OFFICIAL METHODS OF ANALYSIS OF AOAC INTERNATIONAL SIXTEENTH EDITION VOLUME I

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EDITED BY PATRICIA CUNNIFF

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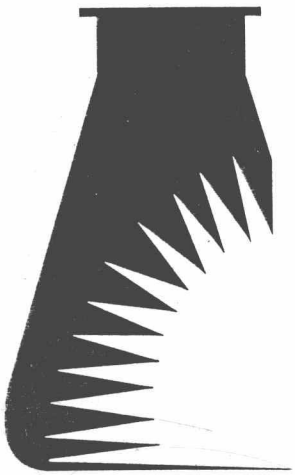

Official Methods of Analysis of AOAC INTERNATIONAL

16th Edition

VOLUME I

Agricultural Chemicals; Contaminants; Drugs

Important Notices

DISCLAIMER

METHODS

Analytical methods and procedures in this compendium have undergone systematic interlaboratory studies to determine the performance characteristics for the intended analytical application. AOAC INTERNATIONAL members and other volunteers have reviewed the analytical results and determined that a particular method is appropriate for the analyte and matrix stated, provided the analysis is conducted by a competent analyst as written. **No warranty, implied or expressed, is made by AOAC INTERNATIONAL on the methods described, their safety, or products mentioned. AOAC, its members, and nonmember volunteers who have aided in the development and validation of methods included in this volume assume no responsibility for any economic, personal injury, or other damage that may occur to individuals or organizations because of use of these methods.**

COMMERCIAL PRODUCTS

Names of manufacturers, suppliers, and trade names are furnished solely as a matter of identification and convenience and reflect the conditions within which each method was developed in the originator's laboratory. Inclusion of this information does *NOT* imply AOAC promotion, approval, endorsement, or certification. The same or equivalent products, instruments, supplies, apparatus, or reagents available from manufacturers and suppliers other than those named, or other brands from other sources, may serve equally well if proper validation indicates their use is satisfactory.

WARNING

Do not perform analyses using AOAC® Official Methods unless you are knowledgeable about their potential dangers or hazards and have received appropriate training. Do not handle instruments, supplies, apparatus, reagents, biohazards, or other products when unfamiliar with their operation or the potential hazards associated with their use. If a method requires the use of potentially hazardous equipment or products, see manufacturer's safety and cautionary instructions. Material Safety Data Sheets (MSDS), or the equivalent, must be read and understood prior to the use of materials specified by a method.

Always use fume hoods, proper ventilation, and protective clothing and equipment where required.

See Appendix B, "Laboratory Safety" for further information on safety.

SURPLUS METHODS

Do *NOT* destroy previous editions of the *Official Methods of Analysis*. They contain surplus methods that are not reprinted in the 16th edition. Surplus methods are satisfactory methods that have been determined not to be in significant current use. These methods retain their official status but are carried only by reference to the edition in which they appear. See page xviii for a more detailed definition of surplus methods.

If you regularly use surplus methods, marked by this symbol "★", **please notify AOAC. Regular use may indicate the method should be reinstated in the current edition.**

REFERENCING AOAC® OFFICIAL METHODS

Each AOAC® Official Method has its own permanent method number that is part of the title block. The paragraph number located in the upper left is only a locator number and not the method number. For example:

39.1.08

AOAC Official Method 991.36
Fat (Crude) in Meat
and Meat Products

991.36 is the permanent number of the method and 39.1.08 is only the paragraph number used to facilitate locating methods. When referencing AOAC® Official Methods, only the permanent number should be referenced as seen in this example:

(1) *Official Methods of Analysis* (1997) 16th Ed., 3rd Revision, 1997, AOAC INTERNATIONAL, Gaithersburg, MD, method 991.36.

REVISIONS

If you did not do so at the time you purchased this 16th Edition of the *Official Methods of Analysis*, it is highly recommended you subscribe to receive periodic revisions by completing and returning the card provided with this edition. You will then automatically receive notice of new revisions and be sent an advance invoice allowing you to purchase the revisions without obligation. If you subscribe to and maintain the revision service, your 16th Edition will always be current.

NOTE: **Individual copies of revisions will be provided only to those who subscribe to the revision service. AOAC will *NOT* retain back copies of revisions for individual sale. If you do not subscribe to the revision service, you will need to buy an entire new book to obtain missing revisions.**

INQUIRIES

Inquiries regarding methods published in this book should be directed to AOAC INTERNATIONAL, Technical Services, 481 N. Frederick Avenue, Suite 500, Gaithersburg, MD 20877-2417 USA. Telephone +1-301-924-7077. Fax +1-301-924-7089. Internet e-mail: aoac@aoac.org

Inquiries regarding purchase of *Official Methods of Analysis* or the revision service, the *Journal of AOAC INTERNATIONAL*, or other AOAC publications should be directed to AOAC INTERNATIONAL, Customer Services, 481 N. Frederick Avenue, Suite 500, Gaithersburg, MD 20877-2417 USA. Telephone +1-301-924-7077. Fax +1-301-924-7089. Internet e-mail: aoac@aoac.org

COMMENTS ON METHODS

AOAC INTERNATIONAL adopts methods that show by their performance data what can be expected of them. As analysts use AOAC® Official Methods, they generate additional information and data concerning applicability, specificity, sensitivity, reliability, and accuracy of the methods. Analysts are requested to advise AOAC about their experiences with the AOAC® Official Methods published in this book. In particular, analysts should notify AOAC of problems in the performance of any method which indicate the method should be revised or restudied. Direct comments to AOAC INTERNATIONAL, Technical Services, 481 N. Frederick Avenue, Suite 500, Gaithersburg, MD 20877-2417 USA. Telephone +1-301-924-7077. Fax +1-301-924-7089. Internet e-mail: aoac@aoac.org

Preface to the 16th Edition

The 16th edition of the *Official Methods of Analysis,* 3rd revision, current through the March 1997 Supplement, includes over 206 new and 403 newly revised methods not included in the 15th edition. Many of the existing methods have been significantly revised since the 15th edition to extend their applicability or to improve their accuracy or both. Additional methods have been declared surplus and removed from the compendium. These actions represent the Association's response to the AOAC mandate to keep pace with the practical needs of regulatory, industry, and research chemists and microbiologists.

