 16, consists of a flowmeter (*a*), capable of measuring flow rates from 500 to 1000 mL/min, connected via a three-way stopcock (*b*), with a Teflon plug, to 125-mL gas washing bottles (*c* and *d*), followed by a drying tube (*e*), and finally a suitable quartz liquid absorption cell (*f*), terminating with a vent (*g*) to a fume hood.

> **Note**: The absorption cell will vary in optical pathlength depending on the type of mercury detection instrument used.

Bottle *c* is fitted with an extra-coarse fritted bubbler (Corning 31770 125 EC, or equivalent), and the bottle is marked with a 60-mL calibration line. The drying tube *e* is lightly packed with magnesium perchlorate. Bottle *c* is used for the test solution, and bottle *d*, which remains empty throughout the procedure, is used to collect water droplets.

Alternatively, an apparatus embodying the principle of the assembly described and illustrated may be used. The aerating medium may be either compressed air or compressed nitrogen.

Standard Preparation Transfer 1.71 g of mercuric nitrate [$Hg(NO_3)\cdot H_2O$] into a 1000-mL volumetric flask, dissolve in a mixture of 100 mL of water and 2 mL of nitric acid, dilute to volume with water, and mix. Discard after 1 month. Transfer 10.0 mL of this solution into a second 1000-mL volumetric flask, acidify with 5 mL of a 1:5 sulfuric acid solution, dilute

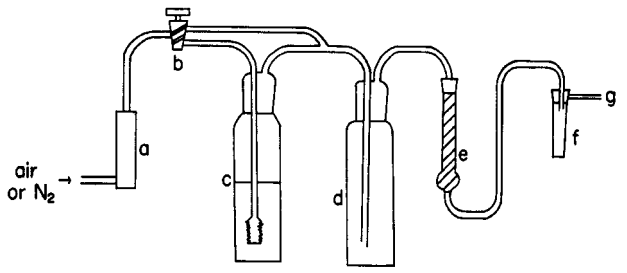


FIGURE 16 Aeration Apparatus for Mercury Limit Test.

to volume with water, and mix. Discard after 1 week. On the day of use, transfer 10.0 mL of the second solution into a 100-mL volumetric flask, acidify with 5 mL of 1:5 sulfuric acid, dilute to volume with water, and mix. Each milliliter of this solution contains 1 μg of mercury. Transfer 2.0 mL of this solution (2 μg Hg) into a 50-mL beaker, and add 20 mL of water, 1 mL of a 1:5 sulfuric acid solution, and 1 mL of a 1:25 solution of potassium permanganate. Cover the beaker with a watch glass, boil for a few seconds, and cool.

Sample Preparation Prepare as directed in the individual monograph.

Procedure Assemble the aerating apparatus as shown in Fig. 16, with bottles *c* and *d* empty and stopcock *b* in the bypass position. Connect the apparatus to the absorption cell (*f*) in the instrument, and adjust the air or nitrogen flow rate so that in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a baseline reading at 253.6 nm, following the manufacturer's instructions for operating the instrument.

Treat the *Standard Preparation* as follows: Destroy the excess permanganate by adding a 1:10 solution of hydroxylamine hydrochloride, dropwise, until the solution is colorless. Immediately wash the solution into bottle *c* with water, and dilute to the 60-mL mark with water. Add 2 mL of 10% stannous chloride solution (prepared fresh each week by dissolving 10 g of $SnCl_2 \cdot 2H_2O$ in 20 mL of warm hydrochloric acid and diluting with 80 mL of water), and immediately reconnect bottle *c* to the aerating apparatus. Turn stopcock *b* from the bypass to the aerating position, and continue the aeration until the absorption peak has been passed and the recorder pen has returned to the baseline. Disconnect bottle *c* from the aerating apparatus, discard the *Standard Preparation* mixture, wash bottle *c* with water, and repeat the foregoing procedure using the *Sample Preparation*; any absorbance produced by the *Sample Preparation* does not exceed that produced by the *Standard Preparation*.

Method II

Dithizone Extraction Solution Dissolve 30 mg of dithizone in 1000 mL of chloroform, add 5 mL of alcohol, and mix. Store in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1:100 nitric acid, discarding the nitric acid. Discard the solution after 1 month.

Diluted Dithizone Extraction Solution Just before use, dilute 5 mL of *Dithizone Extraction Solution* with 25 mL of chloroform.

Hydroxylamine Hydrochloride Solution Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 mL, transfer the solution into a separator, add a few drops of thymol blue TS, and then add ammonium hydroxide until a yellow color develops. Add 10 mL of a 1:25 solution of sodium diethyldithiocarbamate, mix, and allow to stand for 5 min. Extract the solution with successive 10- to 15-mL portions of chloroform until a 5-mL test portion of the chloroform extract does not develop a yellow color when shaken with a dilute solution of cupric sulfate. Add 2.7 *N* hydrochloric acid until the extracted solution is pink, adding one or two more drops of thymol blue TS, if necessary, then dilute to 100 mL with water, and mix.

Mercury Stock Solution Transfer 135.4 mg of mercuric chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with 1 *N* sulfuric acid, and mix. Dilute 5.0 mL of this solution to 500.0 mL with 1 *N* sulfuric acid. Each milliliter contains the equivalent of 10 μg of mercury.

Diluted Standard Mercury Solution On the day of use, transfer 10.0 mL of *Mercury Stock Solution* into a 100-mL volumetric flask, dilute to volume with 1 *N* sulfuric acid, and mix. Each milliliter contains the equivalent of 1 μg of mercury.

Sodium Citrate Solution Dissolve 250 g of sodium citrate dihydrate in 1000 mL of water.

Sample Solution Dissolve 1 g of sample in 30 mL of 1.7 *N* nitric acid by heating on a steam bath. Cool to room temperature in an ice bath, stir, and filter through S and S No. 589, or equivalent, filter paper that has been previously washed with 1.7 *N* nitric acid, followed by water. Add 20 mL of *Sodium Citrate Solution* and 1 mL of *Hydroxylamine Hydrochloride Solution* to the filtrate.

Procedure (**Note**: Because mercuric dithizonate is light sensitive, perform this procedure in subdued light.) Prepare a control containing 3.0 mL of *Diluted Standard Mercury Solution* (3 μg Hg), 30 mL of 1.7 *N* nitric acid, 5 mL of *Sodium Citrate Solution*, and 1 mL of *Hydroxylamine Hydrochloride Solution*. Treat the control and the *Sample Solution* as follows: Using a pH meter, adjust the pH of each solution to 1.8 with ammonium hydroxide, and transfer the solutions into different separators. Extract each with two 5-mL portions of *Dithizone Extraction Solution*, and then extract again with 5 mL of chloroform, discarding the aqueous solutions. Transfer the combined extracts from each separator into different separators, add 10 mL of 1:2 hydrochloric acid to each, shake well, and discard the chloroform layers. Extract the acid solutions with about 3 mL of chloroform, shake well, and discard the chloroform layers. Add 0.1 mL of 0.05 *M* disodium EDTA and 2 mL of 6 *N* acetic acid to each separator, mix, and then slowly add 5 mL of ammonium hydroxide. Stopper the separators, and cool under a stream of cold water, and dry the outside of the separators. To avoid loss, carefully pour the solutions through the tops of the separators into separate beakers, and using a pH meter, adjust the pH of both solutions to 1.8 with 6 *N* ammonium hydroxide. Return the sample and control solutions to their original separators, add 5.0 mL of *Diluted Dithizone Extraction Solution*, and shake vigorously. Any color developed in the *Sample Solution* does not exceed that in the control.

NICKEL LIMIT TEST

Method I

Atomic Absorption System Apparatus Use a suitable atomic absorption spectrometer equipped with a nickel hollow cathode lamp and an air–acetylene flame to measure the absorbance of the *Blank Preparation*, the *Standard Preparations*, and the *Test Preparation* as directed under *Procedure* (below).

Test Preparation Dissolve 20.0 g of sample in strong acetic acid TS, and dilute to 150.0 mL with the same solvent. Add 2.0 mL of a saturated solution of ammonium pyrrolidinedithiocarbamate (about 10 g/L of water), and 10.0 mL of methyl isobutyl ketone, and shake for 30 s. Protect from bright light. Allow the two layers to separate, and use the methyl isobutyl ketone layer.

Blank Preparation Prepare in the same manner as in the *Test Preparation*, but omit the sample.

Standard Preparations Prepare three *Standard Preparations* in the same manner as in the *Test Preparation*, but add 0.5 mL, 1.0 mL, and 1.5 mL, respectively, of 10 mg/kg nickel standard solution TS in addition to 20.0 g of sample.

Procedure Zero the instrument with the *Blank Preparation*. Concomitantly determine the absorbances of each of the *Standard Preparations* and of the *Test Preparation* at least three times each, and record the average of the steady readings for each. Between each measurement, aspirate the *Blank Preparation*, and ascertain that the reading returns to its initial blank value.

Calculation Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of nickel in the *Test Preparation*. Alternatively, plot on a graph the mean of the readings against the added quantity of nickel. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of nickel in the *Test Preparation*.

Method II

Atomic Absorption System Apparatus Use a suitable atomic absorption spectrometer equipped with a nickel hollow cathode lamp and an air–acetylene flame to measure the absorbance of the *Blank Preparation*, the *Standard Preparations*, and the *Test Preparation* as directed under *Procedure* (below).

Test Preparation Dissolve 20.0 g of sample in strong acetic acid TS, and dilute to 150.0 mL with the same solvent. Add 2.0 mL of a saturated solution of ammonium pyrrolidinedithiocarbamate (about 10 g/L of water) and 10.0 mL of methyl isobutyl ketone, and shake for 30 s. Protect from bright light. Allow the two layers to separate, and use the methyl isobutyl ketone layer.

Blank Preparation Prepare in the same manner as in the *Test Preparation*, but omit the sample.

Standard Preparation Prepare three *Standard Preparations* in the same manner as in the *Test Preparation*, but add 0.5 mL, 1.0 mL, and 1.5 mL, respectively, of 10 mg/kg nickel standard solution TS in addition to 20.0 g of sample.

Procedure Zero the instrument with the *Blank Preparation*. Concomitantly determine the absorbances of each of the *Standard Preparations* and of the *Test Preparation* at least three times each, and record the average of the steady readings for each. Between each measurement, aspirate the *Blank Preparation*, and ascertain that the reading returns to its initial blank value.

Calculation Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of nickel in the *Test Preparation*. Alternatively, plot on a graph the mean of the readings against the added quantity of nickel. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of nickel in the *Test Preparation*.

PHOSPHORUS LIMIT TEST

Reagents

Ammonium Molybdate Solution (5%) Dissolve 50 g of ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 900 mL of warm water, cool to room temperature, dilute to 1000 mL with water, and mix.

Ammonium Vanadate Solution (0.25%) Dissolve 2.5 g of ammonium metavanadate, NH_4VO_3, in 600 mL of boiling water, cool to 60° to 70°, and add 20 mL of nitric acid. Cool to room temperature, dilute to 1000 mL with water, and mix.

Zinc Acetate Solution (10%) Dissolve 120 g of zinc acetate dihydrate, $Zn(C_2H_3O_2)_2\cdot 2H_2O$, in 880 mL of water, and filter through Whatman No. 2V or equivalent filter paper before use.

Nitric Acid Solution (29%) Add 300 mL of nitric acid (sp. gr. 1.42) to 600 mL of water, and mix.

Standard Phosphorus Solution (100 μg P in 1 mL) Dissolve 438.7 mg of monobasic potassium phosphate, KH_2PO_4, in water in a 1000-mL volumetric flask, dilute to volume with water, and mix.

Standard Curve Pipet 5.0, 10.0, and 15.0 mL of the *Standard Phosphorus Solution* into separate 100-mL volumetric flasks. To each of these flasks, and to a fourth, blank flask, add in the order stated 10 mL of *Nitric Acid Solution*, 10 mL

of *Ammonium Vanadate Solution*, and 10 mL of *Ammonium Molybdate Solution*, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1-cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument to zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg of phosphorus (P) per 100 mL.

Treated Sample Place 20 to 25 g of the starch sample in a 250-mL beaker, add 200 mL of a 7:3 methanol:water mixture, disperse the sample, and agitate mechanically for 15 min. Recover the starch by vacuum filtration in a 150-mL medium-porosity fritted-glass or Büchner funnel, and wash the wet cake with 200 mL of the methanol:water mixture. Reslurry the wet cake in the solvent, and wash it a second time in the same manner. Dry the filter cake in an air oven at a temperature below 50°, then grind the sample to 20-mesh or finer, and blend thoroughly. Determine the amount of dry substance by drying a 5-g portion in a vacuum oven, not exceeding 100 mm Hg, at 120° for 5 h.

> **Note**: The treatment outlined above is satisfactory for starch products that are insoluble in cold water. For pregelatinized starch and other water-soluble starches, prepare a 1% to 2% aqueous paste, place it in a cellophane tube, and dialyze against running distilled water for 30 h to 40 h. Precipitate the starch by pouring the solution into 4 volumes of acetone per volume of paste while stirring. Recover the starch by vacuum filtration in a medium-porosity fritted-glass or Büchner funnel, and wash the filter cake with absolute ethanol. Dry the filter cake, and determine the amount of dry substance as directed for water-insoluble starches.

Sample Preparation Transfer about 10 g of the *Treated Sample*, calculated on the dry-substance basis and accurately weighed, into a Vycor dish, and add 10 mL of *Zinc Acetate Solution* in a fine stream, distributing the solution uniformly in the sample. Carefully evaporate to dryness on a hot plate, then increase the heat, and carbonize the sample on the hot plate or over a gas flame. Ignite in a muffle furnace at 550° until the ash is free from carbon (about 1 to 2 h), and cool. Wet the ash with 15 mL of water, and slowly wash down the sides of the dish with 5 mL of *Nitric Acid Solution*. Heat to boiling, cool, and quantitatively transfer the mixture into a 200-mL volumetric flask, rinsing the dish with three 20-mL portions of water and adding the rinsings to the flask. Dilute to volume with water, and mix. Transfer an accurately measured aliquot (V, in mL) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-mL volumetric flask, and add 50 mL of water to a second flask to serve as a blank. To each flask add in the order stated 10 mL of *Nitric Acid Solution*, 10 mL of *Ammonium Vanadate Solution*, and 10 mL of *Ammonium Molybdate Solution*, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min.

Procedure Determine the absorbance of the *Sample Preparation* in a 1-cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. From the *Standard Curve*, determine the mg of phosphorus in the aliquot taken, recording this value as a. Calculate the amount, in mg/kg, of phosphorus (P) in the original sample by the equation

$$\text{mg/kg P} = (a \times 200 \times 1000)/(V \times W),$$

in which W is the weight, in g, of the sample taken.

SELENIUM LIMIT TEST

Reagents and Solutions

2,3-Diaminonaphthalene Solution On the day of use, dissolve 100 mg of 2,3-diaminonaphthalene ($C_{10}H_{10}N_2$) and 500 mg of hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) in sufficient 0.1 *N* hydrochloric acid to make 100 mL.

Selenium Stock Solution Transfer 40.0 mg of powdered metallic selenium into a 1000-mL volumetric flask, and dissolve in 100 mL of 1:2 nitric acid, warming gently on a steam bath to effect solution. Cool, dilute to volume with water, and mix.

Selenium Standard Solution Pipet 5.0 mL of *Selenium Stock Solution* into a 200-mL volumetric flask, dilute to volume with water, and mix. Each milliliter of this solution contains the equivalent of 1 μg of selenium (Se).

Method I

Standard Preparation Pipet 6.0 mL of *Selenium Standard Solution* into a 150-mL beaker, add 50 mL of 0.25 *N* nitric acid, and mix.

Sample Preparation Using a 1000-mL combustion flask and 25 mL of 0.5 *N* nitric acid as the absorbing liquid, proceed as directed under *Oxygen Flask Combustion*, Appendix I, using the amount of sample specified in the individual monograph (and the magnesium oxide or other reagent, where specified).

> **Note**: If the sample contains water of hydration or more than 1% of moisture, dry it at 140° for 2 h before combustion, unless otherwise directed.

Upon completion of combustion, place a few milliliters of water in the cup or lip of the combustion flask, loosen the stopper of the flask, and rinse the stopper, sample holder, and sides of the flask with about 10 mL of water. Transfer the solution, with the aid of about 20 mL of water, into a 150-mL beaker, heat gently to boiling, boil for 10 min, and cool.

Procedure Treat the *Sample Preparation*, the *Standard Preparation*, and 50 mL of 0.25 *N* nitric acid, to serve as the blank, similarly and in parallel as follows: Add a 1:2 solution of ammonium hydroxide to adjust the pH of the solution to 2.0 ± 0.2. Dilute with water to 60.0 mL, and transfer to a low-actinic separator with the aid of 10.0 mL of water, adding the 10.0 mL of rinsings to the separator. Add 200 mg of

hydroxylamine hydrochloride, swirl to dissolve, immediately add 5.0 mL of *2,3-Diaminonaphthalene Solution*, insert the stopper, and swirl to mix. Allow the solution to stand at room temperature for 100 min. Add 5.0 mL of cyclohexane, shake vigorously for 2 min, and allow the layers to separate. Discard the aqueous phases, and centrifuge the cyclohexane extracts to remove any traces of water. Determine the absorbance of each extract in a 1-cm cell at the maximum at about 380 nm with a suitable spectrophotometer, using the extract from the blank to set the instrument. The absorbance of the extract from the *Sample Preparation* is not greater than that from the *Standard Preparation* when a 200-mg sample is tested, or not greater than one-half the absorbance of the extract from the *Standard Preparation* when a 100-mg sample is tested.

Method II

Standard Preparation Pipet 6.0 mL of *Selenium Standard Solution* into a 150-mL beaker, add 50 mL of 2 *N* hydrochloric acid, and mix.

Sample Preparation Transfer the amount of sample specified in the individual monograph into a 150-mL beaker, dissolve in 25 mL of 4 *N* hydrochloric acid, swirling if necessary to effect solution, heat gently to boiling, and digest on a steam bath for 15 min. Remove from heat, add 25 mL of water, and allow to cool to room temperature.

Procedure Place the beakers containing the *Standard Preparation* and the *Sample Preparation* in a fume hood, and to a third beaker, add 50 mL of 2 *N* hydrochloric acid to serve as the blank. Cautiously add 5 mL of ammonium hydroxide to each beaker, mix, and allow the solution to cool. Treat each solution, similarly and in parallel, as directed under *Procedure* in *Method I*, beginning with ''Add a 1:2 solution of ammonium hydroxide. . . . ''

C. OTHERS

ALGINATES ASSAY

In a suitable closed system, liberate the carbon dioxide from the uronic acid groups of about 250 mg of the test sample by heating with hydrochloric acid, and sweep the carbon dioxide, by means of an inert gas, into a titration vessel containing excess standardized sodium hydroxide. Any suitable system may be used as long as it provides precautions against leakage and overheating of the reaction mixture, adequate sweeping time, avoidance of entrainment of hydrochloric acid, and meets the requirements of the *System Suitability Test*. One suitable system, with accompanying procedure, is given below.

Apparatus The apparatus is shown in Fig. 17. It consists essentially of a soda lime column, *A*, a mercury valve, *B*, connected through a side arm, *C*, to a reaction flask, *D*, by means of a rubber connection. Flask *D* is a 100-mL round-bottom, long-neck boiling flask, resting in a suitable heating mantle, *E*.

The reaction flask is provided with a reflux condenser, *F*, to which is fitted a delivery tube, *G*, of 40-mL capacity, having a stopcock, *H*. The reflux condenser terminates in a trap, *I*, containing 25 g of 20-mesh zinc or tin, which can be connected with an absorption tower, *J*.

The absorption tower consists of a 45-cm tube fitted with a medium-porosity fritted glass disk sealed to the inner part above the side arm and having a delivery tube sealed to it extending down to the end of the tube. A trap, consisting of a bulb of approximately 100-mL capacity, is blown above the fritted disk and the outer portion of a ground spherical joint is sealed on above the bulb. A 250-mL Erlenmeyer flask, *K*, is connected to the bottom of the absorption tower. The top of the tower is connected to a soda lime tower, *L*, which is connected to a suitable pump to provide vacuum and air supply, the choice of which is made by a three-way stopcock, *M*. The volume of air or vacuum is controlled by a capillary-tube regulator or needle valve, *N*.

All joints are a size 35/25 ground spherical type.

Standard D-Glucurono-6,3-lactone This chemical ($C_6H_8O_6$) is available as a reference standard with an assay of 100.0 ± 1.0% (24.99 ± 0.25% CO_2) from Aldrich Chemical Co.

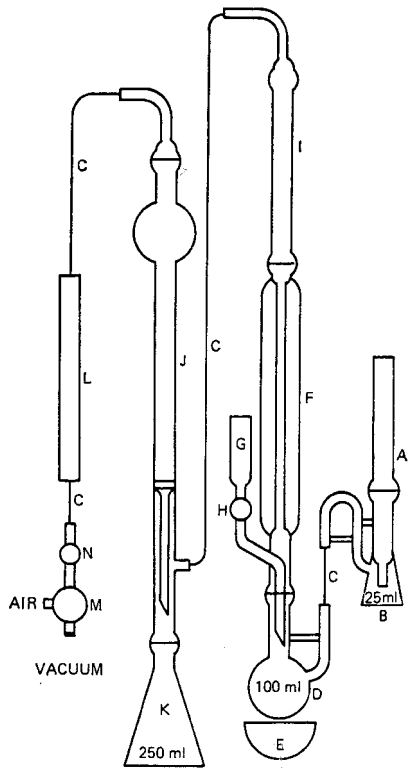


FIGURE 17 Apparatus for Alginates Assay.

System Suitability Test Transfer about 250.0 mg of *Standard D-Glucurono-6,3-lactone*, accurately weighed, into the reaction flask, *D*, and carry out the *Procedure* described below. The system is considered suitable when the net titration results in a calculation of $\%CO_2$ in a range of 24.73 to 25.26, which is equivalent to a range of 98.95 to 101.06% *D-Glucurono-6,3-lactone*.

Procedure Transfer about 250 mg of sample, accurately weighed, into the reaction flask, *D*, add 25 mL of 0.1 *N* hydrochloric acid, insert several boiling chips, and connect the flask to the reflux condenser, *F*, using syrupy phosphoric acid as a lubricant.

> **Note**: Stopcock grease may be used for the other connections.

Check the system for air leaks by forcing mercury up into the inner tube of the mercury valve, *B*, to a height of about 5 cm. Turn off the pressure using the stopcock, *M*. If the mercury level does not fall appreciably after 1 to 2 min, the apparatus may be considered to be free from leaks. Draw carbon dioxide-free air through the apparatus at a rate of 3000 to 6000 mL/h. Raise the heating mantle, *E*, to the flask, heat the sample to boiling, and boil gently for 2 min. Turn off and lower the mantle, and allow the sample to cool for 15 min. Charge the delivery tube, *G*, with 23 mL of hydrochloric acid. Disconnect the absorption tower, *J*, rapidly transfer 25.0 mL of 0.25 *N* sodium hydroxide into the tower, add 5 drops of *n*-butanol, and reconnect the absorption tower. Draw carbon dioxide-free air through the apparatus at the rate of about 2000 mL/h, add the hydrochloric acid to the reaction flask through the delivery tube, raise the heating mantle, and heat the reaction mixture to boiling. After 2 h, discontinue the current of air and heating. Force the sodium hydroxide solution down into the flask, *K*, using gentle air pressure, and then rinse down the absorption tower with three 15-mL portions of water, forcing each washing into the flask with air pressure. Remove the flask, and add to it 10 mL of a 10% solution of barium chloride ($BaCl_2 \cdot 2H_2O$). Stopper the flask, shake gently for about 2 min, add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid. Perform a blank determination (see *General Provisions*). Each milliliter of 0.25 *N* sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO_2). Calculate the results on the dried basis.

α-AMINO NITROGEN (AN) DETERMINATION

Transfer 7 to 25 g of sample, accurately weighed, into a 500-mL volumetric flask with the aid of several 50-mL portions of warm, ammonia-free water, dilute to volume with water, and mix. Neutralize 20.0 mL of the solution with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide, using phenolphthalein TS as the indicator, and add 10 mL of freshly prepared phenolphthalein–formol solution (50 mL of 40% formaldehyde containing 1 mL of 0.05% phenolphthalein in 50% alcohol neutralized exactly to pH 7 with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide). Titrate with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide to a distinct red color, add a small, but accurately measured, volume of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide in excess, and back titrate to neutrality with 0.2 *N* hydrochloric acid. Conduct a blank titration using the same reagents, with 20 mL of water in place of the test solution. Each milliliter of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide is equivalent to 2.8 mg of α-amino nitrogen.

AMMONIA NITROGEN (NH_3-N) DETERMINATION

> **Caution**: Provide adequate ventilation.

> **Note**: Use nitrogen-free reagents, where available, or reagents very low in nitrogen content.

Transfer between 700 mg and 2.2 g of sample into a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass. If desired, wrap the sample, if solid or semisolid, in nitrogen-free filter paper to facilitate the transfer.

Add about 200 mL of water, and mix. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets, or a 2:5 sodium hydroxide solution, down the inside of the flask so that it forms a layer under the solution, using a sufficient amount (usually about 25 g of solid sodium hydroxide) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser that has a delivery tube extending well beneath the surface of a measured excess of 0.5 *N* hydrochloric or sulfuric acid contained in a 500-mL flask. Add 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and heat until all of the ammonia has distilled, collecting at least 150 mL of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*), substituting 2 g of sucrose for the sample, and make any necessary correction. Each milliliter of 0.5 *N* acid consumed is equivalent to 7.003 mg of ammonia nitrogen.

> **Note**: If it is known that the substance to be determined has a low nitrogen content, 0.1 *N* acid and alkali may be used, in which case each milliliter of 0.1 *N* acid consumed is equivalent to 1.401 mg of nitrogen.

Calculate the percent ammonia nitrogen by the formula

$$(NH_3\text{-}N/S) \times 100,$$

in which NH_3-*N* is the weight, in milligrams, of ammonia nitrogen, and *S* is the weight, in milligrams, of sample.

BENZENE (in Paraffinic Hydrocarbon Solvents)

Apparatus (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph, equipped with a column, or equivalent, that will elute *n*-decane before benzene under the conditions of the *System Suitability Test* (below). Column materials and conditions that have been found suitable for this method are listed in the accompanying tables. See Fig. 18 for a typical chromatogram obtained with column No. 5.

Reagents

Isooctane 99 mole percent minimum containing less than 0.05 mole percent aromatic material.

Benzene 99.5 mole percent minimum.

Internal Standard *n*-Decane and either *n*-undecane or *n*-dodecane according to the requirement of the *System Suitability Test*.

Reference Solution A Prepare a standard solution containing 0.5% by weight each of the *Internal Standard* and of benzene in isooctane.

Reference Solution B Prepare a standard solution containing about 0.5% by weight each of *n*-decane, of *Internal Standard*, and of benzene in isooctane.

Calibration Select the instrument conditions necessary to give the desired sensitivity. Inject a known volume of *Reference Solution A*, and change the attenuation, if necessary, so that the benzene peak is measured with a chart deflection of not less than 25% or more than 95% of full scale. When choosing the attenuation, consider all unresolved peaks to represent a single compound. There may be tailing of the nonaromatic peak, but do not use any conditions that lead to a depth of the valley ahead of the benzene peak (A) less than 50% of the weight of the benzene peak (B) as depicted in Fig. 19.

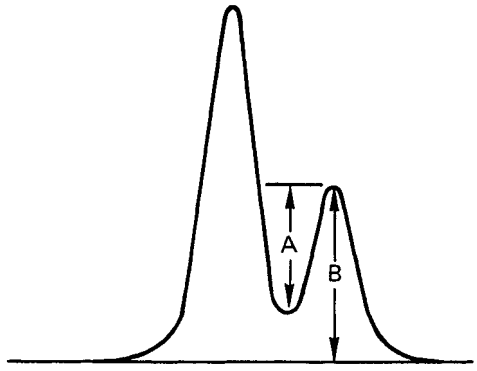


FIGURE 19 Illustration of *A/B* Ratio.

If there is tailing of the nonaromatic material, construct a baseline by drawing a line from the bottom of the valley ahead of the benzene peak to the point of tangency after the peak (see Fig. 20). Measure the areas of the benzene peak and the internal standard peak by any of the following means: triangulation, planimeter, paper cutout, or mechanical or electronic integrator. Do not use integrators on peaks without a constant baseline, unless the integrator has provision for making baseline corrections with accuracy at least as good as that of manual methods.

Calculate a response factor for benzene (R_b) relative to the *Internal Standard* by the formula

$$A_i/W_i \times B_v/A_b,$$

in which A_i is the area of the *Internal Standard* peak in arbitrary units corrected for attenuation; W_i is the weight percent of *Internal Standard* in *Reference Solution A*; A_b is the area of the benzene peak in arbitrary units corrected for attenuation; and W_b is the weight percent of benzene in *Reference Solution A*.

Procedure Place approximately 0.1 mL of *Internal Standard* into a tared 25-mL volumetric flask, weigh on an analytical balance, dissolve in and dilute to volume with the sample to be analyzed.

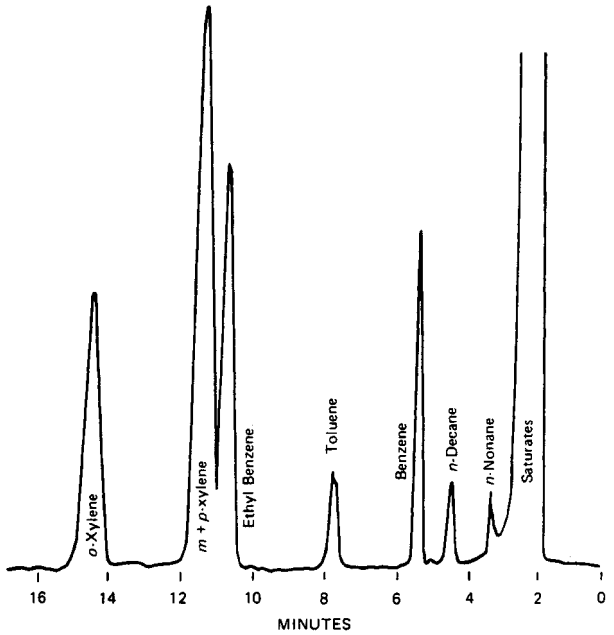


FIGURE 18 Typical Chromatogram for the Determination of Benzene in Hexanes Using Column No. 5.

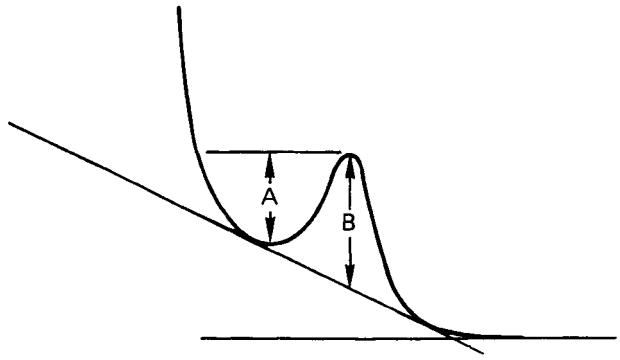


FIGURE 20 Illustration of *A/B* Ratio for a Small Component Peak on the Tail of a Large Peak.

Using the exact instrumental conditions that were used in the calibration, inject the same volume of sample containing the *Internal Standard*. Before measuring the area of the *Internal Standard* and benzene peaks, change the attenuation to ensure at least 25% chart deflection.

Measure the area of the *Internal Standard* and benzene peaks in the same manner as was used for the calibration. Calculate the weight percent of benzene in the sample (W_B) by the formula

$$(A_b \times R_b \times W_i \times 100)/(A_i \times S),$$

in which A_b is the area of the benzene peak corrected for attenuation; R_b is the relative response factor for benzene; W_i is the weight, in grams, of *Internal Standard* added; A_i is the area of the *Internal Standard* peak corrected for attenuation; and S is the weight, in grams of the sample taken.

System Suitability Test Inject the same volume of *Reference Solution B* as in the *Calibration* and record the chromatogram. *n*-Decane must be eluted before benzene, and the ratio of *A* to *B* (Fig. 19) must be at least 0.5 where *A* is equal to the depth of the valley between the *n*-decane and benzene peaks and *B* is equal to the height of the benzene peak.

Column Materials and Conditions for the Determination of Benzene in Hexanes

Column No.	1	2	3	4	5	6	7
Liquid phase	CEF	PEF 200	CEF	DEGS	TCEPE	TCEPE	DEGS
Length, ft	15	6	16	10	15	100	12
m		4.5	2	5	3.1	—	313.7
Diameter, in (mm)							
Inside	0.07(1.8)	—	0.07	0.18(4.5)	0.06(1.5)	0.01(.254)	
Outside	1/8(3.2)	1/4(6.4)	1/8	—	—	—	1/8
Weight, percent	17	30	20	20	10	—	20
Solid support	Chromosorb P	Chromosorb P	Chromosorb P	Chromosorb P	Chromosorb P	Capillary	Chromosorb P
Mesh	60–80	60–80	60–80	80–100	60–80	—	80–100
Treatment	AW	AW	AW	none	AW	none	AW Sil
Inlet, deg	200	210	250	260	250	275	260
Detector, deg	200	155	250	200	175	250	240
Column, deg	115	95	90	100	115	95	65
Carrier gas	N_2	He	He	He	N_2	N_2	He
Flow rate, cm^3/min	30	60	60	60	1	3	52
Detector	FI	TC	FI	FI	FI	FI	FI
Recorder, mV	5	1	1	1	10	1	1
Sample, l	5	10	1	2	5	0.8	5
Split	9 + 1	—	—	—	100 + 1	100 – 1	—
Area	Tri	EI	DI	Tri Plan	EI	EI	Tri

Abbreviations Used in Table

AW—Acid washed; CEF—*N*,*N*-Bis(2-cyanoethyl)formamide; DEGS—Diethylene Glycol Succinate; DI—Disk integrator; EI—Electronic integrator; FI—Flame ionization; Sil—Silanized; TC—Thermal conductivity; TCEPE—Tetracyanoethylated Pentaerythritrol; Tri—Triangulation.

Retention Times in Minutes for Selected Hydrocarbons Under the Conditions for the Determination of Benzene in Hexanes

Column No.	1	2	3	4	5	6	7
Benzene	3.4	2.0	6.5	6.7	5.4	6.1	6.7
Toluene	4.4	3.2	9.0	10.3	7.8	7.0	10.3
Ethylbenzene	5.4	5.2	11.5	14.8	10.8	8.0	14.8
p-*m*-Xylenes	5.8	—	12.5	—	11.4	8.5	—
o-Xylene	7.5	6.8	17.0	16.1	14.5	10.0	—
n-Undecane	3.0	2.8	3.5	—	—	—	—
n-Dodecane	—	—	—	12.8	8.5	6.5	—

COLORS[1]

Chromium

Standards

Standard Chromium Solution (1000 mg/kg) Transfer 2.829 g of $K_2Cr_2O_7$, accurately weighed (National Institute of Standards and Technology No. 136) into a 1-L volumetric flask; dissolve in and dilute to volume with water.

Standard Colorant Solution Transfer 62.5 g of colorant previously shown to be free of chromium to a 1-L volumetric flask; dissolve in and dilute to volume with water.

Apparatus Use any suitable atomic absorption spectrophotometer equipped with a fast response recorder and capable of measuring the radiation absorbed at 357.9 nm.

Instrument Parameters *Wavelength setting*: 357.9 nm; *optical passes*: 5; *lamp current*: 8 mA; *lamp voltage*: 500 v; *fuel*: hydrogen; *oxidant*: air; *recorder*: 1 mv with a scale expansion of 5 or 10. Alternatively, follow the instructions supplied with the instrument.

Procedure Set the instrument at the optimum conditions for measuring chromium as directed by the manufacturer's instructions. Prepare a series of seven standard chromium solutions containing Cr at approximately 5, 10, 15, 20, 40, 50, and 60 mg/kg by appropriate dilutions of the *Standard Chromium Solution* into 100-mL volumetric flasks; add 80 mL of the *Standard Colorant Solution*, and dilute each flask to volume with water.

Transfer 5 g of the colorant to be analyzed to a 100-mL volumetric flask; dissolve in and dilute to volume with water. Prepare a calibration curve using the series of standards, and using this curve, determine the chromium content of the colorant samples.

Ether Extracts

Caution: Isopropyl ether forms explosive peroxides. To ensure the absence of peroxides, perform the following test: Prepare a colorless solution of ferrous thiocyanate by mixing equal volumes of 0.1 *N* ferrous sulfate and 0.1 *N* ammonium thiocyanate. Using titanous chloride, carefully discharge any red coloration due to ferric ions. Add 10 mL of ether to 50 mL of the solution, and shake vigorously for 2 to 3 min. A red color indicates the presence of peroxides. If redistillation is necessary, the usual precautions against peroxide detonation should be observed. Immediately before use, pass the ether through a 30-cm column of chromatography-grade aluminum oxide to remove peroxides and inhibitors.

[1]To be used or sold for use to color food that is marketed in the United States, color additives must be from batches that have been certified by the U.S. Food and Drug Administration (FDA). If color additives are not from FDA-certified batches, they are not permitted color additives for food use in the United States, even if they are compositionally equivalent. The FD&C names can be applied only to FDA-certified batches of these color additives.

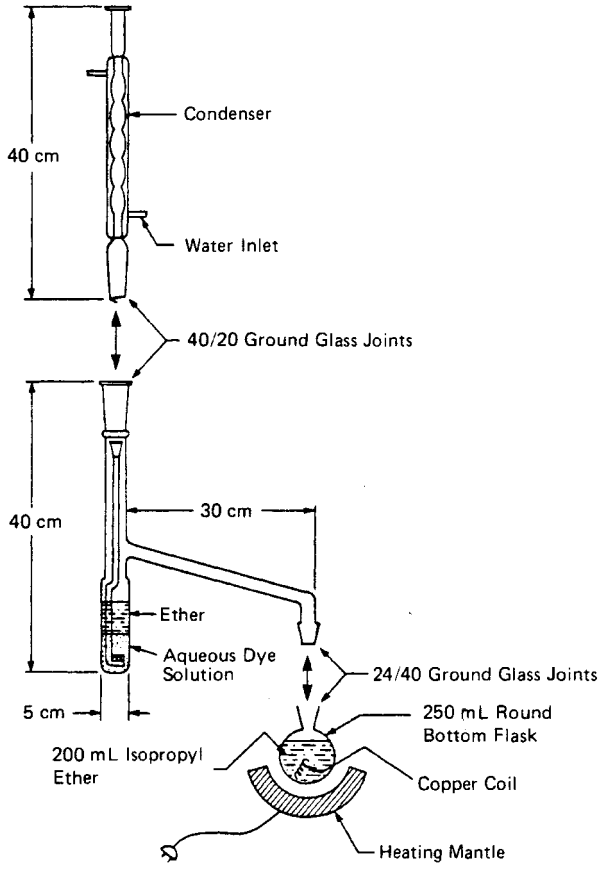


FIGURE 21 Upward Displacement-Type Liquid–Liquid Extractor with Sintered-Glass Diffuser.

Apparatus Use an upward displacement-type liquid–liquid extractor, as shown in Fig. 21, with a sintered-glass diffuser and a working capacity of 200 mL. Suspend a piece of bright copper wire through the condenser, and place a small coil of copper wire (about 0.5 g) in the distillation flask.

Alkaline Ether Extract Transfer 5 g of the colorant to a beaker, and dissolve in 150 mL of water. Add 2 mL of 2.5 *N* NaOH solution, transfer the solution into the extractor; and dilute to approximately 200 mL with water. Add 200 mL of ether to the distillation flask, and extract for 2 h with a reflux rate of about 15 mL/min. Set the extracted colorant solution aside. Transfer the ether extract into a separatory funnel, and wash with two 25-mL portions of 0.1 *N* NaOH followed by two 25-mL portions of water. Reduce the volume of the ether extract to about 5 mL by distillation (in portions) from a tared flask containing a small piece of clean copper coil.

Acid Ether Extract Add 5 mL of 3 *N* hydrochloric acid to the extracted colorant solution set aside in the alkaline ether extract procedure above, mix, and extract with ether as directed above. Wash the ether extract with two 25-mL portions of 0.1 *N* hydrochloric acid and water. Transfer the washed ether in portions to the flask containing the evaporated alkaline extract, and carefully remove all the ether by distillation. Dry the residue in an oven at 85° for 20 min. Then allow the flask

to cool in a desiccator for 30 min, and weigh. Repeat drying and cooling until a constant weight is obtained. The increase in weight of the tared flask, expressed as a percentage of the sample weight, is the combined ether extract.

Leuco Base

Reagents and Solutions

Cupric Chloride Solution Transfer 10.0 g of $CuCl_2 \cdot 2H_2O$ to a 1-L volumetric flask; dissolve in and dilute to volume with dimethylformamide (DMF).

Sample Solution Prepare as directed in the individual monograph.

Procedure

Solution 1 Pipet 50 mL of DMF into a 250-mL volumetric flask, cover, and place in the dark.

Solution 2 Pipet 10 mL of the *Sample Solution* into a 250-mL volumetric flask, add 50 mL of DMF, and place in the dark.

Solution 3 Pipet 50 mL of *Cupric Chloride Solution* into a 250-mL volumetric flask, and gently bubble air through the solution for 30 min.

Solutions 4a and 4b Pipet 10 mL of the *Sample Solution* into each of two 250-mL volumetric flasks, add 50 mL of *Cupric Chloride Solution* to each, and bubble air gently through the solutions for 30 min.

Dilute all of the solutions nearly to volume with water; incubate for 5 to 10 min, but no longer, in a water bath cooled with tap water; and dilute to volume. Record the spectrum for each solution between 500 nm and 700 nm using an absorbance range of 0 to 1 and a 1-cm pathlength cell; record all spectra on the same spectrogram.

Curve No.	Solution in Sample Cell	Solution in Reference Cell
I	1	1
II	1	2
III	3	3
IVa	3	4a
IVb	3	4b

Calculation

$$\% \text{ Leuco Base} = \frac{[(\text{IV} - \text{III}) - (\text{II} - \text{I})] \times 2500}{a \times W \times r},$$

in which the Roman numerals I through IV represent the absorbance readings for solutions of the corresponding Arabic numerals (above) at the wavelength maximum; a is the absorptivity (for Fast Green, $a = 0.156$ at 625 nm; for Brillant Blue, $a = 0.164$ at 630 nm); W is the weight, in grams, of the sample taken; and r is the ratio of the molecular weights of colorant and leuco base (for Fast Green, $r = 0.9712$; for Brillant Blue, $r = 0.9706$).

Mercury

Apparatus The apparatus used for the direct microdetermination of mercury is shown in Fig. 22. It consists of a quartz combustion tube designed to hold a porcelain combustion boat (60 × 10 × 8 mm) and a small piece of copper oxide wire. The combustion tube is placed in a heavy-duty hinged combustion tube furnace (Lindburg Type 70T, or equivalent), and it is connected by clamped ball-joints at one end to a source of nitrogen and connected to a series of three traps at the other. The traps are constructed of a linear array of 18- × 2-mm Pyrex tubes connected by clamped ball-joints and extend from the connection at the combustion tube. Trap I contains anhydrous calcium sulfate packed between quartz-wool plugs, trap II contains Ascarite packed between cotton plugs, and trap III contains aluminum oxide packed between cotton plugs. The nitrogen flow forces the mercury through the combustion tube, the three traps, and a section of Tygon tube to a mercury vapor meter (Beckman model K-23, or equivalent). The mercury released from a sample during combustion is quantitated by comparing the recorder response with that given by a series of mercury standards.

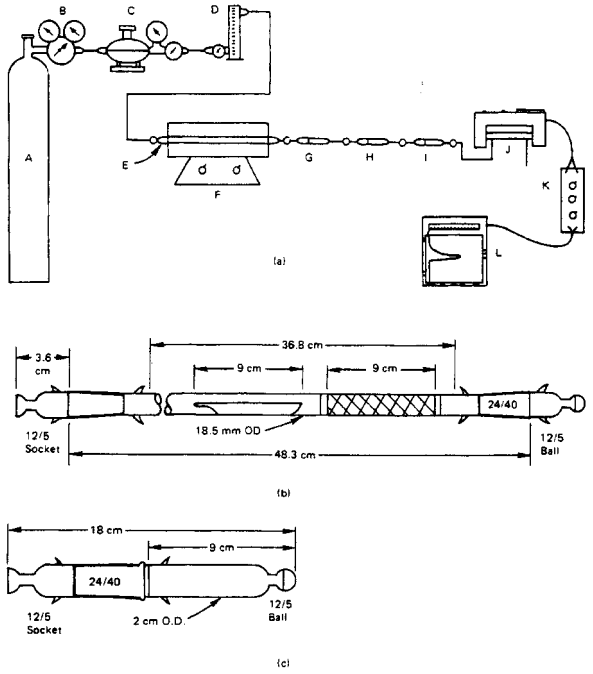


FIGURE 22 (a) Schematic Diagram of Apparatus for Photometric Mercury Vapor M Method:

A. Tank of nitrogen	G. Dehydrite trap
B. Two-stage pressure regulator	H. Ascarite trap
C. Low-pressure regulator	I. Aluminum oxide trap
D. Flowmeter	J. Mercury vapor meter
E. Combustion tube	K. Atenuator
F. Combustion-tube furnace	L. Recorder

(b) Quartz Combustion Tube with Boat and Copper Oxide Packing; (c) Schematic Diagram of Trap Used to Contain Ascarite, Dehydrite, and Aluminum Oxide.

Reagents and Equipment

Absorbent Cotton

Aluminum Oxide Anhydrous.

Calcium Sulfate Anhydrous, dehydrate, or equivalent.

Asbestos Pads, (1 × 0.5 × 1 cm) Preheated at 800° for 1 h.

Ascarite 20- to 30-mesh.

Copper Oxide Wire Preheated at 850° for 2 h.

Nitrogen Purified grade.

Quartz Wool

Sodium Carbonate Anhydrous, fine granular.

Standard Solution Transfer approximately 1.35 g of reagent-grade mercurous chloride, accurately weighed, into a 1-L volumetric flask. Dissolve in and dilute to volume with water. When diluted 100-fold, the solution contains 0.01 μg Hg per microliter (*Diluted Standard Solution*).

Procedure Preheat the furnace to 650°, and adjust the nitrogen flow to 1 L/min.

Blank Analysis Place a square piece of preheated asbestos pad in the combustion boat, and cover it with sodium carbonate. Stop the nitrogen flow, disconnect the ball-joint, quickly insert the boat into the combustion tube with large forceps, and reconnect the joint. Note the time, allow the boat to sit in the tube with no nitrogen flow for exactly 1 min, and then restart the flow of nitrogen. Mercury elutes almost immediately with the reinstated nitrogen flow; note the recorder response. Allow about 30 s between runs.

Calibration Determine the recorder response after the application to the asbestos pad of 1, 2, and 3 μL of the *Diluted Standard Solution*.

Sample Analysis Transfer 25 mg of colorant, accurately weighed, to the combustion boat, and cover the sample completely with sodium carbonate. Follow the procedure used for the *Blank Analysis* above, and calculate the mercury content using the standard curve.

Trap Problems (1) Some colorants (e.g., Brillant Blue and Fast Green) may give a response that is symmetrically dissimilar to the Hg peak. If such a response ''carries over'' to the next sample, then the aluminum oxide trap may need to be changed. (2) If the recorder response is of inadequate sensitivity (peak height induced by 0.01 μg less than 0.5 cm), then the traps are packed too tightly. Remove or redistribute packing first in the aluminum oxide trap, then try the other traps. (3) The traps will need changing periodically as indicated by a change in the physical appearance of the trap material or by chart responses of different retention times or different symmetry from that of mercury standards. (4) If two or more standards are run in succession, a later sample might give an erroneous mercury response. Run blanks and then repeat the sample analysis to confirm the validity of the response.

Sodium Chloride

Dissolve approximately 2 g of colorant, accurately weighed, in 100 mL of water, and add 10 g of activated carbon that is free of chloride and sulfate. Boil gently for 2 to 3 min. Cool to room temperature, add 1 mL of 6 *N* nitric acid, and stir. Dilute to volume with water in a 200-mL volumetric flask, and then filter through dry paper. Repeat the treatment with 2-g portions of carbon until no color is adsorbed onto filter paper dipped into the filtrate.

Transfer 50 mL of filtrate to a 250-mL flask. Add 2 mL of 6 *N* nitric acid, 5 mL of nitrobenzene, and 10 mL of standardized 0.1 *N* silver nitrate solution. Shake the flask until the silver chloride coagulates. Prepare a saturated solution of ferric ammonium sulfate, and add just enough concentrated nitric acid to discharge the red color; add 1 mL of this solution to the 250-mL flask to serve as the indicator. Titrate with 0.1 *N* ammonium thiocyanate solution that has been standardized against the silver nitrate solution until the color persists after shaking for 1 min. Calculate the weight percent of sodium chloride, *P*, by the equation

$$P = [(V \times N)/W] \times 22.79,$$

in which *V* is the net volume, in milliliters, of silver nitrate solution required; *N* is the normality of the silver nitrate solution; and *W* is the weight, in grams, of the sample taken. The factor 22.79 incorporates a total volume of 195 mL because 10 g of activated carbon occupies 5 mL.

Sodium Sulfate

Place 25 mL of the decolorized filtrate obtained from the *Sodium Chloride* test (above) into a 125 mL Erlenmeyer flask, and add 1 drop of a 0.5% phenolphthalein solution in 50% ethanol. Add 0.05 *N* sodium hydroxide, dropwise, until the solution is alkaline to pH paper, and then add 0.002 *N* hydrochloric acid until the indicator is decolorized. Add 25 mL of ethanol and about 0.2 g of tetrahydroquinone sulfate indicator. Titrate with 0.03 *N* barium chloride solution to a red endpoint. Make a blank determination.

Calculate the weight percent, *P*, of sodium sulfate by the equation

$$P = [(V - B) \times N/W] \times 55.4,$$

in which *V* is the volume, in milliliters, of barium chloride solution required to titrate the sample; *B* is the volume, in milliliters, of barium chloride solution required for the blank; *N* is the normality of the barium chloride solution; and *W* is the weight, in grams, of the sample taken. The factor 55.4 incorporates a total volume of 195 mL because 10 g of activated carbon occupies 5 mL.

Total Color

Method I (Spectrophotometric)

Pipet 10.0 mL of the dissolved colorant into a 250-mL Erlenmeyer flask containing 90 mL of 0.04 *N* ammonium acetate, and mix well. Determine the net absorbance of the solution relative to water at the wavelength maximum given for each color. Calculate the percentage of colorant present using the following equation, which presumes a 1-cm pathlength cell:

$$\% \text{ total color} = (A \times 100)/(a \times W),$$

in which *A* is the absorbance; *a* is the absorptivity; and *W* is the weight, in grams, of the sample taken.

Method II (Titration with Titanium Chloride)

Apparatus The apparatus for determining total color by titration with titanium chloride ($TiCl_3$) is shown in Fig. 23.

It consists of a storage bottle, *A*, of 0.1 *N* titanium chloride titrant maintained under hydrogen produced by a Kipp generator; an Erlenmeyer flask, *B*, equipped with a source of CO_2 or N_2 to maintain an inert atmosphere in which the reaction takes place; a stirrer; and the buret, *C*.

Reagents and Solutions

Titanium Chloride Solution (0.1 *N*) Transfer 73 mL of commercially prepared 20% $TiCl_3$ solution into a storage bottle, and carefully add 82 mL of concentrated HCl per L of final solution. Mix well, and bubble CO_2 or N_2 through the solution for 1 h. Before standardizing, maintain the solution under a hydrogen atmosphere for at least 16 h using a Kipp generator.

Potassium Dichromate Solution (0.1 *N*, primary standard) Transfer 4.9032 g of $K_2Cr_2O_7$ (National Institute of Standards and Technology No. 136) to a 1-L volumetric flask; dissolve in and dilute to volume with water.

Ammonium Thiocyanate (50%) Transfer 500 g of NH_4SCN, ACS certified, to a 1-L volumetric flask; dissolve in about 600 mL of water, warming if necessary; and dilute to volume.

Ferrous Ammonium Sulfate $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, ACS certified.

Sodium Bitartrate

Standardization of the Titanium Chloride Solution Drain any standing titanium chloride ($TiCl_3$) from the feed lines and buret, and refill with fresh solution. Add 3.0 g of *Ferrous Ammonium Sulfate* to a wide-mouth Erlenmeyer flask followed by 200 mL of water, 25 mL of 50% sulfuric acid, 25 mL of *0.1* N *Potassium Dichromate Solution* (by pipet), and 2 or 3 boiling chips. Boil the solution vigorously on a hot plate for 30 s to remove dissolved air, then quickly transfer the flask to the titration apparatus, securely connect the stopper assembly, and start the carbon dioxide flow and stirrer. Pass carbon dioxide over the solution for 1 min before beginning the titration.

Add the *0.1* N *Titanium Chloride Solution* at a fast, steady drip to within 1 mL of the estimated endpoint (about 20 mL). Reduce the carbon dioxide flow, remove the solid-glass rod from the stopper assembly, pipet 10 mL of *Ammonium Thiocyanate* (50%) into the flask, insert the glass rod, and increase the carbon dioxide flow. Continue titrating slowly until the endpoint: A color change from brown-red to light green is observed. Perform a blank determination using the same reagents and quantities, and calculate the normality, *N*, of the *0.1* N *Titanium Chloride Solution* on the basis of three titrations by the equation

$$N = (V_r \times N_r/V_t - V_b),$$

in which V_r is the volume, in milliliters, of *0.1* N *Potassium Dichromate* used; N_r is the normality of the *0.1* N *Potassium Dichromate*; V_t is the volume, in milliliters, of *0.1* N *Titanium Chloride Solution* used; and V_b is the volume, in milliliters, of titanium dichloride used in the blank titration.

Procedure Transfer the quantity of colorant prescribed in the individual monograph into a 500-mL wide-mouth Erlenmeyer flask and add 21 to 22 g of *Sodium Bitartrate* (sodium citrate for Sunset Yellow), 275 mL of water, and two or three boiling chips. Boil the solution vigorously on a hot plate for 30 s to remove dissolved air, then quickly transfer the flask to the titration apparatus, securely connect the stopper assembly, and start the carbon dioxide flow and stirrer. Pass carbon dioxide over the solution for 1 min before beginning the titration.

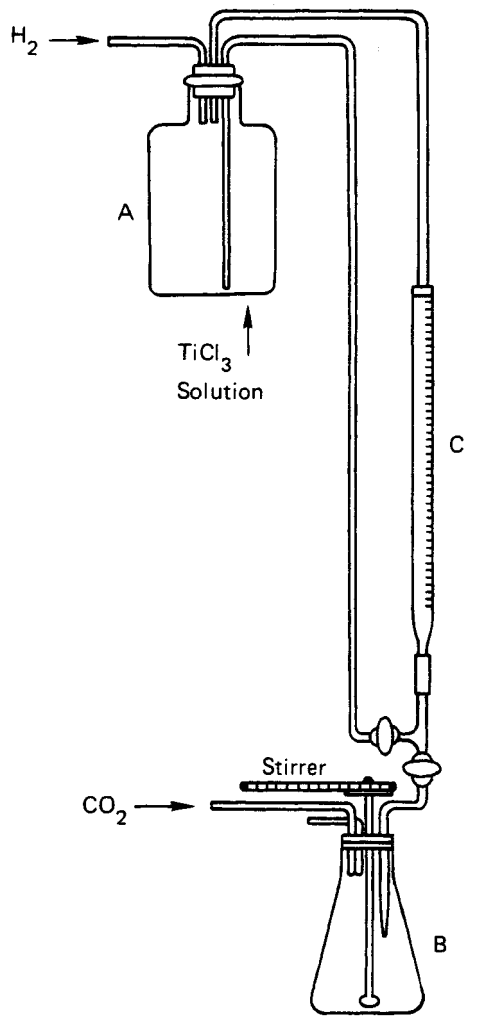


FIGURE 23 Titanous Chloride Titration Apparatus.

Titrate the sample until the color lightens, wait 20 s, and then continue the addition with about 2 s between drops. When the color is almost completely bleached, wait 20 s, and then continue the addition with 5 s between drops. A complete color change indicates the endpoint. Perform a blank determination using the same reagents and quantities, and calculate the total color, *T*, in percent and on the basis of three titrations, by the equation

$$T = [(V_t - V_b)/(W \times F_s)] \times 100 \times N,$$

in which V_t is the volume of titrant used; V_b is the volume of titrant required to produce the endpoint in a blank; *N* is the normality of the titrant; *W* is the weight, in grams, of the sample taken, and F_s is a factor derived from the stoichiometry

of the reaction characteristics of each colorant and is given in the individual monograph.

Method III (Gravimetric)

Transfer approximately 0.5 g of colorant, accurately weighed, to a 400-mL beaker, add 100 mL of water, and heat to boiling. Add 25 mL of 1:50 hydrochloric acid, and bring to a boil. Wash down the sides of the beaker with water, cover, and keep on a steam bath for several hours or overnight. Cool to room temperature, and quantitatively transfer the precipitate into a tared filtering crucible with 1:100 hydrochloric acid. Wash the precipitate with two 15-mL portions of water, and dry the crucible for 3 h at 135°. Cool in a desiccator, and weigh. Calculate the total color, P, in weight percent, by the equation

$$P = [(W_p \times F)/W_s] \times 100,$$

in which W_p is the weight, in grams, of the precipitate; F is the gravimetric conversion factor given in the individual monograph; and W_s is the original weight, in grams, of the sample taken.

Uncombined Intermediates and Products of Side Reactions

Method I

Sample Solution Transfer approximately 2 g of colorant to a 100-mL volumetric flask; dissolve in and dilute to volume with water.

Apparatus Pack a 2.5- × 45-cm glass column with approximately 20 g of cellulose (Whatman CF-11 grade, or equivalent) that has been slurried in the eluant and from which the fines have been removed by decantation. Equilibrate the column thoroughly with the eluant, 35% ammonium sulfate.

Procedure Pipet 5 mL of *Sample Solution* into a beaker containing 5 g of cellulose that has been slurried in eluant and from which the fines have been removed by decantation. Stir the mixture thoroughly, add 10 g of ammonium sulfate, and stir until uniformly mixed. Mix the slurry with 15 mL of eluant, and apply it to the column. Allow the fluid to enter the column, and wash the beaker with eluant until the sample is quantitatively transferred. Elute the column with approximately 500 mL of 35% ammonium sulfate, and collect a total of eight 60-mL fractions. Divide each collected fraction in half and add 0.5 mL of NH_4OH to one half and 0.5 mL of HCl to the other.

Calculation After identifying each intermediate and side product by comparing spectra of the fractions with commercial standards, calculate the concentration, C, of each using the equation

$$C = A/(a \times b),$$

in which A is the absorbance at the wavelength of maximal absorption; b is the cell pathlength, in centimeters; and a is the absorptivity given in the individual monograph.

Method II

Apparatus Use a suitable high-performance liquid chromatography system (see *Chromatography*, Appendix IIA) equipped with a dual wavelength detector system such that the effluent can be monitored serially at 254 nm and 325 to 385 nm (wide-band pass). Use a 1-m × 2.1-mm (id) column, or equivalent, packed with a strong anion-exchange resin (Dupont No. 830950405, or equivalent).

Operating Conditions The operating conditions required may vary depending on the system used. The following conditions have been shown to give suitable results for Allura Red, Tartrazine, and Sunset Yellow.

Allura Red

Primary Eluant: 0.01 *M* aqueous $Na_2B_4O_7$.

Secondary Eluant: 0.20 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.

Sample Size: 20 µL of a 0.25% solution.

Flow Rate: 0.60 mL/min.

Gradient: Linear, in two phases: 0% to 18% in 40 min, 18% to 62% in 8 min more, then hold for 18 min more at 62%.

Temperature: 50°.

Pressure: 1000 psi.

Order of Elution: (1) Cresidinesulfonic acid (CSA); (2) unknown; (3) Schaeffer's salt (SS); (4) unknown; (5) 4,4′-diazoaminobis(5-methoxy-2-methylbenzenesulfonic acid) (DMMA); (6) unknown; (7) Allura Red; (8) 6,6′-oxybis(2-naphthalenesulfonic acid) (DONS).

Tartrazine

Primary Eluant: 0.01 *M* aqueous $Na_2B_4O_7$.

Secondary Eluant: 0.10 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.

Sample Size: 50 µL of a 0.15% solution, prepared within 13 min of injection.

Flow Rate: 1.00 mL/min.

Gradient: Exponential at 4%/min: 0.95%.

Temperature: 50°.

Pressure: 1000 psi.

Order of Elution: (1) Phenylhydrazine-*p*-sulfonic acid (PHSA); (2) sulfanilic acid (SA); (3) 1-(4-sulfophenyl)-3-ethylcarboxy-5-hydroxypyrazolone (PY-T); (4) 1-(4-sulfophenyl)-3-carboxy-5-hydroxypyrazolone (EEPT); (5) 4,4′-(diazoamino)-dibenzenesulfonic acid (DAADBSA).

Sunset Yellow

Primary Eluant: 0.01 *M* aqueous $Na_2B_4O_7$.

Secondary Eluant: 0.20 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.

Sample Size: 5 µL of a 1% solution.

Flow Rate: 0.50 mL/min.

Gradient: Linear in four phases: 0% to 11% in 10 min; hold 25 min; 11% to 38% in 10 min; 38% to 42% in 10 min; 42% to 98% in 20 min; hold 20 min.

Temperature: 50°.

Pressure: 1000 psi.

Order of Elution: (1) Sulfanilic acid (SA); (2) Schaeffer's salt (SS); (3) 4,4′-(diazoamino)-dibenzenesulfonic acid (DAADBSA); (4) *R*-salt dye; (5) Sunset Yellow; (6) 6,6′-oxybis(2-naphthalenesulfonic acid) (DONS).

Standard Solutions

Allura Red Prepare a solution containing 0.25 g of colorant, 0.5 mg of CSA, 0.75 mg of SS, 0.25 mg of DMMA, and 1.25 mg of DONS in a 100-mL volumetric flask. Dissolve in and dilute to volume with 0.1 *M* $Na_2B_4O_7$.

Tartrazine Prepare a solution containing 0.15 g of colorant and 0.3 mg each of PHSA, SA, PY-T, EEPT, and DAADBSA in a 100-mL volumetric flask. Dissolve in and dilute to volume with 0.1 *M* $Na_2B_4O_7$.

Sunset Yellow Prepare a solution containing 0.25 g of colorant, 0.5 mg of SA, 0.75 mg of SS, 0.25 mg of DAADBSA, and 1.25 mg of DONS in a 100-mL volumetric flask. Dissolve in and dilute to volume with 0.1 *M* $Na_2B_4O_7$.

Test Solutions Prepare at least four test solutions, each containing the colorant, and one impurity, accurately weighed, dissolved in 0.1 *M* $Na_2B_4O_7$, and diluted to volume in a 100-mL volumetric flask. The solutions should encompass the range of concentrations, evenly spaced, given below for each constituent:

Allura Red (250 mg) CSA (0.05 to 0.5 mg); SS (0.05 to 0.75 mg); DONS (0.5 to 2.5 mg); DMMA (0.025 to 0.25 mg). Inject 20 µL of each solution.

Tartrazine (150 mg) SA (7.5 to 300 µg); PY-T (7.5 to 300 µg); EEPT (7.5 to 300 µg); DAADBSA (7.5 to 300 µg). Inject 50 µL of each solution.

Sunset Yellow (250 mg) SA (0.05 to 0.5 mg); SS (0.05 to 0.75 mg); DONS (0.5 to 2.5 mg); DAADBSA (0.05 to 0.25 mg). Inject 20 µL of each solution.

System Suitability

Resolution Elute the column, or equivalent, with the gradient specified under *Operating Conditions* until a smooth baseline is obtained. Inject an aliquot of the *Standard Solution*. The resolution of the eluted components matches or exceeds that shown for the corresponding colorant (see Figs. 24, 25, and 26). After determining that the column, or equivalent, will give the required resolution, allow it to rest for 2 weeks before use.

