Official Methods of Analysis

of AOAC INTERNATIONAL

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Current through Revision 1, 2006

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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 INTERNATIONAL, 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 the 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 when required.

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

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:

49.2.18A
AOAC Official Method 2005.08
Aflatoxins in Corn, Raw Peanuts, and Peanut Butter

2005.08 is the permanent number of the method and 49.2.18A is the section number used to facilitate locating methods. Permanent numbers indicate the year the method was approved (in this case 2005) followed by the order in

which the method was approved that year (in this example, it is the 8th method approved in 2005). The first set of numbers in the section number indicates the chapter (in this case, Chapter 49), the second set indicates the subchapter (in this case, subchapter 2), and the last set indicates the order in which the method appears in the subchapter (in this case, it is the 18th method).

When referencing *AOAC® Official Methods*℠, only the permanent number should be referenced as seen in this example:

(1) *Official Methods of Analysis of AOAC INTERNATIONAL* (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method **2005.08**

REVISIONS

AOAC publishes annual revisions to the *Official Methods of Analysis of AOAC INTERNATIONAL*. Revisions contain new methods approved since the last publication and revisions to existing methods. You will automatically receive notice of the availability of new revisions and be sent an advance invoice allowing you to purchase the revisions without obligation. If you maintain the revision service, your 18th Edition will be current.

For additional revisions received after the first printing (2005) of the 18th Edition, visit AOAC's Web site at **www.aoac.org** for the *Official Methods of Analysis* online. These revisions will be included in a subsequent printing.

(*Note:* Individual copies of revisions will be provided on an annual basis only. AOAC INTERNATIONAL will *not* retain back copies of revisions for sale. If you do not purchase revisions on an annual basis, 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, Official Methods Program, 481 N. Frederick Ave, Suite 500, Gaithersburg, MD 20877-2417, USA. Telephone +1-301-924-7077. Fax +1-301-924-7089. E-mail: aoac@aoac.org. Please note that due to the time necessary to answer any technical questions regarding an *AOAC Official Method*℠, AOAC staff provides technical assistance regarding methods in OMA to AOAC members only.

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, Fulfillment, 481 N. Frederick Ave, Suite 500, Gaithersburg, MD 20877-2417, USA. Telephone +1-301-924-7077. Fax +1-301-924-7089. 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 INTERNATIONAL about their experiences with the *AOAC® Official Methods*℠ published in this book. In particular, analysts should notify AOAC INTERNATIONAL of problems in the performance of an *Official Method*℠, which may indicate the method should be revised or restudied. Direct comments to AOAC INTERNATIONAL, Official Methods Program, 481 N. Frederick Ave, Suite 500, Gaithersburg, MD 20877-2417, USA. Telephone +1-301-924-7077. Fax +1-301-924-7089. E-mail: aoac@aoac.org.

Preface

Electronic publishing arrived just in time to save the *Official Methods of Analysis of AOAC INTERNATIONAL* from its own success. Each of the two volumes of the previous 17th Edition has grown to an almost unmanageable size. Now, for the first time, the results of 122 years of review and approval of collaboratively studied methods are captured on the Internet. The older methods are still there, but their use is probably confined to teaching "agricultural chemistry." The properties of methods do not deteriorate with age and the "classical" analytes still need to be determined. But the change in emphasis with the regulatory winds is obvious: Microbiology and nutrition have blossomed as regulatory emphasis has shifted from economics to safety and health. New drug and food additive approval, based on preclearance of manufacturing operations and continuous quality control as well as safety and efficacy, have reduced the need for regulatory control through market sampling and analysis.

Most notable has been the shift from classical stoichiometric chemistry, based on the balance and buret, to calibration chemistry, based on an instrumental comparison of a response of an analyte with that of a standard. This shift was initiated by the remarkable separation powers of chromatography allowing an analyte to be separated from interfering components before being measured by the instrument. Chromatography moved analytical chemistry from the realm of gram quantities into microgram quantities but not without unrecognized sampling problems. The sensitivity, stability, and speed of modern electronics permit the performance of analytical work automatically, from the measurement of the test portion, through detection, amplification, and interpretation of the signal, to the printing of the analytical report.

The analytical problem has shifted from measurement to control. Much of the analytical operation has moved from an operator to a black box in a computer. Changes in physical properties, such as light intensities or ion conductances, are measured automatically and converted into analytical reports continuously, changing the laboratory into an automated factory. But the facility for automated performance allows the responsibility for reliability to easily shift from the analyst to the instrument. This is also true of the blind application of computer programs with no review of the applicability of the program to the problem. The computer has the ability not only to extract hidden information from a jungle of background, but also to formulate spurious peaks that it has been programmed to guess ought to be there.

AOAC initiated the procedure of validation of methods through interlaboratory studies. These studies produce results from a single sample of method performance in the hands of an assumed random sample of laboratories. Unfortunately, time and expense rarely permit performing additional studies. Therefore, the initial studies usually stand as the sole published evidence of satisfactory interlaboratory performance. AOAC members are investigating surrogates for this necessary, but lengthy and costly procedure.

Seventy-seven new methods have been added to this edition, predominantly microbiological or chromatographic in nature, all of them subjected to the rigors of an interlaboratory study. Many of these methods incorporate internal controls to ensure that the reactions are proceeding as intended. Most appealing is the introduction of system suitability specifications into chromatographic systems that permit flexibility without sacrificing reliability. For over a century, the guiding principle in the application of standard methods has been to follow instructions to the letter to obtain results equivalent to those originally obtained. But the competition for improvements in systems advanced the science of separation and

detection so rapidly that suitability specifications for introducing flexibility without sacrificing performance had to be invented.

Internal controls require that the methods meet repeatability performance specifications. An appreciable fraction of the new microbiological methods are screening tests involving preassembled immunoassays kits. Relatively quickly, these kits separate laboratory samples that can be discarded as negative from those that presumably contain pathogenic organisms, requiring the application of confirmatory tests. These kits also invariably contain the requirement for accompanying positive and negative controls that provide concurrent assurance of proper performance.

This edition joins the universal movement toward the use of the international system (SI) of units, many of which are unfamiliar to U.S. scientists. During a transition period, both the common system as well as the SI system will be given. Note that the term "normality" is being replaced by "moles per liter." Another editorial change being introduced is to move away from the tendency to designate anything being worked on as the "sample." Instead, the sequence of "laboratory sample" → "test sample" → "test portion" is being introduced. This vocabulary is being used by the International Union Of Pure and Applied Chemistry (IUPAC) and the International Organization for Standardization (ISO), which does not permit the unmodified term "sample" to be used in conjunction with subsequent chemical operations.

An important feature of the 18th Edition is the international source of many of the methods, with many countries and international organizations contributing their expertise to method standardization. It is also gratifying to see the introduction of quality control features into the methods, which provide the analyst with guides to proper performance. On the other hand, the ease with which results are obtained from computers also permits the introduction of unanticipated errors, detected only by the unreasonableness of the results. In the absence of a blueprint of what is to be expected, gross errors may be made. The introduction of quality assurance principles into the laboratory may assist in minimizing such occurrences.