What are the dominant factors driving the development of new methods? Specificity continues to drive the development and adoption of many new AOAC® Official Methods and modifications to current Official Methods. Methods providing specificity by means of instrumental separation and quantitation of analytes, such as those based on gas or liquid chromatography, continue to be the predominant ones adopted. However, methods that depend on immunoassay or a particular enzyme's activity for their specificity are certainly becoming commonplace in the analytical laboratory as well. Newer, highly specific techniques, such as SNIF NMR and GC/MS have also been adopted as AOAC® Official Methods, a trend that will certainly continue in the future.

With the increased attention to food safety from a microbiological perspective, due in part to the emerging knowledge and discovery of new food pathogens, the emphasis is on rapid, specific tests to ensure that safety will increase dramatically in the near future. Tests that specifically identify and quantitate particular pathogenic organisms to the exclusion of nonpathogenic ones will be in high demand, especially if the tests are rapid and cost effective. Positive identifications or assurance of negative identification using techniques such as GC/MS for fingerprinting of microbial byproducts and DNA fingerprinting of the microbes themselves will be important tools for future analysts.

As methods based on new technologies become sufficiently rugged and useful for application in multiple laboratory settings, AOAC INTERNATIONAL, through its cadre of volunteer scientists, will continue to evaluate, validate, and adopt those methods suitable for Official Method status.

As for other changes to this 16th edition of *Official Methods of Analysis*, a new chapter on Infant Formula and Medical Diets, Chapter 50, has been added to the 16th edition. Methods in this new chapter were split out from other chapters and combined to accommodate the current and anticipated level of method development in this area.

Additional attention has been given to the safety notices within each chapter. Each General Referee was asked to review all methods in the chapter for adequacy of the safety precautions. As a consequence of this, numerous safety statements have been added. Appendix B, an overview of laboratory safety, has also been significantly revised to include a section on Safe Handling of Microorganisms.

The user will quickly notice several major changes in format from the 15th edition. The most obvious are the loose-leaf binder, the addition of method locator numbers, and the removal of many of the abbreviations. Both the loose-leaf format and the method locator numbers have been introduced as a result of user input from AOAC-conducted surveys as well as significant discussion within the committee structure. The loose-leaf format will allow for incorporation of new methods, the removal of surplus methods, the addition of changes within adopted methods, and the noting of actions taken, all on an on-going basis. When such changes occur, the locator number may change but not the permanent number, and the

chapter or section will be reprinted at modest cost to subscribers. Thus, each user will be able to have the most current version of the *Official Methods of Analysis* at all times.

The permanent number indicates the year that the method was adopted by the Association. The year determines the first three numbers with the next digits being simply the sequence in which the method was adopted within a given year.

To further aid our users, most of the abbreviations have been eliminated from this edition. Only those internationally used technical abbreviations have been retained and are listed in the *Definition of Terms and Explanatory Notes*. This change will make the *Official Methods of Analysis* easier to use by scientists in countries around the world.

The *Official Methods of Analysis* is available in electronic format on CD-ROM. The user will is able to perform full-text searches and link, through hypertext linking, to referenced and other methods almost instantaneously.

Many thanks go to all those individuals who worked as a team to bring this 16th edition, 3rd revision current through March 1997 Supplement, together. Specific thanks go to the Associate Referees, General Referees, collaborators, and Methods Committee members who researched, perfected, validated, and reviewed each method. The General Referees, as Associate Chapter Editors, reviewed each chapter twice in an attempt to ensure that all needed changes were made. The changes within each chapter were then tabulated and submitted to the appropriate Methods Committee for approval. Throughout this process, the AOAC Official Methods Board and the Editorial Board provided guidance and support.

Patricia A. Cunniff
Editor

Jonathan W. DeVries
Chairman
Official Methods Board

ABOUT THE ASSOCIATION

MISSION AND METHODS VALIDATION PROGRAMS

The mission of AOAC INTERNATIONAL (formerly the Association of Official Analytical Chemists), an association of scientists and organizations in the public and private sectors, is to promote methods validation and quality measurements in the analytical sciences. To further its mission, the Association's primary programs focus on the validation of chemical and microbiological analytical methods. These validation programs are: the AOAC® Official Methods Program, the program of choice when the highest level of confidence is desired; the AOAC® Peer Verified Methods Program, used when speed of validation is essential and a lesser degree of confidence is acceptable; and the AOAC® Performance Tested Test Kit Program used to test the performance of test kits. The actual work of validation is largely accomplished by over 800 volunteers, expert scientists, working in their industry, government, and academic laboratories worldwide.

The methods found here in *Official Methods of Analysis* have been validated within the AOAC® Official Methods Program. Candidates for AOAC® Official Method status are subjected to collaborative study by eight or more laboratories, according to internationally recognized standards and receive rigorous scientific review of performance results (see page xxii for additional details of the AOAC® Official Methods Validation Program).

AOAC validated and recognized methods are used by government, industry, and academia throughout the world for analysis of a variety of commodities—particularly those related to food, agriculture, public health and safety, and the environment.

COOPERATIVE ACTIVITIES

AOAC INTERNATIONAL has established joint committees, liaisons, and representation with numerous scientific organizations worldwide. The Association is officially represented at meetings of working groups, subcommittees, and ad hoc committees of the International Dairy Federation (IDF), the International Organization for Standardization (ISO), the Collaborative International Pesticides Analytical Council (CIPAC), the International Union of Pure and Applied Chemistry (IUPAC), and the European Committee for Standardization (CEN). Such arrangements enable AOAC to express its basic policies on the development of internationally acceptable methods of analysis and provide secretariats with basic information regarding AOAC's philosophy and procedures.

AOAC-appointed Liaison Officers coordinate AOAC activities with national, state, provincial, municipal, local agencies and industries and their affiliated organizations, and other method organizations that have oral or written cooperative agreements with AOAC.