Calibration Inject the designated volume of each *Test Solution* onto a conditioned column, and prepare a standard curve corresponding to each unreacted intermediate and side reaction product. Determine the area, A, for each peak from the integrator if an automated system is used or by multiplying the peak height by the width at one-half the height. The peak height alone may be used for EEPT, PY-T, and DAADBSA. Calculate the concentration, C_i, of each intermediate or side product using the equation

$$C_i = mA_i + b,$$

in which A_i is the area of its corresponding chromatographic peak. Calculate the slope, m, and intercept, b, using the following linear regression equations:

$$m = [N\Sigma C_iA_i - \Sigma C_i\Sigma A_i]/[N\Sigma A_i^2 - (\Sigma A_i)^2],$$

$$b = [\overline{A}]_i - m[\overline{C}]_i,$$

in which $\overline{C}$ and $\overline{A}$ are the calculated averages of the concentrations and peak areas, respectively, used to construct the standard curve for one intermediate or side reaction product. Calculate the correlation coefficient, r, from the following equation:

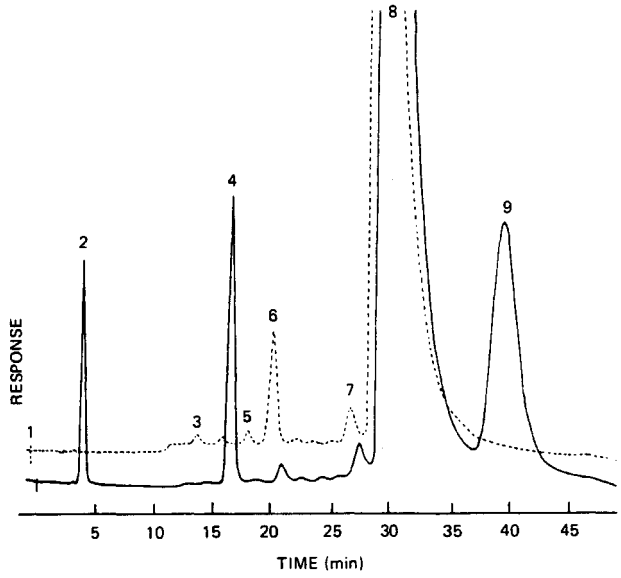


FIGURE 24 Allura Red–Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

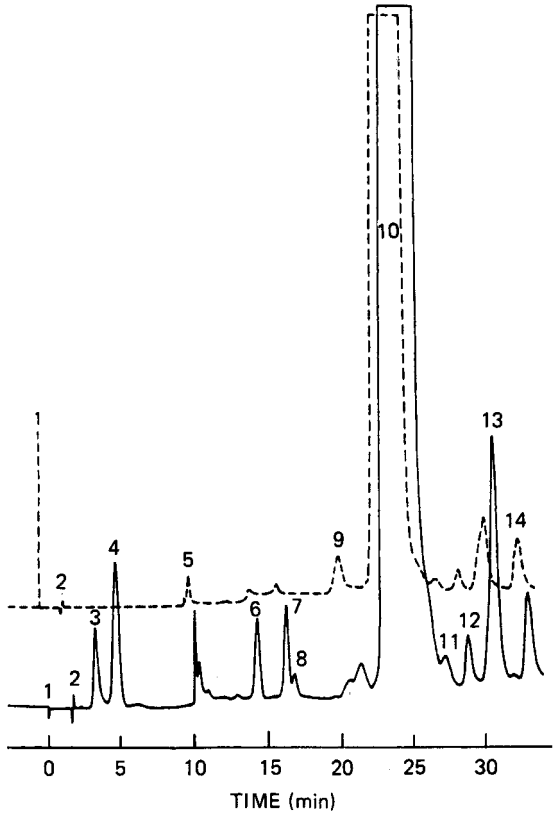


FIGURE 25 Tartrazine–Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

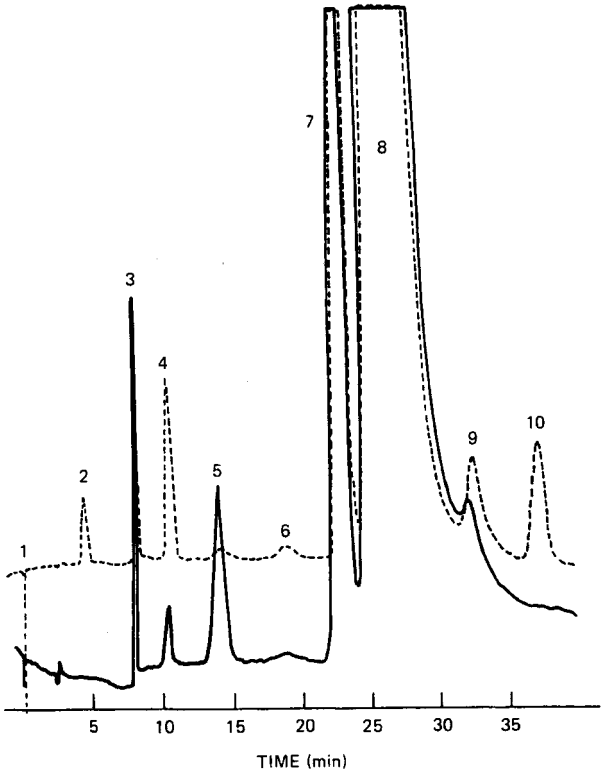


FIGURE 26 Sunset Yellow–Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

$$r = [\Sigma(C_i - \overline{C})(A_i - A)]/[\Sigma(C_i - \overline{C}^2) \times \Sigma(A_i - \overline{A})^2].$$

Each time the system is calibrated, add the new data to those accumulated from previous analyses. The correlation coefficient must be between 0.95 and 1.00 for any single experiment or from accumulated data.

Recalibrate the system after every ten determinations or 2 days, whichever occurs first.

Sample Preparation Prepare as directed in the individual monograph.

Procedure Inject the volume of *Sample Preparation* as designated in the monograph into the column. Determine the concentration of intermediates and side reaction products from the peak areas using the slope, m, and intercept, b, calculated under *Calibration* by the equation

$$C_s = mA_s + b,$$

in which C_s is the concentration of the unknown in the *Sample Preparation* and A_s its corresponding peak area.

Loss on Drying (Volatile Matter) Transfer 1.5 to 2.5 g of colorant, accurately weighed, to a tared crucible. Heat in a vacuum oven at 135° for 12 to 15 h. Lower the pressure in the oven to −125 mm Hg, and continue heating for an additional 2 h. Cover the crucible, and allow to cool in a desiccator. Reweigh the crucible when cool. The loss of weight is defined as the volatile matter.

Water-Insoluble Matter Transfer about 1 g of colorant, accurately weighed, to a 250-mL beaker, and add 200 mL of boiling water. Stir to facilitate dissolution of the color.

Tare a filtering crucible equipped with a glass fiber filter (Reeve Angel, No. 5270, or equivalent). Filter the solution with the aid of suction when it has cooled to ambient temperature. Rinse the beaker three times, pouring the rinsings through the crucible. Wash the filter with water until the filtrate is colorless.

Dry the crucible and filter in an oven at 135° for at least 3 h, cool them in a desiccator and reweigh to the nearest 0.1 mg. Calculate the percent water-insoluble matter, I, by the equation

$$I = (W_c/W_s) \times 100,$$

in which W_c is the difference in crucible weight and W_s is the sample weight.

GLUTAMIC ACID

Apparatus Use an ion-exchange amino acid analyzer, equipped with sulfonated polystyrene columns, in which the effluent from the sample is mixed with ninhydrin reagent and the absorbance of the resultant color is measured continuously and automatically at 570 and 440 nm by a recording photometer.

Standard Solution Transfer 1250 ± 2 mg of reagent-grade glutamic acid, accurately weighed, into a 500-mL volumetric flask. Fill the flask half-full with water, add 5 mL of hydrochloric acid to help dissolve the amino acid, dilute to volume with water, and mix. Prepare the standard for analysis by diluting 1 mL of this solution with 4 mL of 0.2 *N* sodium citrate, pH 2.2, buffer. This *Standard Solution* contains 0.5 mg of glutamic acid per milliliter (C_S).

Sample Preparation Dilute 5 mg of sample, accurately weighed, to exactly 5 mL with 0.2 *N* sodium citrate, pH 2.2, buffer. Remove any insoluble material by centrifugation or filtration.

Procedure Using 2-mL aliquots of the *Standard Solution* and *Sample Preparation*, proceed according to the apparatus manufacturer's instructions. From the chromatograms thus obtained, match the retention times produced by the *Standard Preparation* with those produced by the *Sample Solution*, and identify the peak produced by glutamic acid. Record the area of the glutamic acid peak from the sample as A_U, and that from the standards as A_S.

Calculations Calculate the concentration, C_A, in milligrams per milliliter, of glutamic acid in the *Sample Preparation* by the formula

$$A_U \times C_S/A_S,$$

in which C_S is the concentration, in milligrams per milliliter, of glutamic acid in the *Standard Solution*.

Calculate the percent glutamic acid, on the basis of total protein, by the formula

$$(100 \times C_A)/(6.25 \times N_T),$$

in which N_T is the percent total nitrogen determined in the monograph *Assay*, and 6.25 is the conversion factor for protein and amino acids.

Calculate the percent glutamic acid in the sample by the formula

$$100 \times C_A/S_W,$$

in which S_W is the weight, in milligrams, of the sample taken.

HYDROXYPROPOXYL DETERMINATION

Apparatus The apparatus for hydroxypropoxyl group determination is shown in Fig. 27. The boiling flask, *D*, is fitted with an aluminum foil-covered Vigreaux column, *E*, on the side arm and with a bleeder tube through the neck and to the bottom of the flask for the introduction of steam and nitrogen. A steam generator, *B*, is attached to the bleeder tube through tube *C*, and a condenser, *F*, is attached to the Vigreaux column. The boiling flask and steam generator are immersed in an oil bath, *A*, equipped with a thermoregulator such that a temperature of 155° and the desired heating rate may be maintained. The distillate is collected in a 150-mL beaker, *G*, or other suitable container.

Procedure Unless otherwise directed, transfer about 100 mg of the sample, previously dried at 105° for 2 h and accurately weighed, into the boiling flask, and add 10 mL of chromium trioxide solution (60 g in 140 mL of water). Immerse the steam generator and the boiling flask in the oil bath (at room temperature) to the level of the top of the chromium trioxide solution. Start cooling water through the condenser, and pass nitrogen gas through the boiling flask at the rate of one bubble per second. Starting at room temperature, raise the temperature of the oil bath to 155° over a period of not less than 30 min, and maintain this temperature until the end of the determination. Distill until 50 mL of distillate is collected. Detach the condenser from the Vigreaux column, and wash it with water, collecting the washings in the distillate container. Titrate the combined washings and distillate with 0.02 *N* sodium hydroxide to a pH of 7.0, using a pH meter set at the expanded scale.

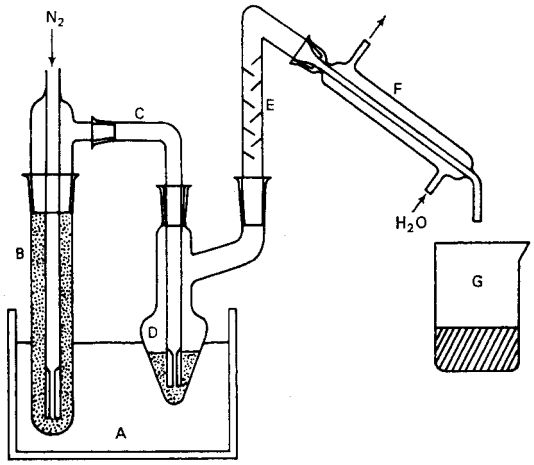


FIGURE 27 Apparatus for Hydroxypropoxyl Determination.

Note: Phenolphthalein TS may be used for this titration if it is also used for all standards and blanks.

Record the volume, V_a, of the 0.02 *N* sodium hydroxide used. Add 500 mg of sodium bicarbonate and 10 mL of 2 *N* sulfuric acid, and then after evolution of carbon dioxide has ceased, add 1 g of potassium iodide. Stopper the flask, shake the mixture, and allow it to stand in the dark for 5 min. Titrate the liberated iodine with 0.02 *N* sodium thiosulfate to the sharp disappearance of the yellow color, confirming the endpoint by the addition of a few drops of starch TS. Record the volume of 0.02 *N* sodium thiosulfate required as Y_a.

Make several reagent blank determinations, using only the chromium trioxide solution in the above procedure. The ratio of the sodium hydroxide titration (V_b) to the sodium thiosulfate titration (Y_b), corrected for variation in normalities, will give the acidity-to-oxidizing ratio, $V_b/Y_b = K$, for the chromium trioxide carried over in the distillation. The factor K should be constant for all determinations.

Make a series of blank determinations using 100 mg of methylcellulose (containing no foreign material) in place of the sample, recording the average volume of 0.02 *N* sodium hydroxide required as V_m and the average volume of 0.02 *N* sodium thiosulfate required as Y_m.

Calculate the hydroxypropoxyl content of the sample, in milligrams, by the formula

$$75.0 \times [N_1(V_a - V_m) - kN_2(Y_a - Y_m)],$$

in which N_1 is the exact normality of the 0.02 *N* sodium hydroxide solution, N_2 is the exact normality of the 0.02 *N* sodium thiosulfate solution, and $k = V_bN_1/Y_bN_2$.

The percentage of substitution, by weight, of hydroxypropoxyl groups, determined as directed above, may be converted to molecular substitution per glucose unit by reference to Fig. 28.

METHOXYL DETERMINATION

Apparatus The apparatus for methoxyl determination, as shown in Fig. 29, consists of a boiling flask, *A*, fitted with a capillary side arm to provide an inlet for carbon dioxide and connected to a column, *B*, which separates aqueous hydriodic acid from the more volatile methyl iodide. After the methyl iodide passes through a suspension of aqueous red phosphorus

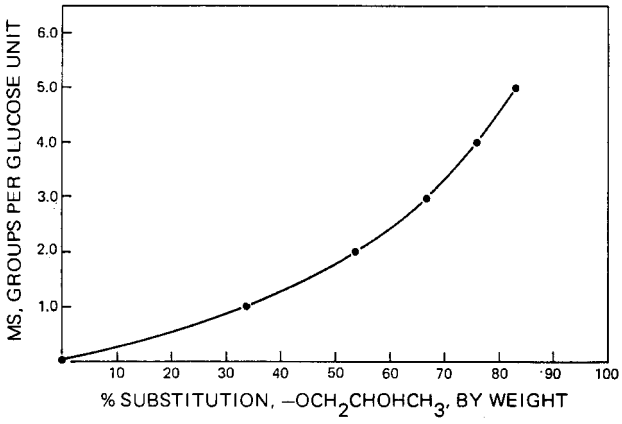


FIGURE 28 Chart for Converting Percentage of Substitution, by Weight, of Hydroxypropoxyl Groups to Molecular Substitution per Glucose Unit.

in the scrubber trap, *C*, it is absorbed in the bromine–acetic acid absorption tube, *D*. The carbon dioxide is introduced from a device arranged to minimize pressure fluctuations and connected to the apparatus by a small capillary containing a small plug of cotton.

Reagents

Acetic Potassium Acetate Dissolve 100 g of potassium acetate in 1000 mL of a mixture consisting of 900 mL of glacial acetic acid and 100 mL of acetic anhydride.

Bromine–Acetic Acid Solution On the day of use, dissolve 5 mL of bromine in 145 mL of the *Acetic Potassium Acetate* solution.

Hydriodic Acid Use special-grade hydriodic acid suitable for alkoxyl determinations, or purify reagent grade as follows: Distill over red phosphorus in an all-glass apparatus, passing a slow stream of carbon dioxide through the apparatus until the distillation is terminated and the receiving flask has completely cooled.

> **Caution**: Use a safety shield, and conduct the distillation in a fume hood.

Collect the colorless, or almost colorless, constant-boiling acid distilling between 126° and 127°. Store the acid in a cool, dark place in small, brown, glass-stoppered bottles previously flushed with carbon dioxide and finally sealed with paraffin.

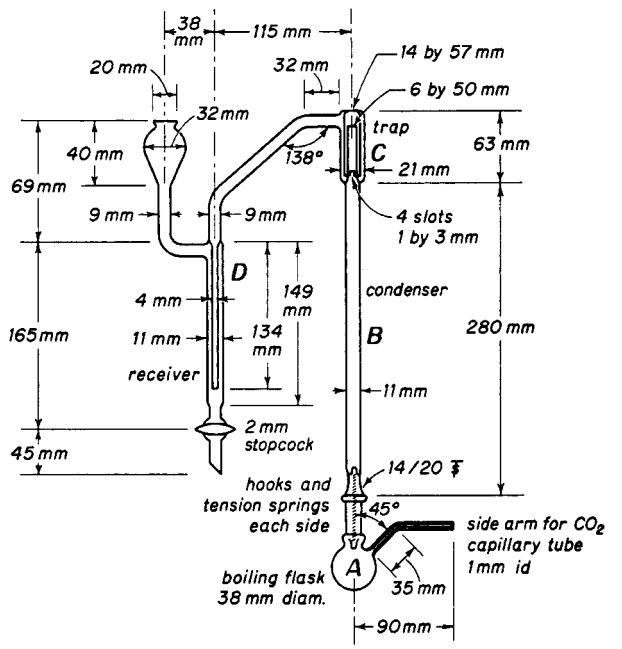


FIGURE 29 Distillation Apparatus for Methoxyl Determination.

Procedure Fill trap *C* half-full with a suspension of about 60 mg of red phosphorus in 100 mL of water, introduced through the funnel on tube *D* and the side arm that connects with the trap at *C*. Rinse tube *D* and the side arm with water, collecting the rinsings in trap *C*, then charge absorption tube *D* with 7 mL of *Bromine–Acetic Acid Solution*. Place the sample, previously accurately weighed in a tared gelatin capsule, into the boiling flask *A*, along with a few glass beads or boiling stones, then add 6 mL of *Hydriodic Acid*. Connect the flask to the condenser, using a few drops of the acid to seal the junction, and begin passing the carbon dioxide through the apparatus at the rate of about two bubbles per second. Heat the flask in an oil bath at 150°, continue the reaction for 40 min, and drain the contents of absorption tube *D* into a 500-mL Erlenmeyer flask containing 10 mL of a 1:4 solution of sodium acetate. Rinse tube *D* with water, collecting the rinsings in the flask, and dilute to about 125 mL with water. Discharge the red-brown color of bromine by adding formic acid, dropwise with swirling, then add 3 drops in excess. Usually a total of 12 to 15 drops of formic acid is required. Allow the flask to stand for 3 min, add 15 mL of 2 *N* sulfuric acid and 3 g of potassium iodide, and titrate immediately with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Perform a blank determination with the same quantities of the same reagents, including the gelatin capsule, and in the same manner, and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 0.517 mg (517 μg) of methoxyl groups (—OCH_3).

NITROGEN DETERMINATION (Kjeldahl Method)

> **Caution**: Provide adequate ventilation in the laboratory, and do not permit accumulation of exposed mercury.
>
> **Note**: All reagents should be nitrogen free, where available, or otherwise very low in nitrogen content.

Method I

Use this method unless otherwise directed in the individual monograph. It is not applicable to certain nitrogen-containing compounds that do not yield their entire nitrogen upon digestion with sulfuric acid.

Nitrites and Nitrates Absent

Unless otherwise directed, transfer from 700 mg to 2.2 g of sample into a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass, wrapping the sample, if solid or semisolid, in nitrogen-free filter paper to facilitate the transfer if desired. Add 700 mg of mercuric oxide or 650 mg of metallic mercury, 15 g of powdered potassium sulfate or anhydrous sodium sulfate, and 25 mL of 93% to 98% sulfuric acid. (If a sample weight greater than 2.2 g is used, increase the sulfuric acid by 10 mL for each additional gram of sample.) Place the flask in an inclined position, and heat gently until frothing ceases, adding a small amount of paraffin, if necessary, to reduce frothing.

> **Caution**: The digestion should be conducted in a fume hood, or the digestion apparatus should be equipped with a fume exhaust system.

Boil briskly until the solution clears, and then continue boiling for 30 min longer (or for 2 h for samples containing organic material). Cool, add about 200 mL of water, mix, and then cool to below 25°. Add 25 mL of sulfide or thiosulfate solution (40 g of K_2S, 40 g of Na_2S, or 80 g of $Na_2S_2O_3 \cdot 5H_2O$ in 1000 mL of water), and mix to precipitate the mercury. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets or a 2:5 solution of sodium hydroxide down the inside of the flask so that it forms a layer under the acid solution, using a sufficient amount (usually about 25 g of solid NaOH) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of a measured excess of 0.5 *N* hydrochloric or sulfuric acid contained in a 500-mL flask. Add from 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and then heat until all of the ammonia has distilled, collecting at least 150 mL of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 *N* sodium hydroxide. Perform a blank determination, substituting 2 g of sucrose for the sample, and make any necessary correction (see *General Provisions*). Each milliliter of 0.5 *N* acid consumed is equivalent to 7.003 mg of nitrogen.

> **Note**: If the substance to be determined is known to have a low nitrogen content, 0.1 *N* acid and alkali may be used, in which case each milliliter of 0.1 *N* acid consumed is equivalent to 1.401 mg of nitrogen.

Nitrites and Nitrates Present

> **Note**: This procedure is not applicable to liquids or to materials having a high chlorine-to-nitrate ratio.

Unless otherwise directed, transfer from 700 mg to 2.2 g of sample into a Kjeldahl flask, and add 40 mL of 93% to 98% sulfuric acid containing 2 g of salicylic acid. Mix thoroughly by shaking, and then allow to stand for 30 min or more, with occasional shaking. Add 5 g of $Na_2S_2O_3 \cdot 5H_2O$, or 2 g of zinc dust (as an impalpable powder, not granules or filings), shake, and allow to stand for 5 min. Heat over a low flame until frothing ceases, then remove the heat, add 700 mg of mercuric oxide (or 650 mg of metallic mercury) and 15 g of powdered potassium sulfate (or anhydrous sodium sulfate), and boil briskly until the solution clears. Continue boiling for 30 min longer (or for 2 h for samples containing organic material), and then continue as directed under *Nitrates and Nitrates Absent*, beginning with ''Cool, add about 200 mL of water. . . .''

Method II (Semimicro)

> **Note**: Automated instruments may be used in place of this manual method, provided the automated equipment has been properly calibrated.

Transfer an accurately weighed or measured quantity of sample, equivalent to about 2 or 3 mg of nitrogen, into the digestion flask of a semimicro Kjeldahl apparatus. Add 1 g of a 10:1 powdered mixture of potassium sulfate:cupric sulfate, using a fine jet of water to wash down any material adhering to the neck of the flask, and then pour 7 mL of sulfuric acid down the inside wall of the flask to rinse it. Cautiously add down the inside of the flask 1 mL of 30% hydrogen peroxide, swirling the flask during the addition.

> **Caution**: Do not add any peroxide during the digestion.

Heat over a free flame or an electric heater until the solution has attained a clear blue color and the walls of the flask are free from carbonized material. Cautiously add 20 mL of water, cool, then add through a funnel 30 mL of a 2:5 solution of sodium hydroxide, and rinse the funnel with 10 mL of water. Connect the flask to a steam distillation apparatus, and immediately begin the distillation with steam. Collect the distillate in 15 mL of a 1:25 solution of boric acid to which has been added 3 drops of methyl red–methylene blue TS and enough water to cover the end of the condensing tube. Continue passing the steam until 80 to 100 mL of distillate has been collected, then remove the absorption flask, rinse the end of the condenser tube with a small quantity of water, and titrate with 0.01 *N* sulfuric acid. Each milliliter of 0.01 *N* acid is equivalent to 140 μg of nitrogen.

When more than 2 or 3 mg of nitrogen is present in the measured quantity of the substance to be determined, 0.02 or 0.1 *N* sulfuric acid may be used in the titration if at least 15 mL of titrant is required. If the total dry weight of the material taken is greater than 100 mg, increase proportionately the quantities of sulfuric acid and sodium hydroxide added before distillation.

SULFUR (by Oxidative Microcoulometry) (Based on ASTM D3120)

> **Note**: All reagents used in this test should be reagent grade; water should be of high purity, and gases must be high-purity grade.

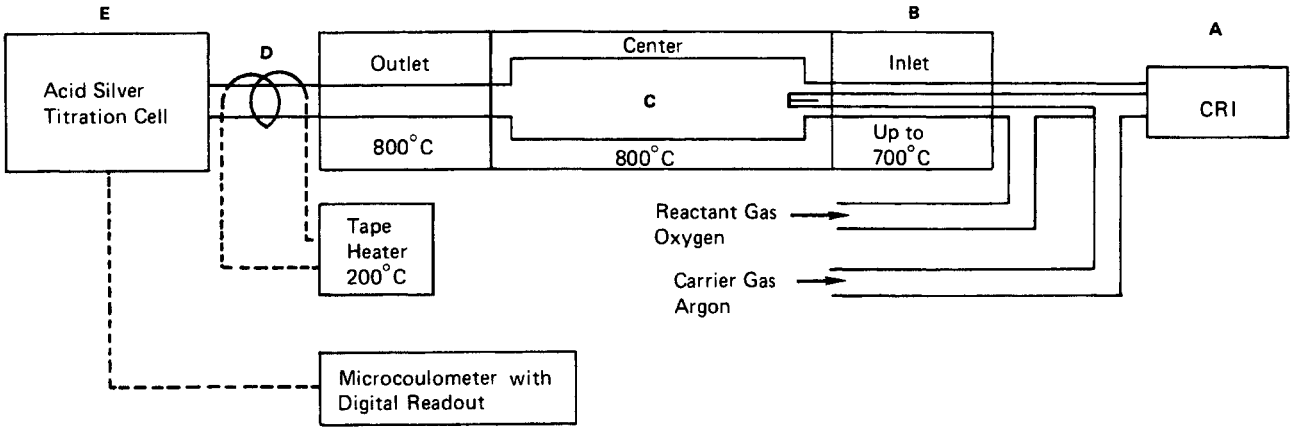


FIGURE 30 Microcoulometric Titrating System for the Determination of Sulfur in Hexanes.

Apparatus Use the Dohrmann Microcoulometric Titrating System (MCTS-30), or equivalent (shown in Fig. 30), unless otherwise specified in an individual monograph. It consists of a constant rate injector, *A*, a pyrolysis furnace, *B*, a quartz pyrolysis tube, *C*, a granular-tin scrubber, *D*, a titration cell, *E*, and a microcoulometer with a digital readout, *F*.

Granular-Tin Scrubber Place 5 g of 20- to 30-mesh granular reagent-grade tin between quartz-wool plugs in an elongated 18/9-12/5 standard-taper adaptor that connects the pyrolysis tube and the titration cell.

Microcoulometer Must have variable attenuation; gain control; and be capable of measuring the potential of the sensing-reference electrode pair, and comparing this potential with a bias potential, amplifying the potential difference, and applying the amplified difference to the working-auxiliary electrode pair to generate a titrant. Also the microcoulometer output voltage signal must be proportional to the generating current.

Pyrolysis Furnace The sample should be pyrolyzed in an electric furnace having at least two separate and independently controlled temperature zones, the first being an inlet section that can maintain a temperature sufficient to volatilize all the organic sample. The second zone is a pyrolysis section that can maintain a temperature sufficient to pyrolyze the organic matrix and oxidize all the organically bound sulfur. A third outlet temperature zone is optional.

Pyrolysis Tube Must be fabricated from quartz and constructed in such a way that a sample, which is vaporized completely in the inlet section, is swept into the pyrolysis zone by an inert gas where it mixes with oxygen and is burned. The inlet end of the tube shall hold a septum for syringe entry of the sample and side arms for the introduction of oxygen and inert gases. The center or pyrolysis section should be of sufficient volume to ensure complete pyrolysis of the sample.

Sampling Syringe A microlitre syringe of 10-μL capacity capable of accurately delivering 1 to 10 μL of sample into the pyrolysis tube. Three-inch × 24-gauge needles are recommended to reach the inlet zone of the pyroloysis furnace.

Titration Cell Must contain a sensor-reference pair of electrodes to detect changes in triiodide ion concentration and a generator anode–cathode pair of electrodes to maintain constant triiodide ion concentration and an inlet for a gaseous sample from the pyrolysis tube. The sensor electrode shall be platinum foil and the reference electrode platinum wire in saturated triiodide half-cell. The generator anode and cathode half-cell shall also be platinum. The titration cell shall be placed on a suitable magnetic stirrer.

Preparation of Apparatus Carefully insert the quartz pyrolysis tube into the furnace, attach the tin scrubber, and connect the reactant and carrier-gas lines. Add the *Cell Electrolyte Solution* (see below) to the titration cell, and flush the cell several times. Maintain an electrolyte level of 3.2 to 6.6 mm above the platinum electrodes. Place the titration cell on a magnetic stirrer, and connect the cell inlet to the tin scrubber outlet. Position the platinum-foil electrodes (mounted on the movable cell head) so that the gas-inlet flow is parallel to the electrodes with the generator anode adjacent to the generator cathode. Assemble and connect the coulometer in accordance with the manufacturer's instructions. Double-wrap the adaptor containing the tin scrubber with heating tape and turn the heating tape on. Adjust the flow of the gases, the pyrolysis furnace temperature, the titration cell, and the coulometer to the desired operating conditions. Typical operating conditions are as follows:

Reactant gas flow (oxygen), cm^3/min	200
Carrier-gas flow (Ar, He), cm^3/min	40
Furnace temperature, °C	
Inlet zone	700 (maximum)
Pyrolysis zone	800 to 1000
Outlet zone	800 (maximum)
Tin-scrubber temperature, °C	200
Titration cell	Stirrer speed set to produce slight vortex
Coulometer	
Bias voltage, mV	160
Gain	50
Constant Rate Injector, μL/s	0.25

The tin scrubber must be conditioned to sulfur, nitrogen, and chlorine before quantitative analysis can be achieved. A solution containing 10 mg/kg butyl sulfide, 100 mg/kg pyridine, and 200 mg/kg chlorobenzene in isooctane has proven

an effective conditioning agent. With a fresh scrubber installed and heated, two 30-μL samples of this conditioning agent injected at a flow rate of 0.5 μL/s produce a steadily increasing response, with final conditioning indicated by a constant reading from the offset during the second injection.

Reagents

Argon or Helium (Argon preferred) High-purity grade, used as the carrier gas. Two-stage gas regulators must be used.

Cell Electrolyte Solution Dissolve 0.5 g of potassium iodide and 0.6 g of sodium azide in 500 mL of high-purity water, add 5 mL of glacial acetic acid and dilute to 1 L. Store in a dark bottle or in a dark place and prepare fresh at least every 3 months.

Oxygen High-purity grade, used as the reactant gas.

Iodine Resublimed, 20-mesh or less, for saturated reference electrode.

Sulfur Standard (approximately 100 mg/kg) Transfer 0.1569 g of *n*-butyl sulfide, accurately weighed, into a tared 500-mL volumetric flask. Dilute to the mark with isooctane, and reweigh. Calculate the sulfur concentration (*S*), in percent, by the formula

$$S = W_b/W_s \times 2.192 \times 10^5,$$

in which W_b is the weight of *n*-butyl sulfide and W_s is the weight of the solution.

Calibration Prepare a calibration standard (approximately 5 mg/kg) by pipetting 5 mL of *Sulfur Standard* into a 10-mL volumetric flask and diluting to volume with isooctane. Fill and clamp the syringe onto the constant-rate injector, push the sliding carriage forward to penetrate the septum with the needle, and zero the meter in case of long-term drift in the automatic baseline zero circuitry. Switch S_1 automatically starts the stepper-motor syringe drive and initiates the analysis cycle. At 2.5 min (before the 3-min meter hold point) set the digital meter with the scan potentiometer to correspond to the sulfur content of the known standard to the nearest 0.01 mg/kg. At the 3-min point, the number displayed on the meter stops, the plunger drive block is retracted to its original position, as preset by switch S_2, and a baseline re-equilibration period equal to the injection period elapses before a ready light and a beeper indicate that a new sample may be injected. Repeat the *Calibration* step a total of at least four times.

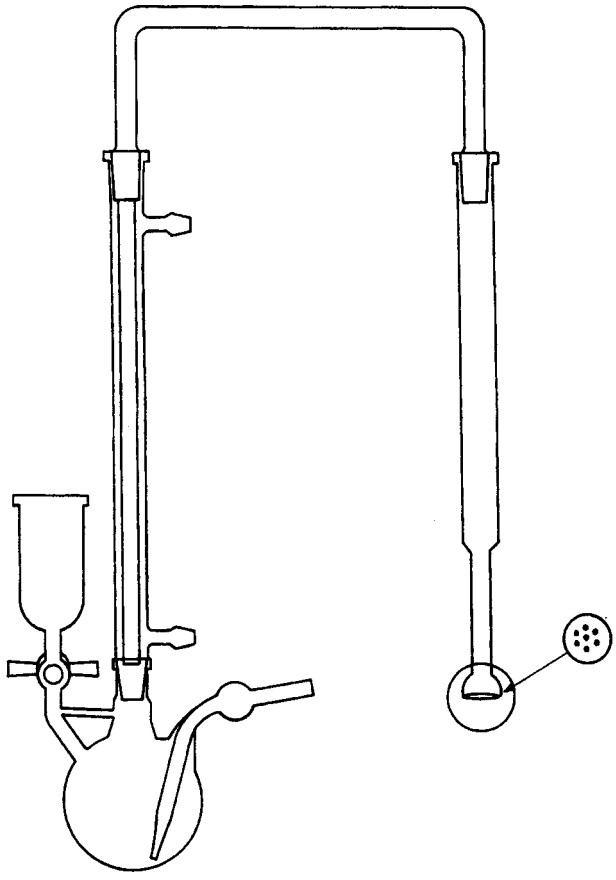


FIGURE 31 Raney Nickel Reduction Apparatus.

Procedure Rinse the syringe several times with sample; then fill it, clamp it onto the constant-rate injector, push the sliding carriage forward to penetrate the septum with the needle, and zero the meter. Turn on switch S_1 to start the stepper-motor syringe drive automatically and initiate the analysis cycle. After the 3-min hold point, the number displayed on the meter corresponds to the sulfur content of the injected sample.

APPENDIX IV: CHEWING GUM BASE POLYMERS

BOUND STYRENE

Abbé-Type Refractometer Use an instrument with fourth decimal place accuracy that can be placed in a nearly horizontal position for measurement of the refractive index of solids. An Amici-type compensating prism for achromatization is necessary unless a sodium vapor lamp is used as a light source.

Ethanol–Toluene Azeotrope Mix 70 volumes of ethanol or of formula 2B ethanol with 30 volumes of toluene, reflux for 4 h over calcium oxide, and then distill, discarding the first and last portions and collecting only that portion distilling within a range of 1°.

Note: Refluxing and distilling are not necessary if anhydrous 2B ethanol or absolute grain alcohol is used.

Sample Preparation Sheet out a sample of the polymer to a thickness of 0.5 mm, and cut the sheeted sample into strips approximately 13 mm wide and 25 mm long. Fasten one strip to each leg of a ''spider,'' consisting of a 13-mm square of sheet aluminum or stainless steel having a Nichrome wire leg about 38 mm long attached to each corner. Place the spider and strips in a 400-mL flask containing 60 mL of *Ethanol–Toluene Azeotrope*, positioning the spider so that each sample strip is in contact on all sides with the solvent. Extract for 1 h at a temperature at which the solvent boils gently, then replace the solvent with another 60-mL portion of *Ethanol–Toluene Azeotrope*, and extract for an additional hour. Remove the spider and sample strips from the flask, and dry them at 100° to constant weight in a vacuum oven at a pressure of about 10 mm Hg.

Caution: The samples must be extracted and dried thoroughly, but avoid overheating, which would cause plasticization.

Remove the extracted and dried strips from the spider, and press the strips between aluminum foil (0.025 to 0.08 mm thick, having good tear strength) at 100° for 3 to 10 min (preferably not more than 5 min), using any suitable apparatus to produce a uniform thickness not exceeding 0.5 mm. If the pressing is done between flat platens without a cavity, use a force between about 500 and 1500 lb, increasing the applied force proportionally if several strips are pressed at one time. If cavity pressing plates are used, close the platens without applying pressure and preheat for 1 min, then apply a force of about 11 tons for 3 min, remove the specimens from the press, and allow them to cool.

Procedure Cut the pressed sample in half with sharp scissors, and peel off one piece of the foil. Cut off a strip about 6 mm wide and 12 mm long with the scissors so that one of the narrower ends is freshly cut.

Check the adjustment of the refractometer by means of a glass test plate pressed firmly against the prism, using a drop of α-bromo-naphthalene as the contact liquid. The small light source should be collimated. The best readings are obtained with the glass test piece if the light is diffused through crumpled tissue paper. After this adjustment, clean the prism well with lens paper moistened with alcohol. The refractive index of the glass test piece and of the test specimen must be measured at a known constant temperature, preferably 25°.

Place the test sample on the prism with the cut edge toward the light source approximately where the edge of the glass test piece was positioned. Remove the tissue paper from the light source, press the specimen firmly on the prism, and wait at least 1 min for the sample to attain temperature equilibrium. The upper prism may be closed lightly on the specimen if adequate light can still be focused on the end of the specimen. Unless the specimen is very thin, however, this operation can damage the prism or its mounting. Adjust the compensating prism until a sharp dividing line between light and dark fields with minimum color is obtained. Test the contact between the specimen and the prism by pressing the test specimen against the prism: There should be no change in the position of the boundary line during this test. Move the hand control from the light into the dark field until the boundary line just reaches the cross hairs, and make at least three readings. If the readings differ by more than 0.0001 refractive index unit, make further readings. Repeat the process of obtaining readings with another portion of the sample having a freshly cut edge, and average the mean values of the two sets of readings thus obtained. If the two mean values do not differ by more than 0.0002 refractive index unit, report the average as the results of the calculations. If necessary, correct the refractive index measurements to 25° using the equation

$$n_{25} = n_t + 0.00037(t - 25),$$

in which n_{25} is the refractive index at 25°, and n_t is the refractive index at the temperature, t, of measurement.

Calculate the percentage of bound styrene in emulsion-polymerized samples by the formula

$$23.50 + 1164(n_{25} - 1.53456) - 3497(n_{25} - 1.53456)^2.$$

Calculate the percentage of bound styrene in solution-polymerized samples by the formula

$$(1212.1212)(n_{25}) - 1838.3636.$$

Alternatively, the percentage of bound styrene may be determined by reference to suitable tables.

MOLECULAR WEIGHT

Polyethylene

Sample Solutions Dissolve 1 g of sample, accurately weighed, in 95 mL of tetrahydronaphthalene, filter into a 100-

mL volumetric flask, dilute to volume with the solvent, and mix (*Solution 1*). Transfer 50.0 mL of *Solution 1* into a tared dish, evaporate on a steam bath for about 1 h, and then evaporate to dryness by heating in a vacuum oven at 70° for 2 h or to constant weight. Calculate the concentration, C_1, in grams per 100 mL, of *Solution 1*. Prepare *Solutions 2* and *3*, respectively, by diluting 5.0-mL and 10.0-mL portions of *Solution 1* to 50.0 mL with the solvent, and then calculate the concentration of each (C_2 and C_3, respectively).

Procedure Determine the flow time, in seconds, of the solvent (t_0) and of the three *Sample Solutions* (t_1, t_2, and t_3, respectively) in a Cannon-Fenske viscometer immersed in a constant-temperature bath maintained at 130°. Calculate the specific viscosity, η_{sp}, of each *Sample Solution* by the formula

$$(t/t_0) - 1,$$

and then calculate the reduced viscosity of each by the formula

$$\eta_{sp}/C.$$

Plot the reduced viscosity of each solution against concentration, and extrapolate to zero concentration to obtain the intrinsic viscosity, [η]. Finally, calculate the molecular weight of the polyethylene by the formula

$$([\eta]/K)^{1/a},$$

in which K is 5.1×10^{-4}, and a is 0.725.

Polyisobutylene (Flory Method)

Sample Solutions Dissolve 1 g of sample, accurately weighed, in 95 mL of diisobutylene, filter into a 100-mL volumetric flask, dilute to volume with solvent, and mix (*Solution 1*). Transfer 50.0 mL of *Solution 1* into a tared dish, evaporate on a steam bath for about 1 h, and then evaporate to dryness by heating in a vacuum oven at 70° for 2 h or to constant weight. Calculate the concentration, C_1, in grams per 100 mL, of *Solution 1*. Prepare *Solutions 2* and *3*, respectively, by diluting 5.0-mL and 10.0-mL portions of *Solution 1* to 50.0 mL with solvent, and then calculate the concentration of each (C_2 and C_3, respectively).

Procedure Determine the flow time, in seconds, of the solvent (t_0) and of the three *Sample Solutions* (t_1, t_2, and t_3, respectively) in a Cannon-Fenske viscometer immersed in a constant-temperature bath maintained at 20°. Calculate the specific viscosity, η_{sp}, of each *Sample Solution* by the formula

$$(t/t_0) - 1,$$

and then calculate the reduced viscosity of each by the formula

$$\eta_{sp}/C.$$

Plot the reduced viscosity of each solution against concentration, and extrapolate to zero concentration to obtain the intrinsic viscosity, [η]. Finally, calculate the molecular weight of the polyisobutylene by the formula

$$([\eta]/K)^{1/a},$$

in which K is 3.60×10^{-4}, and a is 0.64.

Polyvinyl Acetate

Sample Solutions Dissolve 1 g of sample, accurately weighed, in 95 mL of acetone, filter into a 100-mL volumetric flask, dilute to volume with the solvent, and mix (*Solution 1*). Transfer 50.0 mL of *Solution 1* into a tared dish, evaporate on a steam bath for about 1 h, and then evaporate to dryness by heating in a vacuum oven at 70° for 2 h or to constant weight. Calculate the concentration, C_1, in grams per 100 mL, of *Solution 1*. Prepare *Solutions 2* and *3*, respectively, by diluting 5.0-mL and 10.0-mL portions of *Solution 1* to 50.0 mL with solvent, and then calculate the concentration of each (C_2 and C_3, respectively).

Procedure Determine the flow time, in seconds, of the solvent (t_0) and of the three *Sample Solutions* (t_1, t_2, and t_3, respectively) in a Cannon-Fenske viscometer immersed in a constant-temperature bath maintained at 25°. Calculate the specific viscosity, η_{sp}, of each *Sample Solution* by the formula

$$(t/t_0) - 1,$$

and then calculate the reduced viscosity of each by the formula

$$\eta_{sp}/C.$$

Plot the reduced viscosity of each solution against concentration, and extrapolate to zero concentration to obtain the intrinsic viscosity, [η]. Finally, calculate the molecular weight of the polyvinyl acetate by the formula

$$([\eta]/K)^{1/a},$$

in which K is 1.88×10^{-4}, and a is 0.69.

QUINONES

Standard Preparations Transfer 25.0 mg of hydroquinone into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 1.0-, 2.0-, 3.0-, 4.0-, and 6.0-mL aliquots of this solution into a series of 100-mL volumetric flasks, dilute each to volume with water, and mix. Transfer 2.0 mL of each of these solutions and 3.0 mL of water into a series of 25-mL graduates, add 0.5 mL of 0.1 *N* sodium carbonate to each, and continue as directed under *Sample Preparations* (below), beginning with ''. . . shake immediately, then add 1.0 mL of 15% sulfuric acid. . . .''

Sample Preparations Place 30 g of freshly coagulated and washed sample into a 250-mL two-necked flask, add 100 mL of water, and heat at 66° for 2 h.

Caution: Do not boil.

Cool to room temperature, and transfer 5.0 mL of the extract into a 25-mL glass-stoppered graduate. Transfer 5.0 mL of water into a second graduate to serve as the blank. To each graduate add 1.0 mL of 15% sulfuric acid. To the graduate containing the sample extract add 0.5 mL of 0.1 *N* sodium

carbonate, shake immediately, and then add 1.0 mL of 15% sulfuric acid.

> **Note**: The elapsed time for this operation should not exceed 15 s.

Add to each graduate 1.0 mL of 2,4-dinitrophenylhydrazine solution (dissolve 100 mg of 2,4-dinitrophenylhydrazine in 50 mL of carbonyl-free methanol, add 4 mL of hydrochloric acid, and dilute to 100 mL with water), stopper, and heat at 70° in a water bath for 1 h. Cool to room temperature, then add to each graduate 13 mL of water and 5.0 mL of benzene, stopper, and shake vigorously. Allow the phases to separate, and pipet 2.0 mL of the benzene layer from each graduate into corresponding test tubes containing 10 mL of a 1:100 solution of diethanolamine in pyridine. Shake each tube, and allow the color to develop for 10 min.

Procedure Determine the absorbance of the *Sample Preparation* in a 1-cm cell at 620 nm, with a suitable spectrophotometer, against the reagent blank. Determine the absorbance of each *Standard Preparation* in the same manner. Prepare a *Standard Curve* by plotting absorbance of each *Standard Preparation* against micrograms of quinone. From the *Standard Curve*, read the quantity, in micrograms, of quinones (as benzoquinone) in the *Sample Preparation*, and record the value thus obtained as Q. Calculate the quantity of quinones (as benzoquinone), in parts per million, in the sample by the formula

$$20Q/W,$$

in which W is the weight, in grams, of the sample taken.

RESIDUAL STYRENE

Standard Preparation Place 25 mL of carbon disulfide in a 100-mL volumetric flask, cap with a serum stopper, and tare the flask to the nearest 0.1 mg. Using 50-μg syringes, inject 15 μL each of styrene and of alpha-methylstyrene (AMS), reweighing after each addition to obtain the weight of each solution injected. Record the weight, in milligrams, of styrene as w_1 and that of AMS as w_2. Dilute to volume with carbon disulfide, and mix. Pipet 2 mL of this solution into a second 100-mL volumetric flask, dilute to volume with carbon disulfide, and mix. Finally, pipet 25 mL of the diluted solution into a third 100-mL volumetric flask, dilute to volume with carbon disulfide, and mix.

AMS–Solvent Solution Place 25 mL of carbon disulfide into a 100-mL volumetric flask, cap with a serum stopper, and tare the flask to the nearest 0.1 mg. Using a 50-μL syringe, inject 15 μL of AMS, and reweigh to obtain the weight of AMS injected. Dilute to volume with carbon disulfide, and mix. Pipet 2 mL of this solution into a second 100-mL volumetric flask, dilute to volume with carbon disulfide, and mix. Finally, pipet 25 mL of the diluted solution into a third 100-mL volumetric flask, dilute to volume, and mix. Calculate the weight, in grams, of AMS in each milliliter of the final solution, and record the result as w' (approximately 7.5×10^{-7}).

Sample Preparation

Latex Samples Add, with agitation, 100 mL of the latex to a mixture consisting of 15 mL of glacial acetic acid and 10 g of sodium chloride in 500 mL of hot water. Coagulation starts almost immediately. When coagulation is complete, collect the coagulum on a coarse filter or cheesecloth, and wash with 1000 mL of a hot solution prepared with 5.6 g of sodium hydroxide and 1000 mL of water. Wash with hot water until the wash water is free of alkali, then cut the coagulum into small pieces, and dry at 105° for 4 h. Continue as directed under *Solid Samples* (below), beginning with ''Transfer 1.5 g, accurately weighed. . . .''

Solid Samples Cut a piece approximately 2 in. × 3 in. × 5 in. from the corner of a polymer bale, and pass it through a cold mill, set at least ¼ in. open, four times, reversing the sample on each pass. Cut the sample into two pieces at least 1 in. from the edge to expose clean polymer, and then dice approximately 2 g of the clean polymer or cut into small strips. Transfer 1.5 g, accurately weighed, into a 4-oz bottle fitted with a polyethylene cap, add 25.0 mL of the *AMS–Solvent Solution*, cap tightly, and agitate on a mechanical shaker until the polymer dissolves.

> **Note**: Some polymers tend to swell and form viscous cements instead of dissolving cleanly. If this occurs, add 5- to 10-mL increments of carbon disulfide to obtain a mobile slurry, and in the next step increase the volume of methanol by a proportional amount.

Add 25 mL of methanol, cap the bottle, and shake vigorously on the shaker for 30 min. After the contents have settled, decant 10 mL of the coagulant serum into a 1-oz bottle, add 10 mL of water, and stopper with a serum cap. Shake vigorously for 1 min, then turn the bottle upside down, and allow the layers to separate. Withdraw by syringe 1 to 2 mL of the lower (carbon disulfide) layer, and transfer it into a 10-dram vial filled with 1/4 in. of anhydrous sodium sulfate. Seal with a polyethylene cap, shake to mix, and allow to settle.

Procedure (See *Chromatography*, Appendix IIA.) Inject a 10-μL portion of the *Sample Preparation* into a suitable gas chromatograph in which the detector is the hydrogen flame-ionization type and the column is 10-ft × 3/16-in. stainless steel tubing, or equivalent, packed with 25% Ucon 50 HB 2000 on 60- to 80-mesh acid-washed DMCS Chromosorb W, or with equivalent packing materials. Use nitrogen or helium as the carrier gas, flowing at 40 mL/min. The injection port temperature is 240°; the column temperature, 170° isothermal; and the detector temperature, 250°. Adjust the sensitivity of the instrument to give as large a signal as possible for styrene and AMS as is consistent with an acceptable background level. Measure the styrene and AMS peaks by any convenient method, recording the area of the styrene peak as A_1 and that of the AMS peak as A_2.

In the same manner, inject a 10-μL portion of the *Standard Preparation* into the chromatograph, obtain the chromato-

gram, and record the area of the styrene peak as a_1 and that of the AMS peak as a_2. Calculate the styrene factor, F, by the formula

$$(w_1/w_2) \times (a_2/a_1).$$

Calculate the content of residual styrene in the sample taken, in parts per million, by the formula

$$(A_1/A_2) \times F \times 25 \times (w'/W) \times 10^6,$$

in which W is the weight, in grams, of the sample taken.

SAMPLE SOLUTION FOR ARSENIC LIMIT TEST

Transfer 1 g of sample, accurately weighed, into a Kjeldahl flask, rest the open end of the flask in a Kjeldahl fume bulb attached to a water aspirator, add 5 mL of sulfuric acid and 4 mL of 30% hydrogen peroxide, and digest over a small flame. (See *Caution* statement under *Arsenic Limit Test*, Appendix IIIB.) Continue adding the peroxide in 2-mL portions, allowing the reaction to subside between additions, until all organic matter is destroyed, fumes of sulfuric acid are copiously evolved, and the solution becomes colorless. Maintain oxidizing conditions at all times during the digestion by adding peroxide whenever the mixture turns brown or darkens. (The amount of peroxide required to completely digest the samples will vary, but as much as 200 mL may be required in some cases, depending on the nature of the material.) Cool, cautiously add 10 mL of water, again evaporate to strong fuming, and cool. Transfer the solution into an arsine generator flask, wash the Kjeldahl flask and bulb with water, adding the washings to the generator flask, and dilute to 35 mL with water.

SAMPLE SOLUTION FOR LEAD LIMIT TEST

Transfer 3.3 g of sample, accurately weighed, into a porcelain dish or casserole, heat on a hot plate until completely charred, then heat in a muffle furnace at 480° for 8 h or overnight, and cool. Cautiously add 5 mL of nitric acid, evaporate to dryness on a hot plate, then heat again in the muffle furnace at 480° for exactly 15 min, and cool. Extract the ash with two 10-mL portions of water, filtering each extract into a separator. Leach any insoluble material on the filter with 6 mL of *Ammonium Citrate Solution*, 2 mL of *Hydroxylamine Hydrochloride Solution*, and 5 mL of water (see *Lead Limit Test*, Appendix IIIB, for preparation of these solutions), adding the filtered washings to the separator. Continue as directed under *Procedure* in the *Lead Limit Test*, Appendix IIIB, beginning with ''Add 2 drops of phenol red TS to the separator. . . .''

TOTAL UNSATURATION

This method measures total unsaturation in a sample by the multivariate analysis of Fourier transform infrared spectra. It correlates the absorbance in the spectral regions corresponding to two major types of unsaturation with their concentrations. This is an extension of univariate least squares analysis that correlates a single band absorbance height or area with concentration.

Apparatus Use a Fourier transform infrared spectrometer (FTIR), with its associated computer and peripherals, capable of measuring from 4500 to 500 cm^{-1} and of acquiring data with a resolution of at least 2 cm^{-1}. The optics of the instrument must be sealed and desiccated, or, like the sample chamber, must be under continuous dry air or nitrogen gas purge. The spectrometer is equipped with software capable of multicomponent analysis using the partial least squares method (PLS-1, or equivalent). This software is commercially available as an accessory to the spectrometer or as an external software package.

Laboratory Press Use a Carver-type press capable of pressing polymer films.

Sample Preparation Compression-mold a thin film of the sample to roughly a 500-μm thickness at 10 tons and 90° for 30 to 60 s. Do not exceed this time or temperature, as structural changes in unsaturation can occur.

Operating Conditions Collect not less than 64 FTIR spectral scans of the standards and sample in the absorbance mode. Boxcar apodization and 2 cm^{-1} resolution are recommended parameters. Spectral normalization should be done on the 4333 cm^{-1} peak to account for varying sample thicknesses. Use identical operating conditions for the standards and for the sample.

Calibration Assemble a set of at least ten calibration standards available from the given supplier of food-grade butyl rubber (such as Exxon Chemical Co.) that covers the entire unsaturation range expected. Identify characteristic FTIR spectral regions corresponding to the unsaturation components by proton magnetic resonance spectroscopy. These spectral regions may include 1700 to 1600 cm^{-1} C=C stretching, 900 to 600 cm^{-1} vinylic H deformations, and 2200 to 1800 cm^{-1} overtone regions

Collect not less than 64 spectral scans of the standards. Construct a calibration matrix containing infrared absorbance values for unsaturation types in the standards and their known concentrations. Confirm the validity of the calibration matrix model as recommended in the software manual. A recommended method is cross-validation for all standards by sequentially excluding one of the standards from the calibration matrix, then using the remaining standards to predict the concentrations. After validation, determine the optimum number of factors, or loading vectors, needed to minimize the deviation between actual and predicted concentrations. This determination is automated in most multicomponent analysis

packages. For the highest possible precision, a calibration for each rubber grade for each manufacturer is recommended.

Procedure Obtain the FTIR spectra of the sample under identical sample preparation and operating conditions as described above. Determine the amount of unsaturation in the sample using the same multivariate analysis parameters and optimal number of factors that were obtained from the calibration matrix. Sum the different unsaturation amounts to obtain the total unsaturation in the sample.

APPENDIX V: ENZYME ASSAYS

A list of the enzymes covered by the general monograph on *Enzyme Preparations* is shown in the accompanying table. Also incorporated in the table are the trivial names by which each is commonly known, as well as the systematic names of the major components or of the enzyme for which the preparation is standardized, in accordance with the *Recommendations (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes*.

Enzyme Preparations Used in Food Processing

TRIVIAL NAME	CLASSIFICATION	SOURCE	NAMES (IUB)[a]	NO.[a]
α-Amylase	carbohydrase	(1) *Aspergillus niger* var. (2) *Aspergillus oryzae* var. (3) *Rhizopus oryzae* var. (4) *Bacillus subtilis* var. (5) barley malt (6) *Bacillus licheniformis* var. (7) *Bacillus stearothermophilus* (8) *Bacillus subtilis** d-*Bacillus megaterium* (9) *Bacillus subtilis** d-*Bacillus stearothermophilus* (10) *Bacillus licheniformis** d-*Bacillus stearothermophilus*	1,4-α-D-glucan glucanohydrolase	3.2.1.1
β-Amylase	carbohydrase	(1) barley malt (2) barley	1,4-α-D-glucan maltohydrolase	3.2.1.2
Bromelain	protease	pineapples: *Ananas comosus* *Ananas bracteatus* (L)	none	3.4.22.32 3.4.22.33
Catalase	oxidoreductase	(1) *Aspergillus niger* var. (2) bovine liver (3) *Micrococcus lysodeikticus*	hydrogen peroxide: hydrogen peroxide oxidoreductase	1.11.1.6
Cellulase	carbohydrase	(1) *Aspergillus niger* var. (2) *Trichoderma longibrachiatum* (formerly *reesei*)	Endo-1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase	3.2.1.4
Chymosin	protease	(1) *Aspergillus niger* var. *awamori** d-calf prochymosin gene (2) *Escherichia coli* K-12* d-calf prochymosin gene (3) *Kluyveromyces marxianus**	cleaves a single bond in *kappa* casein cleaves a single bond in *kappa* casein	 3.4.23.4
Chymotrypsin	protease	bovine or porcine pancreatic extract	none	3.4.21.1
Ficin	protease	figs: *Ficus* sp.	none	3.4.22.3
α-Galactosidase	carbohydrase	(1) *Mortierella vinacea* var. *raffinoseutilizer* (2) *Aspergillus niger*	α-D-galactoside galactohydrolase	3.2.1.22

TRIVIAL NAME	CLASSIFICATION	SOURCE	NAMES (IUB)[a]	NO.[a]
β-Glucanase	carbohydrase	(1) *Aspergillus niger* var. (2) *Bacillus subtilis* var. (3) *Trichoderma longibrachiatum*	1,3-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase	3.2.1.6
Glucoamylase (Amyloglucosidase)	carbohydrase	(1) *Aspergillus niger* var. (2) *Aspergillus oryzae* var. (3) *Rhizopus oryzae* var. (4) *Rhizopus niveus*	1,4-α-D-glucan glucohydrolase	3.2.1.3
Glucose Isomerase	isomerase	(1) *Actinoplanes missouriensis* (2) *Bacillus coagulans* (3) *Streptomyces olivaceus* (4) *Streptomyces olivochromogenus* (5) *Streptomyces rubiginosus* (6) *Streptomyces murinus* (7) *Microbacterium arborescens*	D-xylose ketoisomerase	5.3.1.5
Glucose Oxidase	oxidoreductase	*Aspergillus niger* var.	β-D-glucose: oxygen 1-oxidoreductase	1.1.3.4
β-D-Glucosidase	carbohydrase	(1) *Aspergillus niger* var. (2) *Trichoderma longibrachiatum*	β-D-glucoside glucohydrolase	3.2.1.21
Hemicellulase	carbohydrase	(1) *Aspergillus niger* var. (2) *Trichoderma longibrachiatum*	(1) α-L-arabinofuranoside arabinofuranohydrolase (2) 1,4-β-D-mannan mannanohydrolase (3) 1,3-β-D-xylan xylanohydrolase (4) 1,5-α-L-arabinan arabinanohydrolase	3.2.1.55 3.2.1.78 3.2.1.32 3.2.1.99
Invertase	carbohydrase	*Saccharomyces* sp. (*Kluyveromyces*)	β-D-fructofuranoside fructohydrolase	3.2.1.26
Lactase	carbohydrase	(1) *Aspergillus niger* var. (2) *Aspergillus oryzae* var. (3) *Saccharomyces* sp. (4) *Candida pseudotropicalis* (5) *Kluyveromyces marxianus* var. *lactis*	β-D-galactoside galactohydrolase	3.2.1.23
Lipase	lipase	(1) edible forestomach tissue of calves, kids, and lambs (2) animal pancreatic tissues (3) *Aspergillus oryzae* var. (4) *Aspergillus niger* var. (5) *Rhizomucor miehei* (6) *Candida rugosa*	(1) carboxylic-ester hydrolase (2) triacylglycerol acylhydrolase	3.1.1.1 3.1.1.3
Maltogenic Amylase	carbohydrase	*Bacillus subtilis** d-*Bacillus stearothermophilus*	1,4-α-D-glucan α-maltohydrolase	3.2.1.133
Pancreatin	mixed carbohydrase, protease, and lipase	bovine and porcine pancreatic tissue	(1) 1,4-α-D-glucan glucanohydrolase (2) triacylglycerol acylhydrolase (3) protease	3.2.1.1 3.1.1.3 3.4.21.4

TRIVIAL NAME	CLASSIFICATION	SOURCE	NAMES (IUB)[a]	NO.[a]
Papain	protease	papaya: *Carica papaya (L)*	none	3.4.22.2 3.4.22.6
Pectinase[b]	carbohydrase	(1) *Aspergillus niger* var.	(1) poly(1,4-α-D-galacturonide) glycanohydrolase	3.2.1.15
		(2) *Rhizopus oryzae* var.	(2) pectin pectylhydrolase	3.1.1.11
			(3) poly(1,4-α-D-glacturonide) lyase	4.2.2.2
			(4) poly(methoxyl-L-galacturonide) lyase	4.2.2.10
Pepsin	protease	porcine or other animal stomach tissue	none	3.4.23.1 3.4.23.2
Phospholipase A_2	lipase	animal pancreatic tissue 2-acylhydrolase	phosphatidylcholine	3.1.1.4
Phytase	phosphatase	*Aspergillus niger* var.	(1) *myo*-inositol-hexakisphosphate-3-phosphohydrolase	3.1.3.8
			(2) orthophosphoric-mono ester phosphohydrolase	3.1.3.2
Protease (general)	protease	(1) *Aspergillus niger* var.	none	3.4.23.18
		(2) *Aspergillus oryzae* var		
		(3) *Bacillus subtilis* var.		3.4.24.28
		(4) *Bacillus licheniformis* var.		3.4.21.62
Pullulanase	carbohydrase	*Bacillus acidopullulyticus*	α-dextrin-6-glucanohydrolase	3.2.1.41
Rennet	protease	(1) fourth stomach of ruminant animals	none	3.4.23.1
		(2) *Endothia parasitica*		3.4.23.4
		(3) *Rhizomucor miehei*		3.4.23.22
		(4) *Rhizomucor pusillus* (Lindt)		3.4.23.23
		(5) *Bacillus cereus*		
Trypsin	protease	animal pancreas	none	3.4.21.4

[a] *Enzyme Nomenclature: recommendations (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology*, Academic Press, New York, 1992.
[b] Usually a mixture of pectin depolymerase, pectin methylesterase, pectin lyase, and pectate lyase.
*The asterisk indicates a genetically modified organism. The donor organism is listed after ''d-.''

The following procedures are provided for application as necessary in determining compliance with the vendor's declared representations for enzyme activity. For all of the procedures use filtered, ultra-high purity water with a resistivity of 16 to 18 megohms.

ACID PHOSPHATASE ACTIVITY

Application and Principle This procedure is used to determine acid phosphatase activity in preparations derived from *Aspergillus niger* var. The test is based on the enzymatic hydrolysis of *p*-nitrophenyl phosphate, followed by the measurement of the released inorganic phosphate.

Reagents and Solutions

Glycine Buffer (0.2 *M*, pH 2.5) Dissolve 15.014 g of glycine (Merck, Catalog No. 4201) in about 800 mL of water. Adjust the pH to 2.5 with 1 *M* hydrochloric acid (consumption should be about 80 mL), and dilute to 1000 mL with water.

Substrate (30 m*M*) Dissolve 1.114 g of *p*-nitrophenyl phosphate (Boehringer, Catalog No. 738 352) in *Glycine Buffer*, and adjust the volume to 100 mL with the buffer. Prepare a fresh substrate solution daily.

TCA Solution Dissolve 15 g of trichloroacetic acid in water, and dilute to 100 mL.

Ascorbic Acid Solution Dissolve 10 g of ascorbic acid in water, and dilute to 100 mL. Store under refrigeration. The solution is stable for 7 days.

Ammonium Molybdate Solution Dissolve 2.5 g of ammonium molybdate [$(NH_4)_6MoO_{24}{\cdot}4H_2O$] (Merck, Catalog No. 1182) in water, and dilute to 100 mL.

1 M Sulfuric Acid Stir 55.6 mL of concentrated sulfuric acid (H_2SO_4) (Merck, Catalog No. 731) into about 800 mL of water. Allow to cool, and make up to 1000 mL with water.

Reagent C Mix 3 volumes of *1 M Sulfuric Acid* with 1 volume of *Ammonium Molybdate Solution*, then add 1 volume of *Ascorbic Acid Solution*, and mix well. Prepare fresh daily.

Standard Phosphate Solution Prepare a 9.0-m*M* phosphate stock solution. Dissolve and dilute 612.4 mg of potassium dihydrogen phosphate (KH_2PO_4) (dried in desiccator with silica) to 500 mL with water in a volumetric flask. Make the following dilutions in water from the stock solution, and use these as standards.

Dilution	Phosphorus Concentration (nmol/mL)	Acid Phosphatase Activity (HFU/mL)
1:100	90	2400
1:200	45	1200
1:400	22.5	600

Pipet 4.0 mL of each dilution into two test tubes. Also pipet 4.0 mL of water into one tube (reagent blank). Add 4.0 mL of *Reagent C*, and mix. Incubate at 500 for 20 min, and cool to room temperature. Measure the absorbances at 820 nm against that of reagent blank. Prepare a standard curve by plotting the absorbances against acid phosphatase activity [HFU (acid phosphatase unit)/mL]. Construct a new standard curve with each series of assays.

Test Preparation Prepare a solution of the enzyme preparation in the *Glycine Buffer* so that 1 mL will contain between 600 and 2400 HFU/mL.

Procedure Pipet 1.9 mL of *Substrate* in two test tubes. Add 2.0 mL of *TCA Solution* to one of the tubes (blank), and mix. Put the tubes without *TCA Solution* in a water bath at 37° and let them equilibrate for 5 min. While using a stopwatch, start the hydrolysis by adding sequentially at proper intervals 0.1 mL of *Test Preparation* to each tube, and mix. After exactly 15 min of incubation, stop the reaction by adding 2.0 mL of *TCA Solution* to each tube. Mix, and cool to room temperature. Add 0.1 mL of *Test Preparation* to the reagent blank tube (kept at room temperature), and mix. If precipitate occurs, separate it by centrifugation for 10 min at 2000 g.

Pipet 0.4 mL of each sample after hydrolysis into separate test tubes. Add 3.6 mL of water to each tube. Add 4.0 mL of *Reagent C*, and mix. Incubate at 50° for 20 min, and cool to room temperature. Determine the absorbance against that of reagent blank at 820 nm.

Calculation One acid phosphatase unit (HFU) is the amount of enzyme that liberates, under the conditions of the assay, inorganic phosphate from *p*-nitrophenyl phosphate at the rate of 1 nmol/min.

Subtract the blank absorbance from the sample absorbance (the difference should be between 0.100 and 1.000). Determine the acid phosphatase activity (HFU/mL) from the standard curve, and multiply by the dilution factor. For the activity of solid samples, use the following equation:

$$\text{HFU/g} = (\text{HFU/mL} \times f)/g,$$

in which f is the dilution factor and g is the weight, in grams, of the sample.

AMINOPEPTIDASE (LEUCINE) ACTIVITY

Application and Principle This procedure is used to determine leucine aminopeptidase activity in enzyme preparations derived from *Lactococcus lactis*. The assay is based on the rate of absorbance change over 5 min at 30°; the change in absorbance is due to liberated *p*-nitroaniline from the hydrolysis of leucine *p*-nitroanilide.

Apparatus

Spectrophotometer Use a spectrophotometer with temperature control, suitable for measuring absorbancies at 410 nm.

Cuvette Use a 10-mm light path, quartz.

Thermometer Use a partial immersion thermometer with a suitable range.

Vortex Mixer Use a standard, variable-speed mixer.

Reagents and Solutions

pH 7.0 Phosphate Buffer (100 m*M*) Dissolve 13.6 g of anhydrous potassium dihydrogen orthophosphate in water, and dilute to 1 L (*Solution A*). Dissolve 22.8 g of dipotassium hydrogen orthophosphate trihydrate in water, and dilute to 1 L (*Solution B*). Slowly add approximately 550 mL of *Solution B* to approximately 400 mL of *Solution A* until the pH of the buffer stabilizes at 7 ± 0.02.