Numerous individuals, volunteer scientists, and professional staff have contributed enthusiastically to this century-old program of method validation. The analytical community is grateful for their continued valuable efforts.

—William Horwitz, Editor

Contents

About the Association

During the past 122 years, AOAC INTERNATIONAL (formerly the Association of Official Analytical Chemists and before that the Association of Official Agricultural Chemists) evolved from a group of chemists in the U.S. Department of Agriculture and the individual states into an independent scientific Association of analytical scientists with members throughout the world. Today, AOAC is the leader in providing validated methods, proficiency test samples, accreditation criteria, and scientific information to industry, government agencies, and academic institutions.

MISSION

AOAC INTERNATIONAL advances the global chemistry and microbiology analytical community by promoting methods validation and quality measurements.

AOAC METHODS VALIDATION PROGRAMS

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*SM Program, the program of choice when the highest level of confidence is desired; the AOAC® *Peer-Verified Methods*SM Program, used when speed of validation is essential and a lesser degree of confidence is acceptable; and the AOAC® *Performance Tested Methods*SM Program used to test the performance of test kits. Over 800 volunteer scientists, working in industry, government, and academic laboratories worldwide largely accomplish the actual work of validation.

The methods found in this 18th Edition of the *Official Methods of Analysis of AOAC INTERNATIONAL* have been validated within the AOAC® *Official Methods*SM Program. Candidates for AOAC® *Official Method*SM 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 xxiv for additional details of the AOAC® *Official Methods*SM Validation Program).

AOAC validated 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. In fact, many of the validated methods in this edition have been adopted by industry and government agencies as *de facto* standards in the operation of their laboratories.

OTHER PROGRAMS

The Association also has several programs that assist laboratory managers in measuring the accuracy of analytical results, improve professional development, and provide the opportunity for scientists to interact.

THE AOAC® LABORATORY PROFICIENCY TESTING PROGRAM

The AOAC® Laboratory Proficiency Testing Program provides an independent assessment of the accuracy and reliability of analytical results in the analysis of a wide range of analytes and matrix. Program modules include Standard Microbiology, HACCP, Nutritional Labeling, and Pesticide Residues in Fruits and Vegetables.

TECHNICAL DIVISIONS

The goals of the AOAC technical divisions are to improve the overall quality of laboratory operations and foster harmonization of laboratory procedures and systems. The Technical Division for Laboratory Management helps laboratory managers improve the operations of their laboratories through the exchange of ideas and through professional development activities. The Technical Division on Reference Materials improves the quality of analytical measurements through the use of reference materials.

TRAINING COURSES

AOAC INTERNATIONAL offers a series of courses that provide hands-on training in specific topical areas, and assist analytical scientists to acquire the skills they need to address daily challenges faced in their laboratories. Current offerings include courses on quality assurance for analytical and microbiological laboratories, ISO 17025, ISO audit systems, and single laboratory validation.

COOPERATIVE ACTIVITIES

AOAC INTERNATIONAL has established joint committees, liaisons, and representation with numerous scientific organizations worldwide. The Association serves as the Secretariat of the InterAgency Meeting, an affiliation of 16 international organizations active in the field of analysis and sampling of food products and associated quality assurance measures in conjunction with the Joint FAO/WHO Codex Alimentarius. The Association also participates in the meetings of the Codex Alimentarius Commission, the International Organization for Standardization (ISO), the European Community for Standardization, the World Health Organization, the Pan American Health Organization, and other international groups, both private and government sponsored. Such arrangements enable AOAC INTERNATIONAL 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 INTERNATIONAL.

MEETINGS AND EXPOSITIONS

The AOAC INTERNATIONAL Annual Meeting and Exposition is recognized worldwide as the most significant meeting for quality assurance and laboratory management professionals dealing with regulated commodities. The annual scientific program includes cutting-edge information from the world's most respected scientists and laboratories. Through the scientific symposia, poster sessions, workshops, forums, and short courses, meeting attendees enhance their analytical expertise, share their research, and strengthen contacts with their colleagues from around the world. The Annual Laboratory Exposition held at the Annual Meeting features over 100 displays of the state of the art in laboratory products and services.

SECTIONS

AOAC INTERNATIONAL has 16 Sections located in North America, Europe, Japan, China, Taiwan, Latin America, and the Caribbean. Sections provide opportunities for AOAC members to gather and share information on a more local level, to build and expand their network of professional contacts, enhance their leadership skills, and gain practical management experience. All Sections are managed by an elected group of local volunteers.

PUBLICATIONS

In addition to the *Official Methods of Analysis of AOAC INTERNATIONAL*, the Association publishes the *Journal of AOAC INTERNATIONAL* and a variety of other publications. AOAC's *Journal* contains original fully refereed research articles and reports on current collaborative study data, including information on inter- and intralaboratory performance precision, which enables the users of the AOAC® *Official Methods*℠ to make informed choices about the appropriate use of a particular method. *Inside Laboratory Management,* a full-color bimonthly magazine contains technical and general articles on laboratory management, regulations, emerging technologies, instrumentation, and other areas pertinent to laboratory procedures, management, and quality control.

AOAC's other publications include manuals, methods compilations, and monographs covering subjects that include quality assurance, statistics, food analysis, agricultural analysis, laboratory management, and pesticide analysis.

AWARDS

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

MEMBERSHIP AND GOVERNANCE

AOAC INTERNATIONAL is an association comprised of nearly 4000 individual and 300 organizational members from more than 90 countries. Individual members are laboratory managers, analytical chemists, microbiologists, toxicologists, forensic scientists, and management executives working in industry, government, and academia. Organizational members are corporations, commercial laboratories, government agencies, and universities.

An elected Board of Directors governs the Association. 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 advance and support the mission of the Association.

For further information about AOAC INTERNATIONAL and its programs and activities, contact the Association at:

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Guide to Method Format

(Method shown is incomplete to allow space for description.)

Locator number identifies method by chapter, subchapter, and sequence within the subchapter for easy cross referencing and access. 4 = chapter 4; .10 = subchapter 10; .03 = the third method found in Chapter 4, subchapter 10. The locator number is not the permanent number and is included only for convenient accessibility.

4.10.03

AOAC Official Method 996.13
Ethoxyquin in Feeds
Liquid Chromatographic Method
First Action 1996
Final Action 1997

Permanent number identifies method by year of adoption or first appearance in *Official Methods of Analysis of AOAC INTERNATIONAL*. 996 = First Action 1996; .13 = sequence of adoption in 1996.

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

(Applicable for determination of 0.5–300 g/g ethoxyquin in dry extruded pet food or meat meal.)

Applicability statement addresses utility and limitations on use of method or other information.

See Table **996.13** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Ethoxyquin is extracted with acetonitrile. Extract is analyzed by isocratic liquid chromatography with fluorescence detection.