MEETINGS

The focal point of AOAC's yearly work is the AOAC INTERNATIONAL Annual Meeting and Exposition where most committees meet, newly elected officials are installed, and the groundwork is laid for future activities. Here members and other scientists also have the opportunity to exchange ideas with colleagues from around the world while updating their technical knowledge through scientific symposia, poster sessions, workshops, forums, short courses, and equipment exhibits.

Conferences focusing on specific topics are also offered by the Association.

SECTIONS

Twelve AOAC INTERNATIONAL Sections and two Subsections, serving analytical scientists in various areas in the United States, Canada, Europe, other Mediterranean countries, Latin America and the Caribbean, offer low-cost, close-to-home meetings and equipment exhibits, training, newsletters, scholarships and other programs. Sections and Subsections are organized and run by locally elected Executive Committees to meet the needs of the scientists working in the areas served.

TECHNICAL DIVISIONS

A Technical Division on Reference Materials is the first such division in an AOAC program to provide opportunities for analytical scientists to work together on specialized interests. The division offers the biannual Biological and Environmental Reference Materials Symposium.

A Technical Division for Laboratory Managers is expected to be approved by Spring 1997. Its focus will be on how to develop and run an efficient, cost effective, and quality laboratory.

TRAINING COURSES

AOAC INTERNATIONAL schedules training courses several times a year on topics of interest to analytical scientists and laboratories. Topics include chemical and microbiological laboratory quality assurance, methods validation, statistical analysis, good laboratory practices, and ISO9000.

PUBLICATIONS

In addition to *Official Methods of Analysis*, the Association publishes the *Journal of AOAC INTERNATIONAL* and a variety of other publications. AOAC's journal contains original research articles and reports on current collaborative study data, including information on inter- and intra-laboratory performance precision which enables the users of the AOAC® Official Methods to make informed choices about the appropriate use of a particular method. Also included in the journal are transactions of the Annual Meeting, committee and referee reports, an annual listing of volunteer positions and incumbents, and all official actions of the Association.

AOAC's other publications include manuals, methods compilations in specific areas of analysis, monographs, food safety posters, and a new monthly magazine, *Inside Laboratory Management.*

AOAC ON-LINE

AOAC's connection to the Internet and the World Wide Web facilitates communication with members and other scientists worldwide. The regularly updated AOAC home page on the Web contains an overview of AOAC's mission and programs; a calendar of upcoming events and deadlines; descriptions and news regarding AOAC's methods validation programs; a catalog of publications offered by AOAC with an on-line order form; the monthly AOAC magazine and abstracts of recent AOAC journal articles; information on sections, the annual meeting, courses, and Individual and Sustaining Membership with an on-line Individual Member application form; and links to other sites of interest to analytical scientists. The AOAC INTERNATIONAL home page is located at http://www.aoac.org, and the main address for Internet e-mail is aoac@aoac.org

AWARDS

Each year, the Association presents a number of awards in recognition of outstanding contributions to analytical methodology in areas of interest to AOAC, meritorious service to the Association, and outstanding work in the AOAC® Official Methods Program. AOAC also awards an annual scholarship to encourage study in fields that support the mission of the Association.

STRUCTURE AND MEMBERSHIP

The organizational structure of AOAC INTERNATIONAL includes the Board of Directors, the Official Methods Board, the Editorial Board, special and standing committees, Referee positions concerned with the validation of methods, liaison positions with other organizations, and a headquarters staff. In addition, a consultant represents the Association and recruit participation in Europe.

The two primary member categories provided for by the AOAC Bylaws are Individual and Sustaining Members. Individual Members include analytical chemists, microbiologists and other biologists, biochemists, and toxicologists, forensic and other scientists. Eligibility for Individual Membership requires a degree in science, an interest in the purpose and goals of the Association, and engagement, directly or indirectly, in analysis or analytical science relevant to the purpose of AOAC INTERNATIONAL. Sustaining Members are government agencies, private firms, universities, associations, and other organizations that provide financial and other support for AOAC INTERNATIONAL's mission.

For further information about AOAC and its programs and activities, contact the Association at:
AOAC INTERNATIONAL, 481 N. Frederick Avenue, Suite 500, Gaithersburg, MD 20877-2417 USA; Telephone +1-301-924-7077; Fax +1-301-924-7089; Internet e-mail aoac@aoac.org.

GUIDE TO METHOD FORMAT*

Locator number identifies method by chapter, subchapter, and sequence within the subchapter for easy cross referencing and location. 7 = chapter seven; .6 = subchapter six; and .14 = the fourteenth method found in chapter seven, subchapter six. The locator number is not the permanent number and is included only for reference.

Chemical names of pesticides and drugs are given at end of pertinent chapter.

Cautionary notes refer to Appendix B, Safety Chapter.

Addresses for suppliers frequently cited throughout book are listed in "Definitions of Terms and Explanatory Notes."

Letters identify main sections for ease of citation and cross-referencing.

Calculation symbols are identified and show correct units.

Chemical Abstracts Service Registry Number. A unique identifier that may be used to search a number of data-retrieval systems.

7.6.14

AOAC Official Method 980.06
Captan in Pesticide Formulations
Liquid Chromatographic Method
First Action 1980
Final Action 1982
AOAC-CIPAC Method

(Method is suitable for technical captan and formulations with captan as only active ingredient.)

A. Principle

Captan is extracted from inerts with solution of diethyl phthalate in CH_2Cl_2. Solution is chromatographed on microparticulate silica gel column, using CH_2Cl_2 as mobile phase. Ratio of captan peak height to diethyl phthalate peak height is calculated from UV response and compared to standard material for quantitation.

(*Caution:* See Appendix B, safety notes on pipets and pesticides.)

B. Apparatus and Reagents

(a) *Liquid chromatograph.*—Able to generate over 1000 psi and measure A at 254 nm.

(b) *Chromatographic column.*—Large bore column containing narrow-range (10 m) porous silica gel particles. Partisil-10, 250 × 4.6 mm id is suitable.