Substrate Solution Dissolve 0.0200 g of leucine *p*-nitroanilide hydrochloride (Sigma Chemical Co., Catalog No. L2158) in 100 mL of *pH 7.0 Phosphate Buffer*.

p-*Nitroaniline Stock Solution* Transfer 156.9 mg of *p*-nitroaniline (Aldrich Chemical Co., Catalog No. 18,531-0) to a 1-L volumetric flask, and dilute to volume with water. This solution is 1.1136 m*M*.

> **Caution**: *p*-Nitroaniline is highly toxic. Avoid breathing its dust; avoid contact with skin, eyes, and clothing. Wash the affected area with water; for eyes seek medical attention.

Standard p-*Nitroaniline Solutions* Prepare the following dilutions of p-*Nitroaniline Stock Solution*: dilute 1 mL of p-*Nitroaniline Stock Solution* to 100 mL with *pH 7.0 Phosphate Buffer* (*Solution 1*, 0.01136 m*M*); dilute 9 mL of *Solution 1* with 3 mL of *pH 7.0 Phosphate Buffer* (*Solution 2*, 0.00852 m*M*); and dilute 5 mL of *Solution 1* with 5 mL of *pH 7.0 Phosphate Buffer* (*Solution 3*, 0.00568 m*M*).

Sample Solution Prepare a solution in *pH 7.0 Phosphate Buffer* that contains between 0.025 and 0.1 unit of aminopeptidase activity per mL.

Procedure Determine the absorbance of each of the three standard *p*-nitroaniline dilutions (solutions *1*, *2*, and *3*) at 410 nm using *pH 7.0 Phosphate Buffer* as the blank.

Pipet 3 mL of *Substrate Solution* into a cuvette, insert a thermometer in each to ensure that the temperature of the solution is correct, and equilibrate in the spectrophotometer to 30° ± 0.2°. Add 150 µL of *Sample Solution* to the equilibrated *Substrate Solution*. Mix, and start recording the absorbance. Continue recording the absorbance for approximately 5 min; it should increase linearly with time. To determine the rate of change of absorbance, ignore the initial 0.5 min of the assay line, and use a period of at least 4 min to estimate the rate of change.

Calculation One aminopeptidase activity unit (*AP*) is defined as the quantity of aminopeptidase required to liberate 1 µmol/min of leucine from leucine *p*-nitroanilide under the conditions of the assay at pH 7.0 and 30°.

For each of the diluted *Standard Solutions—1, 2,* and *3*—plot absorbance against *p*-nitroaniline m*M* concentration. The result is a straight line that passes through the origin. Calculate the millimolar extinction coefficient (ε) of each *Standard* p-*Nitroaniline Solution* using the following formula:

$$\epsilon = A_N/C,$$

in which A_N is the absorbance of the *Standard* p-*Nitroaniline Solution* at 410 nm and *C* is the millimolar concentration of *p*-nitroaniline of that solution. Average the three calculated values; this should result in a value of approximately 8.8. Calculate the activity of each sample taken by the equation:

$$AP/g = (\Delta A \times TCV \times 1000)/(\epsilon \times SV \times C),$$

in which Δ*A* is the rate of change of absorbance per minute; *TCV* is the total cuvette volume (3.150 mL); *SV* is the sample volume (0.150 mL); and *C* is the concentration, in milligrams per milliliter, of the sample.

α-AMYLASE ACTIVITY (NONBACTERIAL)

Application and Principle This procedure is used to determine the α-amylase activity of enzyme preparations derived from *Aspergillus niger* var.; *Aspergillus oryzae* var.; *Rhizopus oryzae* var.; and barley malt. The assay is based on the time required to obtain a standard degree of hydrolysis of a starch solution at 30° ± 0.1°. The degree of hydrolysis is determined by comparing the iodine color of the hydrolysate with that of a standard.

Apparatus

Reference Color Standard Use a special Alpha-Amylase Color Disk (Orbeco Analytical Systems, 185 Marine Street, Farmingdale, NY 11735, Catalog No. 620-S5). Alternatively, prepare a color standard by dissolving 25.0 g of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) and 3.84 g of potassium dichromate in 100 mL of 0.01 *N* hydrochloric acid. This standard is stable indefinitely when stored in a stoppered bottle or comparator tube.

Comparator Use either the standard Hellige comparator (Orbeco, Catalog No. 607) or the pocket comparator with prism attachment (Orbeco, Catalog No. 605AHT). The comparator should be illuminated with a 100-W frosted lamp placed 6 in. from the rear opal glass of the comparator and mounted so that direct rays from the lamp do not shine into the operator's eyes.

Comparator Tubes Use the precision-bored square tubes with a 13-mm viewing depth that are supplied with the Hellige comparator. Suitable tubes are also available from other apparatus suppliers (e.g., Thomas Scientific).

Reagents and Solutions

Buffer Solution (pH 4.8) Dissolve 164 g of anhydrous sodium acetate in about 500 mL of water, add 120 mL of glacial acetic acid, and adjust the pH to 4.8 with glacial acetic acid. Dilute to 1000 mL with water, and mix.

β-Amylase Solution Dissolve into 5 mL of water a quantity of β-amylase, free from α-amylase activity (Sigma Chemical Co., Catalog No. A7005), equivalent to 250 mg of β-amylase with 2000° diastatic power.

Special Starch Use starch designated as ''Starch (Lintner) Soluble'' (Baker Analyzed Reagent, Catalog No. 4010). Before using new batches, test them in parallel with previous lots known to be satisfactory. Variations of more than ±3° diastatic power in the averages of a series of parallel tests indicate an unsuitable batch.

Buffered Substrate Solution Disperse 10.0 g (dry-weight basis) of *Special Starch* in 100 mL of cold water, and slowly pour the mixture into 300 mL of boiling water. Boil and stir for 1 to 2 min, then cool, and add 25 mL of *Buffer Solution*, followed by all of the *β-Amylase Solution*. Quantitatively transfer the mixture into a 500-mL volumetric flask with the aid of water saturated with toluene, dilute to volume with the same solvent, and mix. Store the solution at 30° ± 2° for not less than 18 h nor more than 72 h before use. (This solution is also known as ''buffered limit dextrin substrate.'')

Stock Iodine Solution Dissolve 5.5 g of iodine and 11.0 g of potassium iodide in about 200 mL of water, dilute to 250 mL with water, and mix. Store in a dark bottle, and make a fresh solution every 30 days.

Dilute Iodine Solution Dissolve 20 g of potassium iodide in 300 mL of water, and add 2.0 mL of *Stock Iodine Solution*. Quantitatively transfer the mixture into a 500-mL volumetric flask, dilute to volume with water, and mix. Prepare daily.

Sample Preparation Prepare a solution of the sample so that 5 mL of the final dilution will give an endpoint between 10 and 30 min under the conditions of the assay.

For barley malt, finely grind 25 g of the sample in a Miag-Seck mill (Buhler-Miag, Inc., P.O. Box 9497, Minneapolis, MN 55440). Quantitatively transfer the powder into a 1000-mL Erlenmeyer flask, add 500 mL of a 0.5% solution of sodium chloride, and allow the infusion to stand for 2.5 h at 30° ± 0.2°, agitating the contents by gently rotating the flask at 20-min intervals.

Caution: Do not mix the infusion by inverting the flask. The quantity of the grist left adhering to the inner walls of the flask as a result of agitation must be as small as possible.

Filter the infusion through a 32-cm fluted filter of Whatman No. 1, or equivalent, paper on a 20-cm funnel, returning the first 50 mL of filtrate to the filter. Collect the filtrate until 3 h have elapsed from the time the sodium chloride solution and the sample were first mixed. Pipet 20.0 mL of the filtered infusion into a 100-mL volumetric flask, dilute to volume with the 0.5% sodium chloride solution, and mix.

Procedure Pipet 5.0 mL of *Dilute Iodine Solution* into a series of 13- × 100-mm test tubes, and place them in a water bath maintained at 30° ± 0.1°, allowing 20 tubes for each assay.

Pipet 20.0 mL of the *Buffered Substrate Solution*, previously heated in the water bath for 20 min, into a 50-mL Erlenmeyer flask, and add 5.0 mL of 0.5% sodium chloride solution, also previously heated in the water bath for 20 min. Place the flask in the water bath.

At zero time, rapidly pipet 5.0 mL of the *Sample Preparation* into the equilibrated substrate, mix immediately by swirling, stopper the flask, and place it back in the water bath. After 10 min, transfer 1.0 mL of the reaction mixture from the 50-mL flask into one of the test tubes containing the *Dilute Iodine Solution*, shake the tube, then pour its contents into a *Comparator Tube*, and immediately compare with the *Reference Color Standard* in the *Comparator*, using a tube of water behind the color disk.

Note: Be certain that the pipet tip does not touch the iodine solution; carryback of iodine to the hydrolyzing mixture will interfere with enzyme action and will affect the results of the determination.

In the same manner, repeat the transfer and comparison procedure at accurately timed intervals until the α-amylase color is reached, at which time record the elapsed time. In cases where two comparisons 30 s apart show that one is darker and the other lighter than the *Reference Color Standard*, record the endpoint to the nearest quarter min. Shake out the 13-mm *Comparator Tube* between successive readings. Minimize slight differences in color discrimination between operators by using a prism attachment and by maintaining a 6- to 10-in. distance between the *Comparator* and the operator's eye.

Calculation One α-amylase dextrinizing unit (DU) is defined as the quantity of α-amylase that will dextrinize soluble starch in the presence of an excess of β-amylase at the rate of 1 g/h at 30°.

Calculate the α-amylase dextrinizing units in the sample as follows:

$$\text{DU (solution)} = 24/(W \times T),$$

and

$$\text{DU (dry basis)} = \text{DU (solution)} \times 100/(100 - M),$$

in which W is the weight, in grams, of the enzyme sample added to the incubation mixture in the 5-mL aliquot of the *Sample Preparation* used; T is the elapsed dextrinizing time, in minutes; 24 is the product of the weight of the starch substrate (0.4 g) and 60 min; and M is the percent moisture in the sample, determined by suitable means.

α-AMYLASE ACTIVITY (BACTERIAL)

Application and Principle This procedure is used to determine the α-amylase activity, expressed as bacterial amylase units (BAU), of enzyme preparations derived from *Bacillus subtilis* var., *Bacillus licheniformis* var., and *Bacillus stearothermophilus*. It is not applicable to products that contain β-amylase. The assay is based on the time required to obtain a standard degree of hydrolysis of a starch solution at 30° ± 0.1°. The degree of hydrolysis is determined by comparing the iodine color of the hydrolysate with that of a standard.

Apparatus Use the *Reference Color Standard*, the *Comparator*, and the *Comparator Tubes* as described under α-*Amylase Activity (Nonbacterial)*, described in this Appendix, but use either daylight or daylight-type fluorescent lamps as the light source for the *Comparator*. (Incandescent lamps give slightly lower results.)

Reagents and Solutions

pH 6.6 Buffer Dissolve 9.1 g of potassium dihydrogen phosphate (KH_2PO_4) in sufficient water to make 1000 mL (*Solution A*). Dissolve 9.5 g of dibasic sodium phosphate (Na_2HPO_4) in sufficient water to make 1000 mL (*Solution B*). Add 400 mL of *Solution A* to 600 mL of *Solution B*, mix, and adjust the pH to 6.6, if necessary, by the addition of *Solution A* or *Solution B* as required.

Dilute Iodine Solution Prepare as directed under α-*Amylase Activity (Nonbacterial)*.

Special Starch Use the material described under α-*Amylase Activity (Nonbacterial)*.

Starch Substrate Solution Disperse 10.0 g (dry-weight basis) of *Special Starch* in 100 mL of cold water, and slowly pour the mixture into 300 mL of boiling water. Boil and stir for 1 to 2 min, and then cool while continuously stirring. Quantitatively transfer the mixture into a 500-mL volumetric flask with the aid of water, add 10 mL of *pH 6.6 Buffer*, dilute to volume with water, and mix.

Sample Preparation Prepare a solution of the sample so that 10 mL of the final dilution will give an endpoint between 15 and 35 min under the conditions of the assay.

Procedure Pipet 5.0 mL of *Dilute Iodine Solution* into a series of 13- × 100-mm test tubes, and place them in a water bath maintained at 30° ± 0.1°, allowing 20 tubes for each assay.

Pipet 20.0 mL of the *Starch Substrate Solution* into a 50-mL Erlenmeyer flask, stopper, and allow to equilibrate for 20 min in the water bath at 30°.

At zero time, rapidly pipet 10.0 mL of the *Sample Preparation* into the equilibrated mixture, and continue as directed in the *Procedure* under α-*Amylase Activity (Nonbacterial)*, beginning with ''. . . mix immediately by swirling, stopper the flask. . . .''

Calculation One bacterial amylase unit (BAU) is defined as that quantity of enzyme that will dextrinize starch at the rate of 1 mg/min under the specified test conditions.

Calculate the α-amylase activity of the sample, expressed as BAU, by the formula

$$\text{BAU/g} = 40F/T,$$

in which 40 is a factor (400/10) derived from the 400 mg of starch (20 mL of a 2% solution) and the 10-mL aliquot of *Sample Preparation* used; F is the dilution factor (total dilution volume/sample weight, in grams); and T is the dextrinizing time, in minutes.

CATALASE ACTIVITY

Application and Principle This procedure is used to determine the catalase activity, expressed as Baker Units, of preparations derived from *Aspergillus niger* var., bovine liver, or *Micrococcus lysodeikticus*. The assay is an exhaustion method based on the breakdown of hydrogen peroxide by catalase and the simultaneous breakdown of the catalase by the peroxide under controlled conditions.

Reagents and Solutions

Ammonium Molybdate Solution (1%) Dissolve 1.0 g of ammonium molybdate [$(NH_4)_6MoO_{24}\cdot 4H_2O$] (Merck, Catalog No. 1182) in water, and dilute to 100 mL.

0.250 N *Sodium Thiosulfate* Dissolve 62.5 g of sodium thiosulfate ($Na_2S_2O_3\cdot 5H_2O$) in 750 mL of recently boiled and cooled water, add 3.0 mL of 0.2 *N* sodium hydroxide as a stabilizer, dilute to 1000 mL with water, and mix. Standardize as directed for *0.1* N *Sodium Thiosulfate* (see *Solutions and Indicators*), and, if necessary, adjust to exactly 0.250 *N*.

Peroxide Substrate Solution Dissolve 25.0 g of anhydrous dibasic sodium phosphate (Na_2HPO_4), or 70.8 g of $Na_2HPO_4\cdot 12H_2O$, in about 1500 mL of water, and adjust to pH 7.0 ± 0.1 with 85% phosphoric acid. Cautiously add 100 mL of 30% hydrogen peroxide, dilute to 2000 mL in a graduate, and mix. Store in a clean amber bottle, loosely stoppered. The solution is stable for more than 1 week if kept at 5° in a full container. (With freshly prepared substrate, the blank will require about 16 mL of *0.250* N *Sodium Thiosulfate*. If the blank requires less than 14 mL, the substrate solution is unsuitable and should be prepared fresh again. The sample titration must be between 50% and 80% of that required for the blank.)

Procedure Pipet an aliquot of not more than 1.0 mL of the sample, previously diluted to contain approximately 3.5 Baker Units of catalase, into a 200-mL beaker. Rapidly add 100 mL of *Peroxide Substrate Solution*, previously adjusted to 25°, and stir immediately for 5 to 10 s. Cover the beaker, and incubate at 25° ± 1° until the reaction is completed. Stir vigorously for 5 s, and then pipet 4.0 mL from the beaker into a 50-mL Erlenmeyer flask. Add 5 mL of 2 *N* sulfuric acid to the flask, mix, then add 5.0 mL of 40% potassium iodide solution, freshly prepared, and 1 drop of *Ammonium Molybdate Solution* (1%), and mix. While continuing to mix, titrate rapidly to a colorless endpoint with *0.250* N *Sodium Thiosulfate*, recording the volume, in milliliters, required as *S*. Perform a blank determination with 4.0 mL of *Peroxide Substrate Solution*, and record the volume required, in milliliters, as *B*.

> **Note**: When preparations derived from beef liver are tested, the reaction is complete within 30 min. Preparations derived from *Aspergillus* and other sources may require up to 1 h. In assaying an enzyme of unknown origin, run a titration after 30 min and then at 10-min intervals thereafter. The reaction is complete when two consecutive titrations are the same.

Calculation One Baker Unit is defined as the amount of catalase that will decompose 264 mg of hydrogen peroxide under the conditions of the assay.

Calculate the activity of the sample by the equation

$$\text{Baker Units/g or mL} = 0.4(B - S) \times (1/C),$$

in which C is the milliliters of aliquot of original enzyme preparation added to each 100 mL of *Peroxide Substrate Solution*, or when 1 mL of diluted enzyme is used, C is the dilution factor; B is the volume, in milliliters, as defined above; and S is the milliliters of *0.250* N *Sodium Thiosulfate*, as defined above.

CELLULASE ACTIVITY

Application and Principle This assay is based on the enzymatic hydrolysis of the interior β-1,4-glucosidic bonds of a defined carboxymethyl cellulose substrate at pH 4.5 and at 40°. The corresponding reduction in substrate viscosity is determined with a calibrated viscometer.

Apparatus

Calibrated Viscometer Use a size 100 Calibrated Cannon-Fenske Type Viscometer, or its equivalent (Scientific Products, Catalog No. P2885-100).

Constant-Temperature Glass Water Bath (40° ± 0.1°) Use a constant-temperature glass water bath, or its equivalent (Scientific Products, Catalog No. W3520-10).

Stopwatches Use two stopwatches, *Stopwatch No. 1*, calibrated in $^1/_{10}$ min for determining the reaction time (T_r), and *Stopwatch No. 2*, calibrated in $^1/_5$ s for determining the efflux time (T_t).

Waring Blender Use a two-speed Waring blender, or its equivalent (Scientific Products, Catalog No. 58350-1).

Reagents and Solutions

Acetic Acid Solution (2 *N*) While agitating a 1-L beaker containing 800 mL of water, carefully add 116 mL of glacial acetic acid. Cool to room temperature. Quantitatively transfer the solution to a 1-L volumetric flask, and dilute to volume with water.

Sodium Acetate Solution (2 *N*) Dissolve 272.16 g of sodium acetate trihydrate in approximately 800 mL of water contained in a 1-L beaker. Quantitatively transfer to a 1-L volumetric flask, and dilute to volume with water.

Acetic Acid Solution (0.4 *N*) Transfer 200 mL of *Acetic Acid Solution* (2 *N*) into a 1-L volumetric flask, and dilute to volume with water.

Sodium Acetate Solution (0.4 *N*) Transfer 200 mL of *Sodium Acetate Solution* (2 *N*) into a 1-L volumetric flask, and dilute to volume with water.

Acetate Buffer (pH 4.5) Using a standardized pH meter, add *Sodium Acetate Solution* (0.4 *N*) with continuous agitation to 400 mL of *Acetic Acid Solution* (0.4 *N*) in a suitable flask until the pH is 4.5 ± 0.05.

Sodium Carboxymethylcellulose Use sodium carboxymethylcellulose (Hercules, Inc., CMC Type 7HF).

Sodium Carboxymethylcellulose Substrate (0.2% w/v) Transfer 200 mL of water into the bowl of the Waring blender. With the blender on low speed, slowly disperse 1.0 g (moisture-free basis) of the *Sodium Carboxymethylcellulose* into the bowl, being careful not to splash out any of the liquid. Using a rubber policeman, wash down the sides of the glass bowl with water. Place the top on the bowl and blend at high speed for 1 min. Quantitatively transfer to a 500-mL volumetric flask, and dilute to volume with water. Filter the substrate through gauze before use.

Sample Preparation Prepare an enzyme solution so that 1 mL of the final dilution will produce a relative fluidity change between 0.18 and 0.22 in 5 min under the conditions of the assay. Weigh the enzyme, and quantitatively transfer it to a glass mortar. Triturate with water and quantitatively transfer the mixture to an appropriate volumetric flask. Dilute to volume with water, and filter the enzyme solution through Whatman No. 1 filter paper before use.

Procedure Place the *Calibrated Viscometer* in the 40° ± 0.1° water bath in an exactly vertical position. Use only a scrupulously clean viscometer. (To clean the viscometer, draw a large volume of detergent solution followed by water through the viscometer by using an aspirator with a rubber tube connected to the narrow arm of the viscometer.)

Pipet 20 mL of filtered *Sodium Carboxymethylcellulose Substrate* and 4 mL of *Acetate Buffer* into a 50-mL Erlenmeyer flask. Allow at least two flasks for each enzyme sample and one flask for a substrate blank. Stopper the flasks, and equilibrate them in the water bath for 15 min.

At zero time, pipet 1 mL of the enzyme solution into the equilibrated substrate. Start stopwatch no. 1, and mix the solution thoroughly. Immediately pipet 10 mL of the reaction mixture into the wide arm of the viscometer.

After approximately 2 min, apply suction with a rubber tube connected to the narrow arm of the viscometer, drawing the reaction mixture above the upper mark into the driving fluid head. Measure the efflux time by allowing the reaction mixture to freely flow down past the upper mark. As the meniscus of the reaction mixture falls past the upper mark, start stopwatch no. 2. At the same time, record the reaction time, in minutes, from stopwatch no. 1 (T_r). As the meniscus of the reaction mixture falls past the lower mark, record the time, in seconds, from stopwatch no. 2 (T_t).

Repeat the final step until a total of four determinations is obtained over a reaction time (T_r) of not more than 15 min.

Prepare a substrate blank by pipetting 1 mL of water into 24 mL of buffered substrate. Pipet 10 mL of the reaction mixture into the wide arm of the viscometer. Determine the time (T_s) in seconds required for the meniscus to fall between the two marks. Use an average of five determinations for (T_s).

Prepare a water blank by pipetting 10 mL of equilibrated water into the wide arm of the viscometer. Determine the time (T_w) in seconds required for the meniscus to fall between the two marks. Use an average of five determinations for (T_w).

Calculations One Cellulase Unit (CU) is defined as the amount of activity that will produce a relative fluidity change of 1 in 5 min in a defined carboxymethyl cellulose substrate under the conditions of the assay.

Calculate the relative fluidities (F_r) and the (T_n) values for each of the four efflux times (T_t) and reaction times (T_r) as follows:

$$F_r = (T_s - T_w)/(T_t - T_w),$$

$$T_n = \tfrac{1}{2}(T_t/60 \text{ s/min}) + T_r = (T_t/120) + T_r,$$

in which F_r is the relative fluidity for each reaction time; T_s is the average efflux time, in seconds, for the substrate blank; T_w is the average efflux time, in seconds, for the water blank; T_t is the efflux time, in seconds, of reaction mixture; T_r is the elapsed time, in minutes, from zero time, that is, the time from addition of the enzyme solution to the buffered substrate until the beginning of the measurement of efflux time (T_t); and T_n is the reaction time, in minutes (T_r), plus one-half of the efflux time (T_t), converted to minutes.

Plot the four relative fluidities (F_r) as the ordinate against the four reaction times (T_n) as the abscissa. A straight line should be obtained. The slope of this line corresponds to the relative fluidity change per minute and is proportional to the enzyme concentration. The slope of the best line through a series of experimental points is a better criterion of enzyme activity than is a single relative fluidity value. From the graph, determine the F_r values at 10 and 5 min. They should have a difference in fluidity of not more than 0.22 or less than 0.18. Calculate the activity of the enzyme unknown as follows:

$$\text{CU/g} = [1000(F_{r10} - F_{r5})]/W,$$

in which F_{r5} is the relative fluidity at 5 min of reaction time; F_{r10} is the relative fluidity at 10 min of reaction time; 1000 is the milligrams per gram; and W is the weight, in milligrams, of enzyme added to the reaction mixture in a 1-mL aliquot of enzyme solution.

CHYMOTRYPSIN ACTIVITY

Application and Principle This procedure is used to determine chymotrypsin activity in chymotrypsin preparations derived from purified extracts of porcine or bovine pancreas.

Reagents and Solutions

0.15 M *Phosphate Buffer* (pH 7.0) Dissolve 4.54 g of monobasic potassium phosphate in water, and dilute to 500 mL. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water, and dilute to 500 mL. Mix 38.0 mL of the monobasic potassium phosphate solution with 61.1 mL of the dibasic sodium phosphate solution. Adjust the pH of the mixture to 7.0 by the dropwise addition of the dibasic sodium phosphate solution, if necessary.

Substrate Solution Dissolve 23.7 mg of *N*-acetyl-*L*-tyrosine ethyl ester in about 50 mL of the *0.15* M *Phosphate Buffer* with warming. When the solution has cooled, dilute to 100.0 mL with the *0.15* M *Phosphate Buffer*.

Sample Preparation Dissolve a sufficient amount of sample, accurately weighed, in 0.001 *N* hydrochloric acid to produce a solution containing between 12 and 16 USP Chymotrypsin Units per milliliter. This solution should cause a change in absorbance between 0.008 and 0.012 in a 30-s interval.

Procedure Conduct the assay in a suitable spectrophotometer equipped to maintain a temperature of 24° ± 0.1° in the cell compartment. Determine the temperature before and after measuring the absorbance to ensure that the temperature does not change more than 0.5° during the assay. Pipet 0.2 mL of the 0.001 *N* hydrochloric acid and 3.0 mL of the *Substrate Solution* into a 1-cm cell. Place the cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.200 at 237 nm. Pipet 0.2 mL of the *Sample Preparation* into a second cell, add 3.0 mL of the *Substrate Solution*, and place the cell in the spectrophotometer. Begin timing the reaction from the addition of the *Substrate Solution*. Read the absorbance at 30-s intervals for at least 5 min. Repeat the procedure at least once. If the rate of change fails to remain constant for at least 3 min, repeat the test, and if necessary, use a lower sample concentration. The duplicate determinations at the same sample concentration should match the first determination in rate of absorbance change.

Calculations One USP Chymotrypsin Unit is defined as the activity causing a change in absorbance at the rate 0.0075/min under the conditions of the assay. Determine the average absorbance change per min using only those values within the 3-min portion of the curve where the rate of change is constant. Plot a curve of absorbance against time.

Calculate the number of Chymotrypsin Units per milligram by the formula

$$(A_2 - A_1)/(0.0075TW),$$

in which A_2 is the straight-line initial absorbance reading; A_1 is the straight-line final absorbance reading; T is the elapsed time, in minutes; and W is the weight, in milligrams, of the sample in the volume of solution used to determine the absorbance.

DIASTASE ACTIVITY (DIASTATIC POWER)

Application and Principle This procedure is used to determine the amylase activity of barley malt and other enzyme preparations. The assay is based on a 30-min hydrolysis of a starch substrate at pH 4.6 and 20°. The reducing sugar groups produced on hydrolysis are measured in a titrimetric procedure using alkaline ferricyanide.

Apparatus

Mill Use a laboratory mill of the type Miag-Seck, for fine grinding of malt (Buhler Miag, Inc.).

Reagents and Solutions

Acetate Buffer Solution Dissolve 68 g of sodium acetate ($NaC_2H_3O_2{\cdot}3H_2O$) in 500 mL of 1 *N* acetic acid in a 1000-mL volumetric flask, dilute to volume with water, and mix.

Special Starch Use the material described under α-*Amylase Activity (Nonbacterial)*.

Starch Substrate Solution Disperse 20.0 g (dry-weight basis) of *Special Starch* in 50 mL of water, mix to a fine paste, and pour slowly into 750 mL of boiling water. Boil and stir for 2 min, cool, add 20 mL of *Acetate Buffer Solution*, and mix. Quantitatively transfer into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Acetic Acid–Potassium Chloride–Zinc Sulfate Solution (A-P-Z) Dissolve 70 g of potassium chloride and 20 g of zinc sulfate ($ZnSO_4{\cdot}7H_2O$) in 700 mL of water in a 1000-mL volumetric flask, add 200 mL of glacial acetic acid, dilute to volume with water, and mix.

Alkaline Ferricyanide Solution (0.05 *N*) Dissolve 16.5 g of potassium ferricyanide [$K_3Fe(CN)_6$] and 22 g of anhydrous sodium carbonate in 800 mL of water in a 1000-mL volumetric flask, dilute to volume with water, and mix.

Potassium Iodide Solution Dissolve 50 g of potassium iodide in 50 mL of water in a 100-mL volumetric flask, dilute to volume with water, and mix. Add 2 drops of 50% sodium hydroxide solution, and mix. The solution should be colorless.

Sample Preparation

Malt Samples Grind 30 g of the sample to a fine powder in a Maig-Seck mill. Accurately weigh 25 g of the powder, and transfer it into a 1000-mL Erlenmeyer flask. Add 500 mL of a 0.5% sodium chloride solution, and allow the infusion to stand for 2.5 h at 20° ± 0.2°, agitating the contents by gently rotating the flask at 20-min intervals.

Note: Do not mix the infusion by inverting the flask. The quantity of grist left adhering to the inner walls of

the flask as a result of agitation must be as small as possible. Gently swirl the contents of the flask without splashing them against the walls to mix sufficiently.

Filter the infusion through a 32-cm fluted filter of Whatman No. 1, or equivalent, paper on a 20-cm funnel, returning the first 50 mL of filtrate to the filter. Place a watch glass over the funnel, and use a suitable cover around the stem and over the receiver to reduce evaporation losses during filtration. Collect the filtrate until 30 min of filtration time have elapsed. Pipet 20.0 mL of the filtrate into a 100-mL volumetric flask, dilute to volume with 0.5% sodium chloride solution, and mix.

Other Enzyme Preparations Prepare a solution so that 10 mL of the final dilution will give a diastatic power (DP) value between 2° and 150°.

Procedure Pipet 10.0 mL of the *Sample Preparation* into a 250-mL volumetric flask, and at zero time, add 200 mL of *Starch Substrate Solution*, previously equilibrated for 30 min in a water bath maintained at 20° ± 0.2°. Start the stopwatch at zero time.

Place the mixture in the water bath at 20°, and allow it to cool for exactly 30 min, then add 20.0 mL of 0.5 *N* sodium hydroxide, dilute to volume with water, and mix.

Prepare a blank by adding 20.0 mL of 0.5 *N* sodium hydroxide to a 250-mL volumetric flask, followed by 10.0 mL of the *Sample Preparation*. Swirl to mix, add 200 mL of *Starch Substrate Solution*, dilute to volume with water, and mix.

Pipet 5.0 mL of the sample digestion mixture into a 125-mL Erlenmeyer flask, add 10.0 mL of *Alkaline Ferricyanide Solution*, and swirl to mix. Heat the flask for exactly 20 min in a boiling water bath, and then cool to room temperature. Add 25 mL of *A-P-Z Solution*, followed by 1 mL of *Potassium Iodide Solution*, and swirl to mix. Titrate with 0.05 *N* sodium thiosulfate to the complete disappearance of the blue color, recording the volume, in milliliters, of 0.05 *N* sodium thiosulfate required as *S*.

Treat the blank solution in the same manner as described for the sample, recording the volume, in milliliters, of 0.05 *N* sodium thiosulfate required as *B*.

Calculation One unit of diastase activity, expressed as degrees diastatic power (DP°), is defined as that amount of enzyme contained in 0.1 mL of a 5% solution of the sample enzyme preparation that will produce sufficient reducing sugars to reduce 5 mL of Fehling's solution when the sample is incubated with 100 mL of the substrate for 1 h at 20°.

Note: The definition of the unit does not correspond to the method of the determination.

Calculate the diastase activity, expressed as DP°, of the sample by the formulas

$$\text{DP°, as-is basis} = (B - S) \times 23,$$

and

$$\text{DP°, dry basis} = \text{DP°, as-is basis} \times 100/(100 - M),$$

in which 23 is a factor, determined by collaborative study, required to convert to the units of the definition, and M is the percent moisture of the sample, determined by suitable means.

α-GALACTOSIDASE ACTIVITY

Application and Principle Use this procedure to determine α-galactosidase activity in enzyme preparations derived from *Aspergillus niger* var. The assay is based on a 15-min hydrolysis of *p*-nitrophenyl-α-D-galactopyranoside followed by spectrophotometric measurement of the liberated *p*-nitrophenol.

Reagents and Solutions

Acetate Buffer Dissolve 11.55 mL of glacial acetic acid in water, and dilute to 1 L (*Solution A*). Dissolve 16.4 g of sodium acetate in water, and dilute to 1 L (*Solution B*). Mix 7.5 mL of *Solution A* and 42.5 mL of *Solution B*, and dilute to 200 mL with water. Adjust the pH of this solution to 5.5 with either *Solution A* or *Solution B* as necessary.

Substrate Solution Dissolve 0.210 g of *p*-nitrophenyl-α-D-galactopyranoside (Sigma Chemical Co., Catalog No. 877, or equivalent) in and dilute to 100 mL with *Acetate Buffer*.

Borax Buffer Dissolve 47.63 g of sodium borate decahydrate in warm water. Cool to room temperature. Add 20 mL of 4 *N* sodium hydroxide solution, adjust the pH of the solution to 9.7 with 4 *N* sodium hydroxide, and dilute to 2 L with water.

p-*Nitrophenol Stock Solution* Dissolve 0.0334 g of *p*-nitrophenol (Aldrich Chemical Co., Catalog No. 24,132-6, or equivalent) in and dilute to 1 L with water. This solution contains 0.24 μmol of *p*-nitrophenol per milliliter of water.

Preparation of Standards and Samples

Standards Prepare the following dilutions of p-*Nitrophenol Stock Solution* with water: 100:50 (v/v) (0.16 μmol/mL); 50:100 (v/v) (0.08 μmol/mL); and 25:125 (v/v) (0.04 μmol/mL). Transfer 2.0 mL of the *Substrate Solution* to each of five separate test tubes. Add 1 mL of the p-*Nitrophenol Stock Solution* to the first tube, 1.0 mL of each dilution to the next three tubes, and 1.0 mL of water to the fifth tube. Add 5.0 mL of *Borax Buffer* to each tube, and mix.

Samples Prepare a solution of α-galactosidase sample in *Acetate Buffer* that contains between 0.008 and 0.024 galactosidase units of activity per milliliter.

Procedure Equilibrate the *Substrate Solution* in a water bath at 37° ± 0.2° for at least 15 min. For active samples, transfer 1.0 mL of each sample to separate test tubes and equilibrate in the 37° ± 0.2° water bath. At zero time, add 2.0 mL of *Substrate Solution*, mix, and return to the water bath. After exactly 15.0 min, add 5.0 mL of *Borax Buffer* to each tube, mix, and remove from the water bath.

For sample blanks, transfer, in sequence, 1.0 mL of each sample to separate test tubes, add 5.0 mL of *Borax Buffer*, and mix. Add 2.0 mL of *Substrate Solution* to each tube, and mix.

Measure the absorbance of each standard sample and blank at 405 nm versus that of water. Determine the absorbances of all solutions within 30 min of completing the tests.

Calculations One galactosidase activity unit (GalU) is defined as the quantity of the enzyme that will liberate *p*-nitrophenol at the rate of 1 μmol/min under the conditions of the assay.

Calculate the factor ϵ for the *p*-nitrophenol standards using the following equation:

$$\epsilon = A_N/C,$$

in which A_N is the absorbance of the *p*-nitrophenol standards at 405 nm, and C is the concentration, in millimoles per milliliter, of *p*-nitrophenol.

Because the averaged millimolar extinction coefficient of *p*-nitrophenol at 405 nm is 18.3, ϵ should be approximately 2.29 [or (18.3)/8].

$$\text{GalU/g} = [(A_S - A_B) \times F]/(\epsilon \times T \times M),$$

in which A_S is the sample absorbance; A_B is the blank absorbance; F is the appropriate dilution factor; T is the reaction time, in minutes; M is the weight, in grams, of the sample; and ϵ is a factor calculated above for the *p*-nitrophenol standards (proportional to the millimolar extinction coefficient for *p*-nitrophenol).

β-GLUCANASE ACTIVITY

Application and Principle This procedure is used to determine ß-glucanase activity of enzyme preparations derived from *Aspergillus niger* var. and *Bacillus subtilis* var. The assay is based on a 15-min hydrolysis of lichenin substrate at 40° and at pH 6.5. The increase in reducing power due to liberated reducing groups is measured by the neocuproine method.

Reagents and Solutions

Phosphate Buffer Dissolve 13.6 g of monobasic potassium phosphate in about 1900 mL of water, add 70% sodium hydroxide solution until the pH is 6.5 ± 0.05, then transfer the solution into a 2000-mL volumetric flask, dilute to volume with water, and mix.

Neocuproine Solution A Dissolve 40.0 g of anhydrous sodium carbonate, 16.0 g of glycine, and 450 mg of cupric sulfate pentahydrate in about 600 mL of water. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Neocuproine Solution B Dissolve 600 mg of neocuproine hydrochloride in about 400 mL of water, transfer the solution into a 500-mL volumetric flask, dilute to volume with water, and mix. Discard when a yellow color develops.

Lichenin Substrate Grind 150 mg of lichenin (Sigma Chemical Co., Catalog No. L-6133, or equivalent) to a fine powder in a mortar, and dissolve it in about 50 mL of water at about 85°. After solution is complete (20 to 30 min), add 90 mg of sodium borohydride and continue heating below the boiling point for 1 h. Add 15 g of Amberlite MB-3, or an equivalent ion-exchange resin, and stir continuously for 30 min. Filter with the aid of a vacuum through Whatman No. 1 filter paper, or equivalent, in a Büchner funnel, and wash the paper with about 20 mL of water. Add 680 mg of monobasic potassium phosphate to the filtrate, and refilter through a 0.22-μm Millipore filter pad, or equivalent. Wash the pad with 10 mL of water, and adjust the pH of the filtrate to 6.5 ± 0.05 with 1 *N* sodium hydroxide or 1 *N* hydrochloric acid. Transfer the filtrate into a 100-mL volumetric flask, dilute to volume with water, and mix. Store at 2° to 4° for not more than 3 days.

Glucose Standard Solution Dissolve 36.0 mg of anhydrous dextrose in *Phosphate Buffer* in a 1000-mL volumetric flask, dilute to volume with water, and mix.

Test Preparation Prepare a solution from the enzyme preparation sample so that 1 mL of the final dilution will contain between 0.01 and 0.02 β-glucanase units. Weigh the sample, transfer it into a volumetric flask of appropriate size, dilute to volume with *Phosphate Buffer*, and mix.

Procedure Pipet 2 mL of *Lichenin Substrate* into each of four separate test tubes graduated at 25 mL, and heat the tubes in a water bath at 40° for 10 to 15 min to equilibrate.

After equilibration, add 1 mL of *Phosphate Buffer* to tube 1 (substrate blank), 1 mL of *Glucose Standard Solution* to tube 2 (glucose standard), 4 mL of *Neocuproine Solution A* and 1 mL of the *Test Preparation* to tube 3 (enzyme blank), and 1 mL of the *Test Preparation* to tube 4 (sample). Prepare a fifth tube for the buffer blank, and add 3 mL of *Phosphate Buffer*.

Incubate the five tubes at 40° for exactly 15 min, and then add 4 mL of *Neocuproine Solution A* to tubes 1, 2, 4, and 5. Add 4 mL of *Neocuproine Solution B* to all five tubes, and cap each with a suitably sized glass marble.

Caution: Do not use rubber stoppers.

Heat the tubes in a vigorously boiling water bath for exactly 12 min to develop color, then cool to room temperature in cold water, and adjust the volume of each to 25 mL with water. Cap the tubes with Parafilm, or other suitable closure, and mix by inverting several times.

Determine the absorbance of each solution at 450 nm in 1-cm cells, with a suitable spectrophotometer, against the buffer blank in tube 5.

Calculation One β-glucanase unit (BGU) is defined as that quantity of enzyme that will liberate reducing sugar (as glucose equivalence) at a rate of 1 μmol/min under the conditions of the assay.

Calculate the activity of the enzyme preparation taken for analysis as follows:

$$\text{BGU} = [(A_4 - A_3) \times 36 \times 10^6]/[(A_2 - A_1) \times 180 \times 15 \times \mu\text{g sample}],$$

in which A_4 is the absorbance of the sample (tube 4), A_3 is

the absorbance of the enzyme blank (tube 3), A_2 is the absorbance of the glucose standard (tube 2), A_1 is the absorbance of the substrate blank (tube 1), 36 is the micrograms of glucose in the *Glucose Standard Solution*, 10^6 is the factor converting micrograms to grams, 180 is the weight of 1 μmol of glucose, and 15 is the reaction time, in minutes.

GLUCOAMYLASE ACTIVITY (AMYLOGLUCOSIDASE ACTIVITY)

Application and Principle This procedure is used to determine the glucoamylase activity of preparations derived from *Aspergillus niger* var., but it may be modified to determine preparations derived from *Aspergillus oryzae* var. and *Rhizopus oryzae* var. (as indicated by the variations in the text below). The sample hydrolyzes *p*-nitrophenyl-α-D-glucopyranoside (PNPG) to *p*-nitrophenol (PNP) and glucose at pH 4.3 and 50°.

Use the quantity of PNP liberated per unit of time to calculate the enzyme activity. Measure the PNP liberated against a quantity of a standard preparation of PNP by measuring the absorbance of the solutions at 400 nm after adjusting the pH of the reaction mixture to pH 8.0.

> **Note**: Use a pH of 5.0 when testing preparations derived from *Aspergillus oryzae* var. or *Rhizopus oryzae* var.

Apparatus

Water Bath Use an open, circulating water bath with control accuracy of at least ±0.1°.

Spectrophotometer Use a spectrophotometer suitable for measuring absorbances at 400 nm.

Cuvettes Use 10-mm light-path fused quartz.

Thermometer Use a partial immersion thermometer with a suitable range, graduated in 1/10°.

Timer Use a solid-state timer, model 69240 (GCS Corporation, Precision Scientific Group), or equivalent, accurate to ±0.01 min in 240 min.

Vortex Mixer Use a standard variable-speed mixer.

Reagents and Solutions

p-*Nitrophenol Stock Solution (PNP)* (0.001 *M*) Dissolve 139.11 mg of *p*-nitrophenol previously dried (60°, maximum 4 h) into water, and dilute to 1000 mL.

> **Caution**: Avoid contact with skin. If contact occurs, wash the affected area with water. Work in a well-ventilated area.

Acetate Buffer Solution (0.1 *M*) Dissolve 4.4 g of sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$) in approximately 800 mL of water, add 4.5 mL of acetic acid ($C_2H_4O_2$). Adjust to pH 4.5 ± .05 by adding either sodium acetate or glacial acetic acid as required. Dilute to 1 L.

> **Note**: Use a pH of 5.0 when testing preparations derived from *Aspergillus oryzae* var. or *Rhizopus oryzae* var.

The pH optimum is 5.0 for *Aspergillus oryzae* var.—or *Rhizopus oryzae* var.—derived preparations.

Sodium Carbonate Solution (0.3 *M*) Dissolve 15.9 g of sodium carbonate (Na_2CO_3) in water, and dilute to 500 mL.

p-Nitrophenyl-α-D-glucopyranoside Solution (PNPG) Dissolve 100.0 mg of PNPG (Sigma Chemical Co., Catalog No. N1377) in acetate buffer, and dilute to 100 mL.

Preparation of Standards and Samples

Standards Dilute three portions of *PNP Stock Solution* to produce standards for the standard curve. Add 3 mL of the *PNP Stock Solution* to 125 mL of *Sodium Carbonate Solution*, and dilute to 500 mL with water to produce the first standard, containing 0.006 μmol/mL. Add 2 mL of *PNP Stock Solution* to 25 mL of *Sodium Carbonate Solution*, and dilute to 100 mL with water to produce the second standard, containing 0.02 μmol/mL. Add 5 mL of *PNP Stock Solutions* to 25 mL of *Sodium Carbonate Solution*, and dilute to 100 mL with water to produce the third standard, containing 0.05 μmol/mL.

Sample Solution Dilute 1.00 ± 0.01 g of sample in sufficient *Acetate Buffer Solution* to produce a solution that contains between 0.1 and 0.3 glucoamylase units of activity per milliliter.

Procedure Measure absorbances of each of the three *PNP Standard Solutions* to calculate the molar extinction coefficient. Equilibrate the *PNPG Solution* in a 50° water bath for at least 15 min. For active samples, transfer 2.0 mL of the *Sample Solution* to a test tube. Loosely stopper, and place the tube in the water bath to equilibrate for 5 min. At zero time, add 2.0 mL of *PNPG Solution*, and mix at moderate speed on a vortex mixer. Return the mixture to the water bath. Exactly 10.0 min later, add 3.0 mL of the *Sodium Carbonate Solution*, mix on the vortex, and remove from the water bath.

For sample blanks, transfer 2.0 mL of the *Sample Solution* and 3.0 mL of the *Sodium Carbonate Solution* into a test tube, and mix. Add 2.0 mL of *PNPG Solution*, and mix. Measure the absorbance of each sample and the blank versus water in a 10-mm cell.

> **Note**: Determine the absorbance of the sample and blank solutions not more than 20 min after adding *Sodium Carbonate Solution*.

Calculations One unit of glucoamylase activity is defined as the amount of glucoamylase that will liberate 0.1 μmol/min of *p*-nitrophenol from the *PNPG Solution* under the conditions of the assay.

Calculate the millimolar extinction of the PNP standards using the following equation:

$$\epsilon = A_n/C,$$

in which A_n is the absorbance of the *p*-nitrophenol standard, at 400 nm, and C is concentration, in μmol/mL, of *p*-nitrophenol.

The averaged millimolar extinction coefficient, M, should be approximately 18.2.

$$\text{Glucoamylase } \mu/g = [(A_S - A_B) \times 7 \times F]/\epsilon \times 10 \times 0.10 \times W \times 2,$$

in which A_S is the sample absorbance; A_B is the blank absorbance; F is the appropriate dilution factor; W is the weight of sample, in grams; 7 is the final volume of the test solutions; 10 is the reaction time, in minutes; 0.10 is the amount of PNP liberated, in μmol/min/unit of enzyme; 2 is the sample aliquot, in milliliters, and M is the millimolar extinction coefficient.

GLUCOSE ISOMERASE ACTIVITY

Note: Glucose isomerase activity of the commercial enzyme is usually determined on the enzyme that has been immobilized by binding with a polymer matrix or other suitable material. The following method is designed for use with such preparations.

Application and Principle Use this procedure to determine glucose isomerase preparations derived from *Actinoplanes missouriensis*, *Bacillus coagulans*, *Microbacterium arborescens*, *Streptomyces murinus*, *Streptomyces olivaceus*, *Streptomyces olivochromogenes*, and *Streptomyces rubiginosus*. It is based on measurement of the rate of conversion of glucose to fructose in a packed-bed reactor. The procedure as outlined approximates an initial velocity assay method. Specific conditions are glucose concentration, 45% w/w; pH (inlet), measured at room temperature in the 7.0 to 8.5 range, as specified; temperature, 60.0°; and magnesium concentration, 4×10^{-3} *M*.

The optimum conditions for enzymes from different microbial sources and methods of preparation may vary; therefore, if the manufacturer recommends different pH conditions, buffering systems, or methods of sample preparation, use such variations in the instructions given in the text.

Apparatus

Column Assembly and Apparatus (**Note**: Make all connections with inert tubing, glass, or plastic as appropriate.) The column assembly is shown in Fig. 32. Use a 2.5- × 40-cm glass column provided with a coarse, sintered-glass bottom and a water jacket connected to a constant-temperature water bath, maintained at 60.0°, by means of a circulating pump. Connect the top of the column to a variable-speed peristaltic pump having a maximum flow rate of 800 mL/h. The diameter of the tubing with which the peristaltic pump is fitted should permit variation of the pumping volume from 60 to 150 mL/h. Connect the outlet of the column with a collecting vessel.

Reagents and Solutions

Glucose Substrate Dissolve 539 g of anhydrous glucose and 1.0 g of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) in 700 mL of water or the manufacturer's recommended buffer, previously heated to 50° to 60°. Cool the solution to room temperature, and adjust the pH as specified by the enzyme manufacturer. Transfer the solution to a 1000-mL volumetric flask, dilute to volume with water or the specified buffer, and mix. Transfer to a vacuum flask, and de-aerate for 30 min.

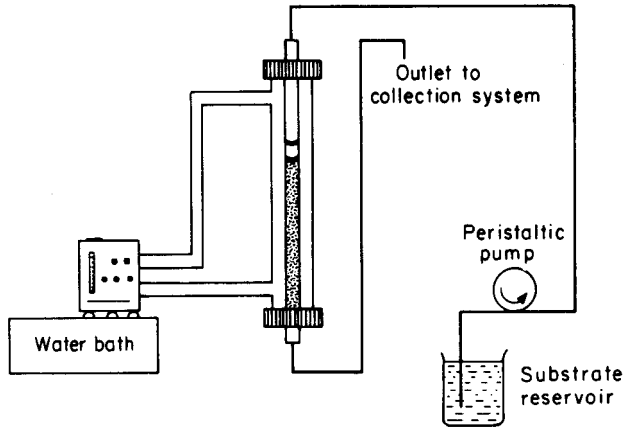


FIGURE 32 Column Assembly for Assay of Immobilized Glucose Isomerase.

Magnesium Sulfate Solution Dissolve 1.0 g of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) in 700 mL of water. Adjust the pH to 7.5 to 8.0 as specified by the manufacturer, using 1 *N* sodium hydroxide, dilute to 1000 mL with water, and mix.

Sample Preparation Transfer to a 500-mL vacuum flask an amount of the sample, accurately weighed in grams or measured in milliliters, as appropriate, sufficient to obtain 2000 to 8000 glucose isomerase units (GI_cU). Add 200 mL of *Glucose Substrate*, stir gently for 15 s, and repeat the stirring every 5 min for 40 min. De-aerate by vacuum for 30 min.

Column Preparation Quantitatively transfer the *Sample Preparation* to the column with the aid of *Magnesium Sulfate Solution* as necessary. Allow the enzyme granules to settle, and then place a porous disk so that it is even with, and in contact with, the top of the enzyme bed. Displace all of the air from the disk. Place a cotton plug about 1 or 2 cm above the disk. (This plug acts as a filter. It ensures proper heating of the solution and traps dissolved gases that may be present in the *Glucose Substrate*.) Connect the tubing from the peristaltic pump with the top of the column, and seal the connection by suitable means to protect the column contents from the atmosphere. Place the inlet tube of the peristaltic pump into the *Glucose Substrate* solution, and begin a downward flow of the *Glucose Substrate* into the column at a rate of at least 80 mL/h. Maintain the flow rate for 1 h at room temperature.

Assay Adjust the flow of the *Glucose Substrate* to such a rate that a fractional conversion of 0.2 to 0.3 will be produced, based on the estimated activity of the sample. Calculate the fractional conversion from optical rotation values obtained on the starting *Glucose Substrate* and the sample effluent, as specified under *Calculations*, below. After establishing the correct flow rate, run the column overnight (16 h minimum), then check the pH of the *Glucose Substrate*, and readjust if necessary to the specified pH. Measure the flow rate, and collect a sample of the column effluent. Cover the effluent sample, allow it to stand for 30 min at room temperature, and then determine the fractional conversion of glucose to fructose (see *Calculations*, below). If the conversion is less than 0.2

or more than 0.3, adjust the flow rate to bring the conversion into this range. If a flow rate adjustment is required, collect an additional effluent sample after allowing the column to re-equilibrate for at least 2 h, and then determine the fractional conversion. Measure the flow rate, and collect an effluent sample. Cover the sample, let it stand at room temperature for 30 min, and determine the fractional conversion.

Calculations

Specific Rotation Measure the optical rotation of the effluent sample and of the starting *Glucose Substrate* at 25.0°, and calculate their specific rotations [see *Optical (Specific) Rotation*, Appendix IIB] by the equation

$$[\alpha] = 100a/lpd,$$

in which a is the corrected observed rotation, in degrees; l is the length of the polarimeter tube, in decimeters; p is the concentration of the test solution, expressed as grams of solute per 100 g of solution; and d is the specific gravity of the solution at 25°.

Fractional Conversion Calculate the fractional conversion, X, by the equation

$$X = (\alpha_E - \alpha_S)/(\alpha_F - \alpha_S),$$

in which α_E is the specific rotation of the column effluent, α_S is the specific rotation of the *Glucose Substrate*, and α_F is the specific rotation of fructose (which, in this case, has been calculated to be −94.54).

Activity The enzyme activity is expressed in glucose isomerase units (GI_cU, the subscript c signifying column process). One GI_cU is defined as the amount of enzyme that converts glucose to fructose at an initial rate of 1 μmol/min, under the conditions specified.

Calculate the glucose isomerase activity by the equation:

$$GI_cU/g \text{ or } mL = (FS/W) \times X_E \times \ln[X_E/(X_E - X)],$$

in which F is the flow rate, in milliliters per minute; S is the concentration of the *Glucose Substrate*, in micromoles per milliliter; X is the fractional conversion, as determined above; X_E is the fractional conversion at equilibrium, or 0.51; and W is the weight or volume of the sample taken, in grams or milliliters, respectively.

GLUCOSE OXIDASE ACTIVITY

Application and Principle This procedure is used to determine glucose oxidase activity in preparations derived from *Aspergillus niger* var. The assay is based on the titrimetric measurement of gluconic acid produced in the presence of excess substrate and excess air.

Reagents and Solutions

Chloride–Acetate Buffer Solution Dissolve 2.92 g of sodium chloride and 4.10 g of sodium acetate in about 900 mL of water. Adjust the pH to 5.1 with either dilute acetic acid or dilute sodium hydroxide solution and dilute to 1000.0 mL.

Sodium Hydroxide Solution (0.1 *N*)

Hydrochloric Acid Solution (0.05 *N*) Standardized.

Phenolphthalein Solution (2% w/v) Solution in methanol.

Octadecanol Solution Saturated solution in methanol.

Substrate Solution Dissolve 30.00 g of anhydrous glucose in 1000 mL of the *Chloride–Acetate Buffer Solution*.

Sample Preparation Dissolve an accurately weighed amount of enzyme preparation in the *Chloride–Acetate Buffer Solution*, and dilute in the buffer solution to obtain an enzyme activity of 5 to 7 activity units per milliliter.

Procedure Transfer 25.0 mL of the *Substrate Solution* to a 32- × 200-mm test tube. To a second 32- × 200-mm test tube transfer 25.0 mL of the *Chloride–Acetate Buffer Solution* (blank). Equilibrate both tubes in a 35° ± 0.1° water bath for 20 min. Add 3.0 mL of the *Sample Preparation* to each test tube, mix, and insert a glass sparger into each tube with a preadjusted air flow of 700 to 750 mL/min. If excessive foaming occurs, add 3 drops of the *Octadecanol Solution* to each tube. After exactly 15 min, remove the sparge and rinse any adhering reaction mixture back into the tube with water. Immediately add 10 mL of the *Sodium Hydroxide Solution* and 3 drops of the *Phenolphthalein Solution* to each tube. Insert a small magnetic stirrer bar, stir, and titrate to the phenolphthalein endpoint with the standardized *0.05* N *Hydrochloric Acid Solution*.

Calculation One Glucose Oxidase Titrimetric unit of activity (GOTu) is the quantity of enzyme that will oxidize 3 mg of glucose to gluconic acid under the conditions of the assay. Determine the enzyme activity using the following equation:

$$GOTu/g = [(B - T) \times N \times 180 \times F]/[3 \times W],$$

in which B is the titration volume, in milliliters, of the blank; T is the titration volume, in milliliters, of the sample; N is the normality of the titrant; 180 is the molecular weight of glucose; F is the sample dilution factor; 3 is from the unit definition; and W is the weight, in grams, of the enzyme preparation contained in each milliliter of the sample solution.

HEMICELLULASE ACTIVITY

Application and Principle This procedure is used to determine hemicellulase activity of preparations derived from *Aspergillus niger* var. The test is based on the enzymatic hydrolysis of the interior glucosidic bonds of a defined locust (carob) bean gum substrate at 40° and pH 4.5. Determine the corresponding reduction in substrate viscosity with a calibrated viscometer.

Apparatus

Viscometer Use a size 100 calibrated Cannon-Fenske Type Viscometer, or equivalent (Scientific Products, Catalog No. 2885-100).

Glass Water Bath Use a constant-temperature glass water bath, maintained at 40° ± 0.1° (Scientific Products, Catalog No. W3520-10).

Stopwatches Use two stopwatches—*Stopwatch No. 1*, calibrated in $^1/_{10}$ min for determining the reaction time (T_r), and *Stopwatch No. 2*, calibrated in $^1/_5$ s for determining the efflux time (T_t).

Reagents and Solutions

Acetate Buffer (pH 4.5) Add 0.2 *N* sodium acetate, with continuous agitation, to 400 mL of 0.2 *N* acetic acid until the pH is 4.5 ± 0.05, as determined by a pH meter.

Locust Bean Gum Use Powdered Type D-200 locust bean gum, or its equivalent (Meer Corp.). Because the substrate may vary from lot to lot, test each lot in parallel with a previous lot known to be satisfactory. Variations of more than ±5% viscosity in the average of a series of parallel tests indicate an unsuitable lot.

Substrate Solution Place 12.5 mL of 0.2 *N* hydrochloric acid and 250 mL of warm water (72° to 75°) in the bowl of a power blender (Waring two-speed, or equivalent, Scientific Products, Catalog No. 58350-1), and set the blender on low speed. Slowly disperse 2.0 g of *Locust Bean Gum*, on a moisture-free basis, into the bowl, taking care not to splash out any of the liquid in the bowl. Wash down the sides of the bowl with warm water, using a rubber policeman, cover the bowl, and blend at high speed for 5 min. Quantitatively transfer the mixture to a 1000-mL beaker, and cool to room temperature. Using a pH meter, adjust the mixture to pH 6.0 with 0.2 *N* sodium hydroxide. Quantitatively transfer the mixture to a 1000-mL volumetric flask, dilute to volume with water, and mix. Filter the substrate through gauze before use.

Sample Preparation Prepare a solution of the sample in water so that 1 mL of the final dilution will produce a change in relative fluidity between 0.18 and 0.22 in 5 min under the conditions specified in the *Procedure*.

Weigh the enzyme preparation, quantitatively transfer it to a glass mortar, and triturate with water. Quantitatively transfer the mixture to an appropriately sized volumetric flask, dilute to volume with water, and mix. Filter through Whatman No. 1 filter paper, or equivalent, before use.

Procedure Scrupulously clean the viscometer by drawing a large volume of detergent solution, followed by water, through the instrument, and place the viscometer, previously calibrated, in the glass water bath in an exactly vertical position. Pipet 20.0 mL of *Substrate Solution* and 4.0 mL of *Acetate Buffer* into a 50-mL Erlenmeyer flask, allowing at least two flasks for each enzyme sample and one flask for a substrate blank. Stopper the flasks, and equilibrate them in the water bath for 15 min. At zero time, pipet 1.0 mL of the *Sample Preparation* into the equilibrated substrate, start timing with stopwatch no. 1, and mix thoroughly. Immediately pipet 10.0 mL of this mixture into the wide arm of the viscometer. After about 2 min, draw the reaction mixture above the upper mark into the driving fluid head by applying suction with a rubber tube connected to the narrow arm of the instrument. Measure the efflux time by allowing the reaction mixture to flow freely down past the upper mark. As the meniscus falls past the upper mark, start stopwatch no. 2, and at the same time, record the reaction time (T_R), in minutes, from stopwatch no. 1. As the meniscus of the reaction mixture falls past the lower mark, record the time (T_T), in seconds, from stopwatch no. 2. Immediately re-draw the reaction mixture above the upper mark and into the driving fluid head. As the meniscus falls freely past the upper mark, restart stopwatch no. 2, and at the same time record the reaction time (T_R), in minutes, from stopwatch no. 1. As the meniscus falls past the lower mark, record the time (T_T), in seconds, from stopwatch no. 2.

Repeat the latter operation, beginning with ''Immediately re-draw the reaction mixture. . . ,'' until a total of four determinations is obtained over a reaction time (T_R) of not more than 15 min.

Prepare a substrate blank by pipetting 1.0 mL of water into a mixture of 20.0 mL of *Substrate Solution* and 4.0 mL of *Acetate Buffer*, and then immediately pipet 10.0 mL of this mixture into the wide arm of the viscometer. Determine the time (T_S), in seconds, required for the meniscus to fall between the two marks. Use an average of five determinations as T_S.

Prepare a water blank by pipetting 10.0 mL of water, previously equilibrated to 40° ± 0.1°, into the wide arm of the viscometer. Determine the time (T_W), in seconds, required for the meniscus to fall between the two marks. Use an average of five determinations as T_W.

Calculation One hemicellulase unit (HCU) is defined as that activity that will produce a relative fluidity change of 1 over a period of 5 min in a locust bean gum substrate under the conditions specified. Calculate the relative fluidities (F_R) and T_N values (see definition below) for each of the four efflux times (T_T) and reaction times (T_R) as follows:

$$F_R = (T_S - T_W)/(T_T - T_W),$$

and

$$T_N = {}^1/_2(T_T/60 \text{ s/min}) + T_R = (T_T/120) + T_R,$$

in which F_R is the relative fluidity for each reaction time; T_S is the average efflux time, in seconds, for the substrate blank; T_W is the average efflux time, in seconds, for the water blank; T_T is the efflux time, in seconds, of the sample reaction mixture; T_R is the elapsed time, in minutes, from zero time, that is, the time from addition of the enzyme solution to the buffered substrate until the beginning of the measurement of the efflux time (T_T); and T_N is the reaction time (T_R), in minutes, plus one-half of the efflux time (T_T) converted to minutes.

Plot the four relative fluidities (F_R) as the ordinate against the four reaction times (T_N) as the abscissa. This should result in a straight line. The slope of the line corresponds to the relative fluidity change per minute and is proportional to the enzyme concentration. The slope of the best line through a series of experimental points is a better criterion of enzyme activity than is a single relative fluidity value. From the curve, determine the F_R values at 10 and 5 min. They should have a difference in fluidity of not more than 0.22 and not less than 0.18. Calculate the activity of the enzyme sample as follows:

$$\text{HCU/g} = 1000(F_{R10} - F_{R5}/W),$$

in which F_{R10} is the relative fluidity at 10 min reaction time; F_{R5} is the relative fluidity at 5 min reaction time; 1000 is milligrams per gram; and W is the weight, in milligrams, of the enzyme sample contained in the 1.0-mL aliquot of *Sample Preparation* added to the equilibrated substrate in the *Procedure*.

INVERTASE ACTIVITY

Application and Principle This procedure is used to determine the invertase activity of enzyme preparations from yeast *Saccharomyces* sp (*Kluyveromyces*). The assay is based on a 30-min hydrolysis of sucrose at 30° ± 0.1° and at pH 4.62. The degree of hydrolysis is determined by measuring the optical rotation of the solution with a polarimeter.

Reagents and Solutions

Acetate Buffer Dissolve 4.0 g of sodium hydroxide (NaOH) in about 900 mL of water, and carefully neutralize with 12.0 g of acetic acid 98% to 100% (CH_3COOH). Cool to room temperature. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. The pH should be 4.62 ± 0.05.

Sucrose Substrate Solution Dissolve 82.152 g of sucrose in about 900 mL of water. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. Use a freshly prepared solution only.

Sodium Carbonate Solution Dissolve 53.0 g of sodium carbonate (Na_2CO_3) in about 400 mL of water, then transfer the solution into a 500-mL volumetric flask, dilute to volume with water, and mix.

Test Preparation Using a 100-mL volumetric flask, prepare a solution from the starting enzyme preparation by weighing a minimum of 1 g of sample accurately to within 1 mg. Dilute with water so that the final solution will contain between 1.3 and 5.3 Invertase Units per 20 mL. Pipet 20.0 mL of this solution into a 100-mL Erlenmeyer flask.

Blank Preparation Pipet 20.0 mL water in a 100-mL Erlenmeyer flask.

Procedure To the flasks containing 20.0 mL of each *Test Preparation* and to the *Blank Preparation*, add 5.00 mL of *Acetate Buffer*. At zero time, and at regular time intervals so that each test sample is analyzed in the same elapsed time, place the flasks containing the *Test Preparations* and the *Blank Preparation* in a circulating water bath maintained at 30.0° ± 0.1°. Equilibrate the samples for 10 min in the water bath. In the same order and with the same time intervals, rapidly pipet 25.00 mL of equilibrated *Sucrose Substrate Solution* into the test flasks. Incubate for 30.0 min, and stir continuously. Terminate the reaction by adding 10.00 mL of *Sodium Carbonate Solution*, and swirl to mix. Place the flasks containing the *Test Preparations* and the *Blank Preparation* in a water bath maintained at 20.0° ± 0.1° for 30 min. Use a polarimeter with an accuracy of at least 0.001 degrees of arc. With the same precision, determine the optical rotation of each solution at 589 nm (sodium lamp), using a 10-cm path-length cell with the thermostat set at 20.0° ± 0.1°. Use water to blank the polarimeter initially.

Calculation One Invertase Unit (INVU) is defined as the quantity of enzyme that will hydrolyze 1.142 μmol of sucrose per minute under the conditions of the assay.

Calculate the invertase units per 20 mL as follows:

$$(R_{bt} - R_{test}) \times 2 \times 1{,}000{,}000/[66.77 - (Glu + Fru)]$$
$$(342.3)(1.142) = INVU/20\ mL,$$

where Glu is (0.525)(52.5) and Fru is (0.525)(−91.315), or simplified:

$$(R_{bt} + R_{test}) \times 58.71 = INVU/20\ mL,$$

in which R_{bt} is the rotation of *Blank Preparation*; R_{test} is the rotation of the *Test Preparation*; 66.77 is the specific rotation of sucrose; 52.50 is the specific rotation of glucose; −91.315 is the specific rotation of fructose; 0.525 is 0.5 corrected for 5% weight increase by hydrolysis; 342.3 is the molecular mass (grams per mole) of sucrose; and 1.142 is the unit definition factor. Specific rotations are valid at the average concentrations in this test.

Invertase Activity in Weighed Samples:

$$INVU/20\ mL \times d/w = INVU/g,$$

Invertase Activity in Pipetted Samples:

$$INVU/20\ mL \times d = INVU/mL,$$

in which d is the total dilution factor and w is the weight of the sample.