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

B. Apparatus

(**a**) *Liquid chromatograph (LC).*—Generating 1500 200 psi; with peak area integrator (manual or computer), isocratic LC pump, and column heater. Operating conditions: injection volume, 20 L; flow rate, 1.3 mL/min; temperature, 35°C; fluorescence detector output, analog to digital conversion; detector settings: excitation, 360 nm; emission, 432 nm.

(**b**) *LC column.*—250 4.6 mm id, C_{18} octadecylsilane, 5 m spherical, 100 Å pore size.

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

C. Reagents

(**a**) *Water.*—LC grade.
(**b**) *Acetonitrile.*—LC grade.

Method may be divided into several descriptive sections.

D. Preparation of Standard Solutions

(**a**) *Ethoxyquin standard stock solution.*—400 g/mL. Weigh the equivalent of 0.1000 g liquid ethoxyquin into 250 mL amber volumetric flask and dilute to volume with acetonitrile. (*Note:* Amount of ethoxyquin needed for preparation of stock solution is based on purity of liquid, e.g., for purity of 93.5%, amount of liquid ethoxyquin = 0.100/0.935 = 0.1070 g.)

H. Calculations

Calculate concentration of ethoxyquin, g/g or ppm, in test sample from calibration curve (using linear regression with line forced through zero intercept) as follows:

Calculation symbols are identified and show correct units.

$$\text{Ethoxyquin, g/g or ppm} = \frac{C \quad 1.5 \quad F}{W}$$

where C = ethoxyquin concentration from LC calibration curve, g/mL; 1.5 = volume of acetonitrile added to test solution, mL; F = dilution factor; W = weight of test portion, g.

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

Reference: *J. AOAC Int.* **80,** 725(1997).

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

CAS-91-53-2 (ethoxyquin) 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline

Revised: March 1998

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 has been adopted Official First Action by the Methods Committee. A method may be adopted Final Action a minimum of 2 years after it has been adopted First Action, and after it has been recommended by the appropriate General Referee and Methods Committee, and voted on by the Official Methods Board.

Sampling, test sample preparation protocol, or other type of instructions 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 INTERNATIONAL.

Reagents

(*2*) Term "H_2O" means distilled or deionized 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 in the United States under 27CFR21 are as follows:

SDA No.	100	Parts alcohol plus
3-A	5	Parts methanol
3-C	5	Isopropyl alcohol
30	10	Parts methanol

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

(*4*) Term "ether" means ethyl ether, peroxide free by the 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	68.0–70.0% HNO_3
Fuming nitric acid	90% HNO_3
Acetic acid	99.7% CH_3COOH
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, are automatically reagent grade and 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 hydrated 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 laboratories, 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 HCl with 2 volumes 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 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. Refer to *Appendix B*, *Laboratory Safety* chapter.

(*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, Bldg 197, Washington, DC 20407, USA):

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 the National Institute of Standards and Technology (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, USA).

(*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 Blender, 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 m; coarse, 40–60; medium, 10–15; fine, 4–5.5; Jena designations and pore diameter are: (*1*) 110 m; (*2*) 45; (*3*) 25; (*4*) 8.

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

Sample

(*23*) Terminology and usage for items and operations colloquially designated with the term "sample": Newly adopted methods will avoid the confusing usage of the term "sample" for anything the analyst is working with. The nomenclature recommended by the International Union of Pure and Applied Chemistry (IUPAC), *Pure & Appl. Chem*. **62**, 1193(1990), for analytical chemistry, based upon the International Organization for Standardization (ISO) recommendations, will be utilized. The critical definitions are:

A *laboratory sample* is the material sent to or received by the laboratory. The laboratory reduces the laboratory sample in size and fineness to a *test sample* (or *analytical sample* if only chemical or microbiological analysis is involved). A *test* (or *analytical*) portion

Table 1. Nominal dimensions of standard test sieves (USA standard series)

Sieve designation			
International standard[a] (ISO)	USA standard	Nominal sieve opening, in.	Nominal wire diameter, mm
12.5 mm[b]	½ in.[b]	0.500	2.80
11.2 mm	7/16 in.	0.438	2.50
9.5 mm	3/8 in.	0.375	2.24
8.0 mm	5/16 in.	0.312	2.00
6.7 mm	0.265 in.	0.265	1.80
6.3 mm	¼ in.[b]	0.250	1.80
5.6 mm	No. 3	0.223	1.60
4.75 mm	No. 4	0.187	1.60
4.00 mm	No. 5	0.157	1.25
3.35 mm	No. 6	0.132	1.00
2.80 mm	No. 7	0.111	0.90
2.36 mm	No. 8	0.0937	0.80
2.00 mm	No. 10	0.0787	0.71
1.70 mm	No. 12	0.0661	0.63
1.40 mm	No. 14	0.0555	0.56
1.18 mm	No. 16	0.0469	0.45
1.00 mm	No. 18	0.0394	0.40
850 m[c]	No. 20	0.0331	0.355
710 m	No. 25	0.0278	0.315
600 m	No. 30	0.0234	0.280
500 m	No. 35	0.0197	0.224
425 m	No. 40	0.0165	0.200
355 m	No. 45	0.0139	0.180
300 m	No. 50	0.0117	0.160
250 m	No. 60	0.0098	0.125
212 m	No. 70	0.0083	0.100
180 m	No. 80	0.0070	0.090
150 m	No. 100	0.0059	0.080
125 m	No. 120	0.0049	0.063
106 m	No. 140	0.0041	0.056
90 m	No. 170	0.0035	0.045
75 m	No. 200	0.0029	0.040
63 m	No. 230	0.0025	
53 m	No. 270	0.0021	

[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 m = 1 mm.

is removed from the test sample for analysis. Once a test portion is measured, by mass or volume, the term "sample" is no longer appropriate. Use "test" or "unknown" as the modifier, e.g., "test solution," not "sample solution."

The operation often called "preparation of sample" applies to the reduction of the laboratory sample to the test sample, and not to the usual analytical steps of solution, separation, purification, or isolation of the analyte.

The term "sample" will be used solely in the statistical sense as a small portion representing a larger quantity, such as a lot or a batch, where the potential exists for a "sampling error" due to the heterogeneity of the parent population. Most samples are removed from a static population, such as a pile of fertilizer, a stack of cases, or a group of containers. In a dynamic situation, however, where the population changes with time as a flowing river, circulating blood, or a moving conveyor belt, the small portion removed should be called a "*specimen*." In these cases, the phenomenon under study and the sampling error are confounded in such a way that they cannot be separated.

See Figure 1 [International Union of Pure and Applied Chemistry, "Nomenclature for Sampling in Analytical Chemistry," *Pure & Appl. Chem*. **62**, 1193(1990)].

Standard Operations

(*24*) Operations specified as "wash (rinse, extract, etc.) with two (three, four, etc.) 10 mL (or other volumes) portions 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.