(e) *Reference standard captan.*—Chevron Chemical Co., PO Box 4010, Richmond, CA 94804.

(f) *Methylene chloride.*—Spectroscopic grade or distilled in glass.

C. Preparation of Standard

(a) *Internal standard solution.*—0.312 mg diethyl phthalate/mL. Weigh ca 156 mg diethyl phthalate and transfer to 500 mL volumetric flask. Dilute to volume with same CH_2Cl_2 to be used ...within 20%.

D. Preparation of Sample

Accurately weigh sample expected to contain 40 mg captan into glass bottle. Centrifuge and filter supernate through glass fiber paper. Prepare fresh sample daily.

E. Determination

Adjust operating parameters to cause captan to elute in 4 6 min. Maintain all parameters constant throughout analysis. Typical values are: flow rate, 2.5 mL ... agree to within 2% of their mean. If not, repeat determination.

F. Calculation

Measure peak heights to 3 significant figures, and calculate ratio for each injection. Average 4 standard ratios, and the 2 sample ratios.

$$\% \text{ Captan} = (R/R')\ (W'/W) \times P$$

where R = average sample ratio (captan peak height/diethyl phthalate peak height); R' = average standard ratio (captan peak height/diethyl phthalate peak height), W = mg sample; W' = mg standard, and P = % purity of standard.

Reference: JAOAC **63,** 1231(1980).

CAS-133-06-2 (captan)

Permanent number identifies method by year of adoption or first appearance in *Official Methods of Analysis.* 980 = first action1980; .06 = sequence of adoption in 1980.

Title may include analyte and matrix, type of method, official status, cooperating organization.

Applicability statement limitations on use of method or other information.

Scientific basis for method analysis.

Specifications for necessary laboratory apparatus and reagent preparations. See also "Definition of Terms and Explanatory Notes."

Method may be divided into several descriptive sections.

References direct the user to the published collaborative study and any subsequent revisions in the method. Other informative references may be included.

*Method shown is incomplete to allow space for description.

Definition of Terms and Explanatory Notes

Official Methods

(1) Official methods are designated first action or final action, and, in a few cases, procedures. A first action method has undergone collaborative study, has been recommended by the appropriate General Referee and Methods Committee, and has been adopted official by the Association members at an annual meeting. A method may be adopted final action a minimum of 2 years after it has been adopted first action, and again, after it has been recommended by the appropriate General Referee and Methods Committee and voted on by the Association members at an annual meeting.

A sample or sample preparation procedure or other type of procedure for which an interlaboratory collaborative study is impractical may be adopted, as above, as a procedure.

All methods in this book—first action, final action, or procedure—are official methods of AOAC.

Reagents

(2) Term "H_2O" means distilled water, except where otherwise specified, and except where the water does not mix with the determination, as in "H_2O bath."

(3) Term "alcohol" means 95% ethanol by volume. Alcohol of strength x% may be prepared by diluting x mL 95% alcohol to 95 mL with H_2O. Absolute alcohol is 99.5% by volume. Formulas of specially denatured alcohols (SDA) used as reagents are as follows:

SDA No.	100	parts alcohol plus
1	5	wood alcohol
2-B	0.5	benzene or rubber hydrocarbon solvent
3-A	5	methanol
12-A	5	benzene
13-A	10	ether
23-A	10	acetone
30	10	methanol

"Reagent" alcohol is 95 parts SDA 3-A plus 5 parts isopropanol.

(4) Term "ether" means ethyl ether, peroxide free by following test: To 420 mL ether in separator, add 9.0 mL 1% NH_4VO_3 in H_2SO_4 (1 + 16). Shake 3 min and let separate. Drain lower layer into 25 mL glass-stoppered graduate, dilute to 10 mL with H_2SO_4 (1 + 16), and mix. Any orange color should not exceed that produced by 0.30 mg H_2O_2 (1 mL of solution prepared by diluting 1 mL 30% H_2O_2 to 100 mL with H_2O) and 9.0 mL 1% NH_4VO_3 in H_2SO_4 (1 + 16). Peroxides may be eliminated by passing ≤700 mL ether through 10 cm column of Woelm basic alumina in 22 mm id tube.

(5) The following listed reagents, unless otherwise specified, have approximate strength stated and conform in purity with Recommended Specifications for Analytical Reagent Chemicals of the American Chemical Society:

	Assay
Sulfuric acid	95.0–98.0% H_2SO_4
Hydrochloric acid	36.5–38.0% HCl
Nitric acid	69.0–71.0% HNO_3
Fuming nitric acid	≥90% HNO_3
Acetic acid	≥99.7% $HC_2H_3O_2$
Hydrobromic acid	47.0–49.0% HBr
Ammonium hydroxide	28–30% NH_3
Phosphoric acid	≥85% H_3PO_4

Where no indication of dilution is given, reagent concentration is the concentration given above.

(6) All other reagents and test solutions, unless otherwise described in the text, conform to requirements of the American Chemical Society. Where such specifications have not been prepared, use highest grade reagent. When anhydrous salt is intended, it is so stated; otherwise the crystallized product is meant.

(7) Unless otherwise specified, phenolphthalein used as indicator is 1% alcohol solution; methyl orange is 0.1% aqueous solution; methyl red is 0.1% alcohol solution.

(8) Directions for standardizing reagents are given in Appendix A, Standard Solutions and Certified Reference Materials.

(9) Unusual reagents not mentioned in reagent sections or cross referenced, other than common reagents normally found in laboratory, are italicized the first time they occur in a method.

(10) Commercially prepared reagent solutions must be checked for applicability to a specific method. They may contain undeclared buffers, preservatives, chelating agents, etc.

(11) In expressions (1 + 2), (5 + 4), etc., used in connection with name of reagent, the first numeral indicates the volume of reagent used, and the second numeral indicates volume of H_2O. For example, HCl (1 + 2) means reagent prepared by mixing 1 volume of HCl with 2 volumes of H_2O. When one of the reagents is a solid, expression means part by weight. The first numeral represents the solid reagent; the second numeral H_2O. Solutions for which the solvent is not specified are aqueous solutions.