LACTASE (NEUTRAL) (β-GALACTOSIDASE) ACTIVITY

Application and Principle This procedure is used to determine the neutral lactase activity of enzyme preparations derived from *Kluyveromyces marxianus* var. *lactis* and *Saccharomyces* sp. The assay is based on a 10-min hydrolysis of an *o*-nitrophenyl-β-D-galactopyranoside (ONPG) substrate at 30.0° ± 0.1° and at pH 6.50.

Reagents and Solutions

Magnesium Solution Dilute 24.65 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in about 950 mL of water. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix.

EDTA Solution Dissolve 1.86 g of disodium EDTA dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$) in about 950 mL of water. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix.

P-E-M Buffer Dissolve 8.8 g of potassium dihydrogen phosphate (KH_2PO_4) and 8.0 g of dipotassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$) in about 900 mL of water. Add 10.0 mL of *Magnesium Solution* and 10.0 mL of *EDTA Solution*. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. The pH should be 6.50 ± 0.05.

Lactase Reference Preparation (Highly concentrated lactase preparation) This preparation can be obtained from Gist-Brocades, Delft, The Netherlands.

ONPG (*o*-nitrophenyl-β-D-galactopyranoside) is validated according to the following procedure:

Validation of New ONPG Transfer 150, 250, and 375 mg of the new *ONPG* into separate 100-mL volumetric flasks, dilute to volume with *P-E-M Buffer*, and mix. Prepare solutions of the *Lactase Reference Preparation* by weighing an amount of *Lactase Reference Preparation* corresponding to 5000 ± 250 Neutral Lactase Units (NLU) accurately to within 1 mg in duplicate in 50-mL volumetric flasks, dissolve in *P-E-M Buffer*, dilute to volume with the same, and mix. Prepare dilutions of this initial solution with *P-E-M Buffer* so that 1 mL of the final dilution will contain 0.0375, 0.0750, and 0.1125 NLU of activity. In duplicate, determine the enzyme activity of the three enzyme concentrations using each of the new *ONPG Substrate* solutions corresponding to 150, 250, and 375 mg and the old *ONPG Substrate* at 250 mg by following the steps in the *Procedure*, below.

Calculation Calculate the enzyme activity following the steps indicated under *Calculation for NLU Activity*, below. Determine the average of the duplicates for each enzyme concentration at each level of *ONPG Substrate* (the maximum allowable difference between these duplicates is 6.5%). Determine the overall average for the three enzyme concentrations (0.0375, 0.0750, and 0.1125) for each *ONPG Substrate* level (150, 250, and 375 mg of *ONPG*).

To determine the overall average of three enzyme concentrations at 150 mg of *ONPG*:

$$X = (A + B + C)/3,$$

in which *A* is the average result of 0.0375 at 150 mg of *ONPG*, *B* is the average result of 0.0750 at 150 mg of *ONPG*, and *C* is the average result of 0.1125 at 150 mg of *ONPG*.

To determine the overall average of three enzyme concentrations at 250 mg of *ONPG*:

$$Y = (D + E + F)/3,$$

in which *D* is the average result of 0.0375 at 250 mg of *ONPG*, *E* is the average result of 0.0750 at 250 mg of *ONPG*, and *F* is the average result of 0.1125 at 250 mg of *ONPG*.

To determine the overall average of three enzyme concentrations at 375 mg of *ONPG*:

$$Z = (G + H + I)/3,$$

in which *G* is the average result of 0.0375 at 375 mg of *ONPG*, *H* is the average result of 0.0750 at 375 mg of *ONPG*, and *I* is the average result of 0.1125 at 375 mg of *ONPG*.

The *ONPG* analyzed is suitable for use when the following specifications are met for each *ONPG* concentration:

1. The average result of each enzyme concentration for each *ONPG* level does not deviate more than 3% from the overall average of the three enzyme concentrations for that level of *ONPG*. For example, *A* or *B* or *C* should not deviate more than 3% from *X*; *D* or *E* or *F* should not deviate more than 3% from *Y*; *G* or *H* or *I* should not deviate more than 3% from *Z*.
2. The overall average of the three enzyme concentrations found for 150 mg of *ONPG* (*X*) should not vary more than 81% to 99% of the overall average of the three enzymes concentrations found for 250 mg of *ONPG* (*Y*). The overall average of the three enzyme concentrations found for 375 mg of *ONPG* (*Z*) should not vary more than 96% to 114% of the overall average of the three enzyme concentrations of 250 mg of *ONPG* (*Y*).
3. The absorbance of each blank is less than 0.050.
4. For each new lot of *ONPG*, the overall average of the three enzyme concentrations found for 250 mg of *ONPG* (*Y*) per 100 mL should be within 5% of the overall average of the three enzyme concentrations found for 250 mg of *ONPG* of the lot in use at that moment.

ONPG Substrate Dissolve 250.0 mg *ONPG* (use lot currently in use) in about 80 mL of *P-E-M Buffer*. Transfer the solution to a 100-mL volumetric flask, dilute to volume with *P-E-M Buffer*, and mix. Prepare, at most, 2 h before incubation.

Sodium Carbonate Solution Dissolve 50.0 g of sodium carbonate anhydrous (Na_2CO_3) and 37.2 g of disodium EDTA dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$) in about 900 mL of water. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Standard o-Nitrophenol Solution Transfer 139.0 mg of *o*-nitrophenol into a 1000-mL volumetric flask, dissolve in 10 mL of 96% ethanol, dilute to volume with water, and mix. Pipet 2-, 4-, 6-, 8-, 10-, 12-, and 14-mL portions of this solution into a series of 100-mL volumetric flasks, add 25 mL of *Sodium Carbonate Solution* to each, dilute each to volume with *P-E-M Buffer*, and mix. The dilutions contain, respectively, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.14 μmol/mL of *o*-nitrophenol.

Determine the absorbance of each dilution at 420 nm in a 1-cm path-length cell, with a suitable spectrophotometer, using water as the blank. For each dilution, plot absorbance against μmol of *o*-nitrophenol (this must result in a straight line through the origin). Divide the absorbance of each dilution by μmol of *o*-nitrophenol to obtain the extinction coefficient (M) at that dilution (the slope of the line is the extinction coefficient). Average the seven values thus calculated (this should result in a value of 4.60 ± 0.05).

Test Preparation Using a volumetric flask, prepare a test solution from the starting enzyme preparation by accurately weighing out a minimum of 1 g of sample to the nearest milligram. Dissolve in *P-E-M Buffer* so that 1 mL of the final

dilution will contain between 0.027 and 0.095 NLU. Transfer 1 mL of this final dilution to a 15- × 150-mm test tube as the *Test Preparation*. Perform in duplicate.

Procedure Equilibrate the test tubes containing each *Test Preparation* in a water bath maintained at 30.0° ± 0.1° for at least 5 but not more than 15 min. At zero time, in the order of the series and at regular time intervals, rapidly pipet 5.00 mL of *ONPG Substrate*, equilibrated at 30.0° ± 0.1°, into the test tubes, and mix by shaking. After a 10.0-min incubation (reaction) time, in the same order and with the same regular intervals, pipet 2.00 mL of *Sodium Carbonate Solution* into each, mix by shaking, and hold at room temperature. Determine the absorbance of each solution within 30 min at 420 nm in a 1-cm cell with a suitable spectrophotometer, using as the blank a solution prepared in the same manner as for the sample except adding *ONPG Substrate* and *Sodium Carbonate Solution* in reverse order.

Calculation for NLU Activity One Neutral Lactase Unit (NLU) is defined as that quantity of enzyme that will liberate 1.30 μmol/min of *o*-nitrophenol under the conditions of the assay. Calculate the activity of the enzyme preparation taken for the analysis as follows:

$$\text{NLU/g} = [(A \times 8 \times f)/(\text{M} \times 10 \times W)]/1.30,$$

in which A is the average of the absorbance readings for the sample, corrected for the sample blank; 8 is the volume, in milliliters, of the incubation mixture after termination; f is the total dilution factor of the sample; M is the extinction coefficient, determined as directed under *Standard o-Nitrophenol Solution*; 10 is the incubation time, in minutes; W is the sample weight, in grams; and 1.30 is the factor used in the unit definition.

LACTASE (ACID) (ß-GALACTOSIDASE) ACTIVITY

Application and Principle This procedure is used to determine lactase activity of enzyme preparations derived from *Aspergillus oryzae* var. The assay is based on a 15-min hydrolysis of an *o*-nitrophenyl-β-D-galactopyranoside substrate at 37° and pH 4.5.

Reagents and Solutions

2.0 N *Acetic Acid* Dilute 57.5 mL of glacial acetic acid to 500 mL with water. Mix well, and store in a refrigerator.

4.0 N *Sodium Hydroxide* Dissolve 40.0 g of sodium hydroxide in sufficient water to make 250 mL.

Acetate Buffer Combine 50 mL of *2.0* N *Acetic Acid* and 11.3 mL of *4.0* N *Sodium Hydroxide* in a 1000-mL volumetric flask, and dilute to volume with water. Verify that the pH is 4.50 ± 0.05, using a pH meter, and adjust, if necessary, with *2.0 N Acetic Acid* or *4.0* N *Sodium Hydroxide*.

*2.0 m*M *o-Nitrophenol Stock* Transfer 139.0 mg of *o*-nitrophenol to a 500-mL volumetric flask, dissolve in 10 mL of USP alcohol (95% ethanol) by swirling, and dilute to volume with 1% sodium carbonate.

o-Nitrophenol Standards

*0.10 m*M *Standard Solution* Pipet 5.0 mL of the *2.0 m*M o-*Nitrophenol Stock* solution into a 100-mL volumetric flask, and dilute to volume with 1% sodium carbonate solution.

*0.14 m*M *Standard Solution* Pipet 7.0 mL of the *2.0 m*M o-*Nitrophenol Stock* solution into a 100-mL volumetric flask, and dilute to volume with 1% sodium carbonate solution.

*0.18 m*M *Standard Solution* Pipet 9.0 mL of the *2.0 m*M o-*Nitrophenol Stock* solution into a 100-mL volumetric flask, and dilute to volume with 1% sodium carbonate solution.

Substrate Transfer 370.0 mg of *o*-nitrophenyl-β-D-galactopyranoside to a 100-mL volumetric flask, and add 50 mL of *Acetate Buffer*. Swirl to dissolve, and dilute to volume with *Acetate Buffer*.

Note: Perform the assay procedure within 2 h of *Substrate* preparation.

Test Preparation Prepare a solution from the test sample preparation such that 1 mL of the final dilution will contain between 0.15 and 0.65 lactase unit. Weigh, and quantitatively transfer the enzyme to a volumetric flask of appropriate size. Dissolve the enzyme in water, swirling gently, and dilute with water if necessary.

Note: Perform the assay procedure within 2 h of dissolution of the *Test Preparation*.

System Suitability Determine the absorbance of the three o-*Nitrophenol Standards* at 420 nm in a 1-cm cell, using a suitable spectrophotometer. Use water to zero the instrument. Calculate the millimolar extinction, M, for each of the o-*Nitrophenol Standards* (0.10, 0.14, and 0.18 m*M*) by the equation

$$\epsilon = A_n/C,$$

in which A_n is the absorbance of each o-*Nitrophenol Standard* at 420 nm and C is the corresponding concentration of *o*-nitrophenol in the standard. M for each standard should be approximately 4.60/m*M*. Perform a linear regression analysis of the absorbance readings of the three o-*Nitrophenol Standards* versus the *o*-nitrophenol concentration in each (0.10, 0.14, and 0.18 m*M*). The r^2 should not be less than 0.99. Determine the mean M of the three o-*Nitrophenol Standards* for use in the calculations below.

Procedure For each sample or blank, pipet 2.0 mL of the *Substrate* solution into a 25- × 150-mm test tube, and equilibrate in a water bath maintained at 37.0° ± 0.1° for approximately 10 min. At zero time, rapidly pipet 0.5 mL of the *Test Preparation* (or 0.5 mL of water as a blank) into the equilibrated substrate, mix by brief (1 s) vortex, and immediately return the tubes to the water bath. After exactly 15 min of incubation, rapidly add 2.5 mL of 10% sodium carbonate solution, and vortex the tube to stop the enzyme reaction. Dilute the samples and blanks to 25.0 mL by adding 20.0 mL of water, and thoroughly mix. Determine the absorbance of

the diluted samples and blanks at 420 nm in a 1-cm cell, using a suitable spectrophotometer. Use water to zero the instrument.

Calculation One lactase unit (ALU) is defined as that quantity of enzyme that will liberate *o*-nitrophenol at a rate of 1 μmol/min under the conditions of the assay.

Calculate the activity (lactase activity per gram) of the enzyme preparation taken for analysis as follows:

$$\text{ALU/g} = [(A_S - B)(25)]/[(\epsilon)(15)(W)],$$

in which A_S is the average of absorbance readings for the *Test Preparation*; B is the average of absorbance readings for the blank; 25 is the final volume, in milliliters, of the diluted incubation mixture; ϵ is the mean absorptivity of the o-*Nitrophenol Standards* per micromole, 15 is the incubation time, in minutes, and W is the weight, in grams, of original enzyme preparation contained in the 0.5-mL aliquot of *Test Preparation* used in the incubation.

LIPASE ACTIVITY

Application and Principle This procedure is used to determine the lipase activity in preparations derived from microbial sources and animal pancreatic tissues. The assay is based on the potentiometric measurement of the rate at which the preparations will catalyze the hydrolysis of tributyrin.

Apparatus

Automatic Recording Titrimeter Use an instrument operating in the pH stat mode and equipped with a jacketed titration cell (Radiometer Titralab, or equivalent).

Constant Temperature Bath Operated at 30° ± 0.1°.

Blender

Reagents and Solutions

0.05 N *Sodium Hydroxide* Dissolve 2.0 g of sodium hydroxide in water, and dilute to 100 mL. Standardize with NIST grade potassium hydrogen phthalate.

Emulsification Reagent Dissolve 17.9 g of sodium chloride and 0.41 g of monobasic potassium phosphate in about 400 mL of water. Add 540 mL of glycerol and, with vigorous stirring, add 6.0 g of gum arabic (Sigma, Catalog No. G 9752). Stir until dissolved. Dilute to 1000 mL.

Glycine Buffer (0.1 *M*) Dissolve 7.50 g of glycine (Sigma, Catalog No. G 126) and 3.8 g of sodium hydroxide in about 900 mL water. Adjust the pH to 10.8, and dilute to 1000 mL.

> **Note**: Instead of the *Glycine Buffer*, some enzyme preparations may require the use of 0.01 *M* pH 8.0 *Tris Buffer* prepared as directed for *Tris Buffer* under *Proteolytic Activity, Bacterial (PC)*, except to titrate with 1 *N* hydrochloric acid to pH 8.0.

Substrate Emulsion Transfer 15.9 mL of tributyrin (Sigma, Catalog No. T 8626) to a blender, add 50 mL *Emulsification Reagent* and 235 mL water. Blend for 15 min at maximum speed. Equilibrate in the 30° constant temperature bath for at least 15 min before use. Use within 4 h.

Sample Preparation Dissolve an accurately weighed amount of the enzyme preparation in *Glycine Buffer* (or pH 8.0 *Tris Buffer* if specified) so that each milliliter contains between 2000 and 5000 lipase units per milliliter. Accurately dilute a portion of this solution with water to obtain a final solution containing between 0.5 and 1.5 lipase units per milliliter.

Procedure Fill the titrator buret with the *0.05* N *Sodium Hydroxide* solution, and following the manufacturer's instructions, set the temperature to 30° and the pH set point to 7.0. Transfer 15.0 mL of the *Substrate Emulsion* to the titration cell, and add a small stirrer bar. Add 1.0 mL of the diluted *Sample Preparation*, and actuate the titrator. Record the rate of *0.05* N *Sodium Hydroxide* addition. Stop the titration after a constant (linear) rate of addition has been observed for 5 min. Determine the addition rate, in milliliters per minute, from the linear portion of the recording and record this value as R.

Calculation One lipase unit (LU) is defined as the quantity of enzyme that will liberate 1 μmol of butyric acid per min under the conditions of the test.

Calculate the activity of the enzyme preparation by the formula

$$\text{LU/g} = R \times N \times 1000/W,$$

in which R is the addition rate, in milliliters per minute; N is the normality of the *Sodium Hydroxide* solution; 1000 converts mM to μM; and W is the weight, in grams, of the enzyme preparation contained in 1 mL of the diluted *Sample Preparation*.

LIPASE (MICROBIAL) ACTIVITY FOR MEDIUM- AND LONG-CHAIN FATTY ACIDS

Application and Principle

This procedure is used to determine the lipase activity in preparations derived from microbial sources. The assay is based on the measurement of the amount of free fatty acids formed from an olive oil emulsion in the presence of sodium taurocholate over a fixed time interval. This assay is particularly used for measuring lipase activity in foods.

Reagents and Solutions

Gum Arabic Solution Dissolve 110 g of gum arabic (acacia) (Sigma, Catalog No. G-9752, or equivalent) and 12.5 g of analytical-grade calcium chloride ($CaCl_2 \cdot 2H_2O$) in 800 mL of water in a 1000-mL volumetric flask, and dilute to volume with water. Shake or stir for 30 min at room temperature to

dissolve completely. Centrifuge at 4000 × g for 20 min or filter through a Büchner funnel using Celite as a filter aid. Store the supernatant or filtrate at 4°. Divide into single-use, 24-mL aliquots. The solution is stable for 6 months at –20°.

Substrate Emulsion Place 130 mL of olive oil (Sigma, Catalog No. O-1500, or equivalent) and 400 mL of *Gum Arabic Solution* in a mixer bowl, and cool the mixture to 5° to 10° on ice. Emulsify the mixture with a Waring Blender, or equivalent, operated at high speed for 30 min, keeping the temperature below 30° by repeatedly mixing at high speed for 5 min and turning the blender off for 1 min. Check the quality of the emulsion microscopically: 90% of the droplets should have a diameter equal to or less than 3 μm, and the remaining 10% should not exceed 10 μm. The emulsion is stable for 3 days at 4°.

Reference Standard Solution Dissolve an aliquot of Fungi Lipase-International FIP Standard (International Commission on Pharmaceutical Enzymes F.I.P., Center for Standards of the Federation Internationale Pharmaceutique, Harelbekestraat 72, B-9000 Gent, Belgium) in a 1% sodium chloride solution and dilute it to obtain a solution of 2.4 to 3.6 FIP microbial lipase units per milliliter. Prepare this solution fresh.

0.02 N *Sodium Hydroxide Solution* Prepare daily by diluting 10 mL of analytical-grade 1 *N* sodium hydroxide to 500 mL with recently boiled water.

0.5% Sodium Taurocholate Solution Dissolve 0.5 g of sodium taurocholate (DIFCO, Catalog No. 0278-15-8) in 100 mL of water. Prepare this solution fresh.

Sample Preparation Dissolve an accurately weighed amount of the enzyme preparation in a 1% sodium chloride solution, and dilute to obtain a solution of 2.4 to 3.6 FIP microbial lipase units per milliliter. Prepare this solution fresh.

Procedure (**Note**: Assay the Fungi Lipase-International FIP Standard as an internal standard each time.)

Automatic Titration Use an automatic titration device with a 25 mL ± 0.02 mL buret, a pH meter giving a resolution to 0.01, and a reaction vessel with a capacity of 100 mL. Add 24 mL of *Substrate Emulsion*, 9 mL of water, and 2 mL of *0.5% Sodium Taurocholate Solution* to the reaction vessel. Place the reaction vessel in a water bath preheated to 37° ± 0.5° over a hot plate provided with magnetic stirring, and add a magnet to the reaction vessel. Pre-incubate the reaction vessel at 37° ± 0.5° for 10 to 15 min while stirring at about 300 rpm. Immerse a pH-electrode and the tip of the buret into the solution. If desired, gently blow nitrogen gas onto the solution. Adjust the pH of the solution to 7.0 with *0.02* N *Sodium Hydroxide Solution*. Set the automatic buret to zero. Add 5.0 mL of the enzyme solution while simultaneously starting a timer. Maintain the pH at 7.0 by automatic titration. After 10.0 min, abruptly (within 30 s) bring the pH to 9.0 by manually adding additional *0.02* N *Sodium Hydroxide Solution*. Record the volume of *0.02* N *Sodium Hydroxide Solution* consumed as N_1. Run the test with a blank by setting up the titration in the same manner, except after adjusting the pH to 7.0 with *0.02* N *Sodium Hydroxide Solution*, set the automatic buret to zero, and maintain the pH at 7.0 by automatic titration. After 10.0 min, abruptly (within 30 s) bring the pH to 9.0 as before, and then add 5.0 mL of enzyme solution. Because the enzyme lowers the pH, return the pH to 9.0 by adding *0.02* N *Sodium Hydroxide Solution*. Record the volume of *0.02* N *Sodium Hydroxide Solution* consumed as N_2.

Manual Titration Follow the same procedure as with *Automatic Titration*, but keep the pH at 7.0 with *0.02* N *Sodium Hydroxide Solution* from a 25-mL buret, demarked in 0.02-mL units.

Calculation One unit of enzyme activity (FIP Unit) is defined as that quantity of a standard lipase preparation (Fungi Lipase-International FIP Standard) that liberates the equivalent of 1 μmol of fatty acid per minute from the *Substrate Emulsion* under the described assay conditions. The specific activity is expressed in international FIP units per milligram of the *Sample Preparation*.

The use of an enzyme reference standard of known activity, controlled by the Center for Standards of the Commission, eliminates difficulties from interlaboratory differences in quality of reagents such as the *Gum Arabic Solution*, olive oil, or *Substrate Emulsion* or in the set-up of the experiment. The activity (FIP U/mg) using an enzyme reference standard is calculated by the formula

$$(A \times C)/B,$$

in which A is the specific activity, in units/mg, of the test sample (measured); B is the specific activity, in units/mg, of Fungi Lipase-International FIP Standard (measured); and C is the number of FIP units/mg of Fungi Lipase-International FIP Standard as indicated on the container.

One milliliter of the *0.02* N *Sodium Hydroxide Solution* corresponds with the neutralization of 20 μmol of fatty acids. Five milliliters of enzyme solution liberates $(N_1 - N_2)$ mL × 20 μmol of fatty acids over a 10-min time interval. If the enzyme solution contains W mg of enzyme preparation per milliliter, the specific activity, in units/mg, is calculated as follows:

$$[(N_1 - N_2) \times 20]/(10 \times 5 \times W),$$

in which $(N_1 - N_2)$ is the volume, in milliliters, of the *0.02* N *Sodium Hydroxide Solution* used for the titration.

LYSOZYME ACTIVITY[1]

Application and Principle The purpose of this procedure is to determine the lysozyme activity in purified lysozyme preparations derived from animal or microbial sources. The assay is based on the rate of decrease in absorbance at 450 nm, attributed to the lysis of *Micrococcus lysodeikticus* by lysozyme. The decrease in absorbance is measured using a UV/V spectrophotometer equipped to control the sample temperature at 25°.

Note: Ensure that all glassware and supplies are heat sterilized. The work area should be aseptically clean.

[1]Shugar, D. 1952. The measurement of lysozyme activity and the ultra-violet inactivation of lysozyme. Biochimica et Biophysica Acta. 8:302–309.

Any residual lysozyme contamination will adversely affect the results of the assay.

Reagents and Solutions

Sodium Phosphate Buffer Solution Dissolve 10.4 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$) in 500 mL of sterile, deionized water in a 1000-mL volumetric flask, and dilute to volume. Similarly, dissolve 9.465 g of anhydrous dibasic sodium phosphate (Na_2HPO_4) in sterile, deionized water, and dilute to 1000 mL. Mix 815 mL of the monobasic sodium phosphate solution with 185 mL of the dibasic sodium phosphate solution. Adjust the pH of the buffer to 6.2; when checking the pH, use an aliquot of the buffer to prevent contamination of the solution. Adjust the pH by adding more monobasic or dibasic sodium phosphate solution as needed. The buffer solution may be stored under refrigeration for up to 1 month.

Substrate Solution Add 30 to 40 mg of *Micrococcus lysodeikticus* (Sigma M-3770, or equivalent) to 100 mL of *Sodium Phosphate Buffer Solution* in a 250-mL Erlenmeyer flask, tilt gently to mix, and do not shake. Allow the substrate to incubate at 37° for 30 min before using it. The substrate solution is stable for 2 h at room temperature. Zero a spectrophotometer against air, then measure the absorbance of the substrate solution, which should give a reading of 1.7 ± 0.1 at 450 nm.

Note: If the absorbance is significantly lower than 1.7, do not adjust the concentration. Run the analysis, and check the rate of the reaction. The rate of the decrease in absorbance should range between 0.03 and 0.06 units per min.

Standard Preparation Use a commercial reference standard lysozyme of a specified strength from an animal or microbial source in accordance with the origin of the preparation being measured. Measure 50 mg of the reference standard lysozyme into a 50-mL volumetric flask, and dissolve, with stirring, in approximately 25-mL of *Sodium Phosphate Buffer Solution*. Dilute to volume with *Sodium Phosphate Buffer Solution*, and mix thoroughly. If desired, freeze aliquots of this *Standard Preparation* for subsequent assays. Quantitatively transfer 3 mL of the *Standard Preparation* to a 100-mL volumetric flask, and dilute to volume with *Sodium Phosphate Buffer Solution*.

Sample Preparation Measure 50 mg of sample into a 50-mL volumetric flask. Dissolve the sample, with stirring, in approximately 25 mL of *Sodium Phosphate Buffer Solution*. Dilute to volume with *Sodium Phosphate Buffer Solution*, and mix the solution thoroughly. Quantitatively transfer 3 mL of the solution to a 100-mL volumetric flask, and dilute to volume with *Sodium Phosphate Buffer Solution*.

Procedure Conduct the test in a spectrophotometer equipped to maintain a temperature of 25° in the cell compartment. Perform the test in triplicate for the *Standard Preparation* and for the *Sample Preparation*.

Place a 1-cm cell into the spectrophotometer, and adjust the absorbance to zero. Pipet 2.9 mL of *Substrate Solution* into the cell; the initial absorbance of the solution should be 1.7 ± 0.1 at 450 nm (see *Note* above). Pipet 0.1 mL of the *Standard Preparation* into the substrate, and mix well. Record the decrease in absorbance over 3 min, recording the absorbance value approximately every 15 s. The rate of the decrease in absorbance should be linear, and range between 0.03 and 0.06 per min. Repeat the procedure with the *Sample Preparation*.

Calculation One lysozyme unit is defined as the amount of lysozyme that causes a decrease in absorbance of 0.001 per min at 450 nm, 25°, and pH 6.2, using a suspension of *Micrococcus lysodeikticus* as the substrate.

The assay stabilizes over the first min; disregard the first min of readings in the calculation. Determine the average absorbance change per min using only the linear portion of the curve where the rate of change is constant, usually the final 2 min.

Calculate the number of lysozyme units per mg by the equation

$$\text{lysozyme units} = (A_1 - A_2)/(T \times W \times 0.001),$$

in which A_1 is the initial absorbance reading in the straight-line portion of the curve; A_2 is the final absorbance reading in the straight-line portion of the curve; T is the elapsed time, in min, between the initial and final absorbance readings; W is the weight, in mg, of the lysozyme in the volume of *Sample Preparation* used in the *Assay*; and 0.001 is the decrease in absorbance caused by one unit of lysozyme per min.

MALTOGENIC AMYLASE ACTIVITY

Application and Principle This procedure is used to determine maltogenic amylase activity in preparations derived from *Bacillus subtilis* containing a *Bacillus stearothermophilus* amylase gene. The test is based on a 30-min hydrolysis of maltotriose under controlled conditions and measurement of the glucose formed by high-performance liquid chromatography (HPLC).

Reagents and Solutions

Citrate Buffer, 0.1 M Dissolve 5.255 g of citric acid ($C_6H_8O_7 \cdot H_2O$) in about 150 mL of water. Adjust the pH to 5.0 with 1 *N* sodium hydroxide, and dilute to 250 mL.

Substrate Solution Dissolve 1.00 g of maltotriose (Sigma Chemical Co., Catalog No. M 8378) in *Citrate Buffer* in a 50-mL volumetric flask, and dilute to volume with *Citrate Buffer*.

Sodium Chloride Solution, 1 M Dissolve 29.22 g of sodium chloride in water, and dilute to 500 mL.

Amberlite MB-1 Ion Exchange Resin Air dry at room temperature for about 1 week. Protect from contamination.

Glucose Standards Dissolve 1.80 g of anhydrous glucose in water, and dilute to 1000 mL. Transfer 20.0, 50.0, 75.0, and 100.0 mL to separate 100-mL volumetric flasks, and dilute to volume with water. These solutions contain 0.36,

0.9, 1.35, and 1.80 mg of glucose per mL. Using filtered, degassed water as the mobile phase, equilibrate an HPX 87C column, or equivalent, in a high-performance liquid chromatograph equipped with a differential refractometer. Chromatograph 5-μL portions of the glucose standards, and record the chromatograms. Prepare a standard curve of the glucose concentration versus the peak height.

Sample Preparation Prepare a solution of each sample to contain approximately 7.5 Maltogenic Amylase Units (MANU) per mL. Further dilute an aliquot of each sample so that the final dilution contains 1% by volume of the *Sodium Chloride Solution, 1* M and contains between 0.150 and 0.600 MANU per mL.

Procedure Transfer 2.00 mL of each sample to separate test tubes, and equilibrate in the 37° water bath for at least 10 min. At the same time, equilibrate the *Substrate Solution* in the same water bath. At zero time, transfer 2.0 mL of the equilibrated *Substrate Solution* to the first sample tube, mix thoroughly, and return the tube to the 37° bath. Repeat the process for each sample. After exactly 30.0 min, transfer the test tube to a boiling water bath for 15 min, then remove and cool to room temperature. Add approximately 100 mg of *Amberlite MB-1 Ion Exchange Resin* to each tube, place the tubes on the shaker, and mix for at least 15 min. Filter the treated solution through a 0.45-μm filter. Use a separate filter for each sample. Inject a 5-μL portion of each filtered sample into a previously equilibrated high-performance liquid chromatograph equipped with an HPX 87C column (Biorad, or equivalent) and a differential refractometer. Filtered, degassed water is the mobile phase. Record the elution curve.

Calculation One Maltogenic Amylase Unit (MANU) is defined as the amount of enzyme that will cleave maltotriose at a rate of 1 μmol/min under the conditions of the test. From the elution curve of each sample, determine the glucose concentration (G) in the sample from the previously prepared standard curve. Calculate the MANU/g by the equation

$$\text{MANU/g} = G \times 4 \times F/180.1 \times 30 \times W,$$

in which G is the glucose concentration in the test solution; 4 is the total test solution volume; 30 is the reaction time, in min; F is the dilution factor; and W is the sample weight, in g.

MILK-CLOTTING ACTIVITY

Application and Principle This procedure is to be applied to enzyme preparations derived from either animal or microbial sources.

Apparatus

Bottle-Rotating Apparatus Use a suitable assembly, designed to rotate at a rate of 16 to 18 rpm.

Sample Bottles Use 125-mL, squat, round, wide-mouth bottles (such as Scientific Products, Catalog No. B-7545-125).

Substrate Solution Dissolve 60 g of low-heat, nonfat dry milk (such as Galloway West, Peake Grade A) in 500 mL of a solution, adjusted to pH 6.3 if necessary, containing in each mL 2.05 mg of sodium acetate ($NaC_2H_3O_2$) and 1.11 mg of calcium chloride ($CaCl_2$).

Standard Preparation Use a standard-strength rennet, bovine rennet, microbial rennet (*Endothia parasitica*), or microbial rennet (*Mucor* species), as appropriate for the preparation to be assayed. Such standards, which are available from commercial coagulant manufacturers, should be of known activity. Dilute the standard-strength material 1 to 200 with water, and mix. Equilibrate to 300 before use, and prepare no more than 2 h before use.

Sample Preparation Prepare aqueous solutions or dilutions of the sample to produce a final concentration such that the clotting time, as determined in the *Procedure* below, will be within 1 min of that of the *Standard Preparation*. Prepare no more than 1 h before use.

Procedure Transfer 50.0 mL of the *Substrate Solution* into each of four 125-mL *Sample Bottles*. Place the bottles on the *Bottle-Rotating Apparatus*, and suspend the apparatus in a water bath, maintained at 30° ± 0.5°, so that the bottles are at an angle of approximately 20° to 30° to the horizontal. Immerse the bottles so that the water level in the bath is about equal to the substrate level in the bottles. Begin rotating the apparatus at 16 to 18 rpm, then add 1.0 mL of the *Sample Preparation* to each of two bottles, and record the exact time of addition. Add 1.0 mL of the *Standard Preparation* to each of the other two bottles, recording the exact time.

Observe the rotating bottles, and record the exact time of the first evidence of clotting (i.e., when fine granules or flecks adhere to the sides of the bottle). Variations in the response of different lots of the substrate may cause variations in clotting time; therefore, measure the test samples and standards simultaneously on the same substrate. Average the clotting time, in s, of the duplicate samples, recording the time for the *Standard Preparation* as T_S and that for the *Sample Preparation* as T_U.

Calculation Calculate the activity of the enzyme preparation by the equation

$$\text{Milk-clotting units/mL} = 100 \times (T_S/T_U) \times (D_S/D_U),$$

in which 100 is the activity assigned to the *Standard Preparation*, D_S is the dilution factor for the *Standard Preparation*, and D_U is the dilution factor for the *Sample Preparation*.

Note: The dilution factors should be expressed as fractions; for example, a dilution of 1 to 200 would be expressed as $^1/_{200}$.

PANCREATIN ACTIVITY

Application and Principle These procedures are used to determine the primary enzyme activities in pancreatin preparations.

Reference Standards

USP Sodium Taurocholate Reference Standard (**Caution**: Avoid inhaling airborne particles.) Keep container tightly closed. Dry at 105° for 4 h before using.

USP Pancreatin Reference Standard Keep container tightly closed, and store in a refrigerator. Do not open while cold, and do not dry before using.

Amylase Activity

pH 6.8 Phosphate Buffer On the day of use, dissolve 13.6 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 14.2 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 51 mL of the monobasic potassium phosphate solution with 49 mL of the dibasic sodium phosphate solution. If necessary, adjust by the dropwise addition of the appropriate solution to a pH of 6.8

Substrate Solution On the day of use, stir a portion of purified soluble starch equivalent to 2.0 g of dried substance with 10 mL of water, and add this mixture to 160 mL of water, add it to the hot solution, and heat to boiling, with continuous mixing. Cool to room temperature, and add water to make 200 mL.

Standard Preparation Weigh accurately about 20 mg of USP Pancreatin Reference Standard into a suitable mortar. Add about 30 mL of *pH 6.8 Phosphate Buffer*, and triturate for 5 to 10 min. Transfer the mixture with the aid of *pH 6.8 Phosphate Buffer* to a 50-mL volumetric flask, dilute with *pH 6.8 Phosphate Buffer* to volume, and mix. Calculate the activity, in USP Units of amylase activity per mL, of the resulting solution from the declared potency on the label of the Reference Standard.

Assay Preparation For Pancreatin having about the same amylase activity as the USP Pancreatin Reference Standard, weigh accurately about 40 mg of Pancreatin into a suitable mortar.

> **Note**: For Pancreatin having a different amylase activity, weigh accurately the amount necessary to obtain an *Assay Preparation* having amylase activity per mL corresponding approximately to that of the *Standard Preparation*.

Add about 3 mL of *pH 6.8 Phosphate Buffer*, and triturate for 5 to 10 min. Transfer the mixture with the aid of *pH 6.8 Phosphate Buffer* to a 100-mL volumetric flask, dilute with *pH 6.8 Phosphate Buffer* to volume, and mix.

Procedure Prepare four stoppered, 250-mL conical flasks, and mark them *S*, *U*, *BS*, and *BU*. Pipet into each flask 25 mL of *Substrate Solution*, 10 mL of *pH 6.8 Phosphate Buffer*, and 1 mL of sodium chloride solution (11.7 in 1000), insert the stoppers, and mix. Place the flasks in a water bath maintained at 25° ± 0.1°, and allow them to equilibrate. To flasks *BU* and *BS* add 2 mL of 1 *N* hydrochloric acid, mix, and return the flasks to the water bath. To flasks *U* and *BU* add 1.0-mL portions of the *Assay Preparation*, and to flasks *S* and *BS* add 1.0 mL of the *Standard Preparation*. Mix each, and return the flasks to the water bath. After 10 min, accurately timed from the addition of the enzyme, add 2-mL portions of 1 *N* hydrochloric acid to flasks *S* and *U*, and mix. To each flask, with continuous stirring, add 10.0 mL of 1 *N* iodine VS, and immediately add 45 mL of 0.1 *N* sodium hydroxide. Place the flasks in the dark at a temperature between 15° and 25° for 15 min. To each flask add 4 mL of 2 *N* sulfuric acid, and titrate with 0.1 *N* sodium thiosulfate VS to the disappearance of the blue color. Calculate the amylase activity, in USP Units per mg, taken by the formula

$$100(C_S/W_U)(V_{BU} - V_U)/(V_{BS} - V_S),$$

in which C_S is the amylase activity of the *Standard Preparation*, in USP Units per mL; W_U is the amount, in mg, of Pancreatin taken; and V_U, V_S, V_{BU} and V_{BS} are the volumes, in mL, of 0.1 *N* sodium thiosulfate consumed in the titration of the solutions in flasks, *U*, *S*, *BU*, and *BS*, respectively.

Lipase Activity

Gum Arabic Solution Centrifuge a 1:10 solution of gum arabic until clear. Use only the clear solution.

Olive Oil Substrate Combine 165 mL of the *Gum Arabic Solution*, 20 mL of olive oil, and 15 g of crushed ice in the cup of an electric blender. Cool the mixture in an ice bath to 5°, and homogenize at high speed for 15 min, intermittently cooling in an ice bath to prevent the temperature from exceeding 30°. Test for suitability of mixing as follows: Place a drop of the homogenate on a microscope slide and gently press a cover slide in place to spread the liquid. Examine the entire field under high power (43 × magnification objective lens and 5 × magnification ocular), using an eyepiece equipped with a calibrated micrometer. The substrate is satisfactory if 90% of the particles do not exceed 2 μm in diameter and none exceeds 10 μm in diameter.

Buffer Solution Dissolve 60 mg of tris(hydroxymethyl)-aminomethane and 234 mg of sodium chloride in water to make 100 mL.

Sodium Taurocholate Solution Prepare a solution to contain 80.0 mg of USP Sodium Taurocholate Reference Standard in each mL.

Standard Test Dilution Suspend about 200 mg of USP Pancreatin Reference Standard, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 min, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based on the declared potency on the label of the Reference Standard. Maintain the suspension at 4°, and mix before using. For each determination, withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 200 before pipeting the exact volume.

Assay Test Dilution Suspend about 200 mg of the Pancreatin sample, accurately weighed, in about 3 mL of cold water

in a mortar, triturate for 10 min, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based on the estimated potency of the test material. Maintain the suspension at 4°, and mix before using. For each determination, withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Procedure Mix 10.0 mL of *Olive Oil Substrate*, 8.0 mL of *Buffer Solution*, 2.0 mL of *Sodium Taurocholate Solution*, and 9.0 mL of water in a jacketed glass vessel of about 50-mL capacity, the outer chamber of which is connected to a thermostatically controlled water bath. Cover the mixture, and stir continuously with a mechanical stirring device.

With the mixture maintained at a temperature of 37° ± 0.1°, add 0.1 *N* sodium hydroxide, from a microburet inserted through an opening in the cover, to adjust potentiometrically the pH to 9.20, using a calomel-glass electrode system. Add 1.0 mL of *Assay Test Dilution*, and then continue adding the 0.1 *N* sodium hydroxide for 5 min to maintain the pH at 9.0. Determine the volume of 0.1 *N* sodium hydroxide added after each min.

In the same manner, titrate 1.0 mL of *Standard Test Dilution*.

Calculation From the *Standard Test Dilution*, plot the volume of 0.1 *N* sodium hydroxide titrated against time. Using only the points that fall on the straight-line segment of the curve, calculate the mean acidity released per min by the *Assay Test Dilution*. Taking into consideration dilution factors, calculate the lipase activity of the *Standard Test Dilution*, using the lipase activity of the USP Pancreatin Reference Standard stated on the label.

Protease Activity

Casein Substrate Place 1.25 g of finely powdered casein in a 100-mL conical flask containing 5 mL of water, shake to form a suspension, add 10 mL of 0.1 *N* sodium hydroxide, shake for 1 min, add 50 mL of water, and shake for about 1 h to dissolve the casein. Adjust the pH to about 8.0 ± 0.1, using 1 *N* sodium hydroxide or 1 *N* hydrochloric acid. Transfer the solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Use this substrate on the day it is prepared.

Buffer Solution Dissolve 6.8 g of monobasic potassium phosphate and 1.8 g of sodium hydroxide in 950 mL of water in a 1000-mL volumetric flask, adjust to a pH of 7.5 ± 0.2, using 0.2 *N* sodium hydroxide, dilute with water to volume, and mix. Store this solution in a refrigerator.

Trichloroacetic Acid Solution Dissolve 50 g of trichloroacetic acid in 1000 mL of water. Store this solution at room temperature.

Filter Paper Determine the suitability of the filter paper by filtering a 5-mL portion of *Trichloroacetic Acid Solution* through the paper and measuring the absorbance of the filtrate at 280 nm, using an unfiltered portion of the same *Trichloroacetic Acid Solution* as the blank. The absorbance is not more than 0.04. If the absorbance is more than 0.04, the filter paper may be washed repeatedly with *Trichloroacetic Acid Solution* until the absorbance of the filtrate, determined as above, is not more than 0.04.

Standard Test Dilution Add about 100 mg of USP Pancreatin Reference Standard, accurately weighed, to 100.0 mL of *Buffer Solution*, and mix by shaking intermittently at room temperature for about 25 min. Dilute quantitatively with *Buffer Solution* to produce a concentration of about 2.5 USP Units of protease activity per mL, based on the potency declared on the label of the Reference Standard.

Assay Test Dilution Add about 100 mg of USP Pancreatin Reference Standard, accurately weighed, to 100.0 mL of *Buffer Solution*, and mix by shaking intermittently at room temperature for 25 min. Dilute quantitatively with *Buffer Solution* to obtain a dilution that corresponds in activity to the *Standard Test Dilution*.

Procedure Label test tubes in duplicate S_1, S_2, and S_3 for the standard series, and U for the sample. Pipet into tubes S_1 2.0 mL, into S_2 and U 1.5 mL, and into S_3 1.0 mL of *Buffer Solution*. Pipet into tubes S_1 1.0 mL, into S_2 1.5 mL, and into S_3 2.0 mL of the *Standard Test Dilution*. Pipet into tubes U 1.5 mL of the *Assay Test Dilution*. Pipet into one tube each of S_1, S_2, S_3, and U 5.0 mL of *Trichloroacetic Acid Solution*, and mix. Designate these tubes as S_{1B}, S_{2B}, S_{3B}, and U_B, respectively. Prepare a blank by mixing 3 mL of *Buffer Solution* and 5 mL of *Trichloroacetic Acid Solution* in a separate test tube marked *B*. Place all the tubes in a 40° water bath, insert a glass stirring rod into each tube, and allow temperature equilibration. At zero time, add to each tube, at timed intervals, 2.0 mL of the *Casein Substrate*, preheated to the bath temperature, and mix. Accurately timed, 60 min after the addition of the *Casein Substrate*, stop the reaction in tubes S_1, S_2, S_3, and U by adding 5.0 mL of *Trichloroacetic Acid Solution* at the corresponding time intervals, stir, and remove all the tubes from the bath. Allow to stand for 10 min at room temperature to complete protein precipitation, and filter. The filtrates must be free from haze. Determine the absorbances of each filtrate, in a 1-cm cell, at 280 nm, with a suitable spectrophotometer, using the intake from the blank (tube *B*) to set the instrument.

Calculation Correct the absorbance values for the filtrates from tubes S_1, S_2, and S_3 by subtracting the absorbance values for the filtrates from tubes S_{1B}, S_{2B}, and S_{3B}, respectively, and plot the corrected absorbance values against the corresponding volumes of the *Standard Test Dilution* used. From the curve, using the corrected absorbance value ($U - U_B$ for the USP Pancreatin Reference Standard taken), and taking into consideration the dilution factors, calculate the protease activity, in USP Units, of the USP Pancreatin Reference Standard taken by comparison with that of the standard, using the protease activity stated on the label of USP Pancreatin Reference Standard.

PEPSIN ACTIVITY

Application This procedure is to be applied to preparations derived from porcine or other animal stomachs.

Apparatus

Measuring Vessels Use 100-mL conically shaped measuring vessels complying with the following descriptions: (1) diameters not exceeding 1 cm at the bottom; (2) comply in other respects with the water and sediment tube ASTM Standard Method D96-68; (3) graduated from 0 to 0.5 mL in 0.05-mL graduations, from 2 to 3 mL in 0.1-mL graduations, from 3 to 5 mL in 0.2-mL graduations, from 5 to 10 mL in 1-mL graduations, from 10 to 25 mL in 5-mL graduations, and with graduation marks at 50, 75, and 100 mL.

> **Note**: Measuring vessels other than the type described herein may be used if they are of such design and graduation to permit measurement of the residue with equivalent accuracy.

Reagents and Solutions

Hydrochloric Acid Solution Mix 35 mL of 1.0 *N* hydrochloric acid with 385 mL of water.

Substrate Boil one or more hen eggs for 15 min to provide coagulated albumen (Miles, Inc.), and cool rapidly by immersion in cold water. Remove the shell and pellicle and all of the yolk, and at once rub the albumen through a clean, dry No. 40 sieve, rejecting the first portion that passes through the sieve.

Substrate Preparation Place 10 g of the *Substrate* in each of as many 100-mL wide-mouth bottles as needed for the test, and immediately add 35 mL of *Hydrochloric Acid Solution* (all at one time or in portions). By suitable means, thoroughly disintegrate the particles of albumen. Equilibrate to 52° before use in the *Procedure*, below.

Standard Preparation Dissolve 100 mg of USP Pepsin Reference Standard in 150 mL of *Hydrochloric Acid Solution*. Use this solution within 1 h.

Sample Preparation Dissolve 100 mg of the pepsin sample, or an amount of the enzyme preparation that will provide a solution similar to or slightly stronger than the *Standard Preparation*, in 150 mL of *Hydrochloric Acid Solution*. Use this solution within 1 h.

Procedure Pipet 5.0 mL of the *Standard Preparation* into each of two bottles containing the *Substrate Preparation*. To two or more additional substrate bottles add graduated aliquots of the *Sample Preparation* so that one bottle will contain approximately the same amount, and the others will contain successively lesser amounts, of pepsin as is contained in the 5.0 mL of the *Standard Preparation*, using, for example, 5.0, 4.9, and 4.8 mL. When less than 5.0 mL of the *Sample Preparation* is used, add sufficient *Hydrochloric Acid Solution* to make 5.0 mL of combined *Sample Preparation* plus acid added. At once stopper the bottles securely, invert them three times, and heat in a water bath, maintained at 52° ± 0.5°, for 2.5 h, agitating the contents equally every 10 min by inverting the bottles once. Remove the bottles from the bath, and pour the contents of each into separate measuring vessels.

Transfer the undigested albumen that adheres to the sides of the bottles into the respective measuring vessel with the aid of small portions of water until 50 mL has been used for each. Mix the contents of each vessel, allow them to stand for 30 min, and then read for each the volume of undigested albumen. Average the sediment volumes in the two standard vessels, and note which of the sample vessels contains undigested albumen closest to the average for the standards. Finally, record as *v* the volume, in mL, of *Sample Preparation* that produced the undigested albumen closest to the average produced by the *Standard Preparations*.

Calculation One pepsin unit is defined as that quantity of enzyme that digests 3000 times its weight of coagulated egg albumen under the conditions of the assay.

Calculate the activity of the enzyme preparation by the equation

$$\text{Pepsin units/mg} = 3000 \times (S/u) \times (5.0/v),$$

in which S is the weight, in mg, of USP Pepsin Reference Standard used to make the *Standard Preparation*; u is the weight, in mg, of enzyme preparation taken for analysis; and v is as defined in the *Procedure*.

PHOSPHOLIPASE A_2 ACTIVITY

Application and Principle This procedure is used to determine the phospholipase A_2 activity from extracts of porcine pancreatic tissue. The analysis is performed by potentiometric titration.

Apparatus

Automatic Titrator Use a suitable automatic recording titrator equipped with a stirred, thermostated, controlled-atmosphere titration cell (e.g., Radiometer Autotitrator).

Homogenizer Use a suitable homogenizer (e.g., Biomixer; Fisher Scientific, Catalog No. 11-504-2-4, or equivalent).

Constant-Temperature Water Bath Set at 40° ± 0.1°.

Reagents and Solutions

Calcium Chloride Solution (0.3 *M*) Transfer 4.41 g of calcium chloride dihydrate to a 100-mL volumetric flask, dissolve in, and dilute to volume with water.

Sodium Deoxycholate Solution (0.016 *M*) Dissolve 0.67 g of sodium deoxycholate (Sigma Chemical Co., Catalog No. D6750) in 100 mL of water.

Sodium Hydroxide Solution (0.1 *N*) Use a standardized solution.

Substrate Solution Add the yolk of one fresh egg to 100 mL of deionized water and homogenize until a stable emulsion is obtained. Add 5 mL of the *Calcium Chloride Solution*, and mix.

Sample Preparation Dissolve an accurately weighed amount of enzyme preparation in 0.001 *N* hydrochloric acid, and dilute to obtain an enzyme activity of 10 to 80 units of activity per mL.

Procedure Pre-equilibrate the *Substrate Solution*, the *Sodium Deoxycholate Solution*, and about 50 mL of water to 40° in the water bath. Transfer 10 mL of the *Substrate Solution* to the thermostated titration vessel. Add 5 mL of the *Sodium Deoxycholate Solution* and 10 mL of deionized water. Blanket the cell with nitrogen and equilibrate for approximately 5 min. Using the *Automatic Titrator* filled with *0.1* N *Sodium Hydroxide Solution*, adjust the pH of the solution to 8.0 ± 0.05. Monitor the consumption (if any) of sodium hydroxide for 5 min as a blank. Refill the *Automatic Titrator*. Add 0.1 mL of *Sample Solution* containing between 1 and 8 units of activity and start the *Automatic Titrator*. Record the sodium hydroxide consumption for at least 5 min.

Calculation One phospholipase unit is defined as the quantity of enzyme that produces 1 microequivalent of free fatty acid per min under the conditions of the test. Determine the rate, *R*, of titrant consumption during 0 to 3 min of the reaction.

Note: The recorder trace must be linear during the first 3 min of the reaction.

Determine the rate of titrant consumption (if any) during equilibration (blank) (R_B):

$$\text{Units/g} = (R \times N) - (R_B \times N)/W,$$

in which *R* and R_B are the rates of titrant consumption of the sample and blank, respectively, in μL/min; *N* is the normality of the titrant; and *W* is the weight, in g, contained in 0.1 mL of the *Sample Preparation* taken for the test.

PHYTASE ACTIVITY

Application and Principle This procedure is used to determine the activity of enzymes releasing phosphate from phytate. The assay is based on enzymatic hydrolysis of sodium phytate under controlled conditions by measurement of the amount of ortho phosphate released.

Reagents and Solutions

Note: All glassware must be acid washed, rinsed, and scrupulously cleaned to ensure the absence of phosphate.

Acetate Buffer (pH 5.5) Dissolve 1.76 g of 100% acetic acid ($C_2H_4O_2$), 30.02 g of sodium acetate trihydrate ($C_2H_3O_2Na \cdot 3H_2O$), and 0.147 g of calcium chloride dihydrate in about 900 mL of water. Transfer the solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. The pH should be 5.50 ± 0.05.

Substrate Solution Dissolve 8.40 g of sodium phytate decahydrate ($C_6H_6O_{24}P_6Na_{12} \cdot 10H_2O$) (Sigma Chemical Co.) in 900 mL of *Acetate Buffer*. Adjust the pH to 5.50 ± 0.05 at 37.0° ± 0.1° by adding 4 *M* acetic acid. Cool to ambient temperature. Quantitatively transfer the mixture to a 1000-mL volumetric flask, dilute to volume with *Acetate Buffer*, and mix. Prepare fresh daily.

Nitric Acid Solution (27%) While stirring, slowly add 70 mL of 65% nitric acid to 130 mL of water.

Ammonium Heptamolybdate Solution Dissolve 100 g of ammonium heptamolybdate tetrahydrate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in 900 mL of water in a 1000-mL volumetric flask. Add 10 mL of 25% ammonia solution, dilute to volume with water, and mix. This solution is stable for 4 weeks when stored at ambient temperature and shielded from light.

Ammonium Vanadate Solution Dissolve 2.35 g of ammonium monovanadate (NH_4VO_3) in 400 mL of warm (60°) water. While stirring, slowly add 20 mL of *Nitric Acid Solution* (27%). Cool to ambient temperature. Quantitatively transfer the mixture to a 1000-mL volumetric flask, dilute to volume with water, and mix. This solution is stable for 4 weeks when stored at ambient temperature and shielded from light.

Color/Stop Solution While stirring, add 250 mL of *Ammonium Vanadate Solution* to 250 mL of *Ammonium Heptamolybdate Solution*. Slowly add 165 mL of 65% nitric acid. Cool to ambient temperature. Quantitatively transfer the mixture to a 1000-mL volumetric flask, dilute to volume with water, and mix. Prepare fresh daily.

Potassium Dihydrogen Phosphate Solution Dry a sufficient amount of potassium dihydrogen phosphate (KH_2PO_4) in a vacuum oven at 100° to 104° for 2 h. Cool to ambient temperature in a desiccator over dried silica gel.

In duplicate (solutions A and B), weigh approximately 0.245 g of dried potassium dihydrogen phosphate accurately to within 1 mg and dilute with *Acetate Buffer* to 1 L to obtain solutions containing 1.80 mmol/L of potassium dihydrogen phosphate.

Phytase Reference Preparation (Highly concentrated phytase preparation) This preparation can be obtained from Gist-Brocades, Delft, The Netherlands, with an assigned activity (by collaborative assay), or the activity of the reference preparation can be determined according to *Procedure 2*.

Phytase Reference Solutions, Procedure 1 Weigh an amount of *Phytase Reference Preparation* corresponding with 20,000 phytase units accurately to within 1 mg in duplicate in 200-mL volumetric flasks. Dissolve in and dilute to volume with *Acetate Buffer*, and mix. Dilute with *Acetate Buffer* to obtain dilutions containing approximately 0.01, 0.02, 0.04, 0.06, and 0.08 phytase units per 2.0 mL of the final dilution.

Sample Preparation, Procedure 1 Suspend or dissolve and dilute accurately weighed amounts of sample in *Acetate Buffer* so that 2.0 mL of the final dilution will contain between 0.02 and 0.08 phytase units.

Sample Preparation, Procedure 2 In duplicate, accurately weigh amounts of *Phytase Reference Preparation* and dissolve and dilute in *Acetate Buffer* to obtain dilutions containing 0.06 ± 0.006 phytase units per 2.0 mL of the final dilution.

Procedures

Procedure 1 (Determination of the phytase activity) Transfer 2.00 mL of the *Sample Preparation, Procedure 1,* and the *Phytase Reference Solutions, Procedure 1,* into separate 20- × 150-mm glass test tubes. Using a stopwatch and starting at time equals zero, in the order of the series and within regular time intervals, place the tubes into a 37.0° ± 0.1° water bath and allow their contents to equilibrate for 5 min. At time equals 5 min, in the same order of the series and with the same time intervals, add 4.0 mL of *Substrate Solution* (previously equilibrated to 37.00 ± 0.10) to each test tube. Mix, and replace in the 37.0° ± 0.1° water bath. At time equals 65 min, in the same order and within the same time intervals, terminate the incubation by adding 4.0 mL of *Color/Stop Solution*. Mix, and cool to ambient temperature.

Prepare blanks by transferring 2.00 mL of the *Sample Preparation, Procedure 1,* and the *Phytase Reference Solutions, Procedure 1,* into separate 20- × 150-mm glass test tubes. Using a stopwatch and starting at time equals zero, in the order of the series and within regular time intervals, place the tubes into a 37.0° ± 0.1° water bath and allow them to equilibrate for 5 min. At time equal 5 min, in the same order of the series and within the same time intervals, add 4.0 mL of *Color/Stop Solution*. Mix, and cool to ambient temperature. Next add 4.00 mL of *Substrate Solution* to the blank tubes, and mix.

Centrifuge all test tubes for 5 min at 3000 × g. Determine the absorbance of each solution at 415 nm in a 1-cm pathlength cell with a suitable spectrophotometer, using water to zero the instrument.

Procedure 2 (Determination of the phytase activity of the *Phytase Reference Preparation*) Transfer 2.00 mL of *Sample Preparation, Procedure 2,* and 2.00 mL (three times from *Potassium Dihydrogen Phosphate Solution A* and two times from *B*) of *Potassium Dihydrogen Phosphate Solutions* into separate 20- × 150-mm glass test tubes. Using a stopwatch and starting at time equals zero, in the order of the series and within regular time intervals, place the tubes into a 37.0° ± 0.1° water bath and allow their contents to equilibrate for 5 min. At time equals 5 min, in the same order of the series and within the same time intervals, add 4.0 mL of *Substrate Solution* (previously equilibrated to 37.0° ± 0.1°) to the test tubes. Mix, and replace in the 37.0° ± 0.1° water bath. At time equals 35 min, in the same order and within the same time intervals, terminate the incubation by adding 4.0 mL of *Color/Stop Solution*. Mix, and cool to ambient temperature.

Prepare blanks by transferring 2.00 mL of *Sample Preparation, Procedure 2,* into separate 20- × 150-mm glass test tubes. Prepare *Reagent Blanks* by transferring 2.00 mL of water into a series of five separate 20- × 150-mm glass test tubes. Add 4.0 mL of *Color/Stop Solution* to all blank tubes and mix. Next add 4.0 mL of *Substrate Solution*, and mix. Determine the absorbance of each solution at 415 nm in a 1-cm pathlength cell with a suitable spectrophotometer, using water to zero the instrument.

Calculations

Calculation, Procedure 1 One Phytase (fytase) unit (FTU) is the amount of enzyme that liberates inorganic phosphate at 1 μmol/min from sodium phytate 0.0051 mol/L at 37.00 at pH 5.50 under the conditions of the test. Calculate the corrected absorbance (sample minus blank) for each *Sample Preparation* and *Phytase Reference Solution*. Plot the accurately calculated phytase activity (FTU per 2 mL) of each *Phytase Reference Solution* against the corresponding absorbance. From the curve, determine the phytase activity in each *Sample Preparation* (FTU per 2 mL):

$$\text{Activity (FTU/g)} = (\text{FTU per 2 mL} \times \text{dilution})/\text{sample weight (g)}.$$

Calculation, Procedure 2 Calculate the corrected absorbances A_R for each *Sample Preparation* (absorbance *Phytase Reference Solution* minus corresponding absorbance blank) and for each *Potassium Dihydrogen Phosphate Solution*, A_p (absorbance *Potassium Dihydrogen Phosphate Solution* minus average absorbance reagent blank). Calculate *C*, the phosphate concentration of each *Potassium Dihydrogen Phosphate Solution*:

$$(W \times 1000 \times 2)/MW = C \text{ (mmol/2 mL)}.$$

Calculate the absorbances *D* for each *Potassium Dihydrogen Phosphate Solution* after correction for the amount of potassium dihydrogen phosphate weighed:

$$A_p/C = D\text{(absorbance units/mmol of phosphate per 2 mL)}.$$

Calculate the average of results *D*, giving *E* (maximum allowable difference, 5%).

Calculate the activity for each *Phytase Reference Preparation*:

$$(A_R \times f)/(30 \times R \times E) = \text{FTU/g},$$

in which A_R equals the corrected absorbance of the *Phytase Standard Solution*; *f* equals the total dilution factor of the reference preparation; 30 equals the incubation time, in min; *R* equals sample weight, in g; *E* equals average of *D* factors; *W* equals the weight of potassium dihydrogen phosphate, in g; and *MW* equals the molecular weight of potassium dihydrogen phosphate, 136.09 (g/mol).

PLANT PROTEOLYTIC ACTIVITY

Application and Principle This procedure is used to determine the proteolytic activity of papain, ficin, and bromelain. The assay is based on a 60-min proteolytic hydrolysis of a casein substrate at pH 6.0 and 40°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration; solubilized casein is then measured spectrophotometrically.

Reagents and Solutions

Sodium Phosphate Solution (0.05 *M*) Transfer 7.1 g of anhydrous dibasic sodium phosphate into a 1000-mL volumetric flask, dissolve in about 500 mL of water, dilute to volume with water, and mix. Add 1 drop of toluene as a preservative.

Citric Acid Solution (0.05 *M*) Transfer 10.5 g of citric acid monohydrate into a 1000-mL volumetric flask, dissolve in about 500 mL of water, dilute to volume with water, and mix. Add 1 drop of toluene as a preservative.

Phosphate–Cysteine–EDTA Buffer Solution Dissolve 7.1 g of anhydrous dibasic sodium phosphate in about 800 mL of water, and then dissolve in this solution 14.0 g of disodium EDTA dihydrate and 6.1 g of cysteine hydrochloride monohydrate.

Adjust to pH 6.0 ± 0.1 with 1 *N* hydrochloric acid or 1 *N* sodium hydroxide, then transfer into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Trichloroacetic Acid Solution Dissolve 30 g of trichloroacetic acid in 100 mL of water.

Casein Substrate Solution Disperse 1 g (moisture-free basis) of Hammarsten-grade casein (United States Biochemical Corp., Catalog No. 12840, or equivalent) in 50 mL of *Sodium Phosphate Solution*, and heat for 30 min in a boiling water bath, with occasional agitation. Cool to room temperature, and with rapid and continuous agitation, adjust to pH 6.0 ± 0.1 by the addition of *Citric Acid Solution*.

Note: Rapid and continuous agitation during the addition prevents casein precipitation.

Quantitatively transfer the mixture into a 100-mL volumetric flask, dilute to volume with water, and mix.

Stock Standard Solution Transfer 100.0 mg of USP Papain Reference Standard into a 100-mL volumetric flask, dissolve, and dilute to volume with *Phosphate–Cysteine–EDTA Buffer Solution*, and mix.

Diluted Standard Solutions Pipet 2, 3, 4, 5, 6, and 7 mL of *Stock Standard Solution* into a series of 100-mL volumetric flasks, dilute each to volume with *Phosphate–Cysteine–EDTA Buffer Solution*, and mix by inversion.

Test Solution Prepare a solution from the enzyme preparation so that 2 mL of the final dilution will give a ΔA in the *Procedure* between 0.2 and 0.5. Weigh the sample accurately, transfer it quantitatively to a glass mortar, and triturate with *Phosphate–Cysteine–EDTA Buffer Solution*. Transfer the mixture quantitatively into a volumetric flask of appropriate size, dilute to volume with *Phosphate–Cysteine–EDTA Buffer Solution*, and mix.

Procedure Pipet 5 mL of *Casein Substrate Solution* into each of a series of 25- × 150-mm test tubes, allowing three tubes for the enzyme unknown, six for a papain standard curve, and nine for enzyme blanks. Equilibrate the tubes for 15 min in a water bath maintained at 40° ± 0.1°. Starting the stopwatch at zero time, rapidly pipet 2 mL of each of the *Diluted Standard Solutions*, and 2-mL portions of the *Test Solution*, into the equilibrated substrate. Mix each by swirling, stopper, and place the tubes back in the water bath. After 60.0 min, add 3 mL of *Trichloroacetic Acid Solution* to each tube. Immediately mix each tube by swirling.

Prepare enzyme blanks containing 5.0 mL of *Casein Substrate Solution*, 3.0 mL of *Trichloroacetic Acid Solution*, and 2.0 mL of one of the appropriate *Diluted Standard Solutions* or the *Test Solution*.

Return all tubes to the water bath, and heat for 30.0 min, allowing the precipitated protein to coagulate completely. Filter each mixture through Whatman No. 42, or equivalent, filter paper, discarding the first 3 mL of filtrate. The subsequent filtrate must be perfectly clear. Determine the absorbance of each filtrate in a 1-cm cell at 280 nm, with a suitable spectrophotometer, against its respective blank.

Calculation One papain unit (PU) is defined in this assay as that quantity of enzyme that liberates the equivalent of 1 μg of tyrosine per h under the conditions of the assay.

Prepare a standard curve by plotting the absorbances of filtrates from the *Diluted Standard Solutions* against the corresponding enzyme concentrations, in mg/mL. By interpolation from the standard curve, obtain the equivalent concentration of the filtrate from the *Test Solution*.

Calculate the activity of the enzyme preparation taken for analysis as follows:

$$\text{PU/mg} = (A \times C \times 10)/W,$$

in which A is the activity of USP Papain Reference Standard, in PU per mg; C is the concentration, in mg/mL, of Reference Standard from the standard curve, equivalent to the enzyme unknown; 10 is the total volume, in mL, of the final incubation mixture; and W is the weight, in mg, of original enzyme preparation in the 2-mL aliquot of *Test Solution* added to the incubation mixture.

PROTEOLYTIC ACTIVITY, BACTERIAL (PC)

Application and Principle This procedure is used to determine protease activity, expressed as PC units, of preparations derived from *Bacillus subtilis* var. and *Bacillus licheniformis* var. The assay is based on a 30-min proteolytic hydrolysis of casein at 37° and pH 7.0. Unhydrolyzed casein is removed by filtration, and the solubilized casein is determined spectrophotometrically.

Reagents and Solutions

Casein Use Hammarsten-grade casein (United States Biochemical Corp., Catalog No. 12840, or equivalent).