(*25*) 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. (**b**) *Absorbance*(*s*) (*A*): Negative logarithm to base 10 of the ratio of transmittance (*T*) of test solution 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 in cm and *c* = g/L, or *a* = (*A*/*bc*) 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 the test solution to radiant power incident on solution, 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 test material. If test material is solution, solute transmittance is quantity usually desired and is calculated 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 0.05M KOH and determine absorbance at following wavelengths in 1 cm cell: 230 nm, 0.171; 275 nm, 0.757; 313.2 nm, 0.043; 375 nm, 0.991; 400 nm, 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, USA).

(*26*) 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:

$$b \quad \frac{(X_iY_i) \quad [(X_i \; Y_i)/n]}{X_i^2 \quad [(X_i)^2/n]}$$

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

where = "sum of" the *n* individual values of indicated operation, and $\bar{X}$ and $\bar{Y}$ 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	X_i = 240	Y_i = 3.92	X_i^2 = 1300	(X_iY_i) = 212.1

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

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

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

$$a = 0.56 - 0.0163(34.29) = 0.001$$

Best equation is then:

$$Y = 0.00 + 0.0163X$$

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

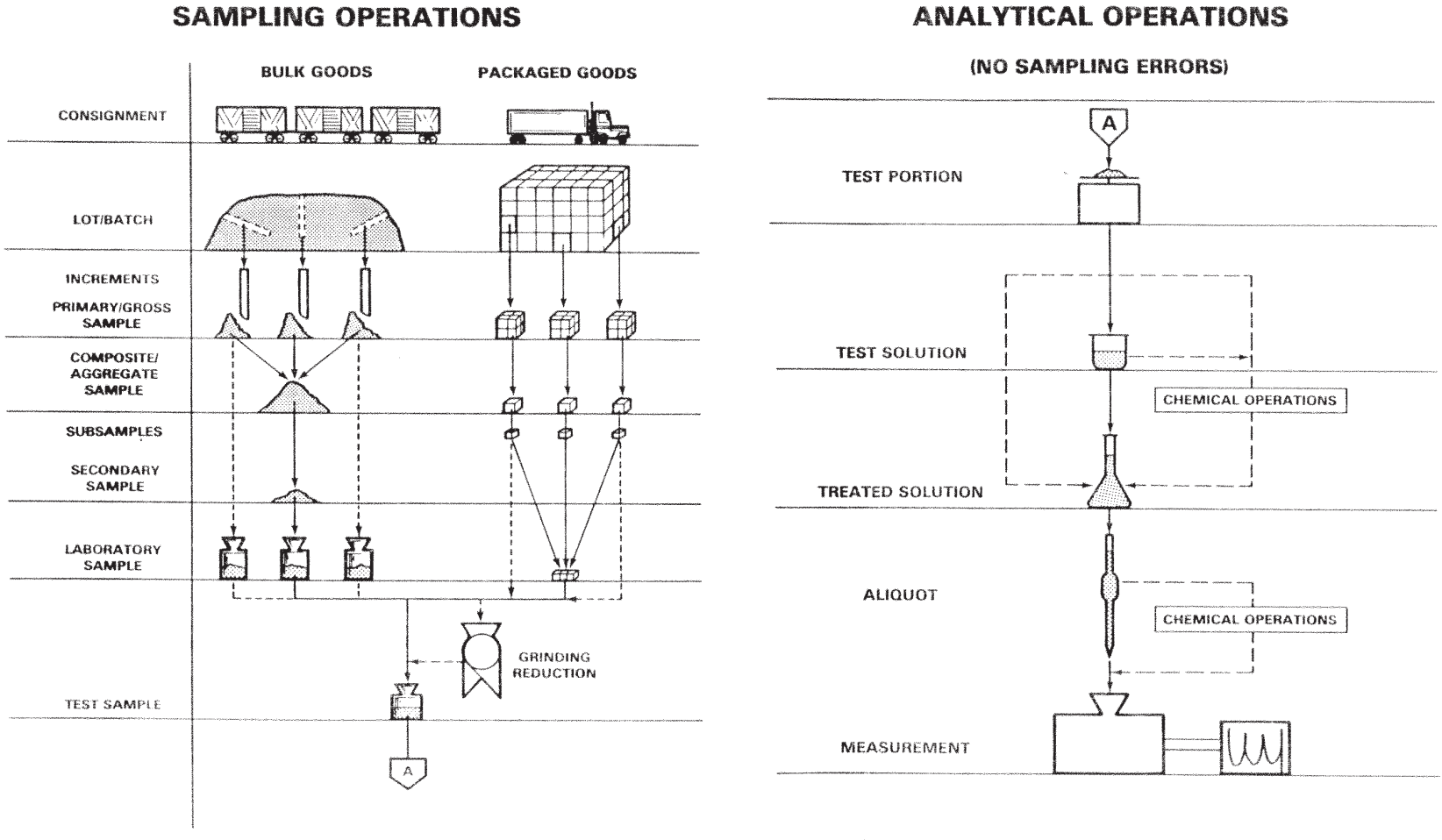

Figure 1. The relationships of the operations involved in sampling and analysis. The lower "A" of the sampling operations continues with the upper "A" of the analytical operations.

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

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.

High correlation coefficients (e.g., >0.99) do not necessarily indicate linearity. This misinformation has been the subject of several reports from the Analytical Methods Committee of the Royal Society of Chemistry [*Analyst* **113**, 1469–1471 (1988); **114**, 753(1989); **119**, 2363–2366(1994)]. Statistically, such a correlation coefficient applies only when both x and y are variables; a standard curve requires that one parameter (concentration) be fixed (known).

When a high correlation is desired between the signal and concentration, use the symbol "r^2" for the relationship as calculation by computer spreadsheet programs.

(*27*) Recovery (R) of analyte from fortified test material by a method of analysis. Fraction of an analyte added to a test sample (fortified test 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 test samples, calculate percent R as follows:

$$\% \text{ Rec} = \frac{C_F \quad C_U}{C_A} \quad 100$$

where C_F = concentration of analyte measured in fortified test sample; C_U = concentration of analyte measured in unfortified test sample; C_A = concentration of analyte *added* to fortified test 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 test sample. Sum of concentration of added analyte plus analyte present before fortification should be in the same range as analyte concentration sought in actual test samples. Addition of analyte must not cause measuring instrument to exceed linear dynamic range of standard curve. Both fortified and unfortified test samples must be treated identically during analysis to minimize experimental bias.

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

Results of Interlaboratory Study

(*29*) Users of methods should consult the report of the collaborative study (reference given with the method) for details as to results of the interlaboratory study.

Editorial Conventions

(*30*) For simplicity, the abbreviations Cl, H, I, N, and O are used rather than their diatomic forms. The charge may not be indicated with the corresponding ion where no ambiguity will result.

(*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, many articles and prepositions have been eliminated.