(12) In making up solutions of definite percentage, it is understood that x g substance is dissolved in H_2O and diluted to 100 mL. Although not theoretically correct, this convention will not result in any appreciable error in any methods given in this book.

(13) Chromic acid cleaning solution is prepared by (*1*) adding 1 L H_2SO_4 to approximately 35 mL saturated aqueous $Na_2Cr_2O_7$ solution; or (*2*) adding 2220 mL (9 lb) H_2SO_4 to approximately 25 mL saturated aqueous CrO_3 solution (170 g/100 mL). Reagents may be technical high grade. Use only after first cleaning by other means (e.g., detergent) and draining. Mixture is expensive and hazardous. Use repeatedly until it is diluted or has a greenish tinge. Discard carefully with copious amounts of H_2O.

(14) All calculations are based on international atomic weights.

Apparatus

(15) Burets, volumetric flasks, and pipets conform to the following U.S. Federal specifications (available from General Services Administration, Specification Section, L'Enfant Plaza, Ste 8100, Washington Navy Yard, Building 197, Washington, DC 20407):

Buret	A-A-51248	May 19, 1965
Flask, volumetric	A-A-51360	February 7, 1977
Pipet, volumetric	A-A-53890	February 24, 1978

See also Appendix V, "Testing of Glass Volumetric Apparatus," in NIST Specification Publication 260—54, "Certification and Use of Acidic Potassium Dichromate Solutions as an Ultraviolet Absorbance Standard SRM935" (available from NIST, Office of Standard Reference Materials, B316 Chemicals, Gaithersburg, MD 20899).

(16) Standard taper glass joints may be used instead of stoppers where the latter are specified or implied for connecting glass apparatus.

(17) Sieve designations, unless otherwise specified, are those described in U.S. Federal Specification RR-S-366e, November 9, 1973 (available from General Services Administration). Designation "100 mesh" (or other number) powder (material, etc.) means material ground to pass through standard sieve No. 100 (or other number). Corresponding international standard and U.S. standard sieves are given in Table 1.

(18) Term "paper" means filter paper, unless otherwise specified.

(19) Term "high-speed blender" designates mixer with 4 canted, sharp-edge, stainless steel blades rotating at the bottom of 4-lobe jar at 10,000–12,000 rpm, or with equivalent shearing action. Suspended solids are reduced to fine pulp by action of blades and by lobular container, which swirls suspended solids into blades. Waring Blendor, or equivalent, meets these requirements.

(20) "Flat-end rod" is glass rod with one end flattened by heating to softening in flame and pressing vertically on flat surface to form circular disk with flat bottom at end.

(21) Designation and pore diameter range of fritted glassware are: extra coarse, 170–220 μm; coarse, 40–60; medium, 10–15; fine, 4–5.5; Jena designations and pore diameter are: 1, 110 μm; 2, 45; 3, 25; 4,8.

(22) Unless otherwise indicated, temperatures are expressed in degrees Centigrade.

Standard Operations

(23) Operations specified as "wash (rinse, extract, etc.) with two (three, four, etc.) 10 mL (or other volumes) portions of H_2O (or other solvent)" mean that the operation is to be performed with indicated volume of solvent and repeated with same volume of solvent until number of portions required have been used.

(24) Definitions of terms used in methods involving spectrophotometry are those given in JAOAC **37**, 54(1954). Most important principles and definitions are:

(a) More accurate instrument may be substituted for less accurate instrument (e.g., spectrophotometer may replace colorimeter) where latter is specified in method. Wavelength specified in method is understood to be that of maximum absorbance (A), unless no peak is present.

Table 1. Nominal Dimensions of Standard Test Sieves (USA Standard Series)

Sieve Designation		Nominal Sieve Opening, inches	Nominal Wire Diameter, mm
International Standard[a] (ISO)	U.S.A. Standard		
12.5 mm[b]	½ in.[b]	0.500	2.67
11.2 mm	7/16 in.	0.438	2.45
9.5 mm	⅜ in.	0.375	2.27
8.0 mm	5/16 in.	0.312	2.07
6.7 mm	0.265 in.	0.265	1.87
6.3 mm	¼ in.[b]	0.250	1.82
5.6 mm	No. 3 ½	0.223	1.68
4.75 mm	No. 4	0.187	1.54
4.00mm	No. 5	0.157	1.37
3.35 mm	No. 6	0.132	1.23
2.80mm	No. 7	0.111	1.10
2.38mm	No. 8	0.0937	1.00
2.00mm	No. 10	0.0787	0.900
1.70mm	No. 12	0.0661	0.810
1.40mm	No. 14	0.0555	0.725
1.18mm	No. 16	0.0469	0.650
1.00mm	No. 18	0.0394	0.580
850μm[c]	No. 20	0.0331	0.510
710μm	No. 25	0.0278	0.450
600μm	No. 30	0.0234	0.390
500μm	No. 35	0.0197	0.340
425μm	No. 40	0.0165	0.290
355μm	No. 45	0.0139	0.247
300μm	No. 50	0.0117	0.215
250μm	No. 60	0.0098	0.180
212μm	No. 70	0.0083	0.152
180μm	No. 80	0.0070	0.131
150μm	No. 100	0.0059	0.110
125μm	No. 120	0.0049	0.091
106μm	No. 140	0.0041	0.076
90μm	No. 170	0.0035	0.064
75μm	No. 200	0.0029	0.053
63μm	No. 230	0.0025	0.044
53μm	No. 270	0.0021	0.037

[a] These standard designations correspond to the values for test sieve apertures recommended by the International Organization for Standardization, Geneva, Switzerland.
[b] These sieves are not in the standard series but they have been included because they are in common usage.
[c] 1000 μm = 1 mm.

(b) *Absorbance(s)* (*A*).—Negative logarithm to base 10 of the ratio of transmittance (T) of sample to that of reference or standard material. Other names that have been used for quantity represented by this term are optical density, extinction, and absorbency.