Tris Buffer (pH 7.0) Dissolve 12.1 g of enzyme-grade (or equivalent) tris(hydroxymethyl)aminomethane in 800 mL of water, and titrate with 1 *N* hydrochloric acid to pH 7.0. Transfer into a 1000-mL volumetric flask, dilute to volume with water, and mix.

TCA Solution Dissolve 18 g of trichloroacetic acid and 19 g of sodium acetate trihydrate in 800 mL of water in a 1000-mL volumetric flask, add 20 mL of glacial acetic acid, dilute to volume with water, and mix.

Substrate Solution Dissolve 6.05 g of enzyme-grade tris(hydroxymethyl)aminomethane in 500 mL of water, add 8 mL of 1 *N* hydrochloric acid, and mix. Dissolve 7 g of *Casein*

in this solution, and heat for 30 min in a boiling water bath, stirring occasionally.

Cool to room temperature, and adjust to pH 7.0 with 0.2 *N* hydrochloric acid, adding the acid slowly, with vigorous stirring, to prevent precipitation of the casein. Transfer the mixture into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Sample Preparation Using *Tris Buffer*, prepare a solution of the sample enzyme preparation so that 2 mL of the final dilution will contain between 10 and 44 bacterial protease units.

Procedure Pipet 10.0 mL of the *Substrate Solution* into each of a series of 25- × 150-mm test tubes, allowing one tube for each enzyme test, one tube for each enzyme blank, and one tube for a substrate blank. Equilibrate the tubes for 15 min in a water bath maintained at 37° ± 0.1°.

Starting the stopwatch at zero time, rapidly pipet 2.0 mL of the *Sample Preparation* into the equilibrated substrate. Mix, and replace the tubes in the water bath. Add 2 mL of *Tris Buffer* (instead of the *Sample Preparation*) to the substrate blank. After exactly 30 min, add 10 mL of *TCA Solution* to each enzyme incubation and to the substrate blank to stop the reaction. Heat the tubes in the water bath for an additional 30 min to allow the protein to coagulate completely.

At the end of the second heating period, shake each tube vigorously, and filter through 11-cm Whatman No. 42, or equivalent, filter paper, discarding the first 3 mL of filtrate.

Note: The filtrate must be perfectly clear.

Determine the absorbance of each sample filtrate in a 1-cm cell, at 275 nm, with a suitable spectrophotometer, using the filtrate from the substrate blank to set the instrument at zero. Correct each reading by subtracting the appropriate enzyme blank reading, and record the value so obtained as A_U.

Standard Curve Transfer 100.0 mg of L-tyrosine, chromatographic-grade or equivalent (Aldrich Chemical Co.), previously dried to constant weight, to a 1000-mL volumetric flask. Dissolve in 60 mL of 0.1 *N* hydrochloric acid. When completely dissolved, dilute the solution to volume with water, and mix thoroughly. This solution contains 100 µg of tyrosine in 1.0 mL. Prepare three more dilutions from this stock solution to contain 75.0, 50.0, and 25.0 µg of tyrosine per mL. Determine the absorbance of the four solutions at 275 nm in a 1-cm cell on a suitable spectrophotometer versus 0.006 *N* hydrochloric acid. Prepare a plot of absorbance versus tyrosine concentration.

Calculation One bacterial protease unit (PC) is defined as that quantity of enzyme that produces the equivalent of 1.5 µg/mL of L-tyrosine per min under the conditions of the assay.

From the *Standard Curve*, and by interpolation, determine the absorbance of a solution having a tyrosine concentration of 60 µg/mL. A figure close to 0.0115 should be obtained. Divide the interpolated value by 40 to obtain the absorbance equivalent to that of a solution having a tyrosine concentration of 1.5 µg/mL, and record the value thus derived as A_S.

Calculate the activity of the sample enzyme preparation by the equation

$$PC/g = (A_U/A_S) \times (22/30W),$$

in which 22 is the final volume, in mL, of the reaction mixture; 30 is the time, in min, of the reaction; and W is the weight, in g, of the original sample taken.

PROTEOLYTIC ACTIVITY, FUNGAL (HUT)

Application and Principle This procedure is used to determine the proteolytic activity, expressed as hemoglobin units on the tyrosine basis (HUT), of preparations derived from *Aspergillus oryzae* var. and *Aspergillus niger* var., and it may be used to determine the activity of other proteases at pH 4.7. The test is based on the 30-min enzymatic hydrolysis of a hemoglobin substrate at pH 4.7 and 40°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration. The quantity of solubilized hemoglobin in the filtrate is determined spectrophotometrically.

Reagents and Solutions

Hemoglobin Use Hemoglobin Substrate Powder (Sigma Chemical Co., Catalog No. H2625) or a similar high-grade material that is completely soluble in water.

Acetate Buffer Solution Dissolve 136 g of sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$) in sufficient water to make 500 mL. Mix 25.0 mL of this solution with 50.0 mL of 1 *M* acetic acid, dilute to 1000 mL with water, and mix. The pH of this solution should be 4.7 ± 0.02.

Substrate Solution Transfer 4.0 g of the *Hemoglobin* into a 250-mL beaker, add 100 mL of water, and stir for 10 min to dissolve. Immerse the electrodes of a pH meter in the solution, and while stirring continuously, adjust the pH to 1.7 by adding 0.3 *N* hydrochloric acid. After 10 min, adjust the pH to 4.7 by adding 0.5 *M* sodium acetate. Transfer the solution into a 200-mL volumetric flask, dilute to volume with water, and mix. This solution is stable for about 5 days when refrigerated.

Trichloroacetic Acid Solution Dissolve 140 g of trichloroacetic acid in about 750 mL of water. Transfer the solution to a 1000-mL volumetric flask, dilute to volume with water, and mix thoroughly.

Sample Preparation Dissolve an amount of the sample in the *Acetate Buffer Solution* to produce a solution containing, in each mL, between 9 and 22 HUT. (Such a concentration will produce an absorbance reading, in the procedure below, within the preferred range of 0.2 to 0.5.)

Procedure Pipet 10.0 mL of the *Substrate Solution* into each of a series of 25- × 150-mm test tubes: one for each enzyme test and one for the substrate blank. Heat the tubes in a water bath at 40° for about 5 min. To each tube, except the substrate blank, add 2.0 mL of the *Sample Preparation*, and begin timing the reaction at the moment the solution is

added; add 2.0 mL of the *Acetate Buffer Solution* to the substrate blank tube. Close the tubes with No. 4 rubber stoppers, and tap each tube gently for 30 s against the palm of the hand to mix. Heat each tube in a water bath at 40° for exactly 30 min, and then rapidly pipet 10.0 mL of the *Trichloroacetic Acid Solution* into each tube. Shake each tube vigorously against the stopper for about 40 s, and then allow to cool to room temperature for 1 h, shaking each tube against the stopper at 10- to 12-min intervals during this period. Prepare enzyme blanks as follows: Heat, in separate tubes, 10.0 mL of the *Substrate Solution* and about 5 mL of the *Sample Preparation* in the water bath for 30 min, then add 10.0 mL of the *Trichloroacetic Acid Solution* to the *Substrate Solution*, shake well for 40 s, and to this mixture add 2.0 mL of the preheated *Sample Preparation*. Shake again, and cool at room temperature for 1 h, shaking at 10- to 12-min intervals.

At the end of 1 h, shake each tube vigorously, and filter through 11-cm Whatman No. 42, or equivalent, filter paper, refiltering the first half of the filtrate through the same paper. Determine the absorbance of each filtrate in a 1-cm cell, at 275 nm, with a suitable spectrophotometer, using the filtrate from the substrate blank to set the instrument to zero. Correct each reading by subtracting the appropriate enzyme blank reading, and record the value so obtained as A_U.

Note: If a corrected absorbance reading between 0.2 and 0.5 is not obtained, repeat the test using more or less of the enzyme preparation as necessary.

Standard Curve Transfer 100.0 mg of L-tyrosine, chromatographic-grade, or equivalent (Aldrich Chemical Co.), previously dried to constant weight, to a 1000-mL volumetric flask. Dissolve in 60 mL of 0.1 *N* hydrochloric acid. When the L-tyrosene is completely dissolved, dilute the solution to volume with water, and mix thoroughly. This solution contains 100 µg of tyrosine in 1.0 mL. Prepare three more dilutions from this stock solution to contain 75.0, 50.0, and 25.0 µg of tyrosine per mL. Determine the absorbance of the four solutions at 275 nm in a 1-cm cell on a suitable spectrophotometer versus 0.006 *N* hydrochloric acid. Prepare a plot of absorbance versus tyrosine concentration. Determine the slope of the curve in terms of absorbance per µg of tyrosine. Multiply this value by 1.10, and record it as A_S. A value of approximately 0.0084 should be obtained.

Calculation One HUT unit of proteolytic (protease) activity is defined as that amount of enzyme that produces, in 1 min under the specified conditions, a hydrolysate whose absorbance at 275 nm is the same as that of a solution containing 1.10 µg/mL of tyrosine in 0.006 *N* hydrochloric acid.

Calculate the HUT per g of the original enzyme preparation by the equation

$$\text{HUT/g} = (A_U/A_S) \times (22/30W),$$

in which 22 is the final volume of the test solution; 30 is the reaction time, in min; and W is the weight, in g, of the original sample taken.

Note: The value for A_S, under carefully controlled and standardized conditions, is 0.0084; this value may be used for routine work in lieu of the value obtained from the standard curve, but the exact value calculated from the standard curve should be used for more accurate results and in cases of doubt.

PROTEOLYTIC ACTIVITY, FUNGAL (SAP)

Application and Principle This procedure is used to determine proteolytic activity, expressed in spectrophotometric acid protease units (SAP), of preparations derived from *Aspergillus niger* var. and *Aspergillus oryzae* var. The test is based on a 30-min enzymatic hydrolysis of a Hammarsten Casein Substrate at pH 3.0 and at 37°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration. The quantity of solubilized casein in the filtrate is determined spectrophotometrically.

Reagents and Solutions

Casein Use Hammarsten-grade casein (United States Biochemical Corp., Catalog No. 12840, or equivalent).

Glycine–Hydrochloric Acid Buffer (0.05 *M*) Dissolve 3.75 g of glycine in about 800 mL of water. Add 1 *N* hydrochloric acid until the solution is pH 3.0, determined with a pH meter. Quantitatively transfer the solution to a 1000-mL volumetric flask, dilute to volume with water, and mix.

TCA Solution Dissolve 18.0 g of trichloroacetic acid and 11.45 g of anhydrous sodium acetate in about 800 mL of water, and add 21.0 mL of glacial acetic acid. Quantitatively transfer the solution to a 1000-mL volumetric flask, dilute to volume with water, and mix.

Substrate Solution Pipet 8 mL of 1 *N* hydrochloric acid into about 500 mL of water, and with continuous agitation, disperse 7.0 g (moisture-free basis) of *Casein* into this solution. Heat for 30 min in a boiling water bath, stirring occasionally, and cool to room temperature. Dissolve 3.75 g of glycine in the solution, and using a pH meter, adjust to pH 3.0 with 0.1 *N* hydrochloric acid. Quantitatively transfer the solution to a 1000-mL volumetric flask, dilute to volume with water, and mix.

Sample Preparation Using *Glycine–Hydrochloric Acid Buffer*, prepare a solution of the sample enzyme preparation so that 2 mL of the final dilution will give a corrected absorbance of enzyme incubation filtrate at 275 nm (ΔA, as defined in the *Procedure*) between 0.200 and 0.500. Weigh the enzyme preparation, quantitatively transfer it to a glass mortar, and triturate with *Glycine–Hydrochloric Acid Buffer*. Quantitatively transfer the mixture to an appropriately sized volumetric flask, dilute to volume with *Glycine–Hydrochloric Acid Buffer*, and mix.

Procedure Pipet 10.0 mL of *Substrate Solution* into each of a series of 25- × 150-mm test tubes, allowing at least two tubes for each sample, one for each enzyme blank, and one

for a substrate blank. Stopper the tubes, and equilibrate them for 15 min in a water bath maintained at 37° ± 0.1°.

At zero time, start the stopwatch, and rapidly pipet 2.0 mL of the *Sample Preparation* into the equilibrated substrate. Mix by swirling, and replace the tubes in the water bath.

Note: Keep the tubes stoppered during incubation.

Add 2 mL of *Glycine–Hydrochloric Acid Buffer* (instead of the *Sample Preparation*) to the substrate blank. After exactly 30 min, add 10 mL of *TCA Solution* to each enzyme incubation and to the substrate blank to stop the reaction. In the following order, prepare an enzyme blank containing 10 mL of *Substrate Solution*, 10 mL of *TCA Solution*, and 2 mL of the *Sample Preparation*. Heat all tubes in the water bath for 30 min, allowing the precipitated protein to coagulate completely.

At the end of the second heating period, cool the tubes in an ice bath for 5 min, and filter through Whatman No. 42 filter paper, or equivalent. The filtrates must be perfectly clear. Determine the absorbance of each filtrate in a 1-cm cell at 275 nm with a suitable spectrophotometer, against the substrate blank. Correct each absorbance by subtracting the absorbance of the respective enzyme blank.

Standard Curve Transfer 181.2 mg of L-tyrosine, chromatographic-grade or equivalent (Sigma Chemical Co.), previously dried to constant weight, to a 1000-mL volumetric flask. Dissolve in 60 mL of 0.1 *N* hydrochloric acid. When the L-tyrosine is completely dissolved, dilute the solution to volume with water, and mix thoroughly. This solution contains 1.00 μmol of tyrosine per 1.0 mL. Prepare dilutions from this stock solution to contain 0.10, 0.20, 0.30, 0.40, and 0.50 μmol/mL. Determine against a water blank the absorbance of each dilution in a 1-cm cell at 275 nm. Prepare a plot of absorbance versus μmol of tyrosine per mL. A straight line must be obtained. Determine the slope and intercept for use in the *Calculation* below. A value close to 1.38 should be obtained. The slope and intercept may be calculated by the least squares method as follows:

$$\text{Slope} = [n\Sigma\ (MA) - \Sigma\ (M)\ \Sigma(A)]/[n\Sigma\ (M^2) - (\Sigma M)^2],$$

$$\text{Intercept} = [\Sigma(A)\ \Sigma(M^2) - \Sigma\ (M)\ \Sigma(MA)]/[n\Sigma\ (M^2) - (\Sigma M)^2],$$

in which n is the number of points on the standard curve, M is the μmol of tyrosine per mL for each point on the standard curve, and A is the absorbance of the sample.

Calculation One spectrophotometric acid protease unit is that activity that will liberate 1 μmol of tyrosine per min under the conditions specified. The activity is expressed as follows:

$$\text{SAP/g} = (\Delta A - I) \times 22/(S \times 30 \times W),$$

in which ΔA is the corrected absorbance of the enzyme incubation filtrate; I is the intercept of the *Standard Curve*; 22 is the final volume of the incubation mixture, in mL; S is the slope of the *Standard Curve*; 30 is the incubation time, in min; and W is the weight, in g, of the enzyme sample contained in the 2.0-mL aliquot of *Sample Preparation* added to the incubation mixture in the *Procedure*.

PULLULANASE ACTIVITY

Application and Principle This procedure is used to determine pullulanase activity derived from *Bacillus acidopullulyticus*. The method is based on measuring the increase in reducing sugars formed by a 30-min hydrolysis of pullulan at 40° and pH 5.0. The increase in reducing sugars is measured spectrophotometrically at 520 nm using a modified Nelson–Somogyi procedure.

Reagents and Solutions

Citrate Buffer (pH 5.0) Dissolve 10.5 g of citric acid monohydrate in 950 mL of water, adjust the pH to 5.0 ± 0.05 using 5 *N* sodium hydroxide, and dilute to 1000 mL.

Nelson's Color Reagent Dissolve 25.0 g of ammonium molybdate tetrahydrate in 300 mL of water. Carefully add 20.0 mL of concentrated sulfuric acid while stirring. Dissolve 3.0 g of sodium arsenate heptahydrate in 25 mL of water. Slowly add this solution to the ammonium molybdate solution with stirring. Dilute this solution to 500 mL with water.

Somogyi's Copper Reagent Dissolve 14.0 g of anhydrous dibasic sodium phosphate and 20.0 g of potassium sodium tartrate tetrahydrate into 250 mL of water. Add 60.0 g of 1 *M* sodium hydroxide solution. Dissolve 4.0 g of cupric sulfate pentahydrate into 25 mL of water. Add this solution to the tartrate solution. Add 90.0 g of anhydrous sodium sulfate while stirring. Dilute the final solution to 500 mL.

Glucose Standards Dissolve 800 mg of previously dried anhydrous D-glucose in 100 mL of the *Citrate Buffer*. Prepare glucose standards containing 16, 40, 80, and 120 μg/mL of glucose.

Pullulan Substrate Dissolve 150 mg of pullulan (Sigma Chemical Co., Catalog No. P-4516, or equivalent) in 49.80 g of the *Citrate Buffer*. Prepare daily.

Sample Preparation Dissolve an accurately weighed amount of the enzyme preparation in *Citrate Buffer* and dilute in *Citrate Buffer* to obtain an enzyme activity of 0.01 to 0.03 activity units per mL.

Procedure Transfer 1.0-mL aliquots of *Pullulan Substrate* to separate 15- × 150-mm test tubes. Insert a one-hole stopper in each tube, and equilibrate for 15 min in a 40° ± 0.1° water bath. At time equals zero and at 30-s intervals, add 1 mL of the respective samples, and mix. Exactly 30.0 min after addition of the samples, add 2.0 mL of *Somogyi's Copper Reagent* to each tube to terminate the reaction. Mix thoroughly, and allow the tubes to come to room temperature. To obtain sample blanks, add the *Somogyi's Copper Reagent* to the sample before adding the substrate.

Prepare a standard curve by adding 2.0 mL of each glucose standard and 2.0 mL of *Somogyi's Copper Reagent* to a test tube, and mix. The blanks and *Glucose Standards* should not be incubated at 40°.

Loosely stopper samples, blanks, and *Glucose Standards* containing *Somogyi's Copper Reagent*, and place them in a vigorously boiling water bath for exactly 25.0 min. Cool in an ice-water bath for approximately 1 min.

Add 2.00 mL of *Nelson's Color Reagent* to each tube, and mix thoroughly to dissolve any red precipitate that might be present. Let the solutions stand for 5 min. Add 2.0 mL of water to each tube, and mix.

Measure the absorbance of all solutions at 520 nm, using water as the reference. Mix the contents of each tube before transferring them to the cuvette.

Calculations One pullulanase unit (PUN) is the amount of activity that under the conditions of the test, will liberate reducing sugars equivalent to 1 μmol of glucose per min. Determine the linear regression line for absorbance versus two times the glucose concentration (μg/mL) in the standards. Use the slope, *I*, in the following equation to determine the activity in the enzyme preparation:

$$\text{PUN/g} = (A_S - A_B)/I \times W \times 180 \times 30,$$

in which A_S is the absorbance of the sample; A_B is the absorbance of the blank; *W* is the weight, in g, of the enzyme preparation contained in the 1.0 mL of *Sample Preparation* taken for analysis; 180 is the molecular weight of glucose; and 30 is the incubation time, in min.

TRANSGLUTAMINASE ACTIVITY
(Glutaminyl-peptide γ-Glutaminyltransferase)

Application and Principle This procedure is used to determine transglutaminase activity in preparations derived from *Streptoverticillium mobaraense* var. The assay is based on the enzymatic formation of a glutamic acid γ-hydroxamate in a glutaminyl residue in the substrate peptide with another substrate, hydroxylamine. The amount of the glutamic acid γ-hydroxamate formed as a red complex with ferric ion in acidic conditions at 37° is measured spectrophotometrically.

Reagents and Solutions

Substrate Solution Transfer 12.110 g of Tris[tri(hydroxymethyl)aminomethane], 3.475 g of hydroxylamine hydrochloride, 1.624 g of glutathione, and 5.060 g of carbobenzyloxyglutaminylglycine into a 500-mL beaker. Add 350 mL of water, and using a magnetic stirrer, mix well. Adjust the pH to 6.0 with appropriate concentrations (usually 1 *N* and 6 *N*) of hydrochloric acid. Quantitatively transfer this mixture into a 500-mL volumetric flask, and dilute to volume with water.

Stopping Solution Prepare a 3 *N* hydrochloric acid solution by diluting concentrated hydrochloric acid (ca. 36%) four-fold with water. Make a 12% trichloroacetic acid (TCA) solution by transferring 12.0 g of TCA into a 100-mL volumetric flask, adding water to dissolve the TCA, and diluting to volume with water. Prepare a 5% solution of ferric chloride ($FeCl_3$) in 0.1 *N* hydrochloric acid by transferring 5.0 g of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) into a 100-mL volumetric flask, adding 0.1 *N* hydrochloric acid to dissolve the ferric chloride, and diluting to volume with 0.1 *N* hydrochloric acid. On the day of use, combine all three solutions (3 *N* hydrochloric acid, 12% TCA, and 5% ferric chloride) in equal volumes in a beaker, and using a magnetic stirrer, mix well.

0.2 M *Tris-HCl Buffer* (pH 6.0) Transfer 18.11 g of Tris-[tri(hydroxymethyl)aminomethane] into a 500-mL beaker. Add 350 mL of water, and using a magnetic stirrer, mix well. Adjust the pH to 6.0 with appropriate concentrations (usually 1 *N* and 6 *N*) of hydrochloric acid. Quantitatively transfer this mixture into a 500-mL volumetric flask, and dilute to volume with water.

Sample Solution Place 100 mg of sample, accurately weighed, into a 100-mL beaker, and add about 45 mL of *0.2* M *Tris-HCl Buffer*. Using a magnetic stirrer, mix well at room temperature for 30 min. Quantitatively transfer the mixture into a 50-mL volumetric flask, and dilute to volume with *0.2* M *Tris-HCl Buffer*.

Procedure

Calibration Curve Transfer 64.8 mg of L-glutamic acid γ-monohydroxamate standard, accurately weighed, into a suitable flask, and add 10 mL of *0.2* M *Tris-HCl Buffer*. Dilute this solution sequentially in five steps each by a geometric factor of 2 with *0.2* M *Tris-HCl Buffer*. Transfer 200 μL of each dilution by pipet into individual test tubes, and incubate at 37° for 1 min. Add 2 mL of *Substrate Solution*, previously incubated at 37° for 10 min, to each tube, and mix vigorously with a vortex mixer. Further incubate the mixtures for exactly 10 min, add 2 mL of *Stopping Solution* to each tube, and start a stopwatch. Mix vigorously with the vortex mixture, and separate any insoluble material by centrifugation at 1500 × g for 10 min at about 25°. Measure the absorbance of the supernatant in each tube at 525 nm exactly 30 min after the addition of the *Stopping Solution*. Plot the absorbance against the amount of L-glutamic acid γ-monohydroxamate, and obtain a standard calibration curve used to calculate the amount of glutamic acid γ-monohydroxamate in carbobenzyloxyglutaminylglycine from the absorbance obtained in the analysis of the samples.

Analysis of Samples Transfer 200 μL of *Sample Solution* by pipet into a test tube, and incubate at 37° for 1 min. Add 2 mL of *Substrate Solution*, previously incubated at 37° for 10 min, and mix vigorously using a vortex mixer. Further incubate the mixture for exactly 10 min, add 2 mL of *Stopping Solution*, and start a stopwatch. Mix vigorously using a vortex mixer, and separate any insoluble material by centrifugation at 1500 × g for 10 min at about 25°. Measure the absorbance of the supernatant at 525 nm exactly 30 min after adding the *Stopping Solution*.

For the blank, place 200 μL of *Sample Solution* into a test tube, and incubate at 37° for 1 min. Add 2 mL of *Stopping Solution*, and mix vigorously using a vortex mixer. Further incubate for exactly 10 min, and add 2 mL of *Substrate Solution*, previously incubated at 37° for 10 min, and start a stopwatch. Mix vigorously using a vortex mixer. Separate any insoluble material by centrifugation at 1500 × g for 10 min. Measure the absorbance of the supernatant at 525 nm exactly 30 min after adding the *Substrate Solution*.

Calculation One unit of enzyme activity is defined as the amount of enzyme that catalyzes the transglutamination of 1 μmol of substrate into product in 1 min under the conditions of the assay. The specific activity of Transglutaminase is defined as

$$\text{Transglutaminase activity (U/g)} = C \times T_S \times 1/S \times {}^{1}/_{10},$$

in which C is the concentration, in micromoles per milliliter, of hydroxamate in the *Sample Solution* (obtained from the standard calibration curve); T_S is the total volume, in milliliters, of *Sample Solution*; S is the mass, in grams, of the sample taken; and 10 is the reaction time in minutes.

Transglutaminase Transfer of acyl groups between the γ-carboxyamide group of peptide-bound glutamine residues and various amines, including the ε-amino group of peptide-bound lysine, to form intra- and inter-molecular ε-(γ-glutamyl)lysine crosslinks.

Trivial Name	Classification	Source	Systematic Name (IUB)	IUB No.
Transglutaminase	acyltransferase or aminotransferase	*Streptoverticillium mobaraense* var.	R-glutaminyl-peptide: amine γ-glutamyltransferase	2.3.2.13

TRYPSIN ACTIVITY

Application and Principle This procedure is used to determine the trypsin activity of trypsin preparations derived from purified extracts of porcine or bovine pancreas.

Reagents and Solutions

Fifteenth Molar Phosphate Buffer (pH 7.6) Dissolve 4.54 g of monobasic potassium phosphate in sufficient water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in sufficient water to make 500 mL of solution.

Mix 13 mL of the monobasic potassium phosphate solution with 87 mL of the anhydrous dibasic sodium phosphate solution.

Substrate Solution Dissolve 85.7 mg of *N*-benzoyl-l-arginine ethyl ester hydrochloride, suitable for use in assaying trypsin, in sufficient water to make 100 mL.

> **Note**: Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the assay using USP Trypsin Reference Standard.

Dilute 10.0 mL of this solution to 100.0 mL with *Fifteenth Molar Phosphate Buffer*. Determine the absorbance of this solution at 253 nm in a 1-cm cell, with a suitable spectrophotometer, using water as the blank and maintaining the cell temperature at 25° ± 0.1°. Adjust the absorbance of the solution, if necessary, by the addition of *Fifteenth Molar Phosphate Buffer* so that it measures not less than 0.575 and not more than 0.585. Use this solution within a period of 2 h.

Sample Preparations Dissolve a sufficient amount of sample, accurately weighed, in 0.001 *N* hydrochloric acid to produce a solution containing about 3000 USP trypsin units in each mL. Prepare three dilutions using 0.001 *N* hydrochloric acid so that the final solutions will contain 12, 18, and 24 USP trypsin units in each 0.2 mL. Use these concentrations in the *Procedure* below.

Procedure Conduct the test in a spectrophotometer equipped to maintain a temperature of 25° ± 0.1° in the cell compartment.

Determine the temperature in the reaction cell before and after the measurement of absorbance to ensure that the temperature does not change by more than 0.5°.

Pipet 0.2 mL of 0.001 *N* hydrochloric acid and 3.0 mL of *Substrate Solution* into a 1-cm cell. Place this cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.050 at 253 nm. Pipet 0.2 mL of the *Sample Preparation* containing 12 USP units into another 1-cm cell. Add 3.0 mL of *Substrate Solution*, and place the cell in the spectrophotometer. At the same time the *Substrate Solution* is added, start a stopwatch, and read the absorbance at 30-s intervals for 5 min. Repeat the procedure with the *Sample Preparations* containing 18 and 24 USP units. Plot curves of absorbance versus time for each concentration, and use only those values that form a straight line to determine the activity of the trypsin. Discard the values on the plateau, and take the average of the results from the three concentration levels as the actual activity of the trypsin.

Calculations One USP trypsin unit is the activity causing a change in the absorbance of 0.003/min under the conditions specified in this assay.

Calculate the number of USP trypsin units per mg at each level by the equation

$$\text{USP trypsin units} = (A_1 - A_2)/(T \times W \times 0.003),$$

in which A_1 is the absorbance straight-line final reading; A_2 is the absorbance straight-line initial reading; T is the elapsed time, in min, between the initial and final readings; and W is the weight, in mg, of trypsin in the volume of solution used in determining the absorbance.

APPENDIX VI: ESSENTIAL OILS AND FLAVORS

ACETALS

Hydroxylamine Hydrochloride Solution Prepare as directed under *Aldehydes*, this Appendix.

Procedure Weigh accurately the quantity of the sample specified in the monograph, and transfer it into a 125-mL Erlenmeyer flask. Add 30 mL of *Hydroxylamine Hydrochloride Solution*, and reflux on a steam bath for exactly 60 min. Allow the condenser to drain into the flask for 5 min after removing the flask from the steam bath. Detach, and rapidly cool the flask to room temperature. Add bromophenol blue TS as the indicator, and titrate with 0.5 *N* alcoholic potassium hydroxide to pH 3.4, or to the same light color as produced in the original hydroxylamine hydrochloride solution on adding the indicator. Calculate the mL of 0.5 *N* alcoholic potassium hydroxide consumed per g of sample (*A*).

Using a separate portion of the sample, proceed as directed under *Aldehydes*, this Appendix. Calculate the mL of 0.5 *N* alcoholic potassium hydroxide consumed per g of sample (*B*).

Calculate the percentage of acetals by the formula

$$(A - B) \times f,$$

in which *f* is the equivalence factor given in the monograph.

ACID VALUE

Dissolve about 10 g of the sample, accurately weighed, in 50 mL of alcohol, previously neutralized to phenolphthalein with 0.1 *N* sodium hydroxide. (Add 50 g of ice when testing cinnamyl formate, citronellyl formate, geranyl formate, isoamyl formate, or linalyl formate.) Add 1 mL of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 10 s, unless otherwise directed in the individual monograph. Calculate the acid value (*AV*) by the formula

$$AV = (5.61 \times S)/W,$$

in which *S* is the number of mL of 0.1 *N* sodium hydroxide consumed in the titration of the sample, and *W* is the weight, in g, of the sample.

ALDEHYDES

Hydroxylamine Hydrochloride Solution Dissolve 50 g of hydroxylamine hydrochloride (preferably reagent grade or freshly recrystallized before using) in 90 mL of water, and dilute to 1000 mL with aldehyde-free alcohol. Adjust the solution to a pH of 3.4 with 0.5 *N* alcoholic potassium hydroxide.

Procedure Weigh accurately the quantity of sample specified in the monograph, and transfer it into a 125-mL Erlenmeyer flask. Add 30 mL of *Hydroxylamine Hydrochloride Solution*, mix thoroughly, and allow to stand at room temperature for 10 min, unless otherwise specified in the monograph. Titrate with 0.5 *N* alcoholic potassium hydroxide to a greenish yellow endpoint that matches the color of 30 mL of *Hydroxylamine Hydrochloride Solution* in a 125-mL flask when the same volume of bromophenol blue TS has been added to each flask, or preferably titrate to a pH of 3.4 using a suitable pH meter. Calculate the percentage of aldehyde (*A*) by the equation

$$A = (S - b)(100e)/W,$$

in which *S* is the number of mL of 0.5 *N* alcoholic potassium hydroxide consumed in the titration of the sample, *b* is the number of mL of 0.5 *N* alcoholic potassium hydroxide consumed in the titration of the blank, *e* is the equivalence factor given in the monograph, and *W* is the weight, in mg, of the sample.

ALDEHYDES AND KETONES

Hydroxylamine Method

Hydroxylamine Solution Dissolve 20 g of hydroxylamine hydrochloride (reagent grade or, preferably, freshly crystallized) in 40 mL of water, and dilute to 400 mL with alcohol. Add, with stirring, 300 mL of 0.5 *N* alcoholic potassium hydroxide, and filter. Use this solution within 2 days.

Procedure Weigh accurately the quantity of the sample specified in the individual monograph, and transfer it into a 250-mL glass-stoppered flask. Add 75.0 mL of *Hydroxylamine Solution* to this flask and to a similar flask for a residual blank titration (see *General Provisions*). If the component to be determined is an *aldehyde*, stopper the flasks and allow them to stand at room temperature for 1 h unless otherwise stated in the monograph. If the component to be determined is a *ketone*, attach the flask to a suitable condenser, and reflux the mixture for 1 h unless otherwise stated in the monograph, and then cool to room temperature. Titrate both flasks to the same greenish yellow endpoint using bromophenol blue TS as the indicator or, preferably, to a pH of 3.4 using a pH meter. (If the indicator is used, the endpoint color must be the same as that produced when the blank is titrated to a pH of 3.4.) Calculate the percentage of aldehyde or ketone by the equation

$$AK = (b - S)(100e)/W,$$

in which *AK* is the percentage of aldehyde or ketone, *b* is the number of mL of 0.5 *N* hydrochloric acid consumed in the residual blank titration, *S* is the number of mL of 0.5 *N* hydrochloric acid consumed in the titration of the sample, *e* is the equivalence factor given in the monograph, and *W* is the weight, in mg, of the sample.

Hydroxylamine *tert*-Butyl Alcohol Method

Hydroxylamine Solution Dissolve 45 g of reagent-grade hydroxylamine hydrochloride in 130 mL of water, add 850 mL of *tert*-butyl alcohol, mix, and using a pH meter, neutralize to a pH of 3.0 to 3.5 with sodium hydroxide.

Caution: Do not heat the solution.

Procedure Weigh accurately the quantity of the sample specified in the individual monograph, and transfer it into a 250-mL glass-stoppered flask. Add 50 mL of the *Hydroxylamine Solution*, or the volume specified in the monograph, mix thoroughly, and allow to stand at room temperature for the time specified in the monograph. Titrate with 0.5 *N* sodium hydroxide to the same pH as the *Hydroxylamine Solution* used. Calculate the percentage of aldehyde or ketone by the equation

$$AK = (S)(100e)/W,$$

in which *AK* is the percentage of aldehyde or ketone, *S* is the number of mL of 0.5 *N* sodium hydroxide consumed in the titration of the sample, *e* is the equivalence factor given in the monograph, and *W* is the weight, in mg, of the sample.

Neutral Sulfite Method

Pipet a 10-mL sample into a 100-mL cassia flask fitted with a stopper, and add 50 mL of a freshly prepared 30 in 100 solution of sodium sulfite. Add 2 drops of phenolphthalein TS, and neutralize with 50% (by volume) acetic acid solution. Heat the mixture in a boiling water bath, and shake the flask repeatedly, neutralizing the mixture from time to time by the addition of a few drops of the 50% acetic acid solution, stoppering the flask to prevent loss of volatile material. After no coloration appears upon the addition of a few more drops of phenolphthalein TS and heating for 15 min, cool to room temperature. When the liquids have separated completely, add sufficient sodium sulfite solution to raise the lower level of the oily layer within the graduated portion of the neck of the flask. Calculate the percentage, by volume, of the aldehyde or ketone by the equation

$$AK = 100 - (V \times 10),$$

in which *AK* is the percentage, by volume, of the aldehyde or ketone in the sample, and *V* is the number of mL of separated oil in the graduated neck of the flask.

CHLORINATED COMPOUNDS

Wind a 1.5- × 5-cm strip of 20-mesh copper gauze around the end of a copper wire. Heat the gauze in a nonluminous flame of a Bunsen burner until it glows without coloring the flame green. Permit the gauze to cool, and re-ignite it several times until a good coat of oxide has formed. With a medicine dropper, apply 2 drops of the sample to the cooled gauze, ignite, and permit it to burn freely in the air. Again cool the gauze, add 2 more drops, and burn as before. Continue this process until a total of 6 drops have been added and ignited. Then hold the gauze in the outer edge of a Bunsen flame adjusted to a height of 4 cm. Not even a transient green color is imparted to the flame. If at any of the additions the sample appears to be instantly vaporized, the test must be repeated from the beginning.

ESTERS

Ester Determination Weigh accurately the quantity of the sample specified in the monograph, and transfer it into a 125-mL Erlenmeyer flask containing a few boiling stones. Add to this flask and, simultaneously, to a similar flask for a residual blank titration (see *General Provisions*) 25.0 mL of 0.5 *N* alcoholic potassium hydroxide. Connect each flask to a reflux condenser, and reflux the mixtures on a steam bath for exactly 1 h, unless otherwise directed in the monograph. Allow the mixtures to cool, add 10 drops of phenolphthalein TS to each flask, and titrate the excess alkali in each flask with 0.5 *N* hydrochloric acid. Calculate the percentage of esters (*E*) in the sample by the equation

$$E = (b - S)(100e)/W,$$

in which *b* is the number of mL of 0.5 *N* hydrochloric acid consumed in the residual blank titration, *S* is the number of mL of 0.5 *N* hydrochloric acid consumed in the titration of the sample, *e* is the equivalence factor given in the monograph, and *W* is the weight, in mg, of the sample.

Ester Determination (High-Boiling Solvent)

0.5 N *Potassium Hydroxide Solution* Dissolve about 35 g of potassium hydroxide in 75 mL of water, add 1000 mL of a suitable grade of monoethyl ether of diethylene glycol, and mix.

Procedure Weigh accurately the quantity of the sample specified in the monograph, and transfer it into a 200-mL Erlenmeyer flask having a standard-taper joint. To this flask and to a similar flask for a residual blank titration (see *General Provisions*) add two glass beads and 25.0 mL of *0.5* N *Potassium Hydroxide Solution*, allowing exactly 1 min for drainage from the buret or pipet. Attach an air condenser to each flask, reflux gently for 1 h, and cool. Rinse down the condensers with about 50 mL of water, then add phenolphthalein TS to each flask, and titrate the excess alkali with 0.5 *N* sulfuric acid to the disappearance of the pink color. Calculate the percentage of esters (*E*) in the sample by the equation

$$E = (b - S)(100e)/W,$$

in which *b* is the number of mL of 0.5 *N* sulfuric acid consumed in the blank determination, *S* is the number of mL of 0.5 *N*

sulfuric acid required in the titration of the sample, e is the equivalence factor given in the monograph, and W is the weight, in mg, of the sample.

Saponification Value Proceed as directed for *Ester Determination* or *Ester Determination (High-Boiling Solvent)*, as specified in the monograph. Calculate the saponification value (SV) by the equation

$$SV = (b - S)(28.05)/W,$$

in which b and S are as defined under *Ester Determination*, and W is the weight, in g, of the sample.

Ester Value If the sample contains no free acids, the saponification value and the ester value are identical. If a determination of the *Acid Value* (AV) is specified in the monograph, calculate the ester value (EV) by the equation

$$EV = SV - AV,$$

in which SV is the saponification value.

LINALOOL DETERMINATION

Transfer a 10-mL sample, previously dried with sodium sulfate, into a 125-mL glass-stoppered Erlenmeyer flask previously cooled in an ice bath. Add to the cooled oil 20 mL of dimethyl aniline (monomethyl-free), and mix thoroughly. To the mixture add 8 mL of acetyl chloride and 5 mL of acetic anhydride, cool for several min, permit to stand at room temperature for another 30 min, then immerse the flask in a water bath maintained at 40° ± 1° for 16 h. Wash the acetylated oil with three 75-mL portions of ice water, followed by successive washes with 25-mL portions of 5% sulfuric acid, until the separated acid layer no longer becomes cloudy or emits an odor of dimethyl aniline when made alkaline. After removal of the dimethyl aniline, wash the acetylated oil first with 10 mL of sodium carbonate TS and then with successive portions of water until the washings are neutral to litmus. Finally, dry the acetylated oil with anhydrous sodium sulfate, and proceed as directed for *Ester Determination* under *Esters*, this Appendix. Calculate the percentage of linalool ($C_{10}H_{18}O$) by the equation

$$L = [7.707(b - S)]/[W - 0.021(b - S)],$$

in which L is the percentage of linalool, b is the number of mL of 0.5 N hydrochloric acid consumed in the residual blank titration, S is the number of mL of 0.5 N hydrochloric acid consumed in the titration of the sample, and W is the weight, in g, of the sample.

Note: When this method is applied to essential oils containing appreciable amounts of esters, perform an *Ester Determination*, this appendix, on a sample of the *original oil* and calculate the percentage of total linalool by the equation

$$L = [7.707(b - S)(1 - 0.0021E)]/[W - 0.21(b - S)],$$

in which L is the percentage of linalool, E is the percentage of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$) in the sample of the original oil, and b, S, and W are as defined in the preceding paragraph.

Note: This entire procedure is applicable only to linalool and linalool-containing oils. It is not intended for the determination of other tertiary alcohols.

PERCENTAGE OF CINEOLE

Temperature	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
24	45.6	45.7	45.9	46.0	46.1	46.3	46.4	46.5	46.6	46.8
25	46.9	47.0	47.2	47.3	47.4	47.6	47.7	47.8	47.9	48.1
26	48.2	48.3	48.5	48.6	48.7	48.9	49.0	49.1	49.2	49.4
27	49.5	49.6	49.8	49.9	50.0	50.2	50.3	50.4	50.5	50.7
28	50.8	50.9	51.1	51.2	51.3	51.5	51.6	51.7	51.8	52.0
29	52.1	52.2	52.4	52.5	52.6	52.8	52.9	53.0	53.1	53.3
30	53.4	53.5	53.7	53.8	53.9	54.1	54.2	54.3	54.4	54.6
31	54.7	54.8	55.0	55.1	55.2	55.4	55.5	55.6	55.7	55.9
32	56.0	56.1	56.3	56.4	56.5	56.7	56.8	56.9	57.0	57.2
33	57.3	57.4	57.6	57.7	57.8	58.0	58.1	58.2	58.3	58.5
34	58.6	58.7	58.9	59.0	59.1	59.3	59.4	59.5	59.6	59.8
35	59.9	60.0	60.2	60.3	60.4	60.6	60.7	60.8	60.9	61.1
36	61.2	61.3	61.5	61.6	61.7	61.9	62.0	62.1	62.2	62.4
37	62.5	62.6	62.8	62.9	63.0	63.2	63.3	63.4	63.5	63.7
38	63.8	63.9	64.1	64.2	64.4	64.5	64.6	64.8	64.9	65.1
39	65.2	65.4	65.5	65.7	65.8	66.0	66.2	66.3	66.5	66.6
40	66.8	67.0	67.2	67.3	67.5	67.7	67.9	68.1	68.2	68.4
41	68.6	68.8	69.0	69.2	69.4	69.6	69.7	69.9	70.1	70.3
42	70.5	70.7	70.9	71.0	71.2	71.4	71.6	71.8	71.9	72.1
43	72.3	72.5	72.7	72.9	73.1	73.3	73.4	73.6	73.8	74.0
44	74.2	74.4	74.6	74.8	75.0	75.2	75.3	75.5	75.7	75.9
45	76.1	76.3	76.5	76.7	76.9	77.1	77.2	77.4	77.6	77.8
46	78.0	78.2	78.4	78.6	78.8	79.0	79.2	79.4	79.6	79.8
47	80.0	80.2	80.4	80.6	80.8	81.1	81.3	81.5	81.7	81.9
48	82.1	82.3	82.5	82.7	82.9	83.2	83.4	83.6	83.8	84.0
49	84.2	84.4	84.6	84.8	85.0	85.3	85.5	85.7	85.9	86.1
50	86.3	86.6	86.8	87.1	87.3	87.6	87.8	88.1	88.3	88.6
51	88.8	89.1	89.3	89.6	89.8	90.1	90.3	90.6	90.8	91.1
52	91.3	91.6	91.8	92.1	92.3	92.6	92.8	93.1	93.3	93.6
53	93.8	94.1	94.3	94.6	94.8	95.1	95.3	95.6	95.8	96.1
54	96.3	96.6	96.9	97.2	97.5	97.8	98.1	98.4	98.7	99.0
55	99.3	99.7	100.0							

PHENOLS

Pipet 10 mL of the oil, which has been subjected to any treatment specified in the monograph, into a 100-mL cassia flask, add 75 mL of 1 N potassium hydroxide, and shake vigorously for 5 min to ensure complete extraction of the phenol by the alkali solution. Allow the mixture to stand for about 30 min, then add sufficient 1 N potassium hydroxide to raise the oily layer into the graduated portion of the flask, stopper the flask, and allow it to stand overnight. Read the volume of insoluble oil to 0.05 mL. Calculate the percentage, by volume, of phenols by the equation

$$P = (10 - V) \times 10,$$

in which P is the percentage, by volume, of phenols, and V is the observed volume, in mL, of insoluble oil.

PHENOLS, FREE

Transfer about 5 g, accurately weighed, of the sample into a 150-mL flask having a standard-taper neck. Pipet exactly 10 mL of a 1:10 solution of acetic anhydride in anhydrous pyridine into the flask, and pipet exactly 10 mL of this solution, preferably measured with the same pipet, into a second 150-mL flask for the residual blank titration (see *General Provisions*). Connect the flasks to condensers, reflux for 1 h, and cool to a temperature below 100°. Add 25 mL of water to each flask through the condensers, and reflux again for 10 min. Cool the flasks, add phenolphthalein TS, and titrate with 0.5 *N* potassium hydroxide. Calculate the percentage of free phenols by the equation

$$\text{Percentage of Free Phenols} = (b - S) \times 100f/W,$$

in which b is the number of mL of 0.5 *N* potassium hydroxide consumed in the residual blank titration, s is the number of mL of 0.5 *N* potassium hydroxide consumed in the titration of the sample, f is the equivalence factor given in the monograph, and W is the weight, in mg, of the sample.

RESIDUE ON EVAPORATION

Weigh accurately the quantity of sample specified in the monograph, and transfer it into a suitable evaporating dish that has previously been heated on a steam bath, cooled to room temperature in a desiccator, and accurately weighed. Weigh the sample in the dish. Heat the evaporating dish containing the sample on the steam bath for the period of time specified in the monograph. Cool the dish and its contents to room temperature in a desiccator, and weigh accurately. Calculate the residue as percentage of the sample used.

SOLUBILITY IN ALCOHOL

Transfer a 1.0-mL sample into a calibrated 10-mL glass-stoppered cylinder graduated in 0.1-mL subdivisions, and add slowly, in small portions, alcohol of the concentration specified in the monograph. Maintain the temperature at 25°, and shake the cylinder thoroughly after each addition of alcohol. When a clear solution is first obtained, record the number of mL of alcohol required. Continue the addition of the alcohol until a total of 10 mL has been added. If opalescence or cloudiness occurs during these subsequent additions of alcohol, record the number of mL of alcohol at which the phenomenon occurs.

TOTAL ALCOHOLS

Unless otherwise stated in the monograph, transfer 10 g of a solid sample, or 10 mL of a liquid sample, accurately weighed, into a 100-mL flask having a standard-taper neck. Add 10 mL of acetic anhydride and 1 g of anhydrous sodium acetate, mix these materials, attach a reflux condenser to the flask, and reflux the mixture for 1 h. Cool, and through the condenser, add 50 mL of water at a temperature between 50° and 60°. Shake intermittently for 15 min, cool to room temperature, transfer the mixture completely to a separator, allow the layers to separate, and then remove and reject the lower, aqueous layer. Wash the oil layer successively with 50 mL of a saturated sodium chloride solution, 50 mL of a 10% sodium carbonate solution, and 50 mL of saturated sodium chloride solution. If the oil is still acid to moistened litmus paper, wash it with additional portions of sodium chloride solution until it is free from acid. Drain off the oil, dry it with anhydrous sodium sulfate, and then filter it.

Transfer the quantity of acetylated oil specified in the monograph, and accurately weighed, into a tared 125-mL Erlenmeyer flask, and add 10 mL of neutral alcohol, 10 drops of phenolphthalein TS, and 0.1 *N* alcoholic potassium hydroxide, dropwise, until a pink endpoint is obtained. If more than 0.20 mL is needed, reject the sample, and wash and test the remaining acetylated oil until its acid content is below this level. Prepare a blank for residual titration (see *General Provisions*), using the same volume of alcohol and indicator, and add 1 drop of 0.1 *N* alkali to produce a pink endpoint. Transfer 25.0 mL of 0.5 *N* alcoholic potassium hydroxide into each of the flasks, reflux them simultaneously for 1 h, cool, and titrate the contents of each flask with 0.5 *N* hydrochloric acid to the disappearance of the pink color. Calculate the percentage of *Total Alcohols* (A) by the equation

$$A = [b - S)(100e)]/[W - 21(b - S)],$$

in which b is he number of milliliters of 0.5 *N* hydrochloric acid consumed in the residual blank titration; S is the number of milliliters of 0.5 *N* hydrochloric acid consumed in the titration of the sample; e is the equivalence factor given in the monograph; and W is the weight, in milligrams, of the sample of the acetylated oil.

ULTRAVIOLET ABSORBANCE OF CITRUS OILS

Transfer the quantity of the sample specified in the monograph into a 100-mL volumetric flask, add alcohol to volume, and

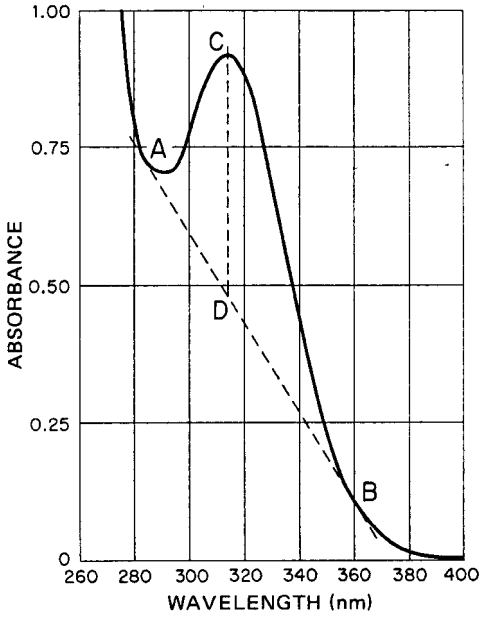


FIGURE 33 Typical Spectrogram of Lemon Oil.

mix. Determine the ultraviolet absorption spectrum of the solution in the range of 260 to 400 nm in a 1-cm cell with a suitable recording or manual spectrophotometer, using alcohol as the blank. If a manual instrument is used, read absorbances at 5-nm intervals from 260 nm to a point about 12 nm from the expected maximum absorbance, then at 3-nm intervals for three readings, and at 1-nm intervals to a point about 5 nm beyond the maximum, and then at 10-nm intervals to 400 nm. From these data, plot the absorbances as ordinates against wavelength on the abscissa, and draw the spectrogram. Draw a baseline tangent to the areas of minimum absorbance, as shown in Fig. 33 (which is typical of lemon oil), joining point *A* in the region of 280 to 300 nm and a second point, *B*, in the region of 355 to 380 nm. Locate the point of maximum absorbance, *C*, and from it drop a vertical line, perpendicular to the abscissa, that intersects line *AB* at *D*. Read from the ordinate the absorbances corresponding to points *C* and *D*, subtract the latter from the former, and correct the difference for the actual weight of oil taken, calculating on the basis of the sample weight specified in the monograph.

VOLATILE OIL CONTENT

This procedure is used, when specified in the individual monograph, for determining the volatile oil content of gums, resins, and essential oils.

Apparatus The apparatus is shown in Fig. 34. It consists of a 1000-mL boiling flask, *A*, attached through a trap, *D*, to a Liebig condenser, *C*, which is connected to a 25-mL collector tube, *B*, graduated in 0.10-mL units.

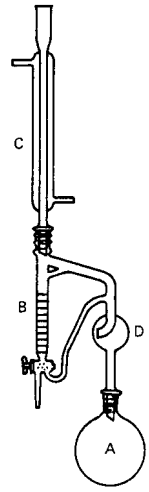


FIGURE 34 Apparatus for Determination of Volatile Oil Content.

Procedure Place 750 mL of water in the boiling flask, boil for 10 min, and cool to 50°. Transfer the specified volume of the sample, prepared as directed in the monograph, into the flask, then immediately attach the remainder of the apparatus to the flask, and boil until the volume of distilled oil collected in the graduated collector tube remains constant. Avoid splashing the contents of the flask in order to prevent contamination of the distillate with nonvolatile material, and do not continue distillation for an extended time after the volume of distillate becomes constant. If the distilled oil is heavier than water, set the stopcock in the closed position to prevent return of the heavy distillate to the flask.

When distillation is complete, allow the contents of the collection tube to settle until the oil and water layers are separated completely. Allow the distillate to cool to room temperature, read its volume, and calculate therefrom the percentage of volatile oil.

Note: When the volatile oil thus collected is to be used in additional tests, as may be specified in the monograph, the oil should be drained off, dried, and filtered before use.

APPENDIX VII: FATS AND RELATED SUBSTANCES

ACETYL VALUE
(Based on AOCS Method Cd 4-40)

The acetyl value is defined as the number of mg of potassium hydroxide required to neutralize the acetic acid obtained by saponifying 1 g of the acetylated sample.

Acetylation Boil 50 mL of the oil or melted fat with 50 mL of freshly distilled acetic anhydride for 2 h under a reflux condenser. Pour the mixture into a beaker containing 500 mL of water, and boil for 15 min, bubbling a stream of nitrogen or carbon dioxide through the mixture to prevent bumping. Cool slightly, remove the water, add another 500 mL of water, and boil again. Repeat for a third time with another 500-mL portion of water, and remove the wash water, which should be neutral to litmus. Transfer the acetylated fat to a separator, and wash with two 200-mL portions of warm water, separating as much as possible of the wash water each time. Transfer the washed sample to a beaker, add 5 g of anhydrous sodium sulfate, and let stand for 1 h, agitating occasionally to assist drying. Filter the oil through a dry filter paper, preferably in an oven at 100° to 110°, and keep the filtered oil in the oven until it is completely dry. The acetylated product should be a clear, brilliant oil.

Saponification Weigh accurately from 2 to 2.5 g each of the acetylated oil and of the original, untreated sample into separate 250-mL Erlenmeyer flasks. Add to each flask 25.0 mL of 0.5 *N* alcoholic potassium hydroxide, and continue as directed in the *Procedure* under *Saponification Value*, in this Appendix, beginning with ''Connect an air condenser. . . .'' Record the saponification value of the untreated sample as *S*, and that of the acetylized oil as *S′*, then calculate the acetyl value of the sample by the formula

$$(S' - S)/(1.000 - 0.00075S).$$

ACID VALUE
(Based on AOCS Methods Te 1a-64 and Cd 3d-63)

The acid value is defined as the number of mg of potassium hydroxide required to neutralize the fatty acids in 1 g of the test substance.

Method I (Commercial Fatty Acids)

Unless otherwise directed, weigh accurately about 5 g of the sample into a 500-mL Erlenmeyer flask, and dissolve it in 75 to 100 mL of hot alcohol, previously boiled and neutralized to phenolphthalein TS with sodium hydroxide. Agitation and further heating may be necessary to effect complete solution of the sample. Add 0.5 mL of phenolphthalein TS, and titrate immediately, while shaking, with 0.5 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Calculate the acid value by the formula

$$56.1V \times N/W,$$

in which *V* is the volume, in mL, and *N* is the normality, respectively, of the sodium hydroxide solution; and *W* is the weight, in g, of the sample taken.

Method II (Animal Fats and Vegetable and Marine Oils)

Prepare a solvent mixture consisting of equal parts, by volume, of isopropyl alcohol and toluene. Add 2 mL of a 1% solution of phenolphthalein in isopropyl alcohol to 125 mL of the mixture, and neutralize with alkali to a faint but permanent pink color. Weigh accurately the appropriate amount of well-mixed liquid sample indicated in the table below, dissolve it in the neutralized solvent mixture, warming if necessary, and shake vigorously while titrating with 0.1 *N* potassium hydroxide to the first permanent pink color of the same intensity as that of the neutralized solvent before mixing with the sample. Calculate the acid value by the formula

$$56.1V \times N/W,$$

in which *V* is the volume, in mL, and *N* is the normality, respectively, of the potassium hydroxide solution; and *W* is the weight, in g, of the sample taken.

Acid Value	Sample Weight (g)
0–1	20
1–4	10
4–15	2.5
15–75	0.5
75 and over	0.1

CHLOROPHYLL
(Based on AOCS Method Cc 13d-55)

Use a reliable spectrophotometer with a sample holder equilibrated at 44° ± 3° to obtain absorbance values at 630, 670, and 710 nm. Calculate the concentration of chlorophyll (*C*) using the following equation:

$$C = [A_{670} - (A_{630}/2) - (A_{710}/2)]/(K \times b),$$

in which *C* is the concentration of chlorophyll, in mg/kg; *A* is the absorbance at the wavelength indicated by the subscript; *K* is the constant for the specific spectrophotometer being used and is equal to 0.1016 for the Beckman Model DU; and *b* is the optical pathlength through the sample, in cm.

COLD TEST
(Based on AOCS Method Cc 11-53)

Filter a sample (200 to 300 mL), and transfer to a clean, dry bottle. Fill the bottle completely, and insert a cork stopper. Seal with paraffin, and equilibrate at 25° in a water bath so that it is completely covered. Next, immerse the bottle in an ice and water bath so it is completely covered. Monitor the bath during the test and replenish the ice frequently to keep the bath at 0°.

After 5.5 h remove the bottle from the bath. The sample must be clear; fat crystals or cloudiness must be totally absent.

COLOR (AOCS-Wesson)
(Based on AOCS Method Cc 13b-45)

Apparatus Use a Lovibond tintometer or the equivalent and a set of color comparison glasses that conform to the AOCS-Wesson Tintometer Color Scale (available from the National Institute of Standards and Technology). A minimum set of glasses consists of

Red	0.1	0.2	0.3	0.4	0.5	0.6	0.8	0.9
	1.0	2.0	2.5	3.0	3.5	4.0	5.0	6.0
	7.0	7.6	8.0	9.0	10.0	11.0	12.0	16.0
	20.0							
Yellow	1.0	2.0	3.0	5.0	10.0	15.0	20.0	35.0
	50.0	70.0						

For making color comparisons, use color tubes of clear, colorless glass with a smooth, flat, polished bottom (length 154 mm; id 19 mm; od 22 mm), and marked to indicate liquid columns of 25.4 and 133.35 mm.

Procedure Add 0.1 g of diatomaceous earth to a 60-g sample, agitate for 2.5 min at room temperature (or 10° to 15° above the melting point if the sample is not liquid), and filter. Adjust the temperature to 25° to 35° (or not more than 100 above the melting point), and fill the color tube to the desired mark. Place the tube in the tintometer (in a dark booth or cabinet), and match the sample color as closely as possible with a standard glass.

FATTY ACID COMPOSITION
(Based on AOCS Methods Ce 1-62, Ce 1b-89, Ce 1e-91)

Apparatus Use a suitable gas chromatograph (see Appendix IIA) equipped with a flame ionization detector (FID) and containing either a 3.05-m × 2- or 4-mm id glass column packed with preconditioned 10%, by weight, DEGS-PS on 100- to 120-mesh diatomaceous earth (Chromosorb WHP, or equivalent) or a 30-m × 0.20- to 0.35-mm id capillary fused silica column, or equivalent, containing a suitable stationary phase.

Operating Conditions The operating conditions may vary with the instrument used, but a suitable chromatogram may be obtained using a temperature program 180° to 215°; inlet temperature (injector), 300°; detector, 300°; and a suitable carrier gas flow.

Standard Solutions Run through the chromatograph a commercially available standard containing a mixture of fatty-acid methyl esters. Fatty acids and methyl esters with a wide range of carbon numbers and double-bond configurations can be purchased. The calculated concentration should compare to that claimed within $\pm 2\,\sigma$, where σ is the standard deviation calculated from at least 10 replicate determinations, preferably made over a period of several days.

Determine that the system is functioning properly: inject into the chromatograph a suitable number of samples of the standard to ensure that the resolution factor, R, defining the efficiency of the separation between methyl stearate and methyl oleate is 0.9 or greater. Calculate R by the equation

$$R = 2(t_2 - t_1)/(w_2 + w_1),$$

in which t_2 and t_1 are the retention times of peak 2 and peak 1, respectively, and w_2 and w_1 are the corresponding widths of the bases of the peaks obtained by extrapolating relatively straight sides of the peaks to the baseline. Baseline separation of the various components in both the standard and the sample preparations is desirable.

Sample Preparation (for fats and oils) (Based on AOCS Method Ce 2-66) Introduce 100 to 1000 mg of the fat into a 50- or 125-mL reaction flask. Add 4 to 10 mL of 0.5 *N* methanolic sodium hydroxide, and add a boiling chip. Attach a condenser, and heat the mixture on a steam bath until the fat globules go into solution. This step should take 5 to 10 min. Add 5 to 12 mL of 12.5% boron fluoride–methanol reagent (this reagent contains 125 g of boron fluoride per L of methanol and is available commercially) through the condenser, and boil for 2 min. Add 2 to 5 mL of heptane through the condenser, and boil for 1 min longer. Remove from heat, remove condenser, and add about 15 mL of saturated sodium chloride solution. Stopper the flask, and shake vigorously for 15 s. Transfer about 1 mL of the heptane solution into a test tube and add a small amount of anhydrous sodium sulfate. The dry heptane solution may then be injected directly into a gas chromatograph.

The methyl esters should be analyzed as soon as possible. They may be kept in an atmosphere of nitrogen in a screw-cap vial at 2° for 24 h. For longer storage, they should be sealed in a glass ampule, subjected first to a vacuum and then backfilled with nitrogen and stored at −20° (freezer).

Procedure Inject an appropriate volume (0.1 μL to 1.0 μL) of sample into the chromatograph. If an automated system is used, follow the manufacturer's instructions; if calculations are to be done manually, proceed as follows:

Calculate the area percent of each component (C_N) by the equation

$$C_N = [A_N/T_S] \times 100,$$

in which A_N is the area of the peak corresponding to component C_N, and T_S is the total area for all detected components [$T_S = \Sigma A_N$].

FREE FATTY ACIDS
(Based on AOCS Method Ca 5a-40)

Unless otherwise directed, accurately weigh the appropriate amount of the sample, indicated in the table below, into a 250-mL Erlenmeyer flask or other suitable container. Add 2 mL of phenolphthalein TS to the specified amount of hot alcohol, neutralize with alkali to the first faint, but permanent, pink color, and then add the hot, neutralized alcohol to the sample container. Titrate with the appropriate normality of sodium hydroxide, shaking vigorously, to the first permanent pink color of the same intensity as that of the neutralized alcohol. The color must persist for at least 30 s. Calculate the percentage of free fatty acids (FFA) in the sample by the formula

$$VNe/W,$$

in which V is the volume and N is the normality of the sodium hydroxide used; W is the weight of the sample, in g; and e is the equivalence factor given in the monograph.

FFA Range (%)	Grams of Sample	Milliliters of Alcohol	Strength of NaOH
0.00–0.2	56.4 ± 0.2	50	0.1 *N*
0.2–1.0	28.2 ± 0.2	50	0.1 *N*
1.0–30.0	7.05 ± 0.05	75	0.25 *N*
30.0–50.0	7.05 ± 0.05	100	0.25–1.0 *N*
50.0–100	3.525 ± 0.001	100	1.0 *N*

FREE GLYCERIN OR PROPYLENE GLYCOL
(Based on AOCS Method Ca 14-56)

Reagents and Solutions Use the *Periodic Acid Solution*, *Potassium Iodide Solution*, and *Chloroform* as described under *1-Monoglycerides*, in this Appendix.

Procedure To the combined aqueous extracts obtained as directed under *1-Monoglycerides*, add 50.0 mL of *Periodic Acid Solution*. Run two blanks by adding 50.0 mL of this reagent solution to two 500-mL glass-stoppered Erlenmeyer flasks, each containing 75 mL of water. Continue as directed in the *Procedure* under *1-Monoglycerides*, beginning with ''. . . and allow to stand for at least 30 min but no longer than 90 min.''

Calculation Calculate the percentage of free glycerin in the original sample by the formula

$$(b - S) \times N \times 2.30/W,$$

or calculate the percentage of free propylene glycol by the formula

$$(b - S) \times N \times 3.81/W,$$

in which b is the number of mL of sodium thiosulfate consumed in the blank determination; S is the number of mL required in the titration of the aqueous extracts from the sample; N is the exact normality of the sodium thiosulfate; W is the weight, in g, of the original sample taken; 2.30 is the molecular weight of glycerin divided by 40; and 3.81 is the molecular weight of propylene glycol divided by 20.

Note: If the aqueous extract contains more than 20 mg of glycerin or more than 30 mg of propylene glycol, dilute the extract in a volumetric flask and transfer a suitable aliquot into a 500-mL glass-stoppered Erlenmeyer flask before proceeding with the test. The weight of the sample should be corrected in the calculation.

HEXANE-INSOLUBLE MATTER

If the sample is plastic or semisolid, soften a portion by warming it at a temperature not exceeding 60°, and then mix it thoroughly. Transfer 100 g of well-mixed sample into a 1500-mL wide-mouth Erlenmeyer flask, add 1000 mL of solvent hexane, and shake until the sample is dissolved. Filter the resulting solution through a 600-mL Corning ''C'' porosity, or equivalent, filtering funnel that previously has been dried at 105° for 1 h, cooled in a desiccator, and weighed. Wash the flask with two successive 250-mL portions of solvent hexane, and pass the washings through the filter. Dry the funnel at 105° for 1 h, cool to room temperature in a desiccator, and weigh. From the gain in weight of the funnel, calculate the percentage of the hexane-insoluble matter in the sample.

HYDROXYL VALUE
(Based on AOCS Methods Cd 4-40 and Cd 13-60)

The hydroxyl value is defined as the number of mg of potassium hydroxide equivalent to the hydroxyl content of 1 g of the unacetylated sample.

Method I

Proceed as directed under *Acetyl Value*, in this Appendix, but calculate the hydroxyl value by the formula

$$(S' - S)/(1.000 - 0.00075S').$$

Method II

Unless otherwise directed, accurately weigh the appropriate amount of the sample indicated in the table below, transfer it into a 250-mL glass-stoppered Erlenmeyer flask, and add 5.0 mL of pyridine–acetic anhydride reagent (mix 3 volumes of freshly distilled pyridine with 1 volume of freshly distilled acetic anhydride).