Manufacturers and Suppliers

Many manufacturers and suppliers may be found by a search of the Internet. **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.**

Abbreviations

(*33*) 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
Å	Angstrom
AA	Atomic absorption
AACC	American Association of Cereal Chemists
ACS	American Chemical Society
amu	Atomic mass unit
AOCS	American Oil Chemists' Society
APHA	American Public Health Association
ASBC	American Society of Brewing Chemists
ASTM	American Society of Testing and Materials
atm.	Atmosphere
AU	Absorbance units
AUFS	Absorbance units full scale
BAM	*Bacteriological Analytical Manual*
Bé	Degree Baumé
bp	Boiling point
Bq	Becquerel
C	Degree Celsius (Centigrade)
ca	About, approximately
Cat. No.	Catalog number
CDC	Centers for Disease Control and Prevention
cfu	Colony forming unit(s)
Ch	Chapter
Ci	Curie(s) (= 3.7 10^{10} Bq)
CI	Color index
CIPAC	Collaborative International Pesticide Analytical Council
cm	Centimeter(s)
concn	Concentration
cP	Centipoise
cpm	Counts per minute
CRM	Certified reference material
*cu in.	Cubic inch(es)
dc	Direct current
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethyl sulfoxide
EDTA	Ethylenedinitrilotetraacetic acid (or -tetraacetate)
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
Exp	Exponential
F	Degrees Fahrenheit [C = (5/9) (F – 32)]
FAO	Food and Agriculture Organization
FDA	U.S. Food and Drug Administration
FEP	Fluorinated ethylene propylene
*fl oz	Fluid ounce (29.54 mL)

Abbreviation	Word
fp	Freezing point
FSD	Full scale deflection
*ft	Foot (30.48 cm)
g	Gram(s)
g	Gravity (in centrifuging)
*gal.	Gallon(s) (3.785 L)
gr.	Grain(s) (1 grain = 64.8 mg)
GC	Gas chromatography
h	Hour(s)
HorRat	Horwitz ratio
HPLC	High performance liquid chromatography
ICC	International Association for Cereal Science and Technology
id	Inner diameter
IgG	Immunoglobulin G
*in.	Inch(es) (2.54 cm)
IR	Infrared
ISO	International Organization for Standardization
kg	Kilogram(s)
kPa	Kilopascal
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)
m	Megaohm
min	Minutes
min.	Minimum
mg	Milligram(s)
mL	Milliliter(s)
mm	Millimeter(s)
mp	Melting point
MS	Mass spectrometer (spectrometry)
MSDS	Material Safety Data Sheet (www.cdc.gov/niosh/ipcs/nicstart.html)
m	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	Nanometer (10^{-9} m); formerly m
No.	Number

Abbreviation	Word
od	Outer diameter
ODS	Octadecylsilane
	Ohm
*oz	Ounce(s) (28.35 g)
p	Pico (10^{-12}) as prefix
Pa	Pascal [1 Newton/m^2; 9.87 10^{-6} atm; 7.5 10^{-3} mm Hg (torr); 1.45 10^{-4} psi]
pCi	Pico Curie(s) = 27.027 Bq
*ppb	Parts per billion (10^{-9})
*ppm	Parts per million (10^{-6})
ppt	Parts per trillion (10^{-12})
*psi	Pounds per square inch (absolute)
*Psig	Pounds per square inch gauge (atmospheric pressure = 0)
*pt	Pint(s) (473 mL)
QAC	Quaternary ammonium compound
*qt	Quart(s) 946 mL
R	Reproducibility value (= 2.8 s_R)
r	Repeatability value (= 2.8 s_r)
®	Trademark name (registered)
R_f	Distance spot moved/distance solvent moved, TLC
rpm	Revolutions per minute
SDF	Special denatured formula (applied to alcohol)
	Sum
s	Second(s)
sq	Square
SRM	Standard Reference Material (CRM of National Institute of Standards and Technology)
T	Transmittance
TLC	Thin-layer chromatography
	Trademark
ton	= 907 kg
U	Unit
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
UV	Ultraviolet
V	Volt(s)

Abbreviation	Word
v/v	Volume per volume
WHO	World Health Organization
w/v	Weight per volume
$\bar{x}$	Mean
^{2}	Chi square
	Beta
	Lambda
	Gamma
	Micro
c	Micro coulomb
m	Micron (0.001 mm); use micrometer (10^{-6} m)
g	Microgram(s) (10^{-6} g)
L	Microliter(s) (10^{-6} L)
	Difference [e.g., $A = (A - A)$]
*	Foot (feet) (1 = 30.48 cm)
*	Inch(es) (1 = 2.54 cm)
/	Per
%	Percent (parts per hundred); percentage
% Rec	Percent recovery
‰	Parts per thousand
>	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

* = Not official SI units; no longer recommended for use in AOAC INTERNATIONAL.

Conversion table for concentration units

	Parts/thousand	Parts/million	Parts/billion	Parts/trillion
%	10	10000	10000000	10000000000
Parts per thousand	1	1000	1000000	1000000000
Parts per million	0.001	1	1000	1000000
Parts per billion	0.000001	0.001	1	1000
Parts per trillion	0.000000001	0.000001	0.001	1

Use: One unit in left column equals the number of units in columns 2–5. Example: 5% = 50 000 parts per million; 2 ppm = 2000 ppb; 5 ppb = 0.005 ppm.

Note: These units are no longer recommended because United States and international usage differ. Use scientific nomenclature 10 000 = E + 4; 0.000 1 = E – 4.

AOAC® *Official Methods*SM Validation Program

AOAC INTERNATIONAL is a unique, nonprofit scientific organization whose primary purpose is to serve the needs of government, industry, and academic laboratories for analytical methods and quality measurement systems. The AOAC® *Official Methods*SM Program is designed to provide methods of analysis with known performance characteristics, such as accuracy, precision, sensitivity, range, specificity, limit of measurement, and similar attributes. A prerequisite of AOAC adoption is validation through interlaboratory collaborative study in independent laboratories under identical conditions. Such validated methods can then be used with confidence by regulatory agencies, regulated industry, product testing laboratories, and academic institutions. The methods are used to determine compliance with government regulations, to maintain quality control and process requirements, to set and evaluate compliance with terms of procurement contracts, to conduct national and international trade, and to support research.

The actual work is done worldwide by appointed volunteers in their professional capacities as scientists of federal, state, provincial, and municipal laboratories; academic and experiment station laboratories; and commercial laboratories. These volunteers contribute time, expertise, and laboratory capability to participate as researchers, methods collaborators, committee members, and advisors.

AOAC INTERNATIONAL has over a century of experience in using the interlaboratory collaborative study as a means of determining the performance characteristics of a method for both general and regulatory use. AOAC's major contribution to analytical science has been to bring the interlaboratory collaborative study technique to a high degree of perfection, and to encourage other methods organizations to harmonize their programs with the AOAC procedure. As stated in the U.S. Code of Federal Regulations (Title 21), it is the policy of the U.S. Food and Drug Administration in its enforcement programs to use the methods of analysis of AOAC INTERNATIONAL as published in the latest edition (18th Ed., 2005) of their publication *Official Methods of Analysis of AOAC INTERNATIONAL*. In addition, in the U.S. Code of Federal Regulations (Title 9), Animal and Animal Products, *Official Methods of Analysis of AOAC INTERNATIONAL* (15th Ed., 1990), is incorporated by reference with the approval of the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR Part 51.