(c) *Absorptivity(ies)* (*a*).—Absorbance per unit concentration and cell length. $a = A/bc$, where b is cm and c is g/L, or $a = (A/bc) \times 1000$, if c is mg/L. Other names that have been used for this or related quantities are extinction coefficient, specific absorption, absorbance index, and $E^{1\%}_{1cm}$.

(d) *Transmittance(s)* (*T*).—Ratio of radiant power transmitted by sample to radiant power incident on sample, when both are measured at same spectral position and with same slit width. Beam is understood to be parallel radiation and incident at right angles to plane parallel surface of sample. If sample is solution, solute transmittance is quantity usually desired and is detected directly as ratio of transmittance of solution in cell to transmittance of solvent in an equal cell. Other names that have been used for this quantity are transmittancy and transmission.

(e) *Standardization.*—Spectrophotometer may be checked for accuracy of wavelength scale by referring to Hg lines: 239.94, 248, 253.65, 265.3, 280.4, 302.25, 313.16, 334.15, 365.43, 404.66, 435.83, 546.07, 578.0, and 1014.0 nm. To check consistency of absorbance scale, prepare solution of 0.0400 g K_2CrO_4/L 0.05*N* KOH and determine absorbance at following wavelengths in 1 cm cell: 230 nm, 0.171; 275, 0.757; 313.2, 0.043; 375, 0.991; 400, 0.396. See NIST Spec. Pub. 378, "Accuracy in Spectrophotometry and Luminescence Measurements," 1973 (available from NIST, Office of Standard Reference Materials, B316, Chemistry, Gaithersburg, MD 20899).

(25) *Least square treatment of data and calculation of regression lines.*—This technique finds the best fitting straight line for set of data such as standard curve. It calculates that straight line for which the sum of squares of vertical deviations (usually *A*) of observations from the line is smaller than corresponding sum of squares of deviation from any other line. Equation of straight line is:

$$Y = a + bX$$

where a is intercept at Y axis ($X = 0$), and b is slope of line.

Least square estimates of constants are:

$$b = \frac{\Sigma(X_iY_i) - [(\Sigma X_i \Sigma Y_i)/n]}{\Sigma X_i^2 - (\Sigma X_i)^2/n}$$

$$a = \overline{Y} - b\overline{X}$$

where Σ = "sum of" the n individual values of indicated operation, and $\overline{X}$ and $\overline{Y}$ are the averages of the X and Y points.

Example: To find "best" straight line relating $A(Y)$ to concentration (X):

Observation No. (i)	Concentration X_i	Absorbance Y_i	X_i^2	X_iY_i
1	80	1.270	6400	101.6
2	60	1.000	3600	60.0
3	40	0.700	1600	28.0
4	30	0.550	900	16.5
5	20	0.250	400	5.0
6	10	0.100	100	1.0
7	0	0.050	0	0.0
Totals:				
$n = 7$	$\Sigma X_i = 240$	$\Sigma Y_i = 3.92$	$\Sigma X_i^2 = 1300$	$\Sigma(X_iY_i) = 212.1$

$$\overline{X} = \Sigma X_i/n = 240/7 = 34.29$$

$$\overline{Y} = \Sigma Y_i/n = 3.92/7 = 0.56$$

$$b = \frac{212.1 - (240)(3.92)/7}{13000 - (240)^2/7} = \frac{77.7}{4771} = 0.0163$$

$$a = 0.56 - 0.0163(34.29) = 0.001 \cong 0$$

Best equation is then:

$$Y = 0.00 + 0.0163X$$

If for sample, $A = 0.82$, corresponding concentration (X) would be:

$$X = (Y - 0.00)/0.0163 = 0.82/0.0163 = 50.3$$

Many scientific and statistical calculators are programmed to perform this calculation. It should be noted that the least square fit of a data set should not be the only criterion used in evaluating the validity of a given data set.

(26) *Recovery (R) of analyte from fortified sample by a method of analysis.*—Fraction of an analyte added to a sample (fortified sample) prior to analysis, which is measured (recovered) by the method. When the *same* analytical method is used to analyze both the unfortified and fortified samples, calculate %R as follows:

$$\%R = [(C_F - C_U)/C_A] \times 100$$

where C_F = concentration of analyte measured in fortified sample;

C_U = concentration of analyte measured in unfortified sample;

C_A = concentration of analyte *added* in fortified sample.

(Note C_A is a calculated value, not a value measured by the method being used.)

Concentration of added analyte should be no less than concentration of analyte in unfortified sample. Sum of concentration of added analyte plus analyte present before fortification should be in the same range as analyte concentration sought in actual samples. Addition of analyte must not cause measuring instrument to exceed linear dynamic range of standard curve. Both fortified and unfortified samples must be treated identically during analysis to minimize experimental bias.

(27) Common safety precautions are given in Appendix B, Laboratory Safety.

Method Performance

(28) Efforts are being made to standardize the symbols and associated definitions for the statistical parameters that will accompany approved methods. Users of the method should consult the report of the collaborative study (reference given with the method) for complete details.

Beginning with methods published in "Changes in Official Methods of Analysis" (1989) JAOAC **72**, 188, the following statistical parameters are shown. Data from some studies may not be amenable to provide these measures of evaluation.

Within-laboratory precision:

s_r repeatability standard deviation

s_R reproducibility standard deviation

Among-laboratories precision:

RSD_r repeatability relative standard deviation

RSD_R reproducibility relative standard deviation

Surplus Methods

(29) ★This symbol indicates a method which has been declared surplus. Such methods are satisfactory methods, having been subjected to collaborative study and review. They are thought not to be in current use for various reasons: The purpose for which the method was developed no longer exists; the product for which the method was developed is no longer marketed; the method has been replaced by other methods; etc. These methods retain their *official* status but are carried only by reference. Any laboratory which uses a surplus

method and wishes the text reprinted in the next edition must so notify AOAC.

Editorial Conventions

(30) For sake of simplicity, the abbreviations Cl and I instead of Cl_2 and I_2 were used for chlorine and iodine in all methods through the 15th edition of *The Official Methods of Analysis*. Similar abbreviations were used in other cases (O, N, H). The same abbreviation may also have been used for the ion where no ambiguity resulted. With the 16th edition, an attempt has been made to indicate whether the element is present as an ion, a monatomic species, or the diatomic element.