Hydroxyl Value	Sample Weight (g)
0–20	10
20–50	5
50–100	3
100–150	2
150–200	1.50
200–250	1.25
250–300	1
300–350	0.75

Pipet 5 mL of the pyridine–acetic anhydride reagent into a second 250-mL flask for the reagent blank. Heat the flasks for 1 h on a steam bath under reflux condensers, then add 10 mL of water through each condenser, heat for 10 min longer, and allow the flasks to cool to room temperature. Add 15 mL of *n*-butyl alcohol, previously neutralized to phenolphthalein TS with 0.5 *N* alcoholic potassium hydroxide, through the condenser, then remove the condensers, and wash the sides of the flasks with 10 mL of *n*-butyl alcohol. To each flask add 1 mL of phenolphthalein TS, and titrate to a faint pink endpoint with 0.5 *N* alcoholic potassium hydroxide, recording the mL required for the sample as *S* and that for the blank as *B*. To correct for free acid, mix about 10 g of the sample, accurately weighed, with 10 mL of freshly distilled pyridine, previously neutralized to phenolphthalein, add 1 mL of phenolphthalein TS, and titrate to a faint endpoint with 0.5 *N* alcoholic potassium hydroxide, recording the mL required as *A*. Calculate the hydroxyl value by the formula

$$[B + (WA/C) - S] \times 56.1N/W,$$

in which *W* and *C* are the weights, in g, of the samples taken for acetylation and for the free acid determination, respectively; and *N* is the exact normality of the alcoholic potassium hydroxide.

IODINE VALUE

(Based on AOCS Method Cd 1d-92)

The iodine value is a measure of unsaturation and is expressed as the number of g of iodine absorbed, under the prescribed conditions, by 100 g of the test substance.

Modified Wijs Method (Acetic Acid/Cyclohexane Method)

Wijs Solution Dissolve 13 g of resublimed iodine in 1000 mL of glacial acetic acid. Pipet 10.0 mL of this solution into a 250-mL flask, add 20 mL of potassium iodide TS and 100 mL of water, and titrate with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Record the volume required as *A*. Set aside about 100 mL of the iodine–acetic acid solution for future use. Pass chlorine gas, washed and dried with sulfuric acid, through the remainder of the solution until a 10.0-mL portion requires not quite twice the volume of 0.1 *N* sodium thiosulfate consumed in the titration of the original iodine solution. A characteristic color change occurs when the desired amount of chlorine has been added. Alternatively, *Wijs Solution* may be prepared by dissolving 16.5 g of iodine monochloride, ICl, in 1000 mL of glacial acetic acid. Store the solution in amber bottles sealed with paraffin until ready for use, and use within 30 days.

Total Halogen Content Pipet 10.0 mL of *Wijs Solution* into a 500-mL Erlenmeyer flask containing 150 mL of recently boiled and cooled water and 15 mL of potassium iodide TS. Titrate immediately with 0.1 *N* sodium thiosulfate, recording the volume required as *B*.

Halogen Ratio Calculate the I/Cl ratio by the formula

$$A/(B - A).$$

The halogen ratio must be between 1.0 and 1.2. If the ratio is not within this range, the halogen content can be adjusted by adding the original solution or by passing more chlorine through the solution.

Note: *Wijs Solution* is commercially available.

Procedure The appropriate weight of the sample, in g, is calculated by dividing the number 25 by the expected iodine value. Melt the sample, if necessary, and filter it through a dry filter paper. Transfer the accurately weighed quantity of sample into a clean, dry, 500-mL glass-stoppered bottle or flask containing 20 mL of glacial acetic acid/cyclohexane, 1:1, v/v, and pipet 25.0 mL of *Wijs Solution* into the flask. The excess of iodine should be between 50% and 60% of the quantity added, that is, between 100% and 150% of the quantity absorbed. Swirl, and let stand in the dark for 1.0 h where the iodine value is <150 and for 2.0 h where the iodine value is ≥150. Add 20 mL of potassium iodide TS and 100 mL of recently boiled and cooled water, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding the titrant gradually and shaking constantly until the yellow color of the solution almost disappears. Add starch TS, and continue the titration until the blue color disappears entirely. Toward the end of the titration, stopper the container and shake it violently so that any iodine remaining in solution in the glacial acetic acid/cyclohexane, 1:1, solution may be taken up by the potassium iodide solution. Concomitantly, conduct two determinations on blanks in the same manner and at the same temperature. Calculate the iodine value by the formula

$$(B - S) \times 12.69N/W,$$

in which $B - S$ represents the difference between the volumes of sodium thiosulfate required for the blank and for the sample,

respectively; *N* is the normality of the sodium thiosulfate; and *W* is the weight, in g, of the sample taken.

MELTING RANGE

Fats of animal and vegetable origin do not exhibit a sharp melting point. For the purpose of this test, melting range is defined as the range of temperature in which the sample becomes a perfectly clear liquid after first passing through a stage of gradual softening, during which it may become opalescent.

Apparatus Use any suitable commercial or other apparatus. Use melting-point capillary tubes—id, 1 mm; od, 2 mm; length, 50 to 80 mm; and open at both ends.

Procedure

Capillary Method (Based on AOCS Method Cc 1-25) Melt the sample and filter it through filter paper; the sample must be absolutely dry. Dip three capillary tubes in the liquid sample so that the oil stands approximately 10 mm high in the tubes, and fuse the end of the tube containing the sample without burning it. Place the tubes containing the liquid sample in a beaker, and equilibrate them at least 16 h at 4° to 10° in a refrigerator. Determine the melting range, using a temperature increase of 0.5° per min when within 10° of the anticipated melting point. The melting ranges of the three samples should be no more than 0.5° apart.

1-MONOGLYCERIDES

(Based on AOCS Method Cd 11-57)

Reagents and Solutions

Periodic Acid Solution Dissolve 5.4 g of periodic acid, H_5IO_6, in 100 mL of water, add 1900 mL of glacial acetic acid, and mix. Store in a light-resistant, glass-stoppered bottle or in a clear, glass-stoppered bottle protected from light.

Chloroform Use chloroform meeting the following test: To each of three 500-mL flasks add 50.0 mL of *Periodic Acid Solution*, then add 50 mL of chloroform and 10 mL of water to two of the flasks and 50 mL of water to the third. To each flask add 20 mL of potassium iodide TS, mix gently, and continue as directed in the *Procedure*, beginning with "... allow to stand at least 1 min. ..." The difference between the volume of 0.1 *N* sodium thiosulfate required in the titrations with and without the chloroform is not greater than 0.5 mL.

Procedure Melt the sample, if not liquid, at a temperature not higher than 10° above its melting point, and mix thoroughly. Transfer an accurately weighed portion of the sample, equivalent to about 150 mg of 1-monoglycerides, into a 100-mL beaker (or weigh a sample equivalent to 20 mg of glycerin or 30 mg of propylene glycol if only *Free Glycerin or Propylene Glycol* is to be determined), and dissolve in 25 mL of chloroform. Transfer the solution, with the aid of an additional 25 mL of chloroform, into a separator, wash the beaker with 25 mL of water, and add the washing to the separator. Stopper the separator tightly, shake vigorously for 30 to 60 s, and allow the layers to separate. (Add 1 to 2 mL of glacial acetic acid to break emulsions formed due to the presence of soap.) Collect the aqueous layer in a 500-mL glass-stoppered Erlenmeyer flask, and extract the chloroform solution again using two 25-mL portions of water. Retain the combined aqueous extracts for the determination of *Free Glycerin or Propylene Glycol* (in this Appendix). Transfer the chloroform to a 500-mL glass-stoppered Erlenmeyer flask, and add 50.0 mL of *Periodic Acid Solution* to this flask and to each of two blank flasks containing 50 mL of chloroform and 10 mL of water. Swirl the flasks during the addition of the reagent, and allow to stand for at least 30 min, but no longer than 90 min. To each flask, add 20 mL of potassium iodide TS, and allow to stand at least 1 min, but no longer than 5 min, before titrating. Add 100 mL of water, and titrate with 0.1 *N* sodium thiosulfate, using a magnetic stirrer to keep the solution thoroughly mixed, to the disappearance of the brown iodine color, then add 2 mL of starch TS and continue the titration to the disappearance of the blue color. Calculate the percentage of 1-monoglycerides[1] in the sample by the formula

$$(B - S) \times N \times 17.927/W,$$

in which *B* is the number of mL of sodium thiosulfate consumed in the blank determination; *S* is the number of mL required in the titration of the sample; *N* is the exact normality of the sodium thiosulfate; *W* is the weight, in g, of the sample taken; and 17.927 is the molecular weight of glyceryl monostearate divided by 20.

TOTAL MONOGLYCERIDES

Preparation of Silica Gel Place about 10 g of 100- to 200-mesh silica gel of a grade suitable for chromatographic work in a tared weighing bottle, cap immediately, and weigh accurately. Remove the cap, dry at 200° for 2 h, cap immediately, and cool for 30 min. Raise the cap momentarily to equalize the pressure, then weigh again, reheat for 5 min at 200°, cool, and reweigh. Repeat this 5-min drying cycle until two consecutive weights agree within 10 mg. Calculate the percentage of water in the original silica gel (*A*) by the formula

$$(\text{loss in wt/sample wt}) \times 100,$$

[1]The monoglyceride may be calculated to some monoester other than glyceryl monostearate by dividing the molecular weight of the monoglyceride by 20 and substituting the value so obtained for 17.927 in the formula, using 17.80, for example, in calculating to the monooleate.

then calculate the amount of water required to adjust the water content to 5% by the formula

$$W \times (5 - A)/95,$$

in which W is the weight, in g, of the undried sample to be used.

Accurately weigh the appropriate amount of the undried silica gel to be used in the determination, transfer to a suitable blender or mixer, and add the calculated amount of water to give a final water content of 5% ± 0.1%. Blend for 1 h to ensure complete water distribution, and store in a sealed container. Determine the water content of the adjusted silica gel as directed above, and readjust if necessary.

Note: Each new lot of silica gel should be checked for suitability by the analysis of a monoglyceride of known composition.

Sample Preparation (**Caution**: To avoid rearrangement of partial glycerides, use extreme caution in applying heat to samples, and do not heat above 50°.)

Samples Melting Below 50° Melt the sample, if necessary, by warming for short periods below 50°, not exceeding a total of 30 min.

Samples Melting Above 50° Grind about 10 g in a mortar and pestle, chilling solid samples, if necessary, in carbon dioxide.

Weigh accurately about 1 g of the prepared sample into a 100-mL beaker, add 15 mL of chloroform, and warm, if necessary, to effect solution. Use only minimal heat, and do not heat above 40°.

Preparation of Chromatographic Column Connect a 19- × 290-mm chromatographic tube, equipped with an outer 19/22 standard-taper joint at the top and a coarse, fritted-glass disk and inner 19/22 standard-taper joint at the bottom, with an adapter consisting of an outer 19/22 joint connected to a Teflon stopcock. Do not grease the joints. Weigh 30 g of the prepared silica gel into a 150-mL beaker, add 50 to 60 mL of petroleum ether, and stir slowly with a glass rod until all air bubbles are expelled. Transfer the slurry to the column through a powder funnel, and open the stopcock, allowing the liquid level to drop to about 2 cm above the silica gel. Transfer any silica gel slurry remaining in the beaker into the column with a minimum amount of petroleum ether, then rinse the funnel and sides of the column. Drain the solvent through the stopcock until the level drops to 2 cm above the silica gel, and remove the powder funnel.

Procedure Carefully add the *Sample Preparation* to the prepared column. Open the stopcock, and adjust the flow rate to about 2 mL/min, discarding the eluate. Rinse the sample beaker with 5 mL of chloroform, and add the rinsing to the column when the level drops to 2 cm above the silica gel. Never allow the column to become dry on top, and maintain a flow rate of 2 mL/min throughout the elution. Avoid interruptions during elution as they may cause pressure buildup and result in leakage through the stopcock or cracks in the silica gel packing.

Attach a 250-mL reservoir separator, provided with a Teflon stopcock and a 19/22 standard-taper drip tip inner joint, to the column. Add 200 mL of benzene, elute, and discard the eluate, which contains the triglycerides fraction. When the level of benzene drops to 2 cm above the silica gel, add 200 mL of a 1:10 mixture of ether in benzene, elute, and discard the eluate, which contains the diglycerides and the free fatty acid fraction. When all of the ether–benzene solvent has been added from the separator and the level in the column drops to 2 cm above the silica gel, add from 250 to 300 mL of ether, and collect the monoglyceride fraction in a tared flask. Rinse the tip of the column into the flask with a few mL of ether, and evaporate to dryness on a steam bath under a stream of nitrogen or dry air. Cool for at least 15 min, weigh, then reheat on the steam bath for 5 min in the same manner. Cool, reweigh, and repeat the 5-min evaporation, cooling, and reweighing procedures until two consecutive weights agree within 2 mg. The weight of the residue represents the total monoglycerides in the sample taken.

OXYETHYLENE DETERMINATION

Apparatus The apparatus for oxyethylene group determination is shown in Fig. 35. It consists of a boiling flask, *A*, fitted with a capillary side tube to provide an inlet for carbon dioxide and connected by a condenser with trap *B*, which contains an aqueous suspension of red phosphorus. The first absorption tube, *C*, contains a silver nitrate solution to absorb ethyl iodide. Absorption tube *D* is fitted with a 1.75-mm spiral rod (23 turns, 8.5-mm rise per turn), which is required to provide a longer contact of the evolved ethylene with the bromine solution. A standard-taper adapter and stopcock are connected to tube *D* to permit the transfer of the bromine solution into a titration flask without loss. A final trap, *E*, containing a potassium iodide solution, collects any bromine swept out by the flow of carbon dioxide.

Dimensions of the apparatus not readily determined from Fig. 35 are as follows: carbon dioxide inlet capillary, 1-mm id; flask *A*, 28-mm diameter, 12/18 standard-taper joint; condenser, 9-mm id; inlet to trap *B*, 2-mm id; inlet to trap *C*, 7/15 standard-taper joint, 2-mm id; trap *C*, 14-mm id; trap *D*, inner tube, 8-mm od, 2-mm opening at bottom of spiral; outer tube, approximately 12.5-mm id; side arm 7 cm from top of inserted spiral, 3.5-mm id, 2-mm opening at bottom.

Reagents

Hydriodic Acid Use special-grade hydriodic acid suitable for alkoxyl determinations, or purify reagent-grade as follows: Distill over red phosphorus in an all-glass apparatus, passing a slow stream of carbon dioxide through the apparatus until the distillation is terminated and the receiving flask has completely cooled.

Caution: Use a safety shield, and conduct the distillation in a hood.

Silver Nitrate Solution Dissolve 15 g of silver nitrate in 50 mL of water, mix with 400 mL of alcohol, and add a few drops of nitric acid.

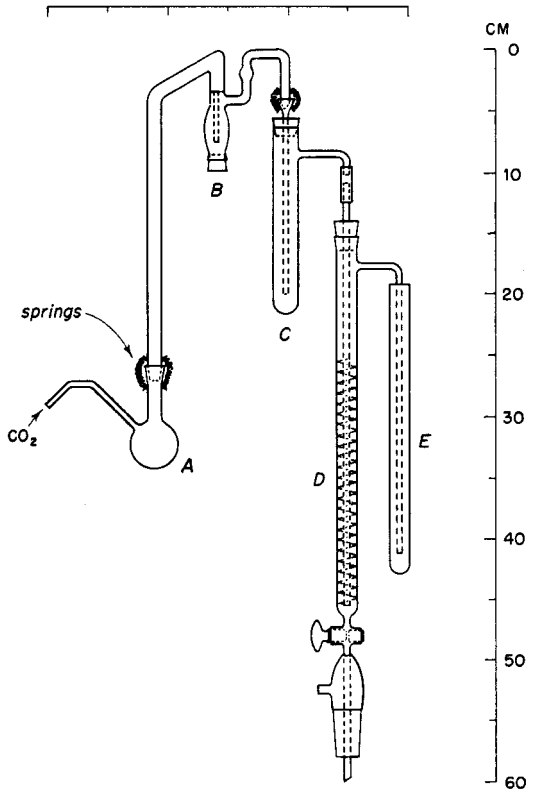


FIGURE 35 Apparatus for Oxyethylene Determination.

Bromine–Bromide Solution Add 1 mL of bromine to 300 mL of glacial acetic acid saturated with dry potassium iodide (about 5 g). Fifteen mL of this solution requires about 40 mL of 0.05 *N* sodium thiosulfate. Store in a brown bottle in a dark place, and standardize at least once a day during use.

Procedure Fill trap *B* with enough of a suspension of 60 mg of red phosphorus in 100 mL of water to cover the inlet tube. Pipet 10 mL of the *Silver Nitrate Solution* into tube *C* and 15 mL of the *Bromine–Bromide Solution* into tube *D*, and place 10 mL of a 1:10 solution of potassium iodide in trap *E*. Transfer an accurately weighed quantity of the sample specified in the monograph into the reaction flask, *A*, and add 10 mL of *Hydriodic Acid* along with a few glass beads or boiling stones. Connect the flask to the condenser, and begin passing carbon dioxide through the apparatus at the rate of about one bubble per s. Heat the flask in an oil bath at 140° to 145°, and continue the reaction at this temperature for at least 40 min. Heating should be continued until the cloudy reflux in the condenser becomes clear and until the supernatant liquid in the silver nitrate tube, *C*, is almost completely clarified. Five min before the reaction is terminated, heat the *Silver Nitrate Solution* in tube *C* in a hot water bath at 50° to 60° to expel any dissolved olefin. At the completion of the decomposition, cautiously disconnect tubes *D* and *C* in the order named, then disconnect the carbon dioxide source and remove the oil bath. Connect tube *D* to a 500-mL iodine flask containing 150 mL of water and 10 mL of a 1:10 solution of potassium iodide, run the *Bromine–Bromide Solution* into the flask, and rinse the tube and spiral with water. Add the potassium iodide solution from trap *E* to the flask, rinsing the side arm and tube with a few mL of water, stopper the flask, and allow to stand for 5 min. Add 5 mL of 2 *N* sulfuric acid, and titrate immediately with 0.05 *N* sodium thiosulfate, using 2 mL of starch TS for the endpoint. Transfer the silver nitrate solution from tube *C* into a flask, rinsing the tube with water, dilute to 150 mL with water, and heat to boiling. Cool, and titrate with 0.05 *N* ammonium thiocyanate, using 3 mL of ferric ammonium sulfate TS as the indicator. Perform a blank determination. Calculate the percentage of oxyethylene groups (—CH_2CH_2O—), as ethylene, by the formula

$$(B - S) \times N \times 2.203/W,$$

in which $B - S$ represents the difference between the volumes of sodium thiosulfate required for the blank and the sample solution, respectively; *N* is the normality of the sodium thiosulfate; *W* is the weight, in g, of the sample taken; and 2.203 is an equivalence factor for oxyethylene. Calculate the percentage of oxyethylene groups, as ethyl iodide, by the formula

$$(B' - S') \times N' \times 4.405/W,$$

in which $B' - S'$ represents the difference between the volumes of ammonium thiocyanate required for the blank and the sample solution, respectively; N' is the normality of the ammonium thiocyanate; and 4.405 is an equivalence factor for oxyethylene. The sum of the values so obtained represents the percentage of oxyethylene groups in the sample taken.

PEROXIDE VALUE

Transfer about 10 g of sample, accurately weighed, into a suitable container, add 30 mL of a 3:2 mixture of glacial acetic acid:chloroform, and mix. Add 1 mL of a saturated solution of potassium iodide, and mix for 1 min. Add 100 mL of water, begin titrating with 0.05 *N* sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value, as milliequivalents of peroxide per kilogram of sample, by the formula

$$(S \times N \times 1000)/W,$$

in which *S* is the net volume, in milliliters, of sodium thiosulfate solution required for the sample; *N* is the exact normality of the sodium thiosulfate solution; and *W* is the weight, in grams, of the sample taken.

REICHERT-MEISSL VALUE
(Based on AOCS Method Cd 5-40)

The Reichert-Meissl value is a measure of soluble volatile fatty acids (chiefly butyric and caproic). It is expressed in

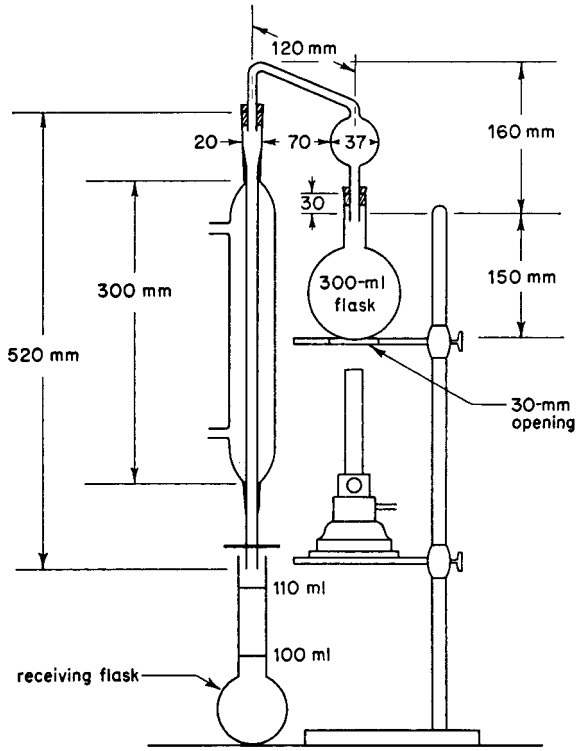


FIGURE 36 Reichert-Meissl Distillation Apparatus. [**Note:** A suitable heating mantle may be substituted for the burner.]

terms of the number of mL of 0.1 *N* sodium hydroxide required to neutralize the fatty acids obtained from a 5-g sample under the specified conditions of the method.

Apparatus Use a glass distillation apparatus of the same dimensions and construction as that shown in Fig. 36.

Reagents

Sodium Hydroxide Solution Prepare a solution containing 50.0% by weight of NaOH, and protect from contact with carbon dioxide. Allow the solution to settle, and use only the clear liquid.

Glycerin–Sodium Hydroxide Mixture Add 20 mL of the *Sodium Hydroxide Solution* to 180 mL of glycerin.

Procedure Unless otherwise directed, accurately weigh about 5 g of the sample, previously melted if necessary, into the 300-mL distillation flask. Add 20.0 mL of the *Glycerin–Sodium Hydroxide Mixture*, and heat until the sample is completely saponified, as indicated by the mixture becoming perfectly clear. Shake the flask gently if any foaming occurs. Add 135 mL of recently boiled and cooled water, dropwise at first to prevent foaming, then add 6 mL of 1:5 sulfuric acid and a few pieces of pumice stone or silicon carbide. Rest the flask on a piece of heat-proof board having a center hole 5 cm in diameter, and begin the distillation, regulating the flame so as to collect 110 mL of distillate in 30 ± 2 min (measure time from the passage of the first drop of distillate from the condenser to the receiving flask), letting the distillate drip into the flask at a temperature not higher than 20°.

When 110 mL has distilled, disconnect the receiving flask, and remove the flame. Mix the contents of the flask with gentle shaking, and immerse almost completely for 15 min in water cooled to 15°. Filter the distillate through dry, 9-cm, moderately retentive paper (S & S No. 589 White Ribbon, or equivalent), add phenolphthalein TS, and titrate 100 mL of the filtrate with 0.1 *N* sodium hydroxide to the first pink color that remains unchanged for 2 to 3 min. Perform a blank determination using the same quantities of the same reagents, and calculate the Reichert-Meissl value by the formula

$$1.1 \times (S - B),$$

in which S is the volume of 0.1 *N* sodium hydroxide required for the sample, and B is the volume required for the blank.

SAPONIFICATION VALUE
(Based on AOCS Methods Tl 1a-64 and Cd 3-25)

The saponification value is defined as the number of mg of potassium hydroxide required to neutralize the free acids and saponify the esters in 1 g of the test substance.

Procedure Melt the sample, if necessary, and filter it through a dry filter paper to remove any traces of moisture. Unless otherwise directed, weigh accurately into a 250-mL flask a sample of such size that the titration of the sample solution after saponification will require between 45% and 55% of the volume of 0.5 *N* hydrochloric acid required for the blank, and add to the flask 50.0 mL of 0.5 *N* alcoholic potassium hydroxide. Connect an air condenser, at least 65 cm in length, to the flask, and reflux gently until the sample is completely saponified (usually 30 min to 1 h). Cool slightly, wash the condenser with a few mL of water, add 1 mL of phenolphthalein TS, and titrate the excess potassium hydroxide with 0.5 *N* hydrochloric acid. Heat the contents of the flask to boiling, again titrate to the disappearance of any pink color that may have developed, and record the total volume of acid required. Perform a blank determination using the same amount of 0.5 *N* alcoholic potassium hydroxide. Calculate the saponification value by the formula

$$56.1(B - S) \times N/W,$$

in which $B - S$ represents the difference between the volumes of 0.5 *N* hydrochloric acid required for the blank and the sample, respectively; N is the normality of the hydrochloric acid; and W is the weight, in g, of the sample taken.

Note: A ''masked phenolphthalein indicator'' may be used with off-color materials. Prepare the indicator by dissolving 1.6 g of phenolphthalein and 2.7 g of methylene blue in 500 mL of alcohol, and adjust the pH with alcoholic alkali solution so that the greenish blue color is faintly tinged with purple. The color change, when going from acid to alkali, is from green to purple.

SOAP

Prepare a solvent mixture consisting of equal parts, by volume, of benzene and methanol, add bromophenol blue TS, and neutralize with 0.5 *N* hydrochloric acid, or use neutralized acetone as the solvent. Accurately weigh the amount of sample specified in the individual monograph, dissolve it in 100 mL of the neutralized solvent mixture, and titrate with 0.5 *N* hydrochloric acid to a definite yellow endpoint. Calculate the percentage of soap in the sample by the formula

$$VNe/W,$$

in which *V* and *N* are the volume and normality, respectively, of the hydrochloric acid; *W* is the weight of the sample, in g; and *e* is the equivalence factor given in the monograph.

SPECIFIC GRAVITY

The specific gravity of a fat or oil is determined at 25°, except when the substance is a solid at that temperature, in which case the specific gravity is determined at the temperature specified in the monograph, and is referred to water at 25°.

Clean a suitable pycnometer by filling it with a saturated solution of chromic acid (CrO_3) in sulfuric acid and allowing it to stand for at least 4 h. Empty the pycnometer, rinse it thoroughly, then fill it with recently boiled water, previously cooled to about 20°, and place in a constant-temperature bath at 25°. After 30 min, adjust the level of water to the proper point on the pycnometer, and stopper. Remove the pycnometer from the bath, wipe dry with a clean cloth free from lint, and weigh. Empty the pycnometer, rinse several times with alcohol and then with ether, allow to dry completely, remove any ether vapor, and weigh. Determine the weight of the contained water at 25° by subtracting the weight of the pycnometer from its weight when full.

Filter the oil or melted sample through filter paper to remove any impurities and the last traces of moisture, and cool to a few degrees below the temperature at which the determination is to be made. Fill the clean, dry pycnometer with the sample, and place it in the constant-temperature bath at the specified temperature. After 30 min, adjust the level of the oil to the mark on the pycnometer, insert the stopper, wipe dry, and weigh. Subtract the weight of the empty pycnometer from its weight when filled with the sample, and divide the difference by the weight of the water contained at 25°. The quotient is the specific gravity at the temperature of observation, referred to water at 25°.

STABILITY (Active Oxygen Method) (Based on AOCS Method Cd 12-57)

Fat stability is the time, in h, required for a sample of fat or oil to attain a peroxide value of 100. This period of time is determined by interpolation between two measurements and is assumed to be an index of resistance to rancidity.

> **Caution**: All equipment must be scrupulously clean (for an acceptable cleaning procedure, see AOCS Official Method Cd 12-57). Do not use chromic acid or other acidic cleaning agents. All receptacles in the heater must be calibrated for temperature under the exact conditions of the test. During the test, the temperature must be monitored in a sample tube containing the recommended quantity of oil.

Apparatus Use a suitable heating block and aeration apparatus, such as shown in the Official and Tentative Methods of the AOCS or in JAOCS *33* (1956), pp. 628–630.

Sampling Remove samples from large containers or processing equipment with sampling devices only of stainless steel, aluminum, nickel, or glass. Solid fat samples should be taken at least 5 cm from the walls of large containers and 2.5 cm from the walls of small containers. If liquid oil is to be poured from a container, clean the spout or lip with an acetone-moistened cloth. Under no circumstances should samples be taken from containers equipped with plastic or enameled tops or paper or wax liners.

Procedure Unless already completely liquid, the sample should be melted at a temperature not more than 10° above its melting point. Pour 20 mL into each of two or more sample tubes ensuring that the sample does not contact the tube where the stopper will later fit. Insert the aeration tube assembly so that the end of the air delivery tube is 5 cm below the surface of the sample. Place the sample tube in a container of vigorously boiling water for 5 min (during this time adjust the air flow rate from the manifold). Remove the tube, wipe dry, and transfer immediately to the constant-temperature heater, maintained at 97.8° ± 0.2°, and connect the aeration tube to the manifold. Determine to the nearest h the time required for the sample to attain a *Peroxide Value* (in this appendix) of 100 milliequivalents (meq) as follows: With 1-g samples determine when the peroxide value is approximately 75 meq and 125 meq, then perform the test on four 5-g samples determining the peroxide value in duplicate at the times corresponding to 75 and 125 meq. Make a second determination on two 5-g samples exactly 1 h after the first pair. Plot these values against aeration time; the AOM stability value in h is given where the line crosses 100 meq.

UNSAPONIFIABLE MATTER (Based on AOCS Method Ca 6a-40)

This procedure determines those substances frequently found dissolved in fatty materials that cannot be saponified by alkali hydroxides but that are soluble in the ordinary fat solvents.

Procedure Accurately weigh 5.0 g of the sample into a 250-mL flask, add a solution of 2 g of potassium hydroxide in 40 mL of alcohol, and boil gently under a reflux condenser for 1 h or until saponification is complete. Transfer the contents of the flask to a glass-stoppered extraction cylinder (approximately 30 cm in length, 3.5 cm in diameter, and graduated at 40, 80, and 130 mL). Wash the flask with sufficient alcohol to make a volume of 40 mL in the cylinder, and complete the transfer with warm and then cold water until the total volume is 80 mL. Finally, wash the flask with a few mL of petroleum ether, add the washings to the cylinder, cool the contents of the cylinder to room temperature, and add 50 mL of petroleum ether.

Insert the stopper, shake the cylinder vigorously for at least 1 min, and allow both layers to become clear. Siphon the upper layer as completely as possible without removing any of the lower layer, collecting the ether fraction in a 500-mL separator. Repeat the extraction and siphoning at least six times with 50-mL portions of petroleum ether, shaking vigorously each time. Wash the combined extracts, with vigorous shaking, with 25-mL portions of 10% alcohol until the wash water is neutral to phenolphthalein, and discard the washings. Transfer the ether extract to a tared beaker, and rinse the separator with 10 mL of ether, adding the rinsings to the beaker. Evaporate the ether on a steam bath just to dryness, and dry the residue to constant weight, preferably at 75° to 80° under a vacuum of not more than 200 mm Hg, or at 100° for 30 min. Cool in a desiccator, and weigh to obtain the uncorrected weight of unsaponifiable matter.

Determine the quantity of fatty acids in the residue as follows: Dissolve the residue in 50 mL of warm alcohol (containing phenolphthalein TS and previously neutralized with sodium hydroxide to a faint pink color), and titrate with 0.02 *N* sodium hydroxide to the same color. Each mL of 0.02 *N* sodium hydroxide is equivalent to 5.659 mg of fatty acids, calculated as oleic acid.

Subtract the calculated weight of fatty acids from the weight of the residue to obtain the corrected weight of unsaponifiable matter in the sample.

VOLATILE ACIDITY

Modified Hortvet-Sellier Method

Apparatus Assemble a modified Hortvet-Sellier distillation apparatus as shown in Fig. 37, using a sufficiently large (approximately 38- × 203-mm) inner Sellier tube and large distillation trap.

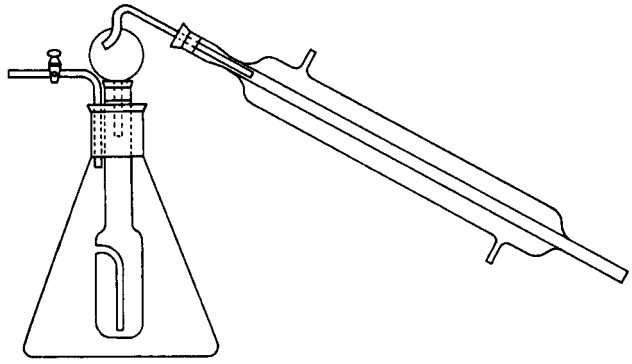


FIGURE 37 Modified Hortvet-Sellier Distillation Apparatus.

Procedure Transfer the amount of sample, accurately weighed, specified in the monograph into the inner tube of the assembly, and insert the tube in the outer flask containing about 300 mL of recently boiled hot water. To the sample add 10 mL of approximately 4 *N* perchloric acid [35 mL (60 g) of 70% perchloric acid in 100 mL of water], and connect the inner tube to a water-cooled condenser through the distillation trap. Distill by heating the outer flask so that 100 mL of distillate is collected within 20 to 25 min. Collect the distillate in 100-mL portions, add phenolphthalein TS to each portion, and titrate with 0.5 *N* sodium hydroxide. Continue the distillation until a 100-mL portion of the distillate requires no more than 0.5 mL of 0.5 *N* sodium hydroxide for neutralization.

Caution: Do not distill to dryness.

Calculate the weight, in mg, of volatile acids in the sample taken by the formula

$$V \times e,$$

in which V is the total volume, in mL, of 0.5 *N* sodium hydroxide consumed in the series of titrations and e is the equivalence factor given in the monograph.

APPENDIX VIII: OLEORESINS

COLOR VALUE

Sample Preparation Transfer 70 to 100 mg of the sample, previously mixed well by shaking and accurately weighed, into a 100-mL volumetric flask, dissolve in acetone, dilute to volume with acetone, and mix. Allow the solution to stand for 2 min, then pipet 10 mL into a second 100-mL volumetric flask, dilute to volume with acetone, and mix.

Procedure Determine the absorbance of the *Sample Preparation* with a suitable spectrophotometer in a 1-cm cell at 460 nm, using acetone as the blank. Record the value obtained as A_S. In the same manner, determine the absorbance of a National Institute of Standards and Technology Standard Glass Filter 930, and record the value obtained as A_F.

> **Note**: The recommended range for absorbance values is between 0.30 and 0.70. Solutions having absorbances greater than 0.70 should be diluted with acetone to one-half the original concentration, and those having absorbances less than 0.30 should be discarded and the *Sample Preparation* prepared with a larger sample. Appropriate adjustments should be made in the sample weight (W) used in the *Calculation* below.

Calculation Determine the instrument correction factor, F, by the formula

$$A_N/A_F,$$

in which A_N is the absorbance of the filter as stated by the National Institute of Standards and Technology. Calculate the color value of the sample by the formula

$$(A_S \times 164 \times F)/W,$$

in which W is the weight, in g, of sample taken.

CURCUMIN CONTENT

Sample Preparation Transfer about 500 mg of sample, accurately weighed, into a 100-mL volumetric flask, and record the weight, in milligrams, as W. Dissolve the sample in about 75 mL of acetone, dilute to volume with acetone, and mix. Pipet a 5-mL portion of this solution into a second 100-mL volumetric flask, dilute to volume with acetone, and mix. Finally, pipet a 1-mL portion of the last solution into a 50-mL volumetric flask, dilute to volume with acetone, and mix.

> **Note**: Protect all solutions from light by using active glassware or by covering the glassware with aluminum foil. Make the absorbance readings as soon as possible after the solutions are prepared.

Procedure Determine the absorbance of the *Sample Preparation* in a 1-cm cell at the wavelength of maximum absorption between 420 and 425 nm with a suitable spectrophotometer, using acetone as the blank. Calculate the percent curcumin in the sample by the formula

$$(A \times 100)(165 \times b \times c),$$

in which A is the absorbance of the *Sample Preparation*; 100 is the conversion to percent; 165 is the absorptivity factor, in liters per gram-centimeter, for curcumin; b is the path length of the cell; and c is the concentration, in grams per liter, of the solution presented to the spectrophotometer.

Calculate c by the formula

$$W \times 5 \times 10^{6},$$

in which W is the starting weight, in milligrams, of the sample, and 5×10^6 is the conversion factor for the dilution schedule.

PIPERINE CONTENT

Stock Standard Solution Purify piperine by repeated crystallization from isopropanol until a product having a melting range of 129° to 130° is obtained. Transfer 100.0 mg of the crystals, accurately weighed, into a 100-mL volumetric flask, dissolve in ethylene dichloride, dilute to volume with ethylene dichloride, and mix. Pipet 10.0 mL of this solution into a second 100-mL volumetric flask, dilute to volume with ethylene dichloride, and mix.

Standard Dilutions Pipet 1.0, 3.0, 5.0, and 10.0 mL of the *Stock Standard Solution* (corresponding to 0.1, 0.3, 0.5, and 1.0 mg of piperine, respectively) into separate 100-mL volumetric flasks, dilute each flask to volume with ethylene dichloride, and mix. Determine the absorbance of each dilution at once, as directed in the *Procedure*.

Sample Preparation Heat a portion of the sample to 100° on a steam bath or in an oven (but not on a hot plate), mix with a glass stirring rod, and transfer 100 mg, accurately weighed, into a 100-mL volumetric flask. Dissolve in ethylene dichloride, dilute to volume with ethylene dichloride, and mix. Pipet 1.0 mL of this solution into a second 100-mL volumetric flask, dilute to volume with ethylene dichloride, and mix. Determine the absorbance of the solution at once, as directed in the *Procedure*.

Procedure Determine the absorbance of the *Sample Preparation* and of each of the *Standard Dilutions* in 1-cm cells at

the wavelength of maximum absorption at about 342 nm with a suitable spectrophotometer, using ethylene dichloride as the blank. Prepare a standard curve of concentration, in mg per 100 mL, versus absorbance for the four *Standard Dilutions*, including the absorbance at zero concentration obtained with the blank. From the standard curve, determine the concentration of piperine in the *Sample Preparation*, and record the value as *C*, in mg per 100 mL. Calculate the percentage of piperine in the sample by the formula

$$100 \times (100C/W),$$

in which *W* is the weight, in mg, of sample taken.

RESIDUAL SOLVENT

This procedure is for the determination of acetone, ethylene dichloride, hexane, isopropanol, methanol, methylene chloride, and trichloroethylene residues.

Distilling Head Use a Clevenger trap designed for use with oils heavier than water. A suitable design is shown in Fig. 38a.

Toluene The toluene used for this analysis should not contain any of the solvents determined by this method. The purity may be determined by gas chromatographic analysis, using one of the following columns or their equivalent: (1) 17% by weight of Ucon 75-H-90,000 on 35/80-mesh Chromosorb W; (2) 20% Ucon LB-135 on 35/80-mesh Chromosorb W; (3) 15% Ucon LB-1715 on 60/80-mesh Chromosorb W; or (4) Porapak Q 50/60 mesh. Follow the conditions described under *Procedure*, and inject the same amount of toluene as will be injected in the analysis of the solvents. If impurities interfering with the test are present, they will appear as peaks occurring before the toluene peak and should be removed by fractional distillation.

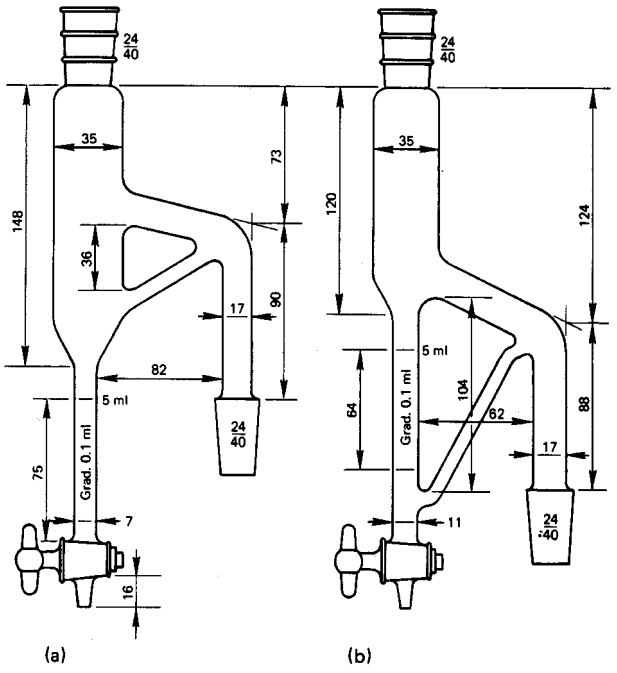


FIGURE 38 Clevenger Traps (all measurements are in mm) (a) Oils Heavier Than Water; (b) Oils Lighter Than Water.

Benzene The benzene used for this analysis should be free from interfering impurities. The purity may be determined as described under *Toluene*.

Detergent and Antifoam Any such products that are free from volatile compounds may be used. If volatile compounds are present, they may be removed by prolonged boiling of the aqueous solutions of the products.

Reference Solution A Prepare a solution in *Toluene* containing 2500 ppm of benzene. If the toluene available contains benzene as the only impurity, the benzene level can be determined by gas chromatography and sufficient benzene added to bring the level to 2500 ppm.

Reference Solution B Prepare a solution containing 0.63% v/w of acetone in water.

Sample Preparation A (all solvents except methanol) Place 50.0 g of the sample, 1.00 mL of *Reference Solution A*, 10 g of anhydrous sodium sulfate, 50 mL of water, and a small amount each of *Detergent* and *Antifoam* in a 250-mL round-bottom flask with a 24/40 ground-glass neck. Attach the *Distilling Head*, a 400-mm water-cooled condenser, and a receiver, and collect approximately 15 mL of distillate. Add 15 g of anhydrous potassium carbonate to the distillate, cool while shaking, and allow the phases to separate. All of the solvents except methanol will be present in the toluene layer, which is used in the *Procedure*. Draw off the aqueous layer for use in *Sample Preparation B*.

Sample Preparation B (methanol only) Place the aqueous layer obtained from *Sample Preparation A* in a 50-mL round-bottom distilling flask with a 24/40 ground-glass neck, add a few boiling chips and 1.00 mL of *Reference Solution B*, and collect approximately 1 mL of distillate, which will contain any methanol from the sample, together with acetone as the internal standard. The distillate is used in the *Procedure*.

Procedure Use a gas chromatograph equipped with a hot-wire detector and a suitable sample-injection system or on-column injection. Under typical conditions, the instrument contains a 1/4-in. (od) × 6- to 8-ft column, or equivalent, maintained isothermally at 70° to 80°. The flow rate of dry carrier gas is 50 to 80 mL/min, and the sample size is 15 to 20 µL (for the hot-wire detector). The column selected for use in the chromatograph depends on the components to be analyzed and, to a certain extent, on the preference of the analyst. The columns 1, 2, 3, and 4, as described under *Toluene*, may be used as follows: (1) This column separates acetone and methanol from their aqueous solution. It may be used for

the separation and analysis of hexane, acetone, and trichloroethylene in the toluene layer from *Sample Preparation A*. The elution order is acetone, methanol, and water, or hexane, acetone, isopropanol plus methylene chloride, benzene, trichloroethylene, and ethylene dichloride plus toluene. (2) This column separates methylene chloride and isopropanol, and ethylene dichloride. The elution order is hexane plus acetone, methylene chloride, isopropanol, benzene, ethylene dichloride, trichloroethylene, and toluene. (3) This is the best general-purpose column, except for the determination of methanol. The elution order is hexane, acetone, benzene, ethylene dichloride, and toluene. (4) This column is used for the determination of methanol, which elutes just after the large water peak.

Calibration Determine the response of the detector for known ratios of solvents by injecting known mixtures of solvents and benzene in toluene. The levels of the solvents and benzene in toluene should be of the same magnitude as they will be present in the sample under analysis.

Calculate the areas of the solvents with respect to benzene, and then calculate the calibration factor, F, as follows:

$$F \text{ (solvent)} = (\text{wt \% solvent/wt \% benzene}) \times (\text{area of benzene/area of solvent}).$$

The recovery of the various solvents from the oleoresin sample, with respect to the recovery of benzene, is as follows: hexane, 52%; acetone, 85%; isopropanol, 100%; methylene chloride, 87.5%; trichloroethylene, 113%; ethylene dichloride, 102%; and methanol, 87%.

Calculation Calculate the ppm of residual solvent (except methanol) by the equation

$$\text{Res. solv.} = \{[43.4 \times F \text{ (solvent)} \times 100]/[\% \text{ recovery of solvent}] \times (\text{area of solvent/area of benzene}),$$

in which 43.4 is the ppm of benzene internal standard, related to the 50-g oleoresin sample taken for analysis. Calculate the ppm of residual methanol by the equation

$$\text{Methanol} = \{[100 \times F \text{ (methanol)}]/0.87\} \times (\text{area of methanol/area of benzene}),$$

in which 100 is the ppm of acetone internal standard, related to the 50-g oleoresin sample taken for analysis.

SCOVILLE HEAT UNITS

Sample Preparation Transfer 200 mg of the sample into a 50-mL volumetric flask, dilute to volume with alcohol, and mix thoroughly by shaking. Allow the insolubles to settle before use.

Sucrose Solution Prepare a suitable volume of a 10% w/v solution of sucrose in water.

Standard Solution Add 0.15 mL of the *Sample Preparation* to 140 mL of the *Sucrose Solution*, and mix. This solution contains the equivalent of 240,000 Scoville Heat Units.

Test Solutions If the oleoresin sample is claimed to contain more than 240,000 Scoville Heat Units, prepare one or more dilutions according to the following table:

Scoville Heat Units	Standard Solution (mL)	Sucrose Solution (mL)
360,000	20	10
480,000	20	20
600,000	20	30
720,000	20	40
840,000	20	50
960,000	20	60
1,080,000	20	70
1,200,000	20	80
1,320,000	20	90
1,440,000	20	100
1,560,000	20	110
1,680,000	20	120
1,800,000	20	130
1,920,000	20	140
2,040,000	20	150

If the oleoresin sample is claimed to contain less than 240,000 Scoville Heat Units, prepare one or more dilutions according to the following table:

Scoville Heat Units	Sample Preparation (mL)	Sucrose Solution (mL)
100,000	0.15	60
117,500	0.15	70
170,000	0.15	100
205,000	0.15	120

Procedure Select five panel members who are thoroughly experienced with this method. Instruct the panelists to swallow 5 mL of the solution corresponding to the claimed content of Scoville Heat Units. The sample passes the test if three of the five panel members perceive a pungent or stinging sensation in the throat.

VOLATILE OIL CONTENT

Weigh accurately an amount of sample sufficient to yield 2 to 5 mL of volatile oil, and transfer with the aid of water into a 1000- or 2000-mL round-bottom shortneck flask with a 24/40 ground-glass neck. Add a magnetic stirring bar and about

500 mL of water, and connect a Clevenger trap of the proper type (see Figs. 38a and 38b) and a 400-mm water-cooled condenser. Heat the flask with stirring, and distill at a rate of 1 to 1.5 drops per s until two consecutive readings taken at 1-h intervals show no change of oil volume in the trap. Cool to room temperature, allow to stand until the oil layer is clear, and read the volume of oil collected, estimating to the nearest 0.02 mL. Calculate the percentage (v/w) of volatile oil in the sample by the formula

$$100(V/W),$$

in which V is the volume, in mL, of oil collected, and W is the weight, in g, of sample taken.

APPENDIX IX: ROSINS AND RELATED SUBSTANCES

ACID NUMBER

The acid number is the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the test substance.

Procedure Unless otherwise directed in the individual monograph, transfer about 4 g of the sample, previously crushed into small lumps and accurately weighed, into a 250-mL Erlenmeyer flask, and add 100 mL of a 1:3 mixture of toluene–isopropyl alcohol, previously neutralized to phenolphthalein TS with sodium hydroxide. Dissolve the sample by shaking or heating gently, if necessary, then add about 0.5 mL of phenolphthalein TS, and titrate with 0.5 *N* or 0.1 *N* alcoholic potassium hydroxide to the first pink color that persists for 30 s. Calculate the acid number by the formula

$$56.1V \times N/W,$$

in which V is the exact volume, in mL, and N is the exact normality, respectively, of the potassium hydroxide solution, and W is the weight, in g, of the sample.

SOFTENING POINT

Drop Method

The *drop softening point* is that temperature at which a given weight of rosin or rosin derivative begins to drop from the bulb of a special thermometer mounted in a test tube that is immersed in a constant-temperature bath.

Apparatus The apparatus illustrated in Fig. 39 consists of the components described in the following paragraphs.

Thermometer Use a special total-immersion softening point thermometer,[1] covering the range from 0° to 250° and graduated in 10 divisions. The bulb should be 15.9 ± 0.8 mm in length and 6.35 ± 0.4 mm in diameter.

Heating Bath Use an 800- to 2000-mL beaker containing a suitable heating medium. For rosins having a softening point below 80°, use water; for those having softening points above 80°, use glycerin or silicone oil, depending upon the temperature range required. Maintain the temperature of the heating medium within ±1° of the temperature specified in the individual monograph. Stir the bath medium constantly during the test with a suitable mechanical stirrer to ensure uniform heating of the medium.

Test Tube Use a standard 22-mm od × 200- to 250-mm test tube with a rim, fitted with a cork stopper as shown in Fig. 39.

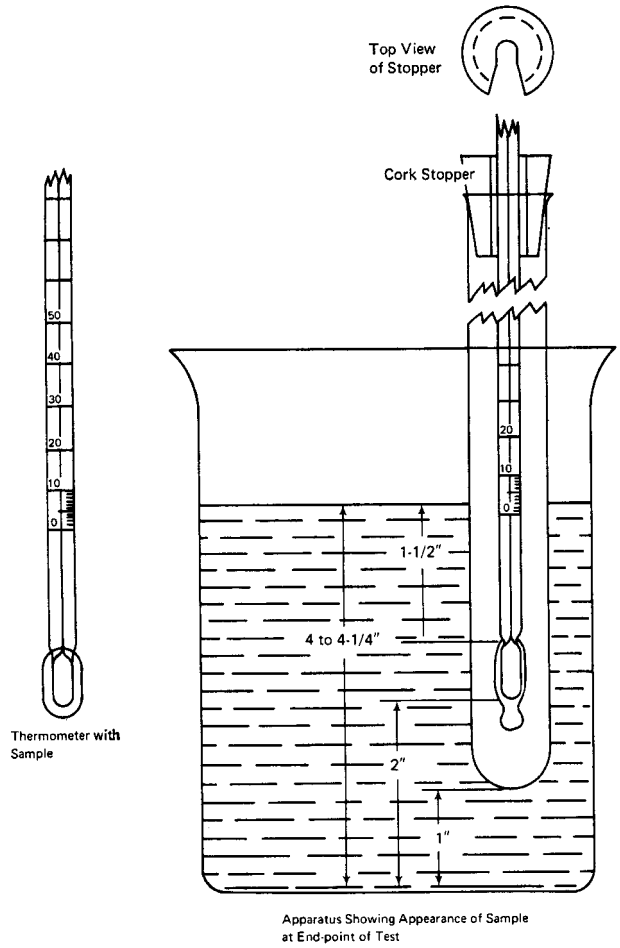


FIGURE 39 Apparatus for Drop Softening Point Determination.

[1] Available from the Walter K. Kessler Co., Inc.

Sample Preparation Place about 20 g of the sample in a 50-mL beaker, and heat it in an oven, on a sand bath or hot plate, or in an oil bath until the sample becomes soft enough to mold on the thermometer bulb. Tare the softening point thermometer, and cautiously warm the bulb over a hot plate until it registers 15° to 20° above the expected softening point of the sample. Immediately dip the thermometer bulb into the melted sample, withdraw, and rotate it to deposit a uniform film of the molten sample over the surface of the bulb, taking care not to extend the film higher than the top of the bulb. Quickly place the thermometer on a balance, and weigh. The weight of the sample on the thermometer bulb should be between 0.5 and 0.55 g. If the weight is low, again dip the bulb in the molten sample; if the weight is high, pull off some of the sample with the fingers. When the correct sample weight has been obtained, mold the sample uniformly around the bulb by rolling it on the palm of the hand or between the fingers. The sample must be of uniform thickness over the bulb, and it must not extend up onto the thermometer stem (see Fig. 39). (If the film of the sample is not uniform when cooled, remove it completely from the bulb and apply a new one. Do not reheat the film and try to remold it.) Allow the film and thermometer to cool to approximately 35° or lower, allowing about 15 min for cooling.

> **Note**: If samples having high softening points crack or ''check'' on the thermometer bulb upon cooling to room temperature, prepare another sample film and cool only to about 50° below the expected softening point.

Procedure Fill the glass beaker to a depth of not less than 101.6 mm or more than 108 mm with a suitable heating medium; support the beaker over a Bunsen burner, hot plate, or other suitable source of heat; and insert the bath stirrer and a bath temperature thermometer. Place the stirrer to one side so that the impeller clears the side of the beaker and is about 12.7 mm above the bottom of the beaker. Start the stirrer, heat the bath to the temperature specified in the individual monograph, and maintain this temperature within ±1° throughout the test.

Insert the prepared sample thermometer in the test tube, supporting it with a notched cork stopper so that the lower end of the bulb is 25.4 mm from the bottom of the test tube. Place the test tube in the bath so that the bottom of the thermometer bulb is 50.8 mm from the bottom of the beaker; the top of the bulb should be about 25.4 to 38.1 mm below the liquid level of the bath. Stir the bath to keep its temperature uniform throughout. Observe the sample thermometer, and record as the softening point the reading at which the elongated drop of sample on the end of the bulb first becomes constricted (see Fig. 39). Report the softening point to the nearest 1.0°.

> **Caution**: If the rosin crystallizes, thus making it difficult to obtain the correct softening point, prepare a new sample by heating the rosin rapidly, yet cautiously, over a flame to a temperature of 160° to 170° to destroy all crystal nuclei. Dip the thermometer bulb into the molten resin, remove it momentarily, and rotate the thermometer to provide a uniform resin film on the bulb as it partially cools in the air. Dip the bulb in the melted sample repeatedly until the proper amount of resin is deposited on the bulb. Do not report results if a crystal-free sample cannot be obtained.

Ring-and-Ball Method

The *ring-and-ball softening point* is the temperature at which a disk of the sample held within a horizontal ring is forced downward a distance of 25.4 mm under the weight of a steel ball as the sample is heated at a prescribed rate in a water, glycerin, or silicone oil (Dow Corning 200 fluid 50 cs or an equivalent is suitable) bath.

Apparatus Ring-and-ball softening point may be determined manually using the apparatus described below. Automated apparatus may be used provided equivalent results are obtained. The calibration of any automated apparatus should be monitored on a regular basis because accurate temperature control is required. The apparatus illustrated in Figs. 40 and 41 consists of the components described in the following paragraphs.

Ring Use a brass-shouldered ring conforming to the dimensions shown in Fig. 40*a*. If desired, the ring may be attached by brazing or other convenient manner to a brass wire of about 13 B & S gauge (1.52 to 2.03 mm in diameter) as shown in Fig. 41*a*.

Ball Use a steel ball, 9.53 mm in diameter, weighing between 3.45 and 3.55 g.

Ball-Centering Guide If desired, center the ball by using a guide constructed of brass and having the general shape and dimensions illustrated in Fig. 40*c*.

Container Use a heat-resistant glass vessel, such as an 800-mL low-form Griffin beaker, not less than 85 mm in diameter and not less than 127 mm in depth from the bottom of the flare.

Support for Ring and Thermometer Use any convenient device for supporting the ring and thermometer, provided that it meets the following requirements: (1) the ring is supported in a substantially horizontal position; (2) when the apparatus shown in Fig. 40*d* is used, the bottom of the ring is 25.4 mm above the horizontal plate below it, the bottom surface of the horizontal plate is 12.7 to 19 mm above the bottom of the container, and the depth of the liquid in the container is not less than 101.6 mm; (3) if the apparatus shown in Fig. 41*e* is used, the bottom of the ring is 25.4 mm above the bottom of the container, with the bottom end of the rod resting on the bottom of the container, and the depth of the liquid in the container is not less than 101.6 mm, as shown in Figs. 41*a*, *b*, and *c*; and (4) in both assemblies, the thermometer is suspended so that the bottom of the bulb is level with the bottom of the ring and within 12.7 mm of, but not touching, the ring.

Thermometers Depending on the expected softening point of the sample, use either an ASTM 15C or 15F low-softening-point thermometer (–2° to 80°) or an ASTM 16C or 16F high-softening-point thermometer (30° to 200°), as described under *Thermometers*, Appendix I.

Stirrer Use a suitable mechanical stirrer rotating between 500 and 700 rpm. To ensure uniform heat distribution in the heating medium, the direction of the shaft rotation should

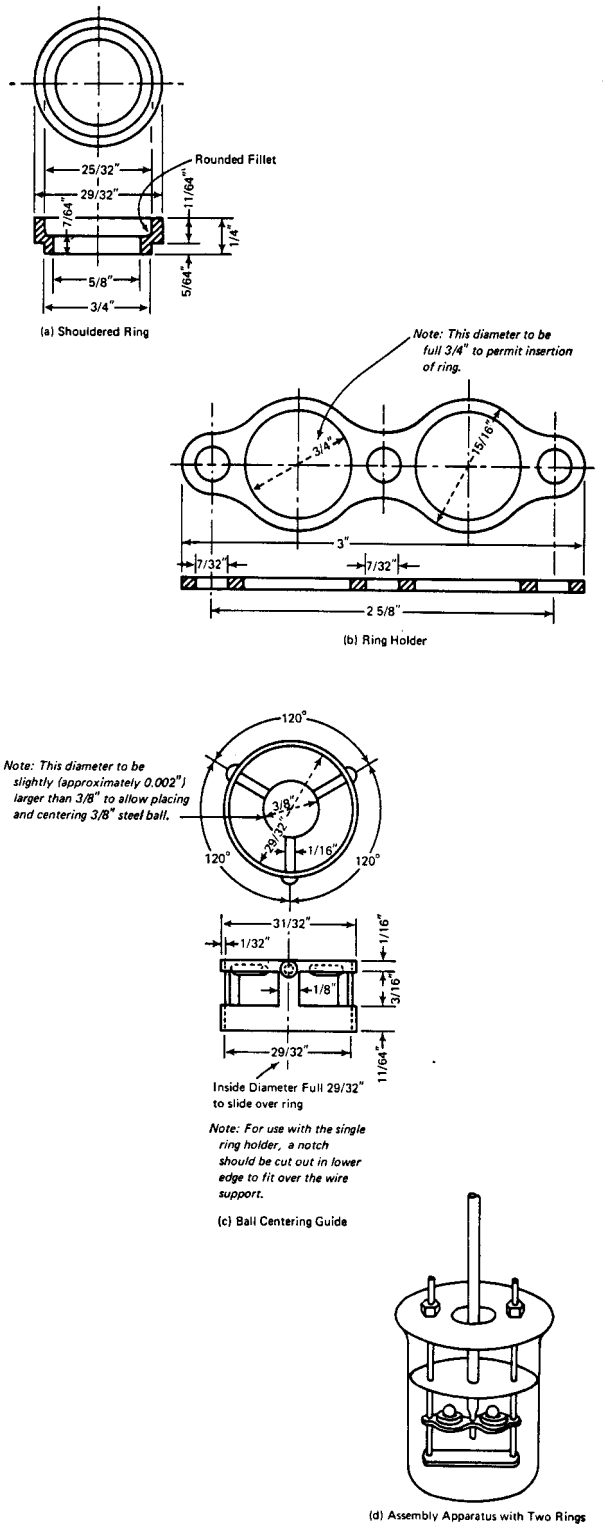


FIGURE 40 Shouldered Ring, Ring Holder, Ball-Centering Guide, and Assembly of Apparatus Showing Two Rings.

move the liquid upward. (See Fig. 41*d* for recommended dimensions.)

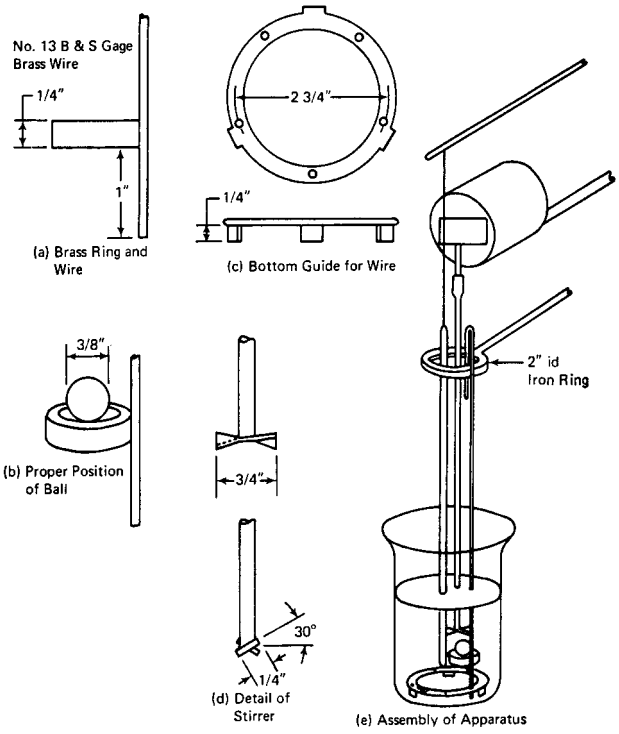


FIGURE 41 Assembly of Apparatus Showing Stirrer and Single-Shouldered Ring.

Sample Preparation Select a representative sample of the material under test consisting of freshly broken lumps free of oxidized surfaces. Immediately before use, scrape off the surface layer of samples received as lumps, avoiding inclusion of finely divided material or dust. The amount of sample taken should be at least twice that necessary to fill the desired number of rings, but in no case less than 40 g. Immediately melt the sample in a clean container, using an oven, hot plate, or sand or oil bath to prevent local overheating. Avoid incorporating air bubbles in the melting sample, which must not be heated above the temperature necessary to pour the material readily without inclusion of air bubbles. The time from the beginning of heating to the pouring of the sample shall not exceed 15 min. Immediately before filling the rings, preheat them to approximately the same temperature at which the sample is to be poured. While being filled, the rings should rest on an aluminum or steel plate. Pour a sufficient amount of the sample into the rings to leave an excess on cooling. Cool for at least 30 min, and then cut the excess material off cleanly with a slightly heated knife or spatula. Use a clean container and a fresh sample if the test is repeated.

Procedure

Materials Having Softening Points above 80° Fill the glass vessel with glycerin to a depth of not less than 101.6 mm and not more than 107.95 mm. The starting temperature of the bath shall be 32°. For resins (including rosin), cool the bath liquid to not less than 27° below the anticipated softening point, but in no case lower than 35°. Position the axis of the stirrer shaft near the back wall of the container, with the

blades clearing the wall and with the bottom of the blades 19 mm above the top of the ring. Unless the ball-centering guide is used, make a slight indentation in the center of the sample by pressing the ball or a rounded rod, slightly heated for hard materials, into the sample at this point. Suspend the ring containing the sample in the bath so that the lower surface of the filled ring is 25.4 mm above the upper surface of the lower horizontal plate (see Fig. 40*d*), which is at least 12.7 mm and not more than 19 mm above the bottom of the glass vessel, or 25.4 mm above the bottom of the container (see Fig. 41*e*). Place the ball in the bath but not on the test specimen. Suspend an ASTM high-softening-point thermometer (16C or 16F) in the bath so that the bottom of its bulb is level with the bottom of the ring and within 12.7 mm of, but not touching, the ring. Maintain the initial temperature of the bath for 15 min. Begin stirring, and continue stirring at 500 to 700 rpm until the determination is complete. Apply heat in such a manner that the temperature of the bath liquid is raised 5° per min, avoiding the effects of drafts by using shields if necessary.

> **Note**: The rate of rise of the temperature should be uniform and should not be averaged over the test period. Reject all tests in which the rate of rise exceeds ±0.5° for any min period after the first three.

Record as the softening point the temperature of the thermometer at the instant the sample touches the lower horizontal plate (see Fig. 40*d*) or the bottom of the container (see Fig. 41*e*). Make no correction for the emergent stem of the thermometer.

Materials Having Softening Points of 80° or Below Follow the above procedure, except use an ASTM low-softening-point thermometer (15C or 15F) and use freshly boiled water cooled to 5° as the heating medium. For resins (including rosins), use water cooled to not less than 27° below the anticipated softening point, but in no case lower than 5°. Report the softening point to the nearest 1.0°.

VISCOSITY

Unless otherwise directed in the individual monograph, transfer the prepared sample into an 8-oz wide-mouth glass jar, 10.8 cm high and 7 cm in inside diameter, equipped with a screw lid. Condition the sample in a water bath at 25° ±0.2° for 30 min (±5 min), taking care to prevent water from coming into contact with the sample. Insert a No. 4 spindle in a Brookfield Model RVF viscometer,[2] or equivalent, and move the jar into place under the spindle, adjusting the elevation of the jar so that the upper surface of the sample is in the center of the shaft indentation and the spindle is in the center of the jar.

> **Note**: Keep the viscometer level at all times during the test procedure.

Set the viscometer to rotate at 20 rpm, and allow the spindle to rotate until a constant dial reading is obtained. The viscosity, in centipoise, is the dial reading on the 0 to 100 scale multiplied by the appropriate factor (for a Brookfield RVF, spindle No. 4, 20 rpm, the factor is 100).

[2]Available from Brookfield Engineering Laboratories, Inc., Stoughton, MA.

APPENDIX X: CARBOHYDRATES (STARCHES, SUGARS, AND RELATED SUBSTANCES)

ACETYL GROUPS

Transfer about 5 g of the sample, accurately weighed, into a 250-mL Erlenmeyer flask, suspend in 50 mL of water, add a few drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a permanent pink endpoint. Add 25.0 mL of 0.45 *N* sodium hydroxide, stopper the flask, and shake vigorously for 30 min, preferably with a mechanical shaker. Remove the stopper, wash the stopper and sides of the flask with a few mL of water, and titrate the excess alkali with 0.2 *N* hydrochloric acid to the disappearance of the pink color, recording the volume, in mL, of 0.2 *N* hydrochloric acid required as *S*. Perform a blank titration of 25.0 mL of 0.45 *N* sodium hydroxide, and record the volume, in mL, of 0.2 *N* hydrochloric acid required as *B*. Calculate the percentage of acetyl groups by the formula

$$\% \text{ Acetyl Groups} = (B - S) \times N \times 0.043 \times 100/W,$$

in which *N* is the exact normality of the hydrochloric acid solution, and *W* is the weight, in g, of the sample.