METHOD SUBMISSION

Several mechanisms exist for submitting methods to AOAC: (*1*) a government agency or an organization may enter into a contract with AOAC for the validation of specific methodology or provide continuous infrastructure support for the review and approval of methods in a particular area of interest; (*2*) a community may be formed comprised of stakeholders from government, industry, and/or academia in a particular area of interest who need validated methods and submit best and most needed methods for AOAC review and approval; (*3*) a company or organization that, for example, has a proprietary product and has an interest in obtaining an economic advantage through the AOAC approval of their method may submit its methods for review and approval, together with a submission fee.

Communities

Methods may be submitted by AOAC analytical communities. Communities bring together analytical scientists in a specific area who share a commitment to consolidate efforts to prioritize method needs, establish performance criteria, gather and evaluate existing methods, help seek funding, and support validation work for methods that are fit-for-purpose. Communities may be able to secure collaborative funding from industry and government in support of much needed methodology. Examples of AOAC analytical communities various stages of development include, but are not limited to, Agricultural Materials, Dietary Supplements, Food Allergens, Homeland Security, and Marine and Freshwater Toxins.

For more on AOAC's various communities, visit our Web site at www.aoac.org and click on "AOAC Analytical Communities."

Contracts and Infrastructure Support

An interested party, usually a government agency, in need of validated methods may build a contractual relationship with AOAC.

For more information about government and industry participation, contact Anita Mishra at amishra@aoac.org or Tel: +1-301-924-7077 ext. 131.

Individual Company or Organization

Methods may be submitted by an individual company or organization that has, for example, a proprietary product and wants to have their method(s) approved by AOAC.

METHOD VALIDATION PROCESS

Method Development and In-House Study

An AOAC-sponsored validation study begins with the appointment of a Study Director, the individual scientist who is responsible for organizing the method study.

He or she selects, develops, or adopts a method to be studied. For a microbiology method, a precollaborative study is required, to be conducted according to AOAC guidelines. In the case of a chemistry method, the Study Director develops the required in-house validation data and collaborative study protocol (design) in accordance with AOAC guidelines.

The recommended study protocol and in-house validation data are reviewed by the General Referee, Committee Statistics and Safety Advisors, up to 2 Methods Committee members, and the Methods Committee Chair. Once an agreement on the study

protocol is reached, the Study Director can begin the interlaboratory collaborative study.

Validation Through Collaborative Study

The Study Director recruits collaborators in laboratories with experience in the type of analysis required in the proposed method. For quantitative methods, AOAC INTERNATIONAL requires valid data from no fewer than 8 laboratories, each analyzing a minimum of 5 materials, as blind duplicates or Youden pairs. For qualitative methods, the minimum criteria are 15 laboratories reporting 2 analyte levels per matrix, 5 replicates per level, and 5 negative controls per matrix.

The Study Director prepares the test materials and, if required, other materials to be supplied to collaborators, such as reference materials, column packings, or monoclonal antibodies, and ships them to the cooperating laboratories along with the method, instructions for conducting the study, and reporting forms.

Collaborators are expected to conduct the test exactly as instructed and according to the method, with no deviations, and return results within the time frame agreed.

Expert Review

The Study Director compiles the data, evaluates the results, and writes the collaborative study report, in accordance with AOAC guidelines. Statistical treatment of the data is considered essential in a rigorous evaluation of the method, and AOAC INTERNATIONAL provides manuals, statistical software, and expert consultation to aid the Study Director.

The report is submitted to the General Referee and Statistics Advisor and then to the Methods Committee and 2 Official Methods Board members for technical review. Methods acceptable through these review levels are then approved for adoption as First Action *AOAC® Official Methods*℠.

Adoption of First Action AOAC® Official Methods℠

A Methods Committee reviews the submitted collaborative study reports, comments, and associated documentation to ensure adherence to the technical review process. Advance notices of the methods to be considered for First Action are published in the Referee section of AOAC's magazine, *Inside Laboratory Management*, and on the AOAC Web site.

Method actions taken by the Methods Committees are published in the Referee section of AOAC's magazine, *Inside Laboratory Management*. The complete text of newly adopted AOAC® *Official Methods*℠ and the reports or summaries of the interlaboratory collaborative studies are published in the *Journal of AOAC INTERNATIONAL*. The adopted methods are added to the compendium, *Official Methods of Analysis of AOAC INTERNATIONAL*.

Adoption of Final Action AOAC® Official Methods℠

First Action AOAC® *Official Methods*℠ are eligible for Final Action status after they have been available in the literature for at least 2 years. If the Association has not received any information as to significant problems in the performance of the method, the General Referee recommends adoption of the method as Final Action, and the method is listed in the Referee section of AOAC's magazine, *Inside Laboratory Management*, and on the AOAC Web site so interested parties may submit comments and data if desired.

A ballot of methods recommended by the General Referees and Methods Committees for Final Action is submitted to the Official Methods Board who votes on the acceptance of the methods as Final Action. Notices of the methods adopted as Final Action AOAC® *Official Methods*℠ are published in the Referee section of AOAC's magazine, *Inside Laboratory Management* and on the AOAC Web site.

Actions Affecting AOAC® Official Methods℠

Methods can be repealed, in which case they lose their official status. Methods are repealed through recommendations initiated by the General Referee and approved by the Methods Committee and Official Methods Board. Notification of the recommendation is made through publication in the Referee section of AOAC's magazine, *Inside Laboratory Management*, so interested parties may submit comments and data on the proposed action.

Adoption of Methods Not Sponsored by AOAC INTERNATIONAL

Methods from other organizations that follow the AOAC harmonized protocol and are formatted in AOAC style may be submitted for AOAC review and adoption as AOAC® *Official Methods*℠. Such methods enter the AOAC process at the point of technical review of the completed collaborative study.

Modifications to AOAC® Official Methods℠

When it is necessary to make a modification in an existing AOAC® *Official Methods*℠, the procedure and extent of validation depend on the extent of the revision, whether editorial, minor, or substantive. These determinations are made by the General Referee and Methods Committee.

Appeals Process

All requests for review of AOAC® *Official Methods*℠ or method action must be submitted in writing. Each request is reviewed similarly to a method; the Official Methods Board then acts on the recommendation of the General Referee and Methods Committee.

COMMITTEE ORGANIZATION

Appointments

The AOAC® *Official Methods*℠ Program is administered by volunteer technical experts appointed by the AOAC President or a designee. Volunteers are generally appointed for 3-year terms, and each position has stated appointment requirements, duties, and responsibilities. Persons appointed as Official Methods Board and Methods Committee members and General Referees must be members of AOAC INTERNATIONAL because of their role in review and recommendation or adoption of AOAC® *Official Methods*℠.

Official Methods Board

The Official Methods Board consists of the Chairs of the 11 Methods Committees plus a Board Chair and Vice Chair. The Board recommends, implements, and promotes uniform policies for the consideration and adoption of AOAC® *Official Methods*℠, including statistical and safety requirements; grants Final Action status for First Action AOAC® *Official Methods*℠, addresses requests for action; and resolves disputes in the AOAC® *Official Methods*℠ Program in accordance with established policies.