(31) Reagents and apparatus referenced with only a letter, e.g., (c), will be found in the reagent or apparatus section of the method.

(32) To conserve space, most of the articles and some prepositions have been eliminated.

Manufacturers and Suppliers

(33) Names and addresses of manufacturers and suppliers, and trade names of frequently mentioned materials, are furnished below solely as a matter of identification and convenience, without implication of approval, endorsement, or certification. The same products available from other suppliers or other brands from other sources may serve equally well if proper tests indicate their use is satisfactory.

Ace Glass, Inc., 1430 Northwest Blvd, Vineland, NJ 08360
Ace Scientific Supply Co., Inc., 40-A Cutter Ln, East Brunswick, NJ 08816
AKZO, see International Salt Co.
Aldrich Chemical Co. Inc., 1001 W. St. Paul Ave, Milwaukee, WI 53233
(ASBC) American Society Brewing Chemists, 3340 Pilot Knob Rd, St. Paul, MN 55121-2097
AMETEK/Mansfield & Green Division, 8600 Somerset Dr, Largo, FL 34643
Analabs Inc., 140 Water St, Norwalk, CT 06854
Analtech Inc., 75 Blue Hen Dr, PO Box 7558, Newark, DE 19714
Alltech-Applied Science Laboratories, 2701 Carolean Industrial Dr, State College, PA 16801
Alston Filteration, PO Box A, Mount Holly Springs, PA 17065
Applied Biosystems, Inc., 850 Lincoln Centre Dr, Foster City, CA 94404
Beckman Industrial, Rosemount Analytical Div., Cedar Grove Operations, 89 Commerce Rd, Cedar Grove, NJ 07009
Beckman Instruments Inc., 2500 Harbor Blvd, PO Box 3100, Fullerton, CA 92634
Becton Dickinson & Co., One Becton Dr, Franklin Lakes, NJ 07417
Becton Dickinson Microbiology Systems, Division of Becton Dickinson & Company, PO Box 243, Cockeysville, MD 21030-0243
Bran & Luebbe, 1025 Bush Pkwy, Buffalo Grove, IL 60089
Brinkmann Instruments Inc., Cantiague Rd, Westbury, NY 11590
Burdick & Jackson Lab Inc., Division Baxter Heathcare Corp, 1953 S. Harvey St, Muskegon, MI 49442
Calbiochem–Navabiochem Corp., 10394 Pacific Center Ct, San Diego, CA 92121
CAMAG Scientific, Inc., 1200 N. 23rd St, Wilmington, NC 28405
Carl Zeiss West Germany, PO Box 1369/1380, D-7082, Oberkochen, Germany
Celite Corporation, 137 W. Central Ave, PO Box 519, Lompoc, CA 93438-0519
CEM Corp., PO Box 200, Matthews, NC 28106
Charm Sciences Inc., 36 Franklin St, Malden MA 02148-4120
Chemical Repository, Midwest Research Institute, 425 Volker Blvd, Kansas City, MO 64110
Corning Glass Works, Lab Product Department, Corning, NY 14830
Curtin Matheson Scientific, Inc., 9999 Veterans Memorial Drive, PO Box 1546, Houston, TX 77038-2499
Difco Laboratories, PO Box 331058, Detroit, MI 48232-7058
Dow Chemical Co., Sample Coordinator, 9001 Bldg., PO Box 1706, Midland, MI 48641-1706
Dynatech Laboratories Inc., 14340 Sullyfield Circle, Chantilly, VA 22021
Eastman Kodak Co., Eastman Organic Chem, 343 State St, Rochester, NY 14650
E.I. DuPont de Nemours & Co. Inc., Electronics Department, Information Storage Division, Barley Mill Plaza 30-1128, Wilmington, DE 19805
Eli Lilly and Co., Lilly Corporate Center, Indianapolis, IN 46285
EM Science, A Division of EM Industries Inc., 480 Democrat Rd, Gibbstown, NJ 08027
Fisher Scientific Co., 1 Reagent Ln, Fair Lawn, NJ 07410
Flow Laboratories, Inc., A Flow General Co., 7655 Old Springhouse Rd, McLean, VA 22102
Foss Food Technology Corp., 10355 W. 70th St, Eden Prairie, MN 55344
Gelman Sciences, Inc., 600 S. Wagner Rd, Ann Arbor, MI 48106
GFS Chemical Inc., PO Box 245, Powell, OH 43065
Hach Chemical Co., PO Box 389, Loveland, CO 80539
Hess & Clark Laboratories, 7th and Orange Sts, Ashland, OH 44805
Hewlett Packard Co., Avondale Division, Route 41, PO Box 900, Avondale, PA 19311-0900
ICN Pharmaceuticals Inc., Life Sciences Group, 26201 Miles Rd, Cleveland, OH 44128
International Salt Co., AKZO, Abington Executive Park, Clarks Summit, PA 18411
Johnson Matthey Catalog Co., PO Box 8247, Ward Hill, MA 01835
J. T. Baker Inc., 222 Red School Ln, Phillipsburg, NJ 08865
Kimble Glass Inc., Crystal Ave, Vineland, NJ 08360
Kontes Glass Co., Spruce St, PO Box 729, Vineland, NJ 08360
Labconco Corp., 8811 Prospect Ave, Kansas City, MO 64132
Lurex Scientific, Inc., 1298 N.W. Blvd, Vineland, NJ 08360
Mallinckrodt Speciality Chemical Co., PO Box 800, Paris, KY 40362-0880
Merk & Co., Chemical Div., PO Box 200-000, Rathway, NJ 07065
Miles Corp., Agricultural Division, 8400 Hawthorne Rd, PO Box 4913, Kansas City, MO 64120-0013
Millipore Corp., 80 Ashby Rd, Bedford, MA 01730
Mitchum Schaefer, Inc., 430 S. Pennsylvania, Indianapolis, IN 46225
(NIST) National Institute of Standards and Technology, Gaithersburg, MD 20899
New York Lab. Supply Company, 510 Hempstead Tnpk, West Hempstead, NY 11552
Orion Research, Inc., 529 Main St, Boston, MA 02149
Pfaltz & Bauer Inc., 172 E. Aurora St, Waterbury, CT 06708
Pierce Chemical Co., PO Box 117, Rockford, IL 61105
Polyscience Corp., 7800 N. Merrimac Ave, PO Box 48312, Niles, IL 60648
Rainin Instrument Co., Mack Rd, Woburn, MA 01801