CRUDE FAT

Apparatus The apparatus consists of a Butt-type extractor,[1] as shown in Fig. 42, having a standard-taper 34/45 female joint at the upper end, to which is attached a Friedrichs- or Hopkins-type condenser, and a 24/40 male joint at the lower end, to which is attached a 125-mL Erlenmeyer flask.

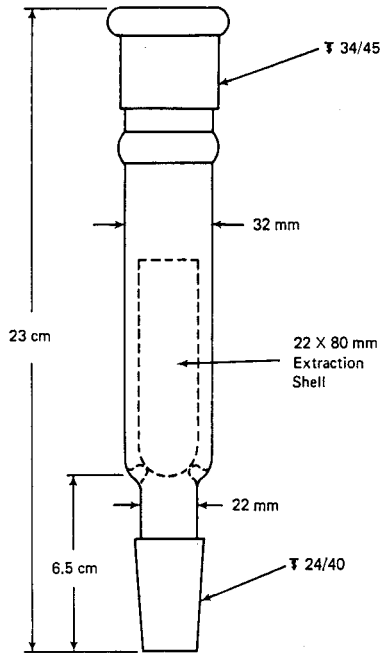


FIGURE 42 Butt-Type Extractor for Crude Fat Determination.

Procedure Transfer about 10 g of the sample, previously ground to 20-mesh or finer and accurately weighed, to a 15-cm filter paper, roll the paper tightly around the sample, and place it in a suitable extraction shell. Plug the top of the shell with cotton previously extracted with hexane, and place the shell in the extractor. Attach the extractor to a dry 125-mL Erlenmeyer flask containing about 50 mL of hexane and to a water-cooled condenser, apply heat to the flask to produce 150 to 200 drops of condensed solvent per min, and extract for 16 h. Disconnect the flask, and filter the extract to remove any insoluble residue. Rinse the flask and filter with a few mL of hexane, combine the washings and filtrate in a tared flask, and evaporate on a steam bath until no odor of solvent remains. Dry in a vacuum for 1 h at 100°, cool in a desiccator, and weigh.

INVERT SUGAR[2]

Assay

Apparatus Mount a ring support on a ringstand 1 to 2 in. above a gas burner, and mount a second ring 6 to 7 in. above the first. Place a 6-in. open-wire gauze on the lower ring to support a 400-mL Erlenmeyer flask, and place a 4-in. watch glass with a center hole on the upper ring to deflect heat. Attach a 50-mL buret to the ringstand so that the tip just passes through the watch glass centered above the flask. Alternatively, a buret with an offset tip may be used in place of a buret with a straight tip extending through the hole in the watch glass. Place an indirectly lighted white surface behind the assembly for observing the endpoint. Alternatively, use a hot titrator/illuminator available from ICUMSA (c/o Central Scientific Laboratories, 445 New Cross Road, London SE 14 6 TA, England).

Mixed Fehling's Solution

Copper Sulfate Solution Dissolve 34.639 g of $CuSO_4 \cdot 5H_2O$ in water; dilute to 500 mL, and filter.

Alkaline Tartrate Solution Dissolve 173 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) and 50 g of NaOH in water, and dilute to 500 mL; allow to stand 2 days, and filter before use.

[1] Available from H.S. Martin & Co., Evanston, IL.

[2] Based on ICUMSA Method GS 4/3-3 (1994).

Just before use, prepare the *Mixed Fehling's Solution* by mixing equal volumes of *Copper Sulfate Solution* and *Alkaline Tartrate Solution*.

Stock Standard Solution Transfer approximately 9.5 g of NF-grade sucrose, accurately weighed, to a 1000-mL volumetric flask; dissolve in 100 mL of water, add 5 mL of hydrochloric acid, and store 3 days at 20° to 25°. Dilute to volume with water. This solution is stable for several months.

Sample Solution Transfer 10 g or a suitable weight of sample, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water so that the final *Sample Solution* contains between 250 and 400 mg of Invert Sugar per 100 mL.

Invert Sugar Solution (0.25 g per 100 mL) Immediately before use in standardizing the *Mixed Fehling's Solution*, pipet 25 mL of *Stock Standard Solution* into a 100-mL volumetric flask, dilute to volume with water, and mix.

Standardized Fehling's Solution To 20 mL of *Mixed Fehling's Solution* in a 400-mL flask containing a few boiling chips add 15 mL of water and 39 mL of *Invert Sugar Solution*. Mix by swirling, heat, and titrate with the *Invert Sugar Solution* as directed under *Procedure*. Adjust the *Mixed Fehling's Solution* for the correct amount of copper (equivalent to 100 mg of invert sugar), and restandardize if the total volume of *Invert Sugar Solution* is more or less than 40 mL.

Procedure

Invert Sugar Conduct a preliminary test to ascertain the volume of water to be added to the 20 mL of *Standardized Fehling's Solution* to obtain a final total volume of 75 mL when the endpoint of the titration is reached. The invert sugar content of the *Sample Solution* should be between 250 and 400 mg per 100 mL so that a titer between 25 and 40 mL is needed to achieve the endpoint. Calculate the amount of water to be added to the *Mixed Fehling's Solution* as the difference

$$75 - [20 \text{ (mL of } \textit{Mixed Fehling's Solution)} + \text{(number of mL of preliminary titer)}].$$

Pipet 20 mL of *Mixed Fehling's Solution* in a 400-mL flask containing a few glass beads or boiling chips, add the required amount of water and mix. Rinse a 50-mL buret, and fill with *Sample Solution*. Rapidly add the *Sample Solution* within 0.5 mL of the endpoint, mix by swirling at room temperature. Immediately place the flask on the wire gauze, adjust the burner flame so that the boiling point of the solution is reached in 2 min. Boil gently but steadily for 2 min. As boiling continues, add 3 to 4 drops of 1% aqueous methylene blue indicator. Complete the titration within 1 min by adding the *Sample Solution* dropwise or in small increments until the blue color disappears. Allow a 5-s reaction time between drops at the end of the titration. Calculate the percent of invert sugar, P_I, in the sample by using the following equation:

$$P_I = f \times 10000/C_S V,$$

in which f is the correction factor for the apparent reducing power of sucrose as seen from the table immediately following this *Assay* ($f = 1$ if no sucrose is present), C_S is the concentration (dry basis), in mg/mL, of the *Sample Solution*, and V is the volume, in mL, of the *Sample Solution* used in the titration.

Sucrose Pipet 100 mL of *Sample Solution* into a 200-mL volumetric flask, and add slowly 10 mL of 2.7 *N* hydrochloric acid, diluted 1:1, while gently swirling the solution; place in a constant-temperature bath maintained at 60°; agitate continuously for 3 min; and allow to sit in the bath for an additional 7 min. Remove the flask from the bath, and cool to 20° as rapidly as possible; dilute to volume with water, and mix well. Continue as directed in the *Procedure* (above) under *Invert Sugar*. Calculate the percent invert sugar present after hydrolysis (P_H) using the equation

$$P_H = 20000/C_S V_H,$$

in which C_S is the concentration, in mg/mL, of sample in the *Sample Solution*, as defined above, and V_H is the volume, in mL, of the hydrolyzed *Sample Solution* used in the titration. If V_H falls outside the limits of 25 to 40 mL, repeat the hydrolysis with a different volume of *Sample Solution*. Calculate the percent sucrose by using the following equation:

$$P_S = 0.95(P_H - P_I),$$

in which P_H and P_I are the percentages of invert sugar determined after and before hydrolysis, respectively.

ICUMSA Table: Sucrose Correction to Be Applied in Lane & Eynon Constant Volume Method

Sucrose in Boiling Mixture, g	Correction Factor (f)	Sucrose in Boiling Mixture, g	Correction Factor (f)
0.5	0.988	5.5	0.900
1.0	0.975	6.0	0.894
1.5	0.962	6.5	0.889
2.0	0.950	7.0	0.884
2.5	0.942	7.5	0.879
3.0	0.934	8.0	0.874
3.5	0.925	8.5	0.870
4.0	0.917	9.0	0.865
4.5	0.912	9.5	0.861
5.0	0.906	10.0	0.856

LACTOSE

Assay

Apparatus Use a suitable high-performance liquid chromatographic system (see *Chromatography*, Appendix IIA) equipped with a differential refractometer detector, a precol-

umn, an online 0.45-μm filter, and a 250-mm × 4.6-mm (id) stainless steel column, or equivalent.

Solid Phase Microparticle silica gel with siloxane bonded cyano-amino moieties (Whatman P-10 carbohydrate, or equivalent) equilibrated and operated at room temperature.

Mobile Phase Acetonitrile–water (80:20) at a flow rate of 2 mL/min.

Reagents

Acetonitrile An appropriate grade for liquid chromatography.

Fructose Internal Standard Solution Prepare a solution of fructose to be used as an internal standard by transferring 50 g of commercial grade β-D(–)fructose powder to a 500-mL volumetric flask, and dissolve in and dilute to volume with water.

Standard Solution Transfer about 2 g of NF-grade anhydrous lactose, accurately weighed, to a 100-mL volumetric flask, add 10 mL of *Fructose Internal Standard Solution,* and dilute to volume with water. Prepare fresh daily.

Water An appropriate grade for liquid chromatography.

System Suitability (See *Chromatography*, Appendix IIA.)

Repeatability Allow the chromatographic system to equilibrate at a flow rate of 2 mL/min, then inject 25-μL aliquots of the *Standard Solution*. The chromatogram should show baseline resolution and a retention time for water of 1 to 2 min; fructose, 2 to 3 min; and lactose, 5 to 6 min. The coefficient of variation for the relative peak heights (lactose peak height/fructose peak height) for ten injections should be ≤0.6% when column equilibration is complete.

Linearity of Detector Response On a monthly basis (or when changes in the system are made), monitor the linearity of detector response by injecting standard lactose solutions containing 1.4%, 1.8%, 2.0%, 2.2%, and 2.6% lactose. Linear regression of the curve generated by plotting peak height versus concentration should give a correlation coefficient of at least 0.999.

Sample Preparation Prepare the sample as directed in the individual monograph. Analysis must be performed within 24 h.

Procedure Inject triplicate 25-μL aliquots of sample and standard solutions. If more than one sample is to be analyzed, inject the standard solution after every third sample. Calculate results using average standard response factors bracketing every three samples (see *Chromatography*, Appendix IIA).

Calculation Calculate the % Lactose (dry basis) by the formula

$$(R_L/R_F) \times (W_L/W_S) \times (100 - M_L/100 - M_S) \times P,$$

in which R_L and R_F are the response factors for lactose and fructose; W_S and W_L are the weights, in g, of the sample and lactose standard in their respective solutions; M_S and M_L are the percentages of moisture in the sample and lactose standard; and P is the purity, in percent, of the lactose standard. Determine the moisture content by drying at 120° for 16 h.

PROPYLENE CHLOROHYDRIN
(2-Chloro-1-propanol)

Special Apparatus

Gas Chromatograph (See *Chromatography*, Appendix IIA.) Use a suitable gas chromatograph. A dual-column, or equivalent, instrument equipped with a flame-ionization detector and an integrator is preferred.

Concentrator Use a Kuderna-Danish concentrator having a 500-mL flask, available from Kontes Glass Co., Vineland, NJ (Catalog No. K-57000), or equivalent.

Pressure Bottles Use 200-mL pressure bottles, with a Neoprene washer, glass stopper, and attached wire clamp, available from Fisher Scientific Co. (Vitro 400, Catalog No. 3-100), or equivalent.

Gas Chromatography Column Use a stainless steel column, or equivalent, 3 m × 3.2 mm (od), packed with 10% Carbowax 20 M on 80/100-mesh Gas Chrom 2, or equivalent. After packing and before use, condition the column overnight at 200°, using a helium flow of 25 mL/min.

Reagents

Diethyl Ether Use anhydrous, analytical reagent-grade diethyl ether, available from Fisher Scientific Co. or J. T. Baker Co., or other suitable sources.

> **Note**: Some lots of diethyl ether contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. If the ether quality is unknown or suspect, concentrate 50 mL to a volume of about 1 mL in the concentrator, and then chromatograph a 2.0-μL portion using the conditions outlined under the *Procedure*. If the chromatogram is excessively noisy and contains signal peaks that overlap or interfere in the measurement of the peaks produced by the propylene chlorohydrin isomers, the ether should be redistilled.

Florisil PR Use 60/100-mesh material, available from Floridin Co., 3 Penn Center, Pittsburgh, PA 15235, or an equivalent product available from Supelco, Bellefonte, PA 16823.

Propylene Chlorohydrins Use 1-Chloro-2-propanol Practical Grade, containing 25% 2-Chloro-1-propanol, available from Aldrich Chemical Company, Milwaukee, WI 53233.

Standard Preparation Draw 25 μL of *Propylene Chlorohydrins* into a 50-μL syringe, weigh accurately, and discharge the contents into a 500-mL volumetric flask partially filled with water. Reweigh the syringe, and record the weight of the chlorohydrins taken. Dilute to volume with water, and mix. This solution contains about 27.5 mg of mixed chlorohydrins, or about 55 μg/mL. Prepare this solution fresh daily.

Sample Preparation Transfer a blended representative 50.0-g sample into a pressure bottle, and add 125 mL of 2 *N* sulfuric acid. Clamp the top in place, and swirl the contents until the sample is completely dispersed. Place the bottle in a boiling water bath, heat for 10 min, then swirl the bottle to

mix the contents, and heat in the bath for an additional 15 min. Cool in air to room temperature, then neutralize the hydrolyzed sample to pH 7 with 25% sodium hydroxide solution, and filter through Whatman No. 1 paper, or equivalent, in a Büchner funnel, using suction. Wash the bottle and filter paper with 25 mL of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, and stir with a magnetic stirring bar for 5 to 10 min, or until the sodium sulfate is completely dissolved. Transfer the solution into a 500-mL separator equipped with a Teflon plug, rinse the flask with 25 mL of water, and combine the washings with the sample solution. Extract with five 50-mL portions of *Diethyl Ether*, allowing at least 5 min in each extraction for adequate phase separation. Transfer the combined ether extracts in a concentrator, place the graduated receiver of the concentrator in a water bath maintained at 50° to 55°, and concentrate the extract to a volume of 4 mL.

> **Note**: Ether extracts of samples may contain foreign residues that interfere with the analysis and/or interpretation of the chromatograms. These residues are believed to be degradation products arising during the hydrolysis treatment. Analytical problems created by their presence can be avoided through application of a cleanup treatment performed as follows: Concentrate the ether extract to about 8 mL, instead of 4 mL specified above. Add 10 g of *Florisil PR*, previously heated to 130° for 16 h just before use, to a chromatographic tube of suitable size, then tap gently, and add 1 g of anhydrous sodium sulfate to the top of the column. Wet the column with 25 mL of *Diethyl Ether*, and quantitatively transfer the concentrated extract to the column with the aid of small portions of the ether. Elute with three 25-mL portions of the ether, collect all of the eluate, transfer it to a concentrator, and concentrate to a volume of 4 mL.

Cool the extract to room temperature, transfer it quantitatively to a 5.0-mL volumetric flask with the aid of small portions of *Diethyl Ether*, dilute to volume with the ether, and mix.

Control Preparations Transfer 50.0-g portions of unmodified (underivatized) waxy corn starch into five separate pressure bottles, and add 125 mL of 2 *N* sulfuric acid to each bottle. Add 0.0, 0.5, 1.0, 2.0, and 5.0 mL of the *Standard Preparation* to the bottles, respectively, giving propylene chlorohydrin concentrations, on the starch basis, of 0, 0.5, 1, 2, and 5 mg/kg, respectively. Calculate the exact concentration in each bottle from the weight of *Propylene Chlorohydrins* used in making the *Standard Preparation*. Clamp the tops in place, swirl until the contents of each bottle are completely dissolved, and proceed with the hydrolysis, neutralization, filtration, extraction, extract concentration, and final dilution as directed under *Sample Preparation*.

Procedure Perform the analysis by gas chromatography with the gas chromatograph and gas chromatography column previously described. The operating conditions may be varied, depending on the column and instrument used. A suitable chromatogram was obtained using a column oven temperature of 110°, isothermal; injection port temperature of 210°; detector temperature of 240°; and hydrogen (30 mL/min), air (350 mL/min), or helium (25 mL/min), as the carrier gas.

Inject 2.0-µL aliquots of each of the concentrated extracts, prepared as directed under *Control Preparations*, allowing sufficient time between injections for signal peaks corresponding to the two chlorohydrin isomers to be recorded (and integrated) and for the column to be purged. Record and sum the signal areas (integrator outputs) from the two chlorohydrin isomers for each of the controls.

Using identical operating conditions, inject a 2.0-µL aliquot of the concentrated extract prepared as directed under *Sample Preparation*, and record and sum the signal areas (integrator outputs) from the sample.

Calculation Prepare a standard curve for the summed signal areas for each of the controls against the calculated propylene chlorohydrin concentrations, in mg/kg, derived from the actual weight of chlorohydrin isomers used. Using the summed signal areas corresponding to the 1-chloro-2-propanol and 2-chloro-1-propanol from the sample, determine the concentration of mixed propylene chlorohydrins, in mg/kg, in the sample by reference to the calibration plot.

> **Note**: After gaining experience with the procedure and demonstrating that the calibration plot derived from the control samples is linear and reproducible, the number of controls can be reduced to one containing about 5 mg/kg of mixed propylene chlorohydrin isomers. The propylene chlorohydrin level in the sample can then be calculated as follows:

$$\text{Propylene chlorohydrins, mg/kg} = (C \times a)/A,$$

in which C is the concentration, in mg/kg, of propylene chlorohydrins (sum of isomers) in the control; a is the sum of the signal areas produced by the propylene chlorohydrin isomers in the sample; and A is the sum of the signal areas produced by the propylene chlorohydrin isomers in the control.

REDUCING SUGARS ASSAY

Apparatus Mount a ring support on a ringstand 1 to 2 in. above a gas burner, and mount a second ring 6 to 7 in. above the first. Place a 6-in. open-wire gauze on the lower ring to support a 250-mL Erlenmeyer flask, and place a 4-in. watch glass with a center hole on the upper ring to deflect heat. Attach a 25-mL buret to the ringstand so that the tip just passes through the watch glass centered above the flask. Place an indirectly lighted white surface behind the assembly for observing the endpoint.

Standardized Fehling's Solution Measure a quantity of *Fehling's Solution A*, add an equal quantity of *Fehling's Solution B*, and mix (see *Cupric Tartrate TS, Alkaline* in the

section on *Solutions and Indicators*). Immediately before use, standardize as follows: Transfer 3.000 g of primary standard dextrose (NIST Standard Reference Material, or equivalent), previously dried in vacuum at 100° for 2 h, into a 500-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Pipet 25 mL of the mixed Fehling's solution into a 200-mL Erlenmeyer flask containing a few glass beads, and titrate with the standard dextrose solution as directed under *Procedure*. Adjust the concentration of *Fehling's Solution A* by dilution or the addition of copper sulfate, so that the titration requires 20.0 mL of the standard dextrose solution.

Procedure Transfer about 3 g of the sample, accurately weighed, into a 500-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Pipet 25.0 mL of *Standardized Fehling's Solution* into a 200-mL Erlenmeyer flask containing a few glass beads, and add the sample solution from a buret to within 0.5 mL of the anticipated endpoint (determined by preliminary titration). Immediately place the flask on the wire gauze of the *Apparatus*, and adjust the burner so that the boiling point will be reached in about 2 min. Bring to a boil, and boil gently for 2 min. As boiling continues, add 2 drops of a 1% aqueous solution of methylene blue, and complete the titration within 1 min by adding the sample solution dropwise or in small increments until the blue color disappears. Record the volume, in mL, of sample solution required as V. Calculate the percentage of reducing sugars, as D-glucose on the dried basis, by the equation

$$\% \text{ Reducing Sugars} = (500 \times 0.12 \times 100)/(V \times W),$$

in which W is the weight, in g, of the sample of dry substance.

SULFUR DIOXIDE DETERMINATION
(Based on AOAC Method 962.16)

Reagents

3% Hydrogen Peroxide Solution Dilute 30% hydrogen peroxide to 3% with water. Just before use, add 3 drops of methyl red TS, and titrate to a yellow endpoint using 0.01 *N* sodium hydroxide. If the endpoint is exceeded, discard the solution and prepare another 3% hydrogen peroxide solution.

Standardized Titrant Prepare a solution of 0.01 *N* sodium hydroxide.

Nitrogen A source of high-purity nitrogen is required with a flow regulator that will maintain a flow of 200 ± 10 mL/min. To guard against the presence of oxygen in the nitrogen, an oxygen scrubbing apparatus or solution such as an alkaline pyrogallol trap may be used. Prepare the pyrogallol trap as follows: Add 4.5 g of pyrogallol to the trap, purge the trap with nitrogen for 2 to 3 min, and add potassium hydroxide solution (65 g of potassium hydroxide added to 85 mL of water) to the trap while maintaining an atmosphere of nitrogen in the trap.

Caution: Exothermic reaction.

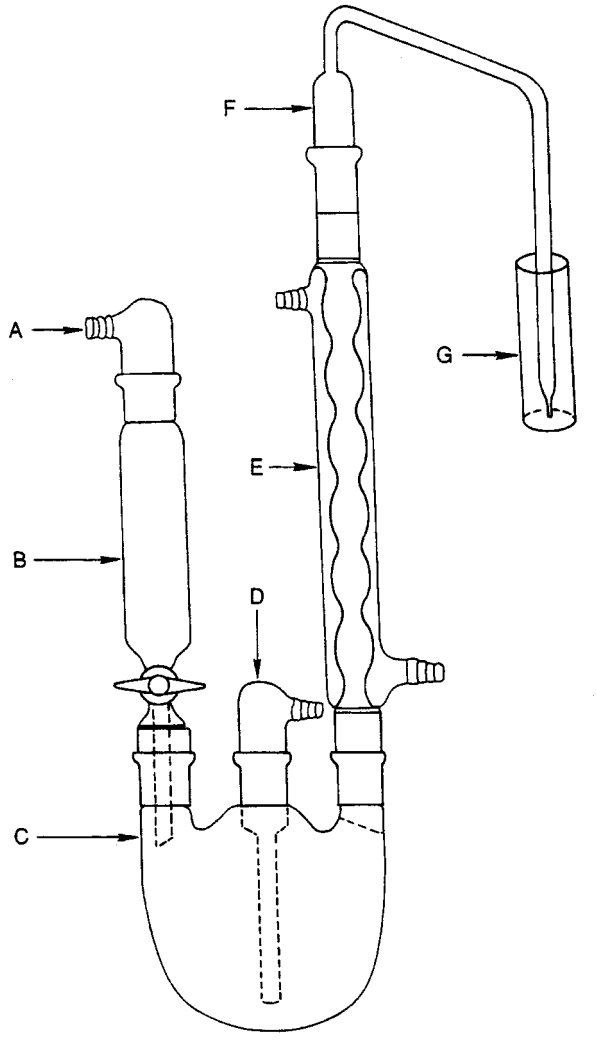


FIGURE 43 The Optimized Monier-Williams Apparatus; Component Identification Is Given in Text (component *F* is depicted in FIGURE 44).

Sample Preparation (for solids) Transfer 50 g of the sample, or a quantity of the sample with a known quantity of sulfur dioxide (500 to 1500 μg of SO_2), to a food processor or blender, if necessary. Add 50 mL of 5% ethanol in water, and briefly grind the mixture, reserving another 50 mL of 5% ethanol in water to rinse the blender jar. Grinding or blending should be continued only until the food is chopped into pieces small enough to pass through the 24/40 joint of a flask (see Fig. 43).

Sample Preparation (for liquids) Mix 50 g of the sample, or a quantity with a known amount of sulfur dioxide (500 to 1500 μg of SO_2), with 100 mL of 5% ethanol in water.

Apparatus The apparatus shown diagrammatically (Fig. 43) is designed to accomplish the selective transfer of sulfur dioxide from the sample in boiling aqueous hydrochloric acid to the *3% Hydrogen Peroxide Solution*. This apparatus is easier to assemble than the official apparatus, and the back-

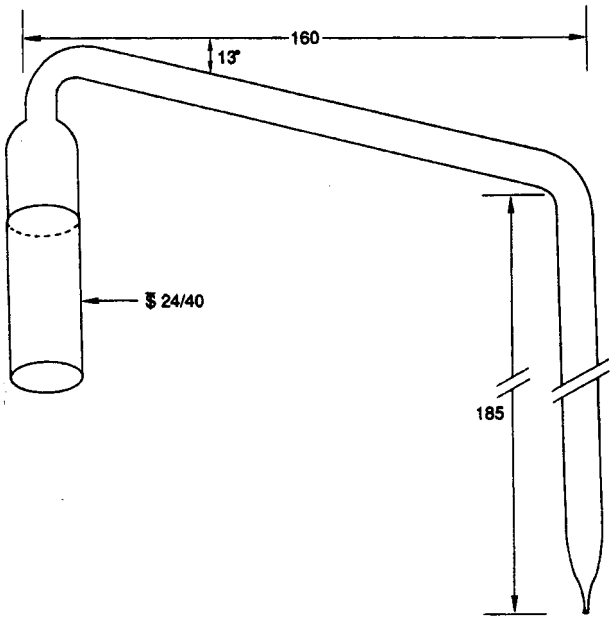


FIGURE 44 Diagram of Bubbler (*F* in FIGURE 43) (lengths are given in mm).

pressure inside the apparatus is limited to the unavoidable pressure due to the height of the *3% Hydrogen Peroxide Solution* above the tip of the bubbler, *F*. Keeping the back-pressure as low as possible reduces the likelihood that sulfur dioxide will be lost through leaks.

Note: Tygon and silicon tubing should be preboiled before use in this procedure.

The apparatus should be assembled as shown in Fig. 43 with a thin film of stopcock grease on the sealing surfaces of all the joints except the joint between the separatory funnel and the flask. Each joint should be clamped together to ensure a complete seal throughout the analysis. The separatory funnel, *B*, should have a capacity of 100 mL or greater. An inlet adapter, *A*, with a hose connector (Kontes K-183000, or equivalent) is required to provide a means of applying a head of pressure above the solution. (A pressure-equalizing dropping funnel is not recommended because condensate, perhaps with sulfur dioxide, is deposited in the funnel and the side arm.) The round-bottom flask, *C*, is a 1000-mL flask with three 24/40 tapered joints. The gas inlet tube, *D* (Kontes K-179000, or equivalent), should be of sufficient length to permit introduction of the nitrogen within 2.5 cm of the bottom of the flask. The Allihn condenser, *E* (Kontes K-431000-2430, or equivalent), has a jacket length of 300 mm. The bubbler, *F*, is fabricated from glass according to the dimensions given in Fig. 44, and it has the same dimensions as a 50-mL graduated cylinder (see Fig. 44). The *3% Hydrogen Peroxide Solution* can be contained in a receiving vessel, *G*, with an id of about 2.5 cm and a depth of 18 cm.

Buret Use a 10-mL buret with overflow tube and hose connections for an Ascarite tube or equivalent air-scrubbing apparatus. This will permit the maintenance of a carbon dioxide-free atmosphere over the *Standardized Titrant*.

Chilled Water Circulator The condenser must be chilled with a coolant, such as 20% methanol–water, at a flow rate so that the condenser outlet temperature is maintained at 5°. A circulating pump equivalent to the Neslab Coolflow 33 is suitable.

Determination Assemble the apparatus as shown in Fig. 43. The flask must be positioned in a heating mantle that is controlled by a power-regulating device such as Variac, or equivalent. Add 400 mL of distilled water to the flask. Close the stopcock of the separatory funnel, and add 90 mL of 4 *N* hydrochloric acid to the separatory funnel. Begin the flow of nitrogen at a rate of 200 ± 10 mL/min. The condenser coolant flow must be initiated at this time. Add 30 mL of *3% Hydrogen Peroxide Solution*, which has been titrated to a yellow endpoint with the *Standardized Titrant*, to the receiving vessel, *G*. After 15 min, the apparatus and the water will be thoroughly deoxygenated, and the apparatus will be ready for sample introduction.

Sample Introduction and Distillation Remove the separatory funnel, and quantitatively transfer the sample in aqueous ethanol to the flask. Wipe the tapered joint clean with a laboratory tissue, apply stopcock grease to the outer joint of the separatory funnel, and return the separatory funnel to the tapered joint flask. The nitrogen flow through the *3% Hydrogen Peroxide Solution* should resume as soon as the funnel is reinserted into the appropriate joint in the flask. Examine each joint to ensure that it is sealed.

Apply a head pressure above the hydrochloric acid solution in the separatory funnel with a rubber bulb equipped with a valve. Open the stopcock in the separatory funnel, and permit the hydrochloric acid solution to flow into the flask. Continue to maintain sufficient pressure above the acid solution to force the solution into the flask. The stopcock may temporarily be closed, if necessary, to pump up the pressure above the acid. To guard against the escape of sulfur dioxide into the separatory funnel, close the stopcock before the last few mL drain out of the separatory funnel.

Apply the power to the heating mantle. Use a power setting that will cause 80 to 90 drops of condensate to return to the flask from the condenser per min. After 1.75 h of boiling, cool the contents of the 1000-mL flask at the condensation rate stated above, and remove the contents of the receiving vessel, *G*.

Titration Add 3 drops of *Methyl Red Indicator*, and titrate the above-mentioned contents with the *Standardized Titrant* to a yellow endpoint that persists for at least 20 s. Calculate the sulfur dioxide content, expressed as μg of sulfur dioxide per g of sample (μg/g or mg/kg) as follows:

$$\text{mg/kg} = (32.03 \times V_B \times N \times 1000)/Wt,$$

in which 32.03 is the milliequivalent weight, in mg, of sulfur dioxide; V_B is the volume, in mL, of sodium hydroxide titrant of normality, *N*, required to reach the endpoint; the factor 1000 converts mg to μg; and *Wt* is the weight, in g, of sample introduced into the 1000-mL flask.

TOTAL SOLIDS

Note: The refractive index, RI, of solutions of various carbohydrates at specific temperatures is directly correlated with the solutions' concentrations (in g/100 g or percent dried solids). The following tables, as required in some monographs in this edition, are provided for the user's convenience.

Apparatus Use a suitable refractometer (see *Refractive Index*, Appendix IIB) equipped with a jacket for water circulation or some other mechanism for maintaining the sample at 20.0° ± 0.1° or some other fixed temperature. Before proceeding with measurements, ensure that the prism has reached the equilibrium temperature.

Standardization To achieve the theoretical accuracy of ±0.0001, calibrate the instrument daily by determining the refractive index of distilled water, which is 1.3330 at 20°, and 1.3325 at 25°.

Procedure Determine the refractive index after ensuring that the sample and prism have reached the equilibrium temperature.

For *Corn Syrups*, *High-Fructose Corn Syrups*, *Liquid Fructose*, and *Maltodextrin*, convert the refractive index to approximate percent solids using the accompanying tables.

Note: These tables cover the approximate total solids levels of these products in commerce. If the ash or dextrose equivalent of the sample differs from the product in the table, use the accompanying ash and dextrose equivalent correction table.

Glucose Syrup (Corn Syrup)

28 DE[a] Glucose Syrup—0.3% Ash

%DS[b]	Ri[c] 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
76.0	1.4888	1.4837	40.98
77.0	1.4915	1.4864	41.49
78.0	1.4943	1.4892	42.00
79.0	1.4971	1.4919	42.51
80.0	1.4999	1.4947	43.01

[a]Dextrose Equivalent
[b]Dry Substance
[c]Refractive Index

36 DE Glucose Syrup—0.3% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
78.4	1.4938	1.4887	42.01
79.4	1.4965	1.4914	42.52
80.4	1.4993	1.4941	43.02
81.4	1.5021	1.4969	43.52
82.4	1.5049	1.4997	44.02

34 DE High-Maltose Glucose Syrup—0.3% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
78.6	1.4933	1.4882	41.99
79.6	1.4960	1.4909	42.49
80.6	1.4988	1.4936	42.99
81.6	1.5015	1.4964	43.49
82.6	1.5043	1.4992	43.99

43 DE High-Maltose Glucose Syrup—0.3% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
78.9	1.4934	1.4883	42.00
79.9	1.4961	1.4910	42.51
80.9	1.4988	1.4937	43.01
81.9	1.5016	1.4964	43.51
82.9	1.5044	1.4992	44.01

43 DE Glucose Syrup—0.3% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
78.7	1.4933	1.4882	42.01
79.7	1.4960	1.4909	42.51
80.7	1.4988	1.4936	43.02
81.7	1.5015	1.4964	43.52
82.7	1.5043	1.4992	44.01

43 DE (Ion-Exchanged) Glucose Syrup—0.03% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
78.8	1.4935	1.4884	41.99
79.8	1.4962	1.4911	42.50
80.8	1.4990	1.4938	43.00
81.8	1.5018	1.4966	43.50
82.8	1.5045	1.4994	43.99

53 DE Glucose Syrup—0.3% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
80.5	1.4962	1.4911	42.64
81.5	1.4989	1.4938	43.14
82.5	1.5016	1.4965	43.64
83.5	1.5044	1.4992	44.13
84.5	1.5072	1.5020	44.63

63 DE Glucose Syrup—0.3% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
81.0	1.4955	1.4904	42.53
82.0	1.4982	1.4931	43.02
83.0	1.5009	1.4958	43.52
84.0	1.5037	1.4985	44.01
85.0	1.5064	1.5012	44.50

63 DE (Ion-Exchanged) Glucose Syrup—0.03% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
81.3	1.4963	1.4912	42.60
82.3	1.4990	1.4939	43.10
83.3	1.5017	1.4965	43.59
84.3	1.5044	1.4993	44.09
85.3	1.5072	1.5020	44.58

66 DE Glucose Syrup—0.3% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
81.0	1.4949	1.4898	42.36
82.0	1.4975	1.4924	42.86
83.0	1.5002	1.4951	43.36
84.0	1.5029	1.4978	43.85
85.0	1.5056	1.5005	44.35

95 DE Glucose Syrup—0.3% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
69.0	1.4598	1.4550	35.46
70.0	1.4621	1.4573	35.96
71.0	1.4644	1.4596	36.46
72.0	1.4668	1.4619	36.96
73.0	1.4692	1.4643	37.45

95 DE (Ion-Exchanged) Glucose Syrup—0.03% Ash

%DS	RI 20°C	RI 45°C	°Baumé at 140°F (60°C) + 1
69.0	1.4597	1.4549	35.39
70.0	1.4620	1.4572	35.89
71.0	1.4644	1.4595	36.39
72.0	1.4667	1.4619	36.89
73.0	1.4691	1.4642	37.38

High-Fructose Corn Syrup Solids

42% High-Fructose Corn Syrup—0.03% Ash

%DS[a]	RI[b] 20°C	RI 45°C
69.0	1.4597	1.4543
70.0	1.4620	1.4565
71.0	1.4643	1.4589
72.0	1.4667	1.4612
73.0	1.4691	1.4635

[a]Dry Substance
[b]Refractive Index

55% High-Fructose Corn Syrup—0.05% Ash

%DS[a]	RI[b] 20°C	RI 45°C
75.0	1.4738	1.4680
76.0	1.4762	1.4704
77.0	1.4786	1.4728
78.0	1.4811	1.4752
79.0	1.4835	1.4776

Liquid Fructose

%DS	RI 20°C	RI 45°C
75.0	1.4732	1.4667
76.0	1.4756	1.4691
77.0	1.4780	1.4715
78.0	1.4805	1.4739
79.0	1.4829	1.4763

Maltodextrin

12 DE[a] Maltodextrin—0.3% Ash

%DS[b]	RI[c] 20°C	RI 45°C	Commercial °Baumé 140°F (60°C) + 1
45.0	1.4149	1.4105	24.57
46.0	1.4171	1.4126	25.13
47.0	1.4193	1.4148	25.68
48.0	1.4215	1.4170	26.24
49.0	1.4237	1.4192	26.79
50.0	1.4260	1.4214	27.34
51.0	1.4282	1.4237	27.89
52.0	1.4305	1.4259	28.44
53.0	1.4328	1.4282	28.99
54.0	1.4351	1.4305	29.53
55.0	1.4375	1.4328	30.08
56.0	1.4398	1.4351	30.62
57.0	1.4422	1.4375	31.16
58.0	1.4446	1.4399	31.71
59.0	1.4470	1.4422	32.24
60.0	1.4494	1.4446	32.78
61.0	1.4519	1.4471	33.32
62.0	1.4544	1.4495	33.85
63.0	1.4569	1.4520	34.39
64.0	1.4594	1.4545	34.92
65.0	1.4619	1.4570	35.45
66.0	1.4644	1.4595	35.98
67.0	1.4670	1.4621	36.51
68.0	1.4696	1.4646	37.04
69.0	1.4722	1.4672	37.56
70.0	1.4748	1.4698	38.08
71.0	1.4775	1.4724	38.61
72.0	1.4801	1.4751	39.13
73.0	1.4828	1.4778	39.65
74.0	1.4855	1.4805	40.16
75.0	1.4883	1.4832	40.68
76.0	1.4910	1.4859	41.19
77.0	1.4938	1.4887	41.71
78.0	1.4966	1.4915	42.22
79.0	1.4994	1.4943	42.73
80.0	1.5023	1.4971	43.24
81.0	1.5051	1.4999	43.74
82.0	1.5080	1.5028	44.25
83.0	1.5110	1.5057	44.75
84.0	1.5139	1.5086	45.26
85.0	1.5168	1.5116	45.76
86.0	1.5198	1.5145	46.26
87.0	1.5228	1.5175	46.76
88.0	1.5259	1.5206	47.25
89.0	1.5289	1.5236	47.75
90.0	1.5320	1.5267	48.24
91.0	1.5351	1.5298	48.73
92.0	1.5382	1.5329	49.23
93.0	1.5414	1.5360	49.72
94.0	1.5446	1.5392	50.21
95.0	1.5478	1.5424	50.69

[a]Dextrose Equivalent
[b]Dry Substance
[c]Refractive Index

Ash and DE[a] Corrections for Corn Syrup and Maltodextrin:[b] Changes in Refractive Index for an increase of. . .

%DS[c]	1% Ash	1 DE
2	0.000000	–0.000001
4	0.000000	–0.000003
6	0.000001	–0.000005
8	0.000002	–0.000007
10	0.000003	–0.000010
12	0.000004	–0.000012
14	0.000006	–0.000015
16	0.000008	–0.000017
18	0.000010	–0.000020
20	0.000013	–0.000023
22	0.000016	–0.000026
24	0.000019	–0.000029
26	0.000022	–0.000033
28	0.000026	–0.000036
30	0.000030	–0.000040
32	0.000034	–0.000044
34	0.000039	–0.000048
36	0.000044	–0.000052
38	0.000049	–0.000057
40	0.000055	–0.000061
42	0.000061	–0.000066
44	0.000068	–0.000071
46	0.000074	–0.000076
48	0.000082	–0.000081
50	0.000089	–0.000087
52	0.000097	–0.000093
54	0.000105	–0.000099
56	0.000114	–0.000105
58	0.000123	–0.000112
60	0.000133	–0.000118
62	0.000143	–0.000125
64	0.000153	–0.000132
66	0.000164	–0.000140
68	0.000175	–0.000147
70	0.000187	–0.000155
72	0.000199	–0.000163
74	0.000212	–0.000172
76	0.000225	–0.000181
78	0.000239	–0.000190
80	0.000253	–0.000199
82	0.000268	–0.000208
84	0.000283	–0.000218

[a]Dextrose Equivalent
[b]Wartman, A. M., et al. J. Chemical and Engineering Data *21*:467, 1976.
[c]Dry Substance

Invert Sugar

For invert sugar, convert the refractive index to approximate percent solids (uncorrected for invert sugar) using the accompanying sucrose table. Correct for invert sugar by using the following formula:

$$D = (S + C) + (P_1 \times 0.022),$$

in which S is the approximate percent solids determined from the refractive index table for sucrose, C is the temperature correction derived from the accompanying temperature correction table if the refractometer was operated at other than 20°, and P_1 is the percent invert sugar determined as directed under *Assay* for *Invert Sugar* in this Appendix.

Sucrose

International Refractive Index Scale of ICUMSA[a] (1974) for Pure Sucrose Solutions at 20°C and 589 nm[b]

Sucrose g/100 g	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
56	1.4329	4332	4334	4336	4338	4340	4343	4345	4347	4349
57	1.4352	4354	4356	4358	4360	4363	4365	4367	4369	4372
58	1.4374	4376	4378	4380	4383	4385	4387	4389	4392	4394
59	1.4396	4398	4401	4403	4405	4407	4410	4412	4414	4417
60	1.4419	4421	4423	4426	4428	4430	4432	4435	4437	4439
61	1.4442	4444	4446	4448	4451	4453	4455	4458	4460	4462
62	1.4464	4467	4469	4471	4474	4476	4478	4481	4483	4485
63	1.4488	4490	4492	4495	4497	4499	4502	4504	4506	4509
64	1.4511	4513	4516	4518	4520	4523	4525	4527	4530	4532
65	1.4534	4537	4539	4541	4544	4546	4548	4551	4553	4556
66	1.4558	4560	4563	4565	4567	4570	4572	4575	4577	4579
67	1.4582	4584	4586	4589	4591	4594	4596	4598	4601	4603
68	1.4606	4608	4610	4613	4615	4618	4620	4623	4625	4627
69	1.4630	4632	4635	4637	4639	4642	4644	4647	4649	4652
70	1.4654	4657	4659	4661	4664	4666	4669	4671	4674	4676
71	1.4679	4681	4683	4686	4688	4691	4693	4696	4698	4701
72	1.4703	4706	4708	4711	4713	4716	4718	4721	4723	4726
73	1.4728	4730	4733	4735	4738	4740	4743	4745	4748	4750
74	1.4753	4756	4758	4761	4763	4766	4768	4771	4773	4776
75	1.4778	4781	4783	4786	4788	4791	4793	4796	4798	4801
76	1.4804	4806	4809	4811	4814	4816	4819	4821	4824	4826
77	1.4829	4832	4834	4837	4839	4842	4844	4847	4850	4852
78	1.4855	4857	4860	4862	4865	4868	4870	4873	4875	4878
79	1.4881	4883	4886	4888	4891	4894	4896	4899	4901	4904
80	1.4907	4909	4912	4914	4917	4920	4922	4925	4928	4930
81	1.4933	4935	4938	4941	4943	4946	4949	4951	4954	4957
82	1.4959	4962	4964	4967	4970	4972	4975	4978	4980	4983
83	1.4986	4988	4991	4994	4996	4999	5002	5004	5007	5010
84	1.5012	5015	5018	5020	5023	5026	5029	5031	5034	5037
85	1.5039									

[a]Adapted from ''Refractometry and Tables—Official'' (ICUMSA SPS-3 1994), International Commission for Uniform Methods of Sugar Analysis (ICUMSA), c/o British Sugar Technical Centre, Colney, Norwich NR4 7UB, England.

[b]No rounding has been carried out; therefore, values given may be too low by a maximum of 1×10^{-4}.

Temperature Corrections for Refractometric Sucrose Solutions with Measurements at 20° and 589 nm

Temperature (°C)	Measured Sucrose (% solids)																	
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85
	Subtract from the measured value																	
15	0.29	0.30	0.32	0.33	0.34	0.35	0.36	0.37	0.37	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.37	0.37
16	0.24	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.30	0.30	0.31	0.31	0.31	0.31	0.31	0.30	0.30	0.30
17	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.22	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.22
18	0.12	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
19	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07
	Add to the measured value																	
21	0.06	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07
22	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15
23	0.20	0.21	0.21	0.22	0.22	0.23	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.23	0.23	0.23	0.23	0.22
24	0.27	0.28	0.29	0.29	0.30	0.30	0.31	0.31	0.31	0.32	0.32	0.32	0.32	0.31	0.31	0.31	0.30	0.30
25	0.34	0.35	0.36	0.37	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.40	0.40	0.39	0.39	0.38	0.38	0.37
26	0.42	0.43	0.44	0.45	0.46	0.46	0.47	0.47	0.48	0.48	0.48	0.48	0.48	0.47	0.47	0.46	0.46	0.45
27	0.50	0.51	0.52	0.53	0.54	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	0.55	0.55	0.54	0.53	0.52
28	0.58	0.59	0.60	0.61	0.62	0.63	0.64	0.64	0.64	0.65	0.65	0.64	0.64	0.63	0.63	0.62	0.61	0.60
29	0.66	0.67	0.68	0.70	0.71	0.71	0.72	0.73	0.73	0.73	0.73	0.73	0.72	0.72	0.71	0.70	0.69	0.67
30	0.74	0.76	0.77	0.78	0.79	0.80	0.81	0.81	0.82	0.82	0.81	0.81	0.80	0.80	0.79	0.78	0.76	0.75
31	0.83	0.84	0.85	0.87	0.88	0.89	0.89	0.90	0.90	0.90	0.90	0.89	0.89	0.88	0.87	0.86	0.84	0.82
32	0.92	0.93	0.94	0.96	0.97	0.98	0.98	0.99	0.99	0.99	0.99	0.98	0.97	0.96	0.95	0.93	0.92	0.90
33	1.01	1.02	1.03	1.05	1.06	1.07	1.07	1.08	1.08	1.08	1.07	1.07	1.06	1.04	1.03	1.01	1.00	0.98
34	1.10	1.11	1.13	1.14	1.15	1.16	1.16	1.17	1.17	1.16	1.16	1.15	1.14	1.13	1.11	1.09	1.07	1.05
35	1.19	1.21	1.22	1.23	1.24	1.25	1.25	1.26	1.26	1.25	1.25	1.24	1.23	1.21	1.19	1.17	1.15	1.13
36	1.29	1.30	1.31	1.33	1.34	1.34	1.35	1.35	1.35	1.34	1.34	1.33	1.31	1.29	1.28	1.25	1.23	1.20
37	1.39	1.40	1.41	1.42	1.43	1.44	1.44	1.44	1.44	1.43	1.43	1.41	1.40	1.38	1.36	1.33	1.31	1.28
38	1.49	1.50	1.51	1.52	1.53	1.53	1.54	1.54	1.53	1.53	1.52	1.50	1.48	1.46	1.44	1.42	1.39	1.36
39	1.59	1.60	1.61	1.62	1.63	1.63	1.63	1.63	1.63	1.62	1.61	1.59	1.57	1.55	1.52	1.50	1.47	1.43
40	1.69	1.70	1.71	1.72	1.73	1.73	1.73	1.73	1.72	1.71	1.70	1.68	1.66	1.63	1.61	1.58	1.54	1.51

SOURCE: Adapted from ''Refractometry and Tables—Official'' (ICUMSA SPS-3 1994), International Commission for Uniform Methods of Sugar Analysis (ICUMSA), c/o British Sugar Technical Centre, Colney, Norwich NR4 7UB, England.

SOLUTIONS AND INDICATORS

The directions given for the preparation of solutions and indicators are for guidance; the use of commercially available ones is acceptable.

COLORIMETRIC SOLUTIONS (CS)

Colorimetric solutions are used in the preparation of colorimetric standards for certain chemicals and for the carbonization tests with sulfuric acid that are specified in several monographs. Directions for the preparation of the primary colorimetric solutions and *Matching Fluids* are given under the test for *Readily Carbonizable Substances*, Appendix IIB. Store the solutions in suitably resistant, tight containers.

Comparison of colors as directed in the *Food Chemicals Codex* tests is preferably made in matched color-comparison tubes or in a suitable colorimeter under conditions that ensure that the colorimetric reference solution and that of the specimen under test are treated alike in all respects.

STANDARD BUFFER SOLUTIONS

Reagent Solutions Before mixing, dry the crystalline reagents, except the boric acid, at 110° to 120°, and use water that has been previously boiled and cooled in preparing the solutions. Store the prepared reagent solutions in chemically resistant glass or polyethylene bottles, and use within 3 months. Discard if molding is evident.

Potassium Chloride, 0.2 *M* Dissolve 14.91 g of potassium chloride (KCl) in sufficient water to make 1000.0 mL.

Potassium Biphthalate, 0.2 *M* Dissolve 40.84 g of potassium biphthalate [$KHC_6H_4(COO)_2$] in sufficient water to make 1000.0 mL.

Potassium Phosphate, Monobasic, 0.2 *M* Dissolve 27.22 g of monobasic potassium phosphate (KH_2PO_4) in sufficient water to make 1000.0 mL.

Boric Acid–Potassium Chloride, 0.2 *M* Dissolve 12.37 g of boric acid (H_3BO_3) and 14.91 g of potassium chloride (KCl) in sufficient water to make 1000.0 mL.

Hydrochloric Acid, 0.2 *M*, and **Sodium Hydroxide, 0.2 *M*** Prepare and standardize as directed under *Volumetric Solutions* in this section.

Procedure To prepare 200 mL of a standard buffer solution having a pH within the range 1.2 to 10.0, place 50.0 mL of the appropriate 0.2 *M* salt solution, prepared as above, in a 200-mL volumetric flask, add the volume of 0.2 *M* hydrochloric acid or of sodium hydroxide specified for the desired pH in the accompanying table, dilute to volume with water, and mix.

Composition of Standard Buffer Solutions

Hydrochloric Acid Buffer		Acid Phthalate Buffer		Neutralized Phthalate Buffer		Phosphate Buffer		Alkaline Borate Buffer	
To 50.0 mL of 0.2 *M* KCl add the mL of HCl specified		To 50.0 mL of 0.2 *M* $KHC_6H_4(COO)_2$ add the mL of HCl specified		To 50.0 mL of 0.2 *M* $KHC_6H_4(COO)_2$ add the mL of NaOH specified		To 50.0 mL of 0.2 *M* KH_2PO_4 add the mL of NaOH specified		To 50.0 mL of 0.2 *M* H_3BO_3-KCl add the mL of NaOH specified	
pH	0.2 *M* HCl (mL)	pH	0.2 *M* HCl (mL)	pH	0.2 *M* NaOH (mL)	pH	0.2 *M* NaOH (mL)	pH	0.2 *M* NaOH (mL)
1.2	85.0	2.2	49.5	4.2	3.0	5.8	3.6	8.0	3.9
1.3	67.2	2.4	42.2	4.4	6.6	6.0	5.6	8.2	6.0
1.4	53.2	2.6	35.4	4.6	11.1	6.2	8.1	8.4	8.6
1.5	41.4	2.8	28.9	4.8	16.5	6.4	11.6	8.6	11.8
1.6	32.4	3.0	22.3	5.0	22.6	6.6	16.4	8.8	15.8
1.7	26.0	3.2	15.7	5.2	28.8	6.8	22.4	9.0	20.8
1.8	20.4	3.4	10.4	5.4	34.1	7.0	29.1	9.2	26.4
1.9	16.2	3.6	6.3	5.6	38.8	7.2	34.7	9.4	32.1
2.0	13.0	3.8	2.9	5.8	42.3	7.4	39.1	9.6	36.9
2.1	10.2	4.0	0.1	—	—	7.6	42.4	9.8	40.6
2.2	7.8	—	—	—	—	7.8	44.5	10.0	43.7
						8.0	46.1	—	—

Dilute all final solutions to 200.0 mL (see *Procedure*). The standard pH values given in this table are considered to be reproducible to within ±0.02 of the pH unit specified at 25°.

STANDARD SOLUTIONS FOR THE PREPARATION OF CONTROLS AND STANDARDS

The following solutions are used in tests for impurities that require the comparison of the color or turbidity produced in a solution of the test substance with that produced by a known amount of the impurity in a control. Directions for the preparation of other standard solutions are given in the monographs or under the general tests in which they are required (see also *Index*).

Ammonium Standard Solution (10 μg NH_4 in 1 mL) Dissolve 296.0 mg of ammonium chloride (NH_4Cl) in sufficient water to make 100.0 mL, and mix. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, dilute to volume with water, and mix.

Barium Standard Solution (100 μg Ba in 1 mL) Dissolve 177.9 mg of barium chloride ($BaCl_2 \cdot 2H_2O$) in water in a 1000-mL volumetric flask, dilute to volume with water, and mix.

Iron Standard Solution (10 μg Fe in 1 mL) Dissolve 702.2 mg of ferrous ammonium sulfate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$] in 10 mL of 2 *N* sulfuric acid in a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 2 *N* sulfuric acid, dilute to volume with water, and mix.

Magnesium Standard Solution (50 μg Mg in 1 mL) Dissolve 50.0 mg of magnesium metal (Mg) in 1 mL of hydrochloric acid in a 1000-mL volumetric flask, dilute to volume with water, and mix.

Phosphate Standard Solution (10 μg PO_4 in 1 mL) Dissolve 143.3 mg of monobasic potassium phosphate (KH_2PO_4) in water in a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, dilute to volume with water, and mix.

TEST SOLUTIONS (TS) AND OTHER REAGENTS

Certain of the following test solutions are intended for use as acid–base indicators in volumetric analyses. Such solutions should be adjusted so that when 0.15 mL of the indicator solution is added to 25 mL of carbon dioxide-free water, 0.25 mL of 0.02 *N* acid or alkali, respectively, will produce the characteristic color change.

In general, the directive to prepare a solution ''fresh'' indicates that the solution is of limited stability and must be prepared on the day of use.

Acetic Acid (approximately 17.5 *N*) Use ACS reagent-grade *Acetic Acid, Glacial* (99.7% of CH_3COOH).

Acetic Acid TS, Diluted (1 *N*) A solution containing about 6% (w/v) of CH_3COOH. Prepare by diluting 60.0 mL of glacial acetic acid, or 166.6 mL of 36% acetic acid (6 *N*), with sufficient water to make 1000 mL.

Acetic Acid TS, Strong (5 *N*) A solution containing 30% (v/v) of CH_3COOH. Prepare by diluting 300.0 mL of glacial acetic acid with sufficient water to make 1000 mL.

Alcohol (*Ethanol; Ethyl Alcohol*; C_2H_5OH) Use ACS reagent-grade *Ethyl Alcohol* (not less than 95.0%, by volume, of C_2H_5OH).

> **Note**: For use in assays and tests involving ultraviolet spectrophotometry, use ACS reagent-grade *Ethyl Alcohol Suitable for Use in Ultraviolet Spectrophotometry*.

Alcohol, Absolute (*Anhydrous Alcohol*; *Dehydrated Alcohol*) Use ACS reagent-grade *Ethyl Alcohol, Absolute* (not less than 99.5%, by volume, of C_2H_5OH).

Alcohol, Diluted A solution containing 41.0% to 42.0%, by weight, corresponding to 48.4% to 49.5%, by volume, at 15.56°, of C_2H_5OH.

Alcohol, 70% (at 15.56°) A 38.6:15 mixture (v/v) of 95% alcohol and water, having a specific gravity of 0.884 at 25°. To prepare 100 mL, dilute 73.7 mL of alcohol to 100 mL with water at 25°.

Alcohol, 80% (at 15.56°) A 45.5:9.5 mixture (v/v) of 95% alcohol and water, having a specific gravity of 0.857 at 25°. To prepare 100 mL, dilute 84.3 mL of alcohol to 100 mL with water at 25°.

Alcohol, 90% (at 15.56°) A 51:3 mixture (v/v) of 95% alcohol and water, having a specific gravity of 0.827 at 25°. To prepare 100 mL, dilute 94.8 mL of alcohol to 100 mL with water at 25°.

Alcohol, Aldehyde-Free Dissolve 2.5 g of lead acetate in 5 mL of water, add the solution to 1000 mL of alcohol contained in a glass-stoppered bottle, and mix. Dissolve 5 g of potassium hydroxide in 25 mL of warm alcohol, cool, and add slowly, without stirring, to the alcoholic solution of lead acetate. Allow to stand for 1 h, then shake the mixture vigorously, allow to stand overnight, decant the clear liquid, and recover the alcohol by distillation. *Ethyl Alcohol FCC*, *Alcohol USP*, or *USSD #3A* or *#30* may be used. If the titration of a 250-mL sample of the alcohol by *Hydroxylamine Hydrochloride TS* does not exceed 0.25 mL of 0.5 *N* alcoholic potassium hydroxide, the above treatment may be omitted.

Alcoholic Potassium Hydroxide TS See *Potassium Hydroxide TS, Alcoholic*.

Alkaline Cupric Tartrate TS (*Fehling's Solution*) See *Cupric Tartrate TS, Alkaline*.

Alkaline Mercuric Potassium Iodide TS (*Nessler's Reagent*) See *Mercuric Potassium Iodide TS, Alkaline*.

Ammonia–Ammonium Chloride Buffer TS (approximately pH 10) Dissolve 67.5 g of ammonium chloride (NH_4Cl) in water, add 570 mL of ammonium hydroxide (28%), and dilute with water to 1000 mL.

Ammonia TS (6 *N* in NH_3) A solution containing between 9.5% and 10.5% of NH_3. Prepare by diluting 400 mL of ammonium hydroxide (28%) with sufficient water to make 1000 mL.

Ammonia TS, Stronger (15.2 *N* in NH_3) (*Ammonium Hydroxide*; *Stronger Ammonia Water*) Use ACS reagent-grade *Ammonium Hydroxide*, which is a practically saturated solution of ammonia in water, containing between 28% and 30% of NH_3.

Ammoniacal Silver Nitrate TS Add 6 *N* ammonium hydroxide, dropwise, to a 1:20 solution of silver nitrate until the precipitate that first forms is almost, but not entirely, dissolved. Filter the solution, and place in a dark bottle.

> **Caution**: *Ammoniacal Silver Nitrate TS* forms explosive compounds on standing. Do not store this solution, but prepare a fresh quantity for each series of determinations. Neutralize the excess reagent and rinse all glassware with hydrochloric acid immediately after completing a test.

Ammonium Acetate TS Dissolve 10 g of ammonium acetate ($NH_4C_2H_3O_2$) in sufficient water to make 100 mL.

Ammonium Carbonate TS Dissolve 20 g of ammonium carbonate and 20 mL of *Ammonia TS* in sufficient water to make 100 mL.

Ammonium Chloride TS Dissolve 10.5 g of ammonium chloride (NH_4Cl) in sufficient water to make 100 mL.

Ammonium Molybdate TS Dissolve 6.5 g of finely powdered molybdic acid (85%) in a mixture of 14 mL of water and 14.5 mL of ammonium hydroxide. Cool the solution, and add it slowly, with stirring, to a well-cooled mixture of 32 mL of nitric acid and 40 mL of water. Allow to stand for 48 h, and filter through a fine-porosity, sintered-glass crucible lined at the bottom with a layer of glass wool. This solution deteriorates upon standing and is unsuitable for use if, upon the addition of 2 mL of *Sodium Phosphate TS* to 5 mL of the solution, an abundant yellow precipitate does not form at once or after slight warming. Store it in the dark. If a precipitate forms during storage, use only the clear, supernatant solution.

Ammonium Oxalate TS Dissolve 3.5 g of ammonium oxalate [$(NH_4)_2C_2O_4 \cdot H_2O$] in sufficient water to make 100 mL.

Ammonium Sulfanilate TS To 2.5 g of sulfanilic acid add 15 mL of water and 3 mL of 6 *N* ammonium hydroxide, and mix. Add, with stirring, more 6 *N* ammonium hydroxide, if necessary, until the acid dissolves, adjust the pH of the solution to about 4.5 with 2.7 *N* hydrochloric acid, using *Bromocresol Green TS* as an outside indicator, and dilute to 25 mL.

Ammonium Sulfide TS Saturate 6 *N* ammonium hydroxide with hydrogen sulfide (H_2S), and add two-thirds of its volume of 6 *N* ammonium hydroxide. Residue upon ignition: not more than 0.05%. The solution is not rendered turbid either by *Magnesium Sulfate TS* or by *Calcium Chloride TS* (*carbonate*). This solution is unsuitable for use if an abundant precipitate of sulfur is present. Store it in small, well-filled, dark amber-colored bottles in a cold, dark place.

Ammonium Thiocyanate TS (1 *N*) Dissolve 8 g of ammonium thiocyanate (NH_4SCN) in sufficient water to make 100 mL.

Anthrone TS Carefully dissolve about 0.1 g of anthrone in 100 g of sulfuric acid. Use a freshly prepared solution.

Antimony Trichloride TS Dissolve 20 g of antimony trichloride ($SbCl_3$) in chloroform to make 100 mL. Filter if necessary.

Barium Chloride TS Dissolve 12 g of barium chloride ($BaCl_2 \cdot 2H_2O$) in sufficient water to make 100 mL.

Barium Diphenylamine Sulfonate TS Dissolve 300 mg of *p*-diphenylamine sulfonic acid barium salt in 100 mL of water.

Barium Hydroxide TS Use a saturated solution of barium hydroxide in recently boiled water. Use a freshly prepared solution.

Benedict's Qualitative Reagent See *Cupric Citrate TS, Alkaline*.

Benzidine TS Dissolve 50 mg of benzidine in 10 mL of glacial acetic acid, dilute to 100 mL with water, and mix.

Bismuth Nitrate TS Reflux 5 g of bismuth nitrate [$Bi(NO_3)_3 \cdot 5H_2O$] with 7.5 mL of nitric acid and 10 mL of water until dissolved, cool, filter, and dilute to 250 mL with water.

Bromine TS (*Bromine Water*) Prepare a saturated solution of bromine by agitating 2 to 3 mL of bromine (Br_2) with 100 mL of cold water in a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum. Store it in a cold place protected from light.

Bromocresol Blue TS Use *Bromocresol Green TS*.

Bromocresol Green TS Dissolve 50 mg of bromocresol green in 100 mL of alcohol, and filter if necessary.

Bromocresol Purple TS Dissolve 250 mg of bromocresol purple in 20 mL of 0.05 *N* sodium hydroxide, and dilute with water to 250 mL.

Bromophenol Blue TS Dissolve 100 mg of bromophenol blue in 100 mL of 1:2 alcohol, and filter if necessary.

Bromothymol Blue TS Dissolve 100 mg of bromothymol blue in 100 mL of 1:2 alcohol, and filter if necessary.

Calcium Chloride TS Dissolve 7.5 g of calcium chloride ($CaCl_2 \cdot 2H_2O$) in sufficient water to make 100 mL.

Calcium Hydroxide TS A solution containing approximately 140 mg of $Ca(OH)_2$ in each 100 mL. To prepare, add 3 g of calcium hydroxide [$Ca(OH)_2$] to 1000 mL of water, and agitate the mixture vigorously and repeatedly for 1 h. Allow the excess calcium hydroxide to settle, and decant or draw off the clear, supernatant liquid.

Calcium Sulfate TS A saturated solution of calcium sulfate in water.

Carr-Price Reagent See *Antimony Trichloride TS*.

Ceric Ammonium Nitrate TS Dissolve 6.25 g of ceric ammonium nitrate [$(NH_4)_2Ce(NO_3)_6$] in 100 mL of 0.25 *N* nitric acid. Prepare the solution fresh every third day.

Chlorine TS (*Chlorine Water*) A saturated solution of chlorine in water. Place the solution in small, completely filled, light-resistant containers. *Chlorine TS*, even when kept from light and air, is apt to deteriorate. Store it in a cold, dark place. For full strength, prepare this solution fresh.

Chromotropic Acid TS Dissolve 50 mg of chromotropic acid or its sodium salt in 100 mL of 75% sulfuric acid (made by cautiously adding 75 mL of 95% to 98% sulfuric acid to 33.3 mL of water).

Cobaltous Chloride TS Dissolve 2 g of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) in 1 mL of hydrochloric acid and sufficient water to make 100 mL.

Cobalt–Uranyl Acetate TS Dissolve, with warming, 40 g of uranyl acetate [$UO_2(C_2H_3O_2)_2 \cdot 2H_2O$] in a mixture of 30 g of glacial acetic acid and sufficient water to make 500 mL. Similarly, prepare a solution containing 200 g of cobaltous acetate [$Co(C_2H_3O_2)_2 \cdot 4H_2O$] in a mixture of 30 g of glacial acetic acid and sufficient water to make 500 mL. Mix the two solutions while still warm, and cool to 20°. Maintain the temperature at 20° for about 2 h to separate the excess salts from solution, and then filter through a dry filter.

Congo Red TS Dissolve 500 mg of congo red in a mixture of 10 mL of alcohol and 90 mL of water.

Copper Sulfate TS Dissolve 12.5 g of cupric sulfate in sufficient water to make 100 mL.

Cresol Red TS Triturate 100 mg of cresol red in a mortar with 26.2 mL of 0.01 *N* sodium hydroxide until solution is complete, then dilute the solution with water to 250 mL.

Cresol Red–Thymol Blue TS Add 15 mL of *Thymol Blue TS* to 5 mL of *Cresol Red TS*, and mix.

Crystal Violet TS Dissolve 100 mg of crystal violet in 10 mL of glacial acetic acid.

Cupric Citrate TS, Alkaline (*Benedict's Qualitative Reagent*) With the aid of heat, dissolve 173 g of sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 117 g of sodium carbonate ($Na_2CO_3 \cdot H_2O$) in about 700 mL of water, and filter through paper, if necessary. In a separate container, dissolve 17.3 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in about 100 mL of water, and slowly add this solution, with constant stirring, to the first solution. Cool the mixture, dilute to 1000 mL, and mix.

Cupric Nitrate TS Dissolve 2.4 g of cupric nitrate [$Cu(NO_3)_2 \cdot 3H_2O$] in sufficient water to make 100 mL.

Cupric Sulfate TS Dissolve 12.5 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) in sufficient water to make 100 mL, and mix.