Methods Committees

The 11 Methods Committees each have 7–11 members plus a Chair, Secretary, and a committee Statistician and Safety Advisor. Each of the Methods Committees guides and supervises the development and validation of analytical methods for the

identification and/or quantitation of analytes from a variety of matrixes; reviews protocols for interlaboratory studies; reviews completed studies and methods; approves methods for First Action; recommends actions on revision, repeal status; recommends scientists for appointment as General Referees; and recommends new General Referee topic areas for study.

General Referees

General Referees are organized along topic lines under appropriate Methods Committees. The General Referee is responsible for a broad area of study (e.g., Fertilizers; Fruits and Fruit Products; Drugs in Feeds; Mycotoxins) and coordinates and guides the activities of a number of Study Directors working on specific methods within the broad topic area. Each General Referee works with the Study Directors on methods development concepts; reviews the reports of Study Directors; recommends appropriate action on methods; and prepares an annual report to the Methods Committee on scientific issues in the designated area.

Study Directors

Study Directors are organized along topic lines under appropriate General Referees and Methods Committees. A Study Director conducts the interlaboratory study of a specific method in a topic area (e.g., a specific drug; a specific food additive; a specific feed component). A Study Director selects test methodology; develops in-house validation data; develops a protocol for the interlaboratory collaborative validation of the method; evaluates the completed study; recommends methods for adoption as First Action *Official Methods*^{SM}; and recommends appropriate First Action methods for adoption as Final Action AOAC® *Official Methods*^{SM}. Study Directors are required to submit an annual status report on the topic to the General Referee.

Topic Advisors and Method Advisors

Topic Advisors are responsible for assisting the General Referee in an assigned subject area. They research their topic area and provide recommendations for new methods that are needed. They provide guidance to Study Directors in designing a collaborative study.

Method Advisors serve as experts on specific methods. They answer technical inquiries about the method and provide recommendations for method modifications based on feedback by method users.

Collaborators

Any scientist experienced in analysis and qualified in the subject matter may collaborate in the study of a method. Collaborators are chosen by the organizer of the collaborative study from laboratories with an interest in the method, including regulatory agencies, industry, commercial laboratories, and universities. A collaborator is expected to analyze materials at times indicated, according to a protocol submitted by the Study Director; follow the method exactly (this is critical); report any unavoidable deviation; perform only the number of determinations requested; and supply raw data, graphs, recorder tracings, photographs, or other documentation.

Safety Committee

Safety Committee members have an interest in the safety and health aspects of the validation and use of analytical methods. The Committee promotes an awareness of safety and health matters within the AOAC membership; serves as a pool of expertise for the AOAC membership in regard to safety matters; submits safety awareness information for publication in *Inside Laboratory Management*; and establishes liaisons with other professional organizations to exchange safety information.

Statistics Committee

Statistics Committee members provide advice on statistical criteria and analysis of validation studies. The Committee develops and recommends harmonized statistical guidelines; encourages greater use of standardized statistical techniques; advises the Official Methods Board on statistical matters; educates AOAC volunteers in proper application of statistical techniques; and encourages greater use of statistical techniques.

Volunteer Participation and Conflicts of Interest

Members of committees, advisors, and referees may be chosen who, because they are experts in the subject area, may have conflicts or apparent conflicts in the performance of their duties. While this will not necessarily disqualify a volunteer from carrying out his or her duties, it is the sense of AOAC INTERNATIONAL that conflicts of interest or even the appearance of conflicts of interest should be avoided. Where it is not practical to eliminate all conflicts, AOAC policy states that these conflicts must be disclosed. All volunteers appointed in the AOAC® *Official Methods*^{SM} Program are required to sign a form accepting their appointment and agreeing to the provisions of the conflict of interest policy.

PRELIMINARY WORK

Purpose and Scope of the Method

The purpose and scope of the method must be decided. A method must be chosen and demonstrated to apply to the matrixes and concentration ranges of interest.

Optimization of New or Available Method

A collaborative study should not be conducted with a nonoptimized method. As much experimentation must be done within a single laboratory as possible with respect to optimization, ruggedness, bias, concentration–response curves, and interferences; the critical steps and variables should be determined and the need for their control emphasized.

Description of the Method

Every step in the analytical method must be described and explained. Performance specifications and system suitability tests, defined critical points, and convenient stopping points must be incorporated. Descriptions of equipment and reagents should be written generically, if possible, to avoid dependence on specific brand names and allow the method user to determine suitability of those items in his or her own laboratory. The detailed method written by the Study Director should then be tested by an analyst not previously associated with its development.

Obtaining Participation

Lists of possible participants can be developed through personal contacts, technical societies, trade associations, literature search, and advertisements in the Referee section of AOAC's magazine, *Inside Laboratory Management*. Laboratories invited to participate should have personnel experienced in the basic techniques employed; experience with the method itself is not a prerequisite for selection.

Laboratories must realize the importance of the study. A large investment is made in testing the method and this probably will be

the only collaborative study of the method that will be performed. Therefore, it is important to have a fair and thorough evaluation of the method.

SUMMARY OF ADOPTION PROCESS

(*1*) A$_{SM}$ method is adopted as a First Action AOAC® *Official Method*^{SM} by a Methods Committee after successful completion of an interlaboratory collaborative study, conducted by a Study Director according to AOAC specifications, and after review and recommendation by the General Referee, Statistical and Safety Advisors, Methods Committee, and 2 Official Methods Board members.

(*2*) A$_{SM}$ method is adopted as a Final Action *AOAC® Official Method*^{SM} after publication of the method and collaborative study report has allowed further use and testing by the scientific community; review and recommendation by the General Referee and Methods Committee; and a vote by the Official Methods Board.

(*3*) First and Final Action AOAC® *Official Methods*^{SM} may also be revised or repealed.

(*4*) Notices of all proposed actions and completed actions for AOAC® *Official Methods*^{SM} are published in the Referee section of AOAC's magazine, *Inside Laboratory Management*, and on the AOAC Web site. Collaborative study reports for new First Action methods are published in the Association journal, *Journal of AOAC INTERNATIONAL*. All First and Final Action AOAC® *Official Methods*^{SM} are published in the compendium, *Official Methods of Analysis of AOAC INTERNATIONAL*, which is updated annually.

HOW CAN YOU GET STARTED?

Scientists who are interested in development and validation of analytical methods should contact AOAC INTERNATIONAL for more detailed information and notify AOAC INTERNATIONAL of their wish for a volunteer appointment. Anyone with the knowledge, interest, and experience in the subject matter field may be appointed as an AOAC Study Director.

WHO IS AVAILABLE TO HELP?

Every appointment comes with information about staff contacts, names and addresses of the assigned General Referee, Statistics Advisor, Safety Advisor, Methods Committee members, and other Study Directors working on methods in similar areas. The Association magazine, *Inside Laboratory Management*, is available as a medium to recruit collaborators.