RCR Scientific Inc., 206 W. Lincoln Ave, Goshen, IN 46526
Rheodyne Inc., PO Box 996, Cotati, CA 94931
Salsbury Laboratories, 200-000 Rockford Rd, Charles City, IA 50616-9984
Sargent-Welch Scientific Inc., 7300 N. Linder Ave, PO Box 1026, Skokie, IL 60077
Schleicher & Schuell Inc., 10 Optical Ave, Keene, NH 03431
Scientific Equipment Products (SEPCO), 2201 Aisquith St, Baltimore, MD 21218
Sigma Chemical Co., 3050 Spruce St, St. Louis, MO 63103
Supelco Inc., Supelco Park, Bellefonte, PA 16823
Thomas Scientific, 99 High Hill Rd, I 295, PO Box 99, Swedesboro, NJ 08085-0099
Tracor Instruments Inc., 6500 Tracor Ln, Austin, TX 78725-2100
Valco Instruments Co., Inc., PO Box 55603, Houston, TX 77255
Whatman Inc., 9 Bridewell Pl, Clifton, NJ 07014
Zeneca Ag Products, Western Research Center, 1200 S. 47th St, PO Box 4023, Richmond, CA 94804-0023

Abbreviations

(34) The following abbreviations, many of which conform with those of *Chemical Abstracts*, are used. In general, principle governing use of periods after abbreviations is that period is used where final letter of abbreviation is not the same as final letter of word it represents.

Abbreviation	Word
a	absorptivity(ies)
A	absorbance(s) throughout (not restricted to formulas; not absorption. $A^{'}$ is used for standard; A_0 is used for blank; 3 digit subscript numerals usually denote wavelength in nm
A	ampere
AA	atomic absorption
ACS	American Chemical Society
AOCS	American Oil Chemists' Society
APHA	American Public Health Association
ASTM	American Society of Testing Materials
atm.	atmosphere
Bé	degree Baumé
bp	boiling point
C	degree Celsius (Centigrade)
ca	about, approximately
Cat. No.	Catalog Number
Ch	Chapter
Ci	curie(s)
CI	Color Index
CIPAC	Collaborative International Pesticide Analytical Council
cm	centimeter(s)
cP	centipoise
cpm	counts per minute
cu in.	cubic inch(es)
dc	direct current
DMF	*N*, *N*-dimethylformamide
DMSO	dimethyl sulfoxide
EDTA	ethylenedinitrilotetraacetic acid (or-tetraacetate)
EPA	Environmental Protection Agency
F	degrees Fahrenheit (°C = (5/9) × (°F – 32)
FAO	Food and Agriculture Organization
Fig.	Figure (illustration)
fl oz	fluid ounce (29.54 mL)
fp	freezing point
ft	foot (30.48 cm)
g	gram(s)
g	gravity (in centrifuging)
gal.	gallon(s) (3.785 L)
gr.	grain(s)
GC	gas chromatography
h	hour(s)
id	inner diameter
in.	inch(es) (2.54 cm)
IR	infrared
ISO	International Organization for Standardization
kg	kilogram(s)
L	liter(s)
LC	liquid chromatography
lb	pound(s) (453.6 g)
m	meter(s); milli—as prefix
m	molal
M	molar (as applied to concentration), not molal
mA	milliampere(s)
mg	milligram(s)
mL	milliliter(s)
mm	millimeter(s)
mp	melting point
mμ	millimicron (10^{-6} mm); use nanometer (nm) (10^{-9} m)
mV	millivolt
MW	molecular weight (molar mass)
N	normal (as applied to concentration; in equations, normality of titrating reagent
N	Newton (10^5 dynes)
n	refractive index
NF	National Formulary
NFPA	National Food Processors Association
NIST	National Institute of Standards and Technology
ng	nanogram (10^{-9} g)
nm	nonometer (10^{-9} m); formally mμ
No.	number
od	outer diameter
oz	ounce(s) (28.35 g)
P	pico (10^{-12}) as prefix
Pa	Pascal (1 Newton/m^2; 9.87 x 10^{-6} atm; 7.5 x 10^{-3} mm Hg (torr); 1.45 x 10^{-4} psi
ppb	parts per billion ($1/10^9$)
ppm	parts per million($1/10^6$)
psi	pounds per square inch (absolute)
psig	pounds per square inch gage (atmospheric pressure = 0)
pt	pint(s) (473 mL)
QAC	quaternary ammonium compound
qt	quart(s) 946 mL
®	Trademark name—(Registered)
R_f	distance spot moved/distance solvent moved, TLC
rpm	revolutions per minute

SDF	special denatured formula (applied to alcohol)
s	second(s)
sq	square
SRM	Standard Reference Material of National Institute of Standards and Technology
T	transmittance
TLC	thin layer chromatography
U	unit
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
UV	ultraviolet
V	volt(s)
WHO	World Health Organization
μm	Micron (0.001 mm); use micrometer (mm)(10^{-6} m)

μg	microgram(s) (10^{-6} g)
μL	microliter(s) (10^{-6} L)
μm	micrometer(s) (10^{-6} m); formerly μ
Δ	difference (e.g., $\Delta A = (A - A')$
′	foot (feet) (1′ = 30.48 cm)
″	inch(es) (1″ = 2.54 χμ)
/	per
%	percent (parts per hundred); percentage
>	more than; greater than; above; exceeds (use with numbers only)
<	less than; under; below (use with numbers only)
≤	equal to or less than
≥	equal to or greater than