Cupric Tartrate TS, Alkaline (*Fehling's Solution*) *The Copper Solution* (*A*): Dissolve 34.66 g of carefully selected, small crystals of cupric sulfate, $CuSO_4 \cdot 5H_2O$, showing no trace of efflorescence or of adhering moisture, in sufficient water to make 500 mL. Store this solution in small, tight containers. *The Alkaline Tartrate Solution* (*B*): Dissolve 173 g of crystallized potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) and 50 g of sodium hydroxide (NaOH) in sufficient water to make 500 mL. Store this solution in small, alkali-resistant containers. For use, mix exactly equal volumes of solutions *A* and *B* at the time required.

Cyanogen Bromide TS Dissolve 5 g of cyanogen bromide in water to make 50 mL.

> **Caution**: Prepare this solution in a hood, as cyanogen bromide volatilizes at room temperature, and the vapor is highly irritating and poisonous.

Denigès' Reagent See *Mercuric Sulfate TS*.

Dichlorophenol–Indophenol TS Warm 100 mg of 2,6-dichlorophenol–indophenol sodium with 100 mL of water. Filter and use within 3 days.

2,7-Dihydroxynaphthalene TS Dissolve 100 mg of 2,7-dihydroxynaphthalene in 1000 mL of sulfuric acid, and allow the solution to stand until the initial color disappears. If the solution is very dark, discard it and prepare a new solution from a different supply of sulfuric acid. This solution is stable for approximately 1 month if stored in a dark bottle.

Diphenylamine TS Dissolve 1 g of diphenylamine in 100 mL of sulfuric acid. The solution should be colorless.

Diphenylcarbazone TS Dissolve about 1 g of diphenylcarbazone ($C_{13}H_{12}N_4O$) in sufficient alcohol to make 100 mL. Store this solution in a brown bottle.

α,α-Dipyridyl TS Dissolve 100 mg of α,α-dipyridyl ($C_{10}H_8N_2$) in 50 mL of absolute alcohol.

Dithizone TS Dissolve 25.6 mg of dithizone in 100 mL of alcohol.

Eosin Y TS (adsorption indicator) Dissolve 50 mg of eosin Y in 10 mL of water.

Eriochrome Black TS Dissolve 200 mg of eriochrome black T and 2 g of hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) in sufficient methanol to make 50 mL, and filter. Store the solution in a light-resistant container and use within 2 weeks.

***p*-Ethoxychrysoidin TS** Dissolve 50 mg of *p*-ethoxychrysoidin monohydrochloride in a mixture of 25 mL of water and 25 mL of alcohol, add 3 drops of hydrochloric acid, stir vigorously, and filter if necessary to obtain a clear solution.

Fehling's Solution See *Cupric Tartrate TS, Alkaline*.

Ferric Ammonium Sulfate TS Dissolve 8 g of ferric ammonium sulfate [$FeNH_4(SO_4)_2{\cdot}12H_2O$] in sufficient water to make 100 mL.

Ferric Chloride TS Dissolve 9 g of ferric chloride ($FeCl_3{\cdot}6H_2O$) in sufficient water to make 100 mL.

Ferric Chloride TS, Alcoholic Dissolve 100 mg of ferric chloride ($FeCl_3{\cdot}6H_2O$) in 50 mL of absolute alcohol. Prepare this solution fresh.

Ferric Sulfate TS, Acid Add 7.5 mL of sulfuric acid to 100 mL of water, and dissolve 80 g of ferrous sulfate in the mixture with the aid of heat. Mix 7.5 mL of nitric acid and 20 mL of water, warm, and add to this the ferrous sulfate solution. Concentrate the mixture until, upon the sudden disengagement of ruddy vapors, the black color of the liquid changes to red. Test for the absence of ferrous iron, and, if necessary, add a few drops of nitric acid and heat again. When the solution is cold, add sufficient water to make 110 mL.

Ferrous Sulfate TS Dissolve 8 g of clear crystals of ferrous sulfate ($FeSO_4{\cdot}7H_2O$) in about 100 mL of recently boiled and thoroughly cooled water. Prepare this solution fresh.

Formaldehyde TS A solution containing approximately 37.0% (w/v) of HCHO. It may contain methanol to prevent polymerization.

Fuchsin–Sulfurous Acid TS Dissolve 200 mg of basic fuchsin in 120 mL of hot water, and allow the solution to cool. Add a solution of 2 g of anhydrous sodium sulfite in 20 mL of water, and then add 2 mL of hydrochloric acid. Dilute the solution with water to 200 mL, and allow to stand for at least 1 h. Prepare this solution fresh.

Hydrochloric Acid (approximately 12 *N*) Use ACS reagent-grade *Hydrochloric Acid* (36.5% to 38.0% of HCl).

Hydrochloric Acid TS, Diluted (2.7 *N*) A solution containing 10% (w/v) of HCl. Prepare by diluting 226 mL of hydrochloric acid (36%) with sufficient water to make 1000 mL.

Hydrogen Peroxide TS A solution containing between 2.5 and 3.5 g of H_2O_2 in each 100 mL. It may contain suitable preservatives, totaling not more than 0.05%.

Hydrogen Sulfide TS A saturated solution of hydrogen sulfide made by passing H_2S into cold water. Store it in small, dark, amber-colored bottles, filled nearly to the top. It is unsuitable unless it possesses a strong odor of H_2S, and unless it produces at once a copious precipitate of sulfur when added to an equal volume of *Ferric Chloride TS*. Store in a cold, dark place.

Hydroxylamine Hydrochloride TS Dissolve 3.5 g of hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) in 95 mL of 60% alcohol, and add 0.5 mL of a 1:1000 solution of bromophenol blue and 0.5 *N* alcoholic potassium hydroxide until a green tint develops in the solution. Then add sufficient 60% alcohol to make 100 mL.

8-Hydroxyquinoline TS Dissolve 5 g of 8-hydroxyquinoline (oxine) in sufficient alcohol to make 100 mL.

Indigo Carmine TS (*Sodium Indigotindisulfonate TS*) Dissolve a quantity of sodium indigotindisulfonate, equivalent to 180 mg of $C_{16}H_8N_2O_2(SO_3Na)_2$, in sufficient water to make 100 mL. Use within 60 days.

Iodine TS Dissolve 14 g of iodine (I_2) in a solution of 36 g of potassium iodide (KI) in 100 mL of water, add 3 drops of hydrochloric acid, dilute with water to 1000 mL, and mix.

Isopropanol [*Isopropyl Alcohol*; *2-Propanol*; $(CH_3)_2CHOH$] Use ACS reagent-grade *Isopropyl Alcohol*.

> **Note**: For use in assays and tests involving ultraviolet spectrophotometry, use ACS reagent-grade *Isopropyl Alcohol Suitable for Use in Ultraviolet Spectrophotometry*.

Isopropanol, Anhydrous (*Dehydrated Isopropanol*) Use isopropanol that has been previously dried by shaking with anhydrous calcium chloride, followed by filtering.

Lead Acetate TS Dissolve 9.5 g of clear, transparent crystals of lead acetate [$Pb(C_2H_3O_2)_2{\cdot}3H_2O$] in sufficient recently boiled water to make 100 mL. Store in well-stoppered bottles.

Lead Subacetate TS Triturate 14 g of lead monoxide (PbO) to a smooth paste with 10 mL of water, and transfer the mixture to a bottle, using an additional 10 mL of water for rinsing. Dissolve 22 g of lead acetate [$Pb(C_2H_3O_2)_2 \cdot 3H_2O$] in 70 mL of water, and add the solution to the lead oxide mixture. Shake it vigorously for 5 min, then set it aside, shaking it frequently during 7 days. Finally, filter, and add enough recently boiled water through the filter to make 100 mL.

Lead Subacetate TS, Diluted Dilute 3.25 mL of *Lead Subacetate TS* with sufficient water, recently boiled and cooled, to make 100 mL. Store in small, well-fitted, tight containers.

Litmus TS Digest 25 g of powdered litmus with three successive 100-mL portions of boiling alcohol, continuing each extraction for about 1 h. Filter, wash with alcohol, and discard the alcohol filtrate. Macerate the residue with about 25 mL of cold water for 4 h, filter, and discard the filtrate. Finally, digest the residue with 125 mL of boiling water for 1 h, cool, and filter.

Magnesia Mixture TS Dissolve 5.5 g of magnesium chloride ($MgCl_2 \cdot 6H_2O$) and 7 g of ammonium chloride (NH_4Cl) in 65 mL of water, add 35 mL of 6 *N* ammonium hydroxide, set the mixture aside for a few days in a well-stoppered bottle, and filter. If the solution is not perfectly clear, filter it before using.

Magnesium Sulfate TS Dissolve 12 g of crystals of magnesium sulfate ($MgSO_4 \cdot 7H_2O$), selected for freedom from efflorescence, in water to make 100 mL.

Malachite Green TS Dissolve 1 g of malachite green oxalate in 100 mL of glacial acetic acid.

Mayer's Reagent See *Mercuric–Potassium Iodide TS*.

Mercuric Acetate TS Dissolve 6 g of mercuric acetate [$Hg(C_2H_3O_2)_2$] in sufficient glacial acetic acid to make 100 mL. Store in tight containers protected from direct sunlight.

Mercuric Chloride TS Dissolve 6.5 g of mercuric chloride ($HgCl_2$) in water to make 100 mL.

Mercuric–Potassium Iodide TS (*Mayer's Reagent*) Dissolve 1.358 g of mercuric chloride ($HgCl_2$) in 60 mL of water. Dissolve 5 g of potassium iodide (KI) in 10 mL of water. Mix the two solutions, and add water to make 100 mL.

Mercuric–Potassium Iodide TS, Alkaline (*Nessler's Reagent*) Dissolve 10 g of potassium iodide (KI) in 10 mL of water, and add slowly, with stirring, a saturated solution of mercuric chloride until a slight red precipitate remains undissolved. To this mixture add an ice-cold solution of 30 g of potassium hydroxide (KOH) in 60 mL of water, then add 1 mL more of the saturated solution of mercuric chloride. Dilute with water to 200 mL. Allow the precipitate to settle, and draw off the clear liquid. A 2-mL portion of this reagent, when added to 100 mL of a 1:300,000 solution of ammonium chloride in ammonia-free water, instantly produces a yellow-brown color.

Mercuric Sulfate TS (*Denigès' Reagent*) Mix 5 g of yellow mercuric oxide (HgO) with 40 mL of water, and while stirring, slowly add 20 mL of sulfuric acid, then add another 40 mL of water, and stir until completely dissolved.

Mercurous Nitrate TS Dissolve 15 g of mercurous nitrate in a mixture of 90 mL of water and 10 mL of 2 *N* nitric acid. Store in dark, amber-colored bottles in which a small globule of mercury has been placed.

Methanol (*Methyl Alcohol*) Use ACS reagent-grade *Methanol*.

Methanol, Anhydrous (*Dehydrated Methanol*) Use *Methanol*.

***p*-Methylaminophenol Sulfate TS** Dissolve 2 g of *p*-methylaminophenol sulfate [$(HOC_6H_4NHCH_3)_2 \cdot H_2SO_4$] in 100 mL of water. To 10 mL of this solution add 90 mL of water and 20 g of sodium bisulfite. Confirm the suitability of this solution by the following test: Add 1 mL of the solution to each of four tubes containing 25 mL of 0.5 *N* sulfuric acid and 1 mL of *Ammonium Molybdate TS*. Add 5 μg of phosphate (PO_4) to one tube, 10 μg to a second, and 20 μg to a third, using 0.5, 1.0, and 2.0 mL, respectively, of *Phosphate Standard Solution*, and allow to stand for 2 h. The solutions in the three tubes should show readily perceptible differences in blue color corresponding to the relative amounts of phosphate added, and the one to which 5 μg of phosphate was added should be perceptibly bluer than the blank.

Methylene Blue TS Dissolve 125 mg of methylene blue in 100 mL of alcohol, and dilute with alcohol to 250 mL.

Methyl Orange TS Dissolve 100 mg of methyl orange in 100 mL of water, and filter if necessary.

Methyl Red TS Dissolve 100 mg of methyl red in 100 mL of alcohol, and filter if necessary.

Methyl Red–Methylene Blue TS Add 10 mL of *Methyl Red TS* to 10 mL of *Methylene Blue TS*, and mix.

Methylrosaniline Chloride TS See *Crystal Violet TS*.

Methyl Violet TS See *Crystal Violet TS*.

Millon's Reagent To 2 mL of mercury in an Erlenmeyer flask add 20 mL of nitric acid. Shake the flask in a hood to break the mercury into small globules. After about 10 min add 35 mL of water, and if a precipitate or crystals appear, add sufficient 1:5 nitric acid (prepared from nitric acid from which the oxides have been removed by blowing air through it until it is colorless) to dissolve the separated solid. Add a 1:10 solution of sodium hydroxide, dropwise, with thorough

mixing, until the curdy precipitate that forms after the addition of each drop no longer redissolves but is dispersed to form a suspension. Add 5 mL more of the dilute nitric acid, and mix well. Prepare this solution fresh.

α-Naphtholbenzein TS Dissolve 0.2 g of α-naphtholbenzein in glacial acetic acid to make 100 mL. *Sensitivity*: Add 100 mL of freshly boiled and cooled water to 0.2 mL of a 1:1000 solution of α-naphtholbenzein in ethanol, and add 0.1 mL of 0.1 *N* sodium hydroxide: a green color develops. Add subsequently 0.2 mL of 0.1 *N* hydrochloric acid: the color of the solution changes to yellow-red.

Naphthol Green TS Dissolve 500 mg of naphthol green B in water to make 1000 mL.

Nessler's Reagent See *Alkaline Mercuric–Potassium Iodide TS*.

Neutral Red TS Dissolve 100 mg of neutral red in 100 mL of 50% alcohol.

Nickel Standard Solution TS (10 mg/kg) Prepare a 0.40% (w/v) solution of analytical reagent-grade nickel chloride ($NiCl_2 \cdot 6H_2O$) with water. Pipet 1.0 mL of the solution into a 100-mL volumetric flask, and dilute to volume with water.

Ninhydrin TS See *Triketohydrindene Hydrate TS*.

Nitric Acid (approximately 15.7 *N*) Use ACS reagent-grade *Nitric Acid* (69.0% to 71.0% of HNO_3).

Nitric Acid TS, Diluted (1.7 *N*) A solution containing about 10% (w/v) of HNO_3. Prepare by diluting 105 mL of nitric acid (70%) with water to make 1000 mL.

Orthophenanthroline TS Dissolve 150 mg of orthophenanthroline ($C_{12}H_8N_2 \cdot H_2O$) in 10 mL of a solution of ferrous sulfate, prepared by dissolving 700 mg of clear crystals of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) in 100 mL of water. The ferrous sulfate solution must be prepared immediately before dissolving the orthophenanthroline. Store the solution in well-closed containers.

Oxalic Acid TS Dissolve 6.3 g of oxalic acid ($H_2C_2O_4 \cdot 2H_2O$) in water to make 100 mL.

Phenol Red TS (*Phenolsulfonphthalein TS*) Dissolve 100 mg of phenolsulfonphthalein in 100 mL of alcohol, and filter if necessary.

Phenolphthalein TS Dissolve 1 g of phenolphthalein in 100 mL of alcohol.

Phenolsulfonphthalein TS See *Phenol Red TS*.

***p*-Phenylphenol TS** On the day of use, dissolve 750 mg of *p*-phenylphenol in 50 mL of *Sodium Hydroxide TS*.

Phosphoric Acid Use ACS reagent-grade *Phosphoric Acid* (not less than 85.0% of H_3PO_4).

Phosphotungstic Acid TS Dissolve 1 g of phosphotungstic acid (approximately $24WO_3 \cdot 2H_3PO_4 \cdot 48H_2O$) in water to make 100 mL.

Picric Acid TS See *Trinitrophenol TS*.

Potassium Acetate TS Dissolve 10 g of potassium acetate ($KC_2H_3O_2$) in water to make 100 mL.

Potassium Chromate TS Dissolve 10 g of potassium chromate (K_2CrO_4) in water to make 100 mL.

Potassium Dichromate TS Dissolve 7.5 g of potassium dichromate ($K_2Cr_2O_7$) in water to make 100 mL.

Potassium Ferricyanide TS (10%) Dissolve 1 g of potassium ferricyanide [$K_3Fe(CN)_6$] in 10 mL of water. Prepare this solution fresh.

Potassium Ferrocyanide TS Dissolve 1 g of potassium ferrocyanide [$K_4Fe(CN)_6 \cdot 3H_2O$] in 10 mL of water. Prepare this solution fresh.

Potassium Hydroxide TS (1 *N*) Dissolve 6.5 g of potassium hydroxide (KOH) in water to make 100 mL.

Potassium Hydroxide TS, Alcoholic Use *0.5* N *Alcoholic Potassium Hydroxide* (see *Volumetric Solutions* in this section).

Potassium Iodide TS Dissolve 16.5 g of potassium iodide (KI) in water to make 100 mL. Store in light-resistant containers.

Potassium Permanganate TS Use *0.1* N *Potassium Permanganate* (see *Volumetric Solutions* in this section).

Potassium Pyroantimonate TS Dissolve 2 g of potassium pyroantimonate in 95 mL of hot water. Cool quickly, and add a solution containing 2.5 g of potassium hydroxide in 50 mL of water and 1 mL of an 8.5:100 solution of sodium hydroxide. Allow to stand for 24 h, filter, and dilute with water to 150 mL.

Potassium Sulfate TS Dissolve 1 g of potassium sulfate (K_2SO_4) in sufficient water to make 100 mL.

Quimociac TS Dissolve 70 g of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in 150 mL of water (*Solution A*). Dissolve 60 g of citric acid in a mixture of 85 mL of nitric acid and 150 mL of water, and cool (*Solution B*). Gradually add *Solution A* to *Solution B*, with stirring, to produce *Solution C*. Dissolve 5.0 mL of natural or synthetic quinoline in a mixture of 35 mL of nitric acid and 100 mL of water (*Solution D*). Gradually add *Solution D* to *Solution C*, mix well, and allow to stand overnight. Filter the mixture, add 280 mL of acetone to the

filtrate, dilute to 1000 mL with water, and mix. Store in a polyethylene bottle.

> **Caution**: This reagent contains acetone. Do not use it near an open flame. Operations involving heating or boiling should be conducted in a well-ventilated hood.

Quinaldine Red TS Dissolve 100 mg of quinaldine red in 100 mL of glacial acetic acid.

Schiff's Reagent, Modified Dissolve 200 mg of rosaniline hydrochloride ($C_{20}H_{20}ClN_3$) in 120 mL of hot water. Cool, add 2 g of sodium bisulfite ($NaHSO_3$) followed by 2 mL of hydrochloric acid, and dilute to 200 mL with water. Store in a brown bottle at 15° or lower.

Silver Nitrate TS Use *0.1* N *Silver Nitrate* (see *Volumetric Solutions* in this section).

Sodium Bisulfite TS Dissolve 10 g of sodium bisulfite ($NaHSO_3$) in water to make 30 mL. Prepare this solution fresh.

Sodium Bitartrate TS Dissolve 1 g of sodium bitartrate ($NaHC_4H_4O_6 \cdot H_2O$) in water to make 10 mL. Prepare this solution fresh.

Sodium Borate TS Dissolve 2 g of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) in water to make 100 mL.

Sodium Carbonate TS Dissolve 10.6 g of anhydrous sodium carbonate (Na_2CO_3) in water to make 100 mL.

Sodium Cobaltinitrite TS Dissolve 10 g of sodium cobaltinitrite [$Na_3Co(NO_2)_6$] in water to make 50 mL, and filter if necessary.

Sodium Fluoride TS Dry about 500 mg of sodium fluoride (NaF) at 200° for 4 h. Weigh accurately 222 mg of the dried sodium fluoride, and dissolve it in sufficient water to make exactly 100 mL. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, dilute to volume with water, and mix. Each mL of this final solution corresponds to 10 μg of fluorine (F).

Sodium Hydroxide TS (1 *N*) Dissolve 4.3 g of sodium hydroxide (NaOH) in water to make 100 mL.

Sodium Indigotindisulfonate TS See *Indigo Carmine TS*.

Sodium Nitroferricyanide TS Dissolve 1 g of sodium nitroferricyanide [$Na_2Fe(NO)(CN)_5 \cdot 2H_2O$] in water to make 20 mL. Prepare this solution fresh.

Sodium Phosphate TS Dissolve 12 g of clear crystals of dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) in water to make 100 mL.

Sodium Sulfide TS Dissolve 1 g of sodium sulfide ($Na_2S \cdot 9H_2O$) in water to make 10 mL. Prepare this solution fresh.

Sodium Tetraphenylborate TS Dissolve 1.2 g of sodium tetraphenylborate in water to make 200 mL. If necessary, stir for 5 min with 1 g of freshly prepared hydrous aluminum oxide, and filter to clarify.

Sodium Thiosulfate TS Use *0.1 N Sodium Thiosulfate* (see *Volumetric Solutions* in this section).

Stannous Chloride TS Dissolve 40 g of reagent-grade stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) in 100 mL of hydrochloric acid.

Starch TS Mix 1 g of a suitable starch with 10 mg of red mercuric oxide and sufficient cold water to make a thin paste. Add 20 mL of boiling water, boil for 1 min with continuous stirring, and cool. Use only the clear solution. Test the sensitivity of the Starch TS by adding 5 mL of Starch TS to 100 mL of water. Add 0.05 mL of freshly prepared 0.1 *N* potassium iodide solution and 1 drop of 50 mg/kg chlorine solution, made by diluting 1 mL of a commercial 5% sodium hypochlorite (NaOCl) solution in 1000 mL of water. The deep blue color produced is discharged by 0.05 mL of 0.1 *N* sodium thiosulfate.

Starch Iodide Paste TS Heat 100 mL of water in a 250-mL beaker to boiling, add a solution of 750 mg of potassium iodide (KI) in 5 mL of water, then add 2 g of zinc chloride ($ZnCl_2$) dissolved in 10 mL of water, and while the solution is boiling, add with stirring a smooth suspension of 5 g of potato starch in 30 mL of cold water. Continue to boil for 2 min, then cool. Store in well-closed containers in a cool place. This mixture must show a definite blue streak when a glass rod dipped in a mixture of 1 mL of 0.1 *M* sodium nitrite, 500 mL of water, and 10 mL of hydrochloric acid is streaked on a smear of the paste.

Sulfanilic Acid TS Dissolve 800 mg of sulfanilic acid (*p*-$NH_2C_6H_4SO_3H \cdot H_2O$) in 100 mL of acetic acid. Store in tight containers.

Sulfuric Acid (approximately 36 *N*) Use ACS reagent-grade *Sulfuric Acid* (95.0% to 98.0% of H_2SO_4).

Sulfuric Acid TS (95%) Add a quantity of sulfuric acid of known concentration to sufficient water to adjust the final concentration to between 94.5% and 95.5% of H_2SO_4. Since the acid concentration may change upon standing or upon intermittent use, the concentration should be checked frequently and solutions assaying more than 95.5% or less than 94.5% discarded or adjusted by adding either diluted or fuming sulfuric acid, as required.

Sulfuric Acid TS, Diluted (2 *N*) A solution containing 10% (w/v) of H_2SO_4. Prepare by cautiously adding 57 mL of sulfuric acid (95% to 98%) or *Sulfuric Acid TS* to about 100 mL of water, then cool to room temperature, and dilute with water to 1000 mL.

Tannic Acid TS Dissolve 1 g of tannic acid (tannin) in 1 mL of alcohol, and add water to make 10 mL. Prepare this solution fresh.

Thymol Blue TS Dissolve 100 mg of thymol blue in 100 mL of alcohol, and filter if necessary.

Thymolphthalein TS Dissolve 100 mg of thymolphthalein in 100 mL of alcohol, and filter if necessary.

Triketohydrindene Hydrate TS (*Ninhydrin TS*) Dissolve 200 mg of triketohydrindene hydrate ($C_9H_4O_3 \cdot H_2O$) in water to make 100 mL. Prepare this solution fresh.

Trinitrophenol TS (*Picric Acid TS*) Dissolve the equivalent of 1 g of anhydrous trinitrophenol in 100 mL of hot water. Cool the solution, and filter if necessary.

Xylenol Orange TS Dissolve 100 mg of xylenol orange in 100 mL of alcohol.

VOLUMETRIC SOLUTIONS

Normal Solutions A normal solution contains 1 g equivalent weight of the solute per L of solution. The normalities of solutions used in volumetric determinations are designated as 1 *N*, 0.1 *N*, 0.05 *N*, etc., in this Codex.

Molar Solutions A molar solution contains 1 g molecular weight of the solute per L of solution. The molarities of such solutions are designated as 1 *M*, 0.1 *M*, 0.05 *M*, etc., in this Codex.

Preparation and Methods of Standardization The details for the preparation and standardization of solutions used in several normalities are usually given only for the one most frequently required. Solutions of other normalities are prepared and standardized in the same general manner as described. Solutions of lower normalities may be prepared accurately by making an exact dilution of a stronger solution, but solutions prepared in this way should be restandardized before use.

Dilute solutions that are not stable, such as 0.01 *N* potassium permanganate and sodium thiosulfate, are preferably prepared by diluting exactly the higher normality with thoroughly boiled and cooled water on the same day they are to be used.

All volumetric solutions should be prepared, standardized, and used at the standard temperature of 25°, if practicable. When a titration must be carried out at a markedly different temperature, the volumetric solution should be standardized at that same temperature, or a suitable temperature correction should be made. Since the strength of a standard solution may change upon standing, the normality or molarity factor should be redetermined frequently.

Although the directions provide only one method of standardization, other methods of equal or greater accuracy may be used. For substances available as certified primary standards, or of comparable quality, the final standard solution may be prepared by weighing accurately a suitable quantity of the substance and dissolving it to produce a specific volume solution of known concentration. Hydrochloric and sulfuric acids may be standardized against a certified primary standard.

In volumetric assays described in this Codex, the number of mg of the test substance equivalent to 1 mL of the primary volumetric solution is given. In general, these equivalents may be derived by simple calculation (see also *Solutions*, in the *General Provisions*).

Ammonium Thiocyanate, 0.1 *N* (7.612 g NH_4SCN per 1000 mL) Dissolve about 8 g of ammonium thiocyanate (NH_4SCN) in 1000 mL of water, and standardize by titrating the solution against *0.1* N *Silver Nitrate* as follows: Transfer about 30 mL of 0.*1* N *Silver Nitrate*, accurately measured, into a glass-stoppered flask. Dilute with 50 mL of water, then add 2 mL of *Ferric Ammonium Sulfate TS* and 2 mL of nitric acid, and titrate with the ammonium thiocyanate solution to the first appearance of a red-brown color. Calculate the normality, and, if desired, adjust the solution to exactly 0.1 *N*. If desired, *0.1* N *Ammonium Thiocyanate* may be replaced by 0.1 *N* potassium thiocyanate where the former is directed in various tests and assays.

Barium Hydroxide, 0.2 *N* [17.14 g $Ba(OH)_2$ per 1000 mL] Dissolve about 36 g of barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$] in 1 L of recently boiled and cooled water, and quickly filter the solution. Keep this solution in bottles with well-fitted rubber stoppers with a soda–lime tube attached to each bottle to protect the solution from carbon dioxide in the air. Standardize as follows: Transfer quantitatively about 60 mL of 0.1 *N* hydrochloric acid, accurately measured, to a flask; add 2 drops of *Phenolphthalein TS*; and slowly titrate with the barium hydroxide solution, with constant stirring, until a permanent pink color is produced. Calculate the normality of the barium hydroxide solution and, if desired, adjust to exactly 0.2 *N* with freshly boiled and cooled water.

> **Note**: Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. Connect the buret used for titrations with barium hydroxide solution directly to the storage bottle, and provide the bottle with a soda–lime tube so that air entering must pass through this tube, which will absorb carbon dioxide. Frequently restandardize standard solutions of barium hydroxide.

Bromine, 0.1 *N* (7.990 g Br per 1000 mL) Dissolve 3 g of potassium bromate ($KBrO_3$) and 15 g of potassium bromide (KBr) in sufficient water to make 1000 mL, and standardize the solution as follows: Transfer about 25 mL of the solution, accurately measured, into a 500-mL iodine flask, and dilute with 120 mL of water. Add 5 mL of hydrochloric acid, stopper the flask, and shake it gently. Then add 5 mL of *Potassium Iodide TS*, restopper, shake the mixture, allow it to stand for 5 min, and titrate the liberated iodine with *0.1* N *Sodium Thiosulfate*, adding *Starch TS* near the end of the titration. Calculate the normality. Store this solution in dark, amber-colored, glass-stoppered bottles.

Ceric Sulfate, 0.1 *N* [33.22 g $Ce(SO_4)_2$ per 1000 mL] Transfer 59 g of ceric ammonium nitrate [$Ce(NO_3)_4 \cdot 2NH_4NO_3 \cdot 2H_2O$] to a beaker, add 31 mL of sulfuric acid, mix, and cautiously add water, in 20-mL portions, until solution is complete. Cover the beaker, let stand overnight, filter through a sintered-glass crucible of fine porosity, add water to make 1000 mL, and mix. Standardize the solution as follows: Weigh accurately 200 mg of primary standard arsenic trioxide (As_2O_3) previously dried at 100° for 1 h, and transfer to a 500-mL Erlenmeyer flask. Wash down the inner walls of the flask with 25 mL of a 2:25 solution of sodium hydroxide, swirl to dissolve the sample, and when solution is complete, add 100 mL of water, and mix. Add 10 mL of 1:3 sulfuric acid and 2 drops each of *Orthophenanthroline TS* and a solution of osmium tetroxide in 0.1 *N* sulfuric acid (1:400), and slowly titrate with the ceric sulfate solution until the pink color is changed to a very pale blue. Calculate the normality. Each 4.946 mg of As_2O_3 is equivalent to 1 mL of *0.1* N *Ceric Sulfate*.

Ceric Sulfate, 0.01 *N* [3.322 g $Ce(SO_4)_2$ per 1000 mL] Dissolve 4.2 g of ceric sulfate [$Ce(SO_4)_2 \cdot 4H_2O$] or 5.5 g of the acid sulfate [$Ce(HSO_4)_4$] in about 500 mL of water containing 28 mL of sulfuric acid, and dilute to 1000 mL. Allow the solution to stand overnight, and filter. Standardize this solution daily as follows: Weigh accurately about 275 mg of hydroquinone ($C_6H_6O_2$), dissolve it in sufficient *0.5 N Alcoholic Sulfuric Acid* to make 500.0 mL, and mix. To 25.0 mL of this solution add 75 mL of 0.5 *N* sulfuric acid, 20 mL of water, and 2 drops of *Diphenylamine TS*. Titrate with the ceric sulfate solution at a rate of about 25 drops per 10 s until an endpoint is reached that persists for 10 s. Perform a blank determination using 100 mL of *0.5* N *Alcoholic Sulfuric Acid*, 20 mL of water, and 2 drops of *Diphenylamine TS*, and make any necessary correction. Calculate the normality of the ceric sulfate solution by the formula

$$0.05W/55.057V,$$

in which W is the weight, in mg, of the hydroquinone sample taken, and V is the volume, in mL, of the ceric sulfate solution consumed in the titration.

Disodium EDTA, 0.05 *M* (16.81 g $C_{10}H_{14}N_2Na_2O_8$ per 1000 mL) Dissolve 18.6 g of disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$) in sufficient water to make 1000 mL, and standardize the solution as follows: Weigh accurately about 200 mg of chelometric standard calcium carbonate ($CaCO_3$), transfer to a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 mL of 2.7 *N* hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute to about 100 mL with water. While stirring, preferably with a magnetic stirrer, add about 30 mL of the disodium EDTA solution from a 50-mL buret, then add 15 mL of *1* N *Sodium Hydroxide* and 300 mg of *Hydroxy Naphthol Blue Indicator*, and continue the titration to a blue endpoint. Calculate the molarity by the formula

$$W/100.09V,$$

in which W is the weight, in mg, of $CaCO_3$ in the sample of calcium carbonate taken, and V is the volume, in mL, of disodium EDTA solution consumed. Each 5.004 mg of $CaCO_3$ is equivalent to 1 mL of *0.05* M *Disodium EDTA*.

For the determination of aluminum in its salts, use *0.05* M *Disodium EDTA* standardized as follows: Transfer 2 g, accurately weighed, of aluminum wire to a 1000-mL volumetric flask, and add 50 mL of a 1:1 hydrochloric acid–water mixture. Swirl the flask to ensure complete wetting of the wire, and allow the reaction to proceed. When dissolution is complete, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 250-mL beaker, add 25.0 mL of the disodium EDTA solution, boil gently for 5 min, and cool. Add in the order given, and with continuous stirring, 20 mL of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 mL of glacial acetic acid in 1000 mL of solution), 50 mL of alcohol, and 2 mL of *Dithizone TS*. Titrate with *0.05* M *Zinc Sulfate* to a bright rose pink color, and perform a blank determination, substituting 10 mL of water for the 10.0 mL of aluminum solution. Each mL of disodium EDTA solution is equivalent to 1.349 mg of aluminum (Al).

Ferrous Ammonium Sulfate, 0.1 *N* [39.21 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ per 1000 mL] Dissolve 40 g of ferrous ammonium sulfate hexahydrate in a previously cooled mixture of 40 mL of sulfuric acid and 200 mL of water, dilute to 1000 mL with water, and mix. On the day of use, standardize the solution as follows: Transfer from 25 to 30 mL of the solution, accurately measured, into a flask, add 2 drops of *Orthophenanthroline TS*, and titrate with *0.1* N *Ceric Sulfate* until the red color is changed to pale blue. From the volume of *0.1* N *Ceric Sulfate* consumed, calculate the normality.

Hydrochloric Acid, 1 *N* (36.46 g HCl per 1000 mL) Dilute 85 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows: Accurately weigh about 1.5 g of primary standard anhydrous sodium carbonate (Na_2CO_3) that has been heated at a temperature of about 270° for 1 h. Dissolve it in 100 mL of water, and add 2 drops of *Methyl Red TS*. Add the acid slowly from a buret, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, and continue the titration until the faint pink color is no longer affected by continued boiling. Calculate the normality. Each 52.99 mg of Na_2CO_3 is equivalent to 1 mL of *1* N *Hydrochloric Acid*.

Hydroxylamine Hydrochloride, 0.5 *N* (35 g $NH_2OH \cdot HCl$ per 1000 mL) Dissolve 35 g of hydroxylamine hydrochloride in 150 mL of water, and dilute to 1000 mL with anhydrous methanol. To 500 mL of this solution add 15 mL of a 0.04% solution of bromophenol blue in alcohol, and titrate with *0.5* N *Triethanolamine* until the solution appears green-blue by transmitted light. *Prepare this solution fresh before each series of analyses*.

Iodine, 0.1 *N* (12.69 g I per 1000 mL) Dissolve about 14 g of iodine (I) in a solution of 36 g of potassium iodide (KI) in 100 mL of water, add 3 drops of hydrochloric acid, dilute with water to 1000 mL, and standardize as follows: Weigh accurately about 150 mg of primary standard arsenic trioxide (As_2O_3) previously dried at 105° for 1 h, and dissolve it in 20 mL of *1* N *Sodium Hydroxide* by warming if necessary. Dilute with 40 mL of water, add 2 drops of *Methyl Orange TS*, and follow with 2.7 *N* hydrochloric acid until the yellow color is changed to pink. Then add 2 g of sodium bicarbonate ($NaHCO_3$), dilute with 50 mL of water, add 3 mL of *Starch TS*, and slowly add the iodine solution from a buret until a permanent blue color is produced. Calculate the normality. Each 4.946 mg of As_2O_3 is equivalent to 1 mL of *0.1* N *Iodine*. Store this solution in glass-stoppered bottles.

Lithium Methoxide, 0.1 *N* (3.797 g CH_3OLi per 1000 mL) Dissolve 600 mg of freshly cut lithium metal in a mixture of 150 mL of anhydrous methanol and 850 mL of benzene. Filter the resulting solution if it is cloudy, and standardize it as follows: Dissolve about 80 mg of benzoic acid (National Institute of Standards and Technology primary standard), accurately weighed, in 35 mL of dimethylformamide, add 5 drops of *Thymol Blue TS*, and titrate with the lithium methoxide solution to a dark blue endpoint.

> **Caution**: Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminum foil while dissolving the benzoic acid sample and during the titration.

Each mL of *0.1* N *Lithium Methoxide* is equivalent to 12.21 mg of benzoic acid.

Mercuric Nitrate, 0.1 *M* [32.46 g $Hg(NO_3)_2$ per 1000 mL] Dissolve about 35 g of mercuric nitrate [$Hg(NO_3)_2 \cdot H_2O$] in a mixture of 5 mL of nitric acid and 500 mL of water, and dilute with water to 1000 mL. Standardize the solution as follows: Transfer an accurately measured volume of about 20 mL of the solution into an Erlenmeyer flask, and add 2 mL of nitric acid and 2 mL of *Ferric Ammonium Sulfate TS*. Cool to below 20°, and titrate with *0.1* N *Ammonium Thiocyanate* to the first appearance of a permanent brown color. Calculate the molarity.

Oxalic Acid, 0.1 *N* (4.502 g $H_2C_2O_4$ per 1000 mL) Dissolve 6.45 g of oxalic acid ($H_2C_2O_4 \cdot 2H_2O$) in sufficient water to make 1000 mL. Standardize by titration against freshly standardized *0.1* N *Potassium Permanganate* as directed under *Potassium Permanganate, 0.1* N. Store this solution in glass-stoppered bottles, protected from light.

Perchloric Acid, 0.1 *N* (10.046 g $HClO_4$ per 1000 mL) Mix 8.5 mL of perchloric acid (70%) with 500 mL of glacial acetic acid and 30 mL of acetic anhydride.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Cool, and add glacial acetic acid to make 1000 mL. Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be combined, and determine the water content by the *Karl Fischer Titrimetric Method*, Appendix IIB. If the water content exceeds 0.05%, add more acetic anhydride, but if the solution contains no titratable water, add sufficient water to make the content between 0.02% and 0.05%. Allow to stand for 1 day, and again determine the water content by titration. Standardize the solution as follows: Weigh accurately about 700 mg of primary standard potassium biphthalate [$KHC_6H_4(COO)_2$], previously dried at 105° for 2 h, and dissolve it in 50 mL of glacial acetic acid in a 250-mL flask. Add 2 drops of *Crystal Violet TS*, and titrate with the perchloric acid solution until the violet color changes to emerald green. Deduct the volume of the perchloric acid consumed by 50 mL of the glacial acetic acid, and calculate the normality. Each 20.42 mg of $KHC_6H_4(COO)_2$ is equivalent to 1 mL of *0.1* N *Perchloric Acid*.

Perchloric Acid, 0.1 *N*, in Dioxane Mix 8.5 mL of perchloric acid (70%) with sufficient dioxane, which has been especially purified by adsorption, to make 1000 mL.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Standardize the solution as follows: Weigh accurately about 700 mg of primary standard potassium biphthalate [$KHC_6H_4(COO)_2$], previously dried at 105° for 2 h, and dissolve in 50 mL of glacial acetic acid in a 250-mL flask. Add 2 drops of *Crystal Violet TS*, and titrate with the perchloric acid solution until the violet color changes to blue-green. Deduct the volume of the perchloric acid consumed by 50 mL of the glacial acetic acid, and calculate the normality. Each 20.42 mg of $KHC_6H_4(COO)_2$ is equivalent to 1 mL of *0.1* N *Perchloric Acid*.

Potassium Acid Phthalate, 0.1 *N* [20.42 g $KHC_6H_4(COO)_2$ per 1000 mL] Dissolve 20.42 g of primary standard potassium biphthalate [$KHC_6H_4(COO)_2$], previously dried at 105° for 2 h, in glacial acetic acid in a 1000-mL volumetric flask, warming on a steam bath if necessary to effect solution and protecting the solution from contamination by moisture. Cool to room temperature, dilute to volume with glacial acetic acid, and mix.

Potassium Dichromate, 0.1 *N* (4.903 g $K_2Cr_2O_7$ per 1000 mL) Dissolve about 5 g of potassium dichromate ($K_2Cr_2O_7$) in 1000 mL of water, transfer quantitatively 25 mL of this solution to a 500-mL glass-stoppered flask, add 2 g of potassium iodide (free from iodate) (KI), dilute with 200 mL of water, add 5 mL of hydrochloric acid, and mix. Allow to stand for 10 min in a dark place, and titrate the liberated iodine with *0.1* N *Sodium Thiosulfate*, adding *Starch TS* as the endpoint is approached. Correct for a blank run on the same quantities of the same reagents, and calculate the normality.

Potassium Hydroxide, 1 *N* (56.11 g KOH per 1000 mL) Prepare and standardize 1 *N* potassium hydroxide by the procedure set forth for *1* N *Sodium Hydroxide*, using 74 g of the potassium hydroxide (KOH) to prepare the solution. Each

204.2 mg of $KHC_6H_4(COO)_2$ is equivalent to 1 mL of *1* N *Potassium Hydroxide*.

Potassium Hydroxide, 0.5 *N*, Alcoholic (**Caution**: The solution may become very hot. Allow it to cool before adding the aldehyde-free alcohol.) Dissolve about 35 g of potassium hydroxide (KOH) in 20 mL of water, and add sufficient aldehyde-free alcohol to make 1000 mL. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant liquid into a suitable, tight container, and standardize as follows: Transfer quantitatively 25 mL of 0.5 *N* hydrochloric acid into a flask, dilute with 50 mL of water, add 2 drops of *Phenolphthalein TS*, and titrate with the alcoholic potassium hydroxide solution until a permanent, pale pink color is produced. Calculate the normality. Store this solution in tightly stoppered bottles protected from light.

Potassium Iodate, 0.05 *M* (10.70 g KIO_3 per 1000 mL) Dissolve 10.700 g of potassium iodate of primary standard quality (KIO_3), previously dried at 110° to constant weight, in sufficient water to make 1000.0 mL.

Potassium Permanganate, 0.1 *N* (3.161 g $KMnO_4$ per 1000 mL) Dissolve about 3.3 g of potassium permanganate ($KMnO_4$) in 1000 mL of water in a flask, and boil the solution for about 15 min. Stopper the flask, allow it to stand for at least 2 days, and filter through a fine-porosity, sintered-glass crucible. If necessary, the bottom of the crucible may be lined with a pledget of glass wool. Standardize the solution as follows: Weigh accurately about 200 mg of sodium oxalate of primary standard quality ($Na_2C_2O_4$), previously dried at 100° to constant weight, and dissolve it in 250 mL of water. Add 7 mL of sulfuric acid, heat to about 70°, and then slowly add the permanganate solution from a buret, with constant stirring, until a pale pink color that persists for 15 s is produced. The temperature at the conclusion of the titration should be not less than 60°. Calculate the normality. Each 6.700 mg of $Na_2C_2O_4$ is equivalent to 1 mL of *0.1* N *Potassium Permanganate*. Potassium permanganate is reduced on contact with organic substances such as rubber; therefore, the solution must be handled in apparatus made entirely of glass or other suitably inert material. Store it in glass-stoppered, amber-colored bottles, and restandardize frequently.

Silver Nitrate, 0.1 *N* (16.99 g $AgNO_3$ per 1000 mL) Dissolve about 17.5 g of silver nitrate ($AgNO_3$) in 1000 mL of water, and standardize the solution as follows: Weigh accurately 100 mg of primary standard sodium chloride, previously dried at 120° for 16 h, into a 150-mL beaker, and dissolve it in 5 mL of water. Add 5 mL of acetic acid, 50 mL of methanol, and 2 or 3 drops of *Eosin Y TS*, and titrate with the silver nitrate solution to the endpoint. Calculate the normality.

Sodium Acetate, 0.1 *N* (8.203 g CH_3COONa per 1000 mL) Dissolve 8.20 g of anhydrous sodium acetate in glacial acetic acid to make 1000 mL, and standardize the solution as follows: To 25.0 mL of the prepared sodium acetate solution, add 50 mL of glacial acetic acid and 1 mL of α-*Naphtholbenzein TS*. Titrate with *0.1* N *Perchloric Acid* until a yellow-brown color changes through yellow to green.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Perform a blank determination, and make any necessary correction. Calculate the normality factor.

Sodium Arsenite, 0.05 *N* (3.248 g $NaAsO_2$ per 1000 mL) Transfer 2.4725 g of arsenic trioxide, which has been pulverized and dried at 100° to constant weight, to a 1000-mL volumetric flask, dissolve it in 20 mL of *1* N *Sodium Hydroxide*, and add *1* N *Sulfuric Acid* or *1* N *Hydrochloric Acid* until the solution is neutral or only slightly acid to litmus. Add 15 g of sodium bicarbonate, dilute to volume with water, and mix.

Sodium Hydroxide, 1 *N* (40.00 g NaOH per 1000 mL) Dissolve about 40 g of sodium hydroxide (NaOH) in about 1000 mL of carbon dioxide-free water. Shake the mixture thoroughly, and allow it to stand overnight in a stoppered bottle. Standardize the clear liquid as follows: Transfer about 5 g of primary standard potassium biphthalate [$KHC_6H_4(COO)_2$], previously dried at 105° for 2 h and accurately weighed, to a flask, and dissolve it in 75 mL of carbon dioxide-free water. If the potassium biphthalate is in the form of large crystals, crush it before drying. To the flask add 2 drops of *Phenolphthalein TS*, and titrate with the sodium hydroxide solution to a permanent pink color. Calculate the normality. Each 204.2 mg of potassium biphthalate is equivalent to 1 mL of *1* N *Sodium Hydroxide*.

> **Note**: Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. Therefore, store them in bottles with well-fitted, suitable stoppers provided with a tube filled with a mixture of sodium hydroxide and lime so that air entering the container must pass through this tube, which will absorb the carbon dioxide. Frequently restandardize standard solutions of sodium hydroxide.

Sodium Hydroxide, 0.5 *N*, Alcoholic (22.5 g NaOH per 1000 mL) (**Caution**: The following solution may become very hot. Allow it to cool before adding the aldehyde-free alcohol.) Dissolve about 22.5 g of sodium hydroxide (NaOH) in 20 mL of water, and add sufficient aldehyde-free alcohol to make 1000 mL. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant liquid into a suitable, tight container, and standardize as follows:

Quantitatively transfer 25 mL of 0 5 *N* hydrochloric acid into a flask, dilute with 50 mL of water, add 2 drops of *Phenolphthalein TS*, and titrate with the alcoholic sodium hydroxide solution until a permanent, pale pink color appears. Calculate the normality. Store this solution in tightly stoppered bottles protected from light.

Sodium Methoxide, 0.1 *N*, in Pyridine (5.40 g CH_3ONa per 1000 mL) Weigh 14 g of freshly cut sodium metal, and cut into small cubes. Place about 0.5 mL of anhydrous methanol

in a round-bottom 120-mL flask equipped with a ground-glass joint, add 1 cube of the sodium metal, and when the reaction subsides, add the remaining sodium metal to the flask. Connect a water-cooled condenser to the flask, and slowly add 100 mL of anhydrous methanol, in small portions, through the top of the condenser. Regulate the addition of the methanol so that the vapors are condensed and do not escape through the top of the condenser. After addition of the methanol is complete, connect a drying tube to the top of the condenser, and allow the solution to cool. Transfer 17.5 mL of this solution (approximately 6 *N*) into a 1000-mL volumetric flask containing 70 mL of anhydrous methanol, and dilute to volume with freshly distilled pyridine. Store preferably in the reservoir of an automatic buret suitably protected from carbon dioxide and moisture. Standardize the solution as follows: Weigh accurately about 400 mg of primary standard benzoic acid, transfer it into a 250-mL wide-mouth Erlenmeyer flask, and dissolve it in 50 mL of freshly distilled pyridine. Add a few drops of *Thymolphthalein TS*, and titrate immediately with the sodium methoxide solution to a blue endpoint. During the titration, direct a gentle stream of nitrogen into the flask through a short piece of 6-mm glass tubing fastened near the tip of the buret. Perform a blank determination (see the *General Provisions*), correct for the volume of sodium methoxide solution consumed by the blank, and calculate the normality. Each 12.21 mg of benzoic acid is equivalent to 1 mL of *0.1* N *Sodium Methoxide in Pyridine*.

Sodium Methoxide, 0.02 *N*, in Toluene (1.08 g CH_3ONa per 1000 mL) Weigh 2.5 g of freshly cut sodium metal, and cut into small cubes. Place about 200 mL of anhydrous methanol in a 1000-mL volumetric flask, chill in an ice bath, and add the cubes one at a time to the methanol. When the last cube is dissolved, dilute to the mark with toluene, and mix. Standardize the solution as follows: Weigh accurately about 20 mg of primary standard benzoic acid, transfer it into a 50-mL conical flask, and dissolve it in 25 mL of dimethylformamide. Add 2 drops of a solution of 100 mg of thymol blue in 10 mL of dimethylformamide, and titrate immediately with the sodium methoxide solution to a blue endpoint. Titrate a blank solution of dimethylformamide in the same manner, correct the volume of sodium methoxide solution consumed by the blank, and calculate the normality. Each 2.442 mg of benzoic acid is equivalent to 1 mL of *0.02* N *Sodium Methoxide in Toluene*.

Sodium Thiosulfate, 0.1 *N* (15.81 g $Na_2S_2O_3$ per 1000 mL) Dissolve about 26 g of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) and 200 mg of sodium carbonate (Na_2CO_3) in 1000 mL of recently boiled and cooled water. Standardize the solution as follows: Weigh accurately about 210 mg of primary standard potassium dichromate, previously pulverized and dried at 120° for 4 h, and dissolve in 100 mL of water in a 500-mL glass-stoppered flask. Swirl to dissolve the sample, remove the stopper, and quickly add 2 g of sodium bicarbonate, 3 g of potassium iodide, and 5 mL of hydrochloric acid. Stopper the flask, swirl to mix, and let stand in the dark for 10 min. Rinse the stopper and inner walls of the flask with water, and titrate the liberated iodine with the sodium thiosulfate solution until the solution is only faint yellow. Add *Starch TS*, and continue the titration to the discharge of the blue color. Calculate the normality.

Sulfuric Acid, 1 *N* (49.04 g H_2SO_4 per 1000 mL) Add slowly, with stirring, 30 mL of sulfuric acid to about 1020 mL of water, allow to cool to 25°, and standardize by titration against primary standard sodium carbonate (Na_2CO_3) as directed under *1* N *Hydrochloric Acid*. Each 52.99 mg of Na_2CO_3 is equivalent to 1 mL of *1* N *Sulfuric Acid*.

Sulfuric Acid, Alcoholic, 5 *N* (245.2 g H_2SO_4 per 1000 mL) Add cautiously, with stirring, 139 mL of sulfuric acid to a sufficient quantity of absolute alcohol to make 1000.0 mL.

Sulfuric Acid, Alcoholic, 0.5 *N* Add cautiously, with stirring, 13.9 mL of sulfuric acid to a sufficient quantity of absolute alcohol to make 1000.0 mL. Alternatively, prepare this solution by diluting 100.0 mL of *5* N *Sulfuric Acid* with absolute alcohol to make 1000.0 mL.

Thorium Nitrate, 0.1 *M* [48.01 g $Th(NO_3)_4$ per 1000 mL] Weigh accurately 55.21 g of thorium nitrate [$Th(NO_3)_4 \cdot 4H_2O$], dissolve it in water, dilute to 1000.0 mL, and mix. Standardize the solution as follows: Transfer 50.0 mL into a 500-mL volumetric flask, dilute to volume with water, and mix. Transfer 50.0 mL of the diluted solution into a 400-mL beaker, add 150 mL of water and 5 mL of hydrochloric acid, and heat to boiling. While stirring, add 25 mL of a saturated solution of oxalic acid, then digest the mixture for 1 h just below the boiling point, and allow to stand overnight. Decant through Whatman No. 42, or equivalent, filter paper, and transfer the precipitate to the filter using about 100 mL of a wash solution consisting of 70 mL of the saturated oxalic acid solution, 430 mL of water, and 5 mL of hydrochloric acid. Transfer the precipitate and filter paper to a tared tall-form porcelain crucible, dry, char the paper, and ignite at 950° for 1.5 h or to constant weight. Cool in a desiccator, weigh, and calculate the molarity of the solution by the formula

$$200W/264.04,$$

in which W is the weight, in g, of thorium oxide obtained.

Triethanolamine, 0.5 *N* [74 g $N(CH_2CH_2OH)_3$ per 1000 mL] Transfer 65 mL (74 g) of 98% triethanolamine into a 1000-mL volumetric flask, dilute to volume with water, stopper the flask, and mix thoroughly.

Zinc Sulfate, 0.05 *M* (8.072 g $ZnSO_4$ per 1000 mL) Dissolve about 15 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) in sufficient water to make 1000 mL, and standardize the solution as follows: Dilute about 35 mL, accurately measured, with 75 mL of water, add 5 mL of *Ammonia–Ammonium Chloride Buffer TS* and 0.1 mL of *Eriochrome Black TS*, and titrate with *0.05* M *Disodium EDTA* until the solution is deep blue. Calculate the molarity.

INDICATORS

The necessary solutions of indicators may be prepared as directed under *Test Solutions (TS) and Other Reagents*. The sodium salts of many indicators are commercially available and may be used interchangeably in water solutions with the alcohol solutions specified for the free indicators.

Useful pH indicators, listed in ascending order of the lower limit of their range, are methyl yellow (pH 2.9 to 4.0), bromophenol blue (pH 3.0 to 4.6), bromocresol green (pH 4.0 to 5.4), methyl red (pH 4.2 to 6.2), bromocresol purple (pH 5.2 to 6.8), bromothymol blue (pH 6.0 to 7.6), phenol red (pH 6.8 to 8.2), thymol blue (pH 8.0 to 9.2), and thymolphthalein (pH 9.3 to 10.5).

Alphazurine 2G Use a suitable grade.

Azo Violet [*4-(p-Nitrophenylazo) Resorcinol*] A red powder, melting at about 193° with decomposition.

Bromocresol Blue Use *Bromocresol Green*.

Bromocresol Green (*Bromocresol Blue; Tetrabromo*-m-*cresolsulfonphthalein*) A white or pale buff-colored powder; slightly soluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 3.8 (yellow) to 5.4 (blue).

Bromocresol Purple (*Dibromo*-o-*cresolsulfonphthalein*) A white to pink, crystalline powder; insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 5.2 (yellow) to 6.8 (purple).

Bromophenol Blue (*Tetrabromophenolsulfonphthalein*) Pink crystals, soluble in alcohol. Insoluble in water; soluble in solutions of alkali hydroxides. Transition interval: from pH 3.0 (yellow) to 4.6 (blue).

Bromothymol Blue (*Dibromothymolsulfonphthalein*) A rose red powder. Insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 6.0 (yellow) to 7.6 (blue).

Cresol Red (o-*Cresolsulfonphthalein*) A red-brown powder. Slightly soluble in water; soluble in alcohol and in dilute solutions of alkali hydroxides. Transition interval: from pH 7.2 (yellow) to 8.8 (blue).

Crystal Violet (*Hexamethyl*-p-*rosaniline Chloride*) Dark green crystals. Slightly soluble in water; sparingly soluble in alcohol and in glacial acetic acid. Its solutions are deep violet.

Sensitiveness Dissolve 100 mg in 100 mL of glacial acetic acid, and mix. Pipet 1 mL of the solution into a 100-mL volumetric flask, and dilute with glacial acetic acid to volume. The solution is violet-blue and does not show a red tint. Pipet 20 mL of the diluted solution into a beaker, and titrate with *0.1* N *Perchloric Acid*, adding the perchloric acid slowly from a microburet. Not more than 0.1 mL of *0.1* N *Perchloric Acid* is required to produce an emerald green color.

> **Caution**: Handle perchloric acid in an appropriate fume hood.

Dithizone (*Diphenylthiocarbazone*) A blue-black powder. Insoluble in water; soluble in alcohol and in chloroform, yielding intensely green solutions even in high dilutions.

Eriochrome Black T [*Sodium 1-(1-Hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonate*] A brown-black powder having a faint metallic sheen. Soluble in alcohol, in methanol, and in hot water.

Sensitiveness To 10 mL of a 1:200,000 solution in a mixture of equal parts (v/v) of methanol and water add a 1:100 solution of sodium hydroxide until the pH is 10. The solution is pure blue and free from cloudiness. Add 0.2 mL of *Magnesium Standard Solution* (10 μg Mg ion). The color of the solution changes to red-violet, and with the continued addition of magnesium ion, it becomes wine red.

***p*-Ethoxychrysoidin Monohydrochloride** [*4-(*p-*Ethoxyphenylazo)*-m-*phenylenediamine Monohydrochloride; 4′-Ethoxy-2,4-diaminoazobenzene Monohydrochloride*] A red powder, insoluble in water. Transition interval: from pH 3.5 (red) to 5.5 (yellow).

Hydroxy Naphthol Blue The disodium salt of 1-(2-naphtholazo-3,6-disulfonic acid)-2-naphthol-4-sulfonic acid deposited on crystals of sodium chloride. Small blue crystals, freely soluble in water. In the pH range between 12 and 13, its solution is red-pink in the presence of calcium ion and deep blue in the presence of excess disodium EDTA.

Suitability for Calcium Determinations Dissolve 300 mg in 100 mL of water, add 10 mL of *1* N *Sodium Hydroxide* and 1.0 mL of a 1:200 calcium chloride solution, and dilute with water to 165 mL. The solution is red-pink. Add 1.0 mL of *0.05* M *Disodium EDTA*. The solution becomes deep blue.

Litmus A blue powder, cubes, or pieces. Partly soluble in water and in alcohol. Transition interval: from approximately pH 4.5 (red) to 8 (blue). Litmus is unsuitable for determining the pH of solutions of carbonates or bicarbonates.

Methylene Blue [*3,7-Bis(dimethylamino)phenazathionium Chloride*] Dark green crystals or a crystalline powder having a bronzelike luster. Soluble in water and in chloroform; sparingly soluble in alcohol.

Methyl Orange (*Helianthin; Tropaeolin D; 4′-Dimethylaminoazobenzene-4-sodium Sulfonate*) An orange-yellow powder or crystalline scales. Slightly soluble in cold water; readily soluble in hot water; insoluble in alcohol. Transition interval: from pH 3.2 (pink) to 4.4 (yellow).

Methyl Red (o-*Carboxybenzeneazodimethylaniline Hydrochloride*) A dark red powder or violet crystals. Sparingly

soluble in water; soluble in alcohol. Transition interval: from pH 4.2 (red) to 6.2 (yellow).

Methyl Red Sodium The sodium salt of *o*-carboxybenzeneazo-dimethylaniline. An orange-brown powder. Freely soluble in cold water and in alcohol. Transition interval: from pH 4.2 (red) to 6.2 (yellow).

Methyl Yellow (p-*Dimethylaminoazobenzene*) Yellow crystals, melting between 114° and 117°. Insoluble in water; soluble in alcohol, in benzene, in chloroform, in ether, in dilute mineral acids, and in oils. Transition interval: from pH 2.9 (red) to 4.0 (yellow).

Murexide Indicator Preparation Add 400 mg of murexide to 40 g of powdered potassium sulfate (K_2SO_4), and grind in a glass mortar to a homogeneous mixture. Alternatively, use tablets containing 0.4 mg of murexide admixed with potassium sulfate or potassium chloride, available commercially.

Naphthol Green B The ferric salt of 6-sodium sulfo-1-isonitroso-1,2-naphthoquinone. A dark green powder, insoluble in water.

Neutral Red (*3-Amino-7-dimethylamino-2-methylphenazine Chloride*) A coarse, red to olive green powder. Sparingly soluble in water and in alcohol. Transition interval: from pH 6.8 (red) to 8.0 (orange).

Phenol Red (*Phenolsulfonphthalein*) A bright to dark red, crystalline powder. Very slightly soluble in water; sparingly soluble in alcohol; soluble in solutions of alkali hydroxides. Transition interval: from pH 6.8 (yellow) to 8.2 (red).

Phenolphthalein White or yellow-white crystals. Practically insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 8.0 (colorless) to 10.0 (red).

Quinaldine Red (*5-Dimethylamino-2-strylethylquinolinium Iodide*) A dark, blue-black powder, melting at about 260° with decomposition. Sparingly soluble in water; freely soluble in alcohol. Transition interval: from pH 1.4 (colorless) to 3.2 (red).

Thymol Blue (*Thymolsulfonphthalein*) A dark, brown-green, crystalline powder. Slightly soluble in water; soluble in alcohol and in dilute alkali solutions. Acid transition interval: from pH 1.2 (red) to 2.8 (yellow). Alkaline transition interval: from pH 8.0 (yellow) to 9.2 (blue).

Thymolphthalein A white to slightly yellow, crystalline powder. Insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 9.3 (colorless) to 10.5 (blue).

Xylenol Orange [*3,3′-Bis-di(carboxymethyl)aminomethyl-o-cresolsulfonphthalein*] An orange powder. Soluble in water and in alcohol. In acid solution it is lemon yellow, and its metal complexes are intensely red. It gives a distinct endpoint in the direct EDTA titration of metals such as bismuth, thorium, scandium, lead, zinc, lanthanum, cadmium, and mercury.

INDICATOR PAPERS AND TEST PAPERS

Indicator papers and test papers are strips of paper of suitable dimension and grade (usually Swedish O filter paper or other makes of like surface, quality, and ash) impregnated with a sufficiently stable indicator solution or reagent.

Treat strong, white filter paper with hydrochloric acid, and wash with water until the last washing shows no acid reaction to *Methyl Red TS*. Then treat with 6 *N* ammonium hydroxide, wash again with water until the last washing is not alkaline toward *Phenolphthalein TS*, and dry thoroughly. Saturate the dry paper with the appropriate indicator solution prepared as directed below, and dry carefully by suspending from glass rods or other inert material in still air free from acid, alkali, and other fumes. Cut the paper into strips of convenient size, and store in well-closed containers protected from light and moisture.

Indicator papers and test papers that are available commercially may be used, if desired.

Acetaldehyde Test Paper Use a solution prepared by mixing equal volumes of a 20% solution of morpholine and a 5% solution of sodium nitroferricyanide. Saturate the prepared filter paper in the mixture, and use the moistened paper without drying.

Cupric Sulfate Test Paper Use *Cupric Sulfate TS*.

Lead Acetate Test Paper Usually about 6 × 80 mm in size. Use *Lead Acetate TS*, and dry the paper at 100°, avoiding contact with metal.

Litmus Paper, Blue Usually about 6 × 50 mm in size. It meets the requirements of the following tests.

Phosphate Place 10 strips in 10 mL of water to which have been added 1 mL of nitric acid and 0.5 mL of 6 *N* ammonium hydroxide. Allow to stand for 10 min, then decant the solution, warm, and add 5 mL of *Ammonium Molybdate TS*. Shake at about 40° for 5 min. No precipitate of phosphomolybdate is formed.

Residue on Ignition Ignite carefully 10 strips of the paper to constant weight. The weight of the residue corresponds to not more than 400 μg per strip of about 3 cm^2.

Rosins, Acids, etc. Immerse a strip of the blue paper in a solution of 100 mg of silver nitrate ($AgNO_3$) in 50 mL of water. The color of the paper does not change in 30 s.

Sensitiveness Drop a 10- to 12-mm strip in 100 mL of 0.0005 *N* hydrochloric acid contained in a beaker, and stir continuously. The color of the paper is changed within 45 s.

Litmus Paper, Red Usually about 6 × 50 mm in size. Red litmus meets the requirements for *Phosphate*, *Residue on Ignition*, and *Rosins, Acids, etc.*, under *Litmus Paper, Blue*.

Sensitiveness Drop a 10- × 12-mm strip into 100 mL of 0.0005 *N* sodium hydroxide contained in a beaker, and stir continuously. The color of the paper changes within 30 s.

Phenolphthalein Paper Use a 1:1000 solution of phenolphthalein in 1:2 alcohol.

Starch Iodate Paper Use a mixture of equal volumes of *Starch TS* and potassium iodate solution (1:20).

Starch Iodide Paper Use a solution of 500 mg of potassium iodide (KI) in 100 mL of freshly prepared *Starch TS*.

DETECTOR TUBES

Ammonia Detector Tube A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for the indicator bromophenol blue. The Draeger Reference Number is CH 20501; the measuring range is 5 to 70 ppm.

> **Note**: Suitable detector tubes are available from National Draeger, Inc., P.O. Box 120, Pittsburgh, PA 15205-0120. Tubes other than those specified in the monograph may be used in accordance with the section entitled *Codex Specifications* in the *General Provisions*.

Carbon Dioxide Detector Tube A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for the indicators hydrazine and crystal violet. The Draeger Reference Number is CH 30801; the measuring range is 0.01% to 0.30%.

Carbon Monoxide Detector Tube A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for the indicators iodine pentoxide, selenium dioxide, and fuming sulfuric acid. The Draeger Reference Number is CH 25601; the measuring range is 5 to 150 ppm.

Chlorine Detector Tube A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for the indicator *o*-toluidine. The Draeger Reference Number is CH 24301; the measuring range is 0.2 to 3 ppm.

Hydrogen Sulfide Detector Tube A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for the indicator, which is a suitable lead salt. The Draeger Reference Number is 6719001; the measuring range is 1 to 20 ppm.

Nitric Oxide–Nitrogen Dioxide Detector Tube A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for an oxidizing layer and the indicator diphenylbenzidine. The Draeger Reference Number is CH 29401; the measuring range is 0.5 to 10 ppm.

Sulfur Dioxide Detector Tube A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for an iodine–starch indicator. The Draeger Reference Number is CH 31701; the measuring range is 1 to 25 ppm.

Water Vapor Detector Tube A fuse-sealed glass tube (Draeger or equivalent) that is designed to allow gas to be passed through it and that contains suitable absorbing filters and support media for the indicator, which consists of a selenium sol in suspension in sulfuric acid. The Draeger Reference Number is CH 67 28531; the measuring range is 5 to 200 mg/m^3.

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Titles of monographs in *Section 2* and those of flavor chemicals in *Section 3* are shown in blue type and are linked. An asterisk indicates a new monograph for the Fifth Edition. For titles of new monographs introduced in the three supplements to the Fourth Edition, please consult the listings under *General Information.*

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