WHERE TO WRITE OR CALL

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Fax: +1-301-924-7089
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METHODS PROGRAM STEPS

Study Design: Study Director
Protocol Review: General Referee, Statistics and Safety Advisors, Methods Committee representatives
Collaborative Study: Study Director and collaborators
Study Report: Study Director
Report Review: General Referee, Statistics Advisor, Methods Committee, and 2 Official Methods Board Members
Method Adoption: Methods Committee
Method Publication: *Official Methods of Analysis of AOAC INTERNATIONAL*
Study Publication: *Journal of AOAC INTERNATIONAL*

COLLABORATIVE STUDY PROCESS

Method Development—In-House: (Method Choice; Method Optimization; Ruggedness Testing)
Protocol Design: Method Write-Up; Choice of Laboratories; Test Materials; Statistical Design
Study Preparation: Participants; Instructions; Preparation and Shipping of Test Samples
Collaborative Study Execution: Collaborative Analyses; Data and Report Submission
Study Analysis: Data Audit; Outliers; Accuracy; Precision; Conclusions
Final Report: Background; Study; Method; Results; Recommendations

COMMITTEE STRUCTURE

Official Methods Board

(A) *Methods Committee on Pesticide and Disinfectant Formulations*: General Referees (CIPAC Studies; Disinfectant Formulations; Fungicides and Rodenticides; Herbicides; Insecticides, Synergists, and Repellents); Study Directors

(B) *Methods Committee on Drugs and Related Topics*: General Referees (Drugs; Drug Residues in Diagnostics and Test Kits; Drug Residues in Foods; Cosmetics; Forensic Sciences); Study Directors

(C) *Methods Committee on Additives, Beverages, and Food Process Related Analytes*: General Referees (Beverage Alcohol; Food Additives; Flavors; Spices and Other Condiments; Color Additives; Filth and Extraneous Materials in Foods and Drugs); Study Directors

(D) *Methods Committee on Natural Toxins and Allergens*: General Referees (Mycotoxins; Food Allergens; Marine and Freshwater Toxins); Study Directors

(E) *Methods Committee on Food Nutrition*: General Referees (Dietary Fiber; Fats and Oils; Infant Formula and Medical Diets; Minerals; Sugars and Sugar Products; Fat-Soluble Vitamins; Water Soluble Vitamins; Nonvitamin Micro-Nutrients); Study Directors

(F) *Methods Committee on Commodity Foods and Commodity Products*: General Referees (Cereals and Cereal Products; Chocolate and Cacao Products; Dairy Chemistry; Fruits and Fruit Products; Meat and Meat Products; Seafoods; Processed Vegetable Products); Study Directors

(G) *Methods Committee on Residues and Related Topics*: General Referees (Metals and Other Elements; Multiclass Multiresidue Methods for Organic Compounds; Single Class Multiresidue for

Organic Compounds; Radioactivity; Pesticides and Other Chemical Contaminants); Study Directors

(H) *Methods Committee on Microbiology*: General Referees (Drug- and Device-Related Microbiology; Food Microbiology—Dairy; Food Microbiology—Nondairy; Genetically Modified Organisms; Microbiological Efficacy Testing of Disinfectants; *Bacillus anthracis*); Study Directors

(I) *Methods Committee on Feeds, Fertilizers, and Related Agricultural Materials*: General Referees (Antibiotics in Feeds; Drugs in Feeds; Feeds; Fertilizers & Agricultural Liming Materials; Nutrients in Soils; Veterinary Analytical Toxicology; Tobacco); Study Directors

(J) *Methods Committee on Environmental Quality*: General Referees (Inorganic Methods; Organic Methods; Environmental Microbiology; Environmental Chemistry; Bioassay Methods); Study Directors

(K) *Methods Committee on Dietary Supplement*: General Referees (Botanicals; Plant Toxins); Study Directors

AOAC COLLABORATIVE STUDY

The following is a summary of the information that is presented in detail in the internationally harmonized document, "Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis," and are given in *Appendix D*. The document is the basis for an AOAC validation study.

Design of the Collaborative Study

General Principles: The design should attempt to identify and to include the possible sources of significant variability that may occur in actual practice, including between days, between runs, and between calibration curves, if these are significant factors. The best measure of within-laboratory variability is obtained by using blind replicates and/or split levels (Youden pairs). The design must take into account how the data will be analyzed statistically.

Laboratories: *Minimum number of laboratories for quantitative analysis*.—A minimum of 8 laboratories submitting valid data is needed for a quantitative method (only in special cases involving very expensive equipment or specialized laboratories may the study be conducted with a minimum of 5 laboratories, with the resulting expansion in the confidence interval for the statistical estimates of the method characteristic). *Minimum number of laboratories for qualitative analysis*.—A minimum of 15 laboratories is needed for qualitative studies reporting on 2 analyte levels per matrix, 5 test samples per level, and 5 negative controls per matrix. It is prudent to include more than the minimum to avoid jeopardizing a study in which results of some laboratories must be discarded.

Test Materials: Minimum number of materials is 5 for quantitative analysis (only when a single level specification is involved for a single matrix may this minimum be reduced to 3). Test materials must be homogeneous (this is critical) and coded at random so that there is no preselection from order of presentation. Analyte levels should be chosen to cover concentration range of interest, especially tolerance limits, specification levels, and likely levels of occurrence.

Materials should be representative of commodities usually analyzed, while being stable and able to withstand the rigors of commercial transportation. Practice test samples should be provided, and reserve test samples should be prepared and preserved to replace lost or damaged items and to permit re-analysis in the case of outliers or abnormal results.

Replication: For within-laboratory variability, independent replication can be ensured by applying one of the following procedures: (*1*) Split levels (Youden pairs). A pair of materials of slightly different concentration obtained either naturally or by diluting (or by fortifying) one portion of the material with a small amount of diluent (or of analyte). (*2*) Split levels for some materials and blind duplicates for other materials in the same study. (*3*) Blind duplicate test samples—randomly coded. (*4*) Independent materials. Although use of known replicates is a common practice, it is preferable to use the same resources for blind replicates or split levels.

Blanks: When the absence of a component is as important as its presence, when determinations must be corrected for the amount of the component or the presence of background in the matrix, or when recovery data are required, provision must be made for the inclusion of blank materials containing "none" (not detected) of the analyte. It is also important to know the variability of the blank and the tendency of the method to produce false positives.

Analysis and Report

AOAC INTERNATIONAL requires the calculation and reporting of percent recovery (% Rec.), HorRat, repeatability (within-laboratory, s_r) and reproducibility (interlaboratory, s_R) standard deviations, and repeatability and reproducibility relative standard deviations (RSD_r and RSD_R, respectively). Specific guidelines and tools are available to aid the Study Director in performing the statistical analysis of the collaborative study data. These include spreadsheet forms for the calculation of performance parameters and a software package for computer calculations from the data.

The final report should contain the purpose of the study and the principles of the method, a brief summary of related work, a description of the collaborative study design, the complete method, and the results and conclusions. The report must also include the names of the study participants and their organizations.