

PART 8

HOW TO WRITE DOCUMENTS IN AOAC STYLE

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QUICK & EASY!

FORMAT OF A COLLABORATIVE STUDY PROTOCOL

- Introduction explaining the purpose of the study and a brief description of the method.
- Design of the collaborative study, e.g., number of collaborators, type and number of test samples to be tested, levels of spiking, etc.
- The method written in AOAC style, i.e., the way methods are published in the *Official Methods of Analysis of AOAC INTERNATIONAL*.
- Letter to collaborators that would include the equipment and reagents requirements, the intended date of beginning of the study, length of time of the study, time frame for reporting results of analyses, etc.
- Forms to be used by collaborators to report results of analyses.
- Basic Format is as follows:
 - TITLE
 - AUTHOR(S)
 - INTRODUCTION
 - COLLABORATORS
 - STUDY DESIGN
 - TEST SAMPLE PREPARATION
 - METHOD
 - REPORTING RAW DATA
 - ANALYZING RAW DATA
 - APPENDICES (or TABLES AND FIGURES)

Collaborative Study protocols and supporting documentation can be directly submitted to AOAC by using our website (www.aoac.org).

FORMAT OF A COLLABORATIVE STUDY PROTOCOL

The following format should be followed when writing a collaborative study protocol:

- (1) **TITLE:** Choose a title. Be explicit and descriptive enough to give an idea of your approach. For example, *Cholesterol Analysis in Foods by Direct Saponification Gas Chromatographic Method.*
- (2) **AUTHOR(S):** Include your name, affiliation, mailing address, phone number, fax number and email address.
- (3) **INTRODUCTION:** Include the following:
 - (a) Background: describe work on the current method or other methods available. If there is a reference method it should be discussed.
 - (b) Goals of the Study.
 - (c) The needs and purpose of the study.
 - (d) A description of your approach to the problem.
 - (e) Intended scope/applicability of method, regarding analyte(s), matrix(ces), and concentration ranges (LOD/LOQ) and whether the method is quantitative or qualitative.
 - (f) A concise but complete explanation of the principles of the method, including the chief chemical reaction or reactions on which it is based, if applicable.
- (4) **COLLABORATORS:** Qualify the collaborators:
 - (a) State whether study is planned in cooperation with any other organization.
 - (b) State number of planned collaborators.
 - (c) Tell how collaborators/laboratories will be selected.
 - (d) List the collaborators who have already agreed to participate with their full contact information (name, title, affiliation, mailing address, phone number, fax number and email addresses) when available
- (5) **STUDY DESIGN:** Outline the design of the study: number of materials, number of blind duplicates or Youden pairs, number of blanks, number of positive and negative controls, where applicable, and number of analyte levels.
- (6) **TEST SAMPLE PREPARATION:** Describe preparation of test samples:
 - (a) What will be the individual materials? What is the matrix, what is the analyte, and at what concentration?
 - (b) How will analyte/matrix combinations be prepared? By spiking or as naturally occurring materials?
 - (c) How will actual content be determined? If using a reference method to determine actual content, how will the reference method be determined to be in control? How will the reference method be selected?
 - (d) What other analytes or contents of interest to this test (interferences, etc.) will be included in materials?
 - (e) Comment on Homogeneity of the samples (see Appendix E of this manual)
 - (f) What will be the blanks, if appropriate?
 - (g) What quality control measures will be followed to assure content of samples?
 - (h) What stability data are available and how is that information used?
 - (i) How will test samples be packaged and how will collaborators handle them upon receipt?
- (7) **METHOD:** Write the method in AOAC style (see Format of AOAC® *Official Method*SM, example of the method, Part 8 and AOAC website):
 - (a) Include applicability statement (matrices, analyte concentrations)
 - (b) Write the method as a series of commands. For example, "add 10 mL," "stir the solution."

- (c) List Apparatus and Reagents as separate sections at the beginning of the method in a list format, apart from the procedural steps. Stock items found in every laboratory that don't need special preparation are listed. Concentrations of reagents and any directions for purification, preparation, and storage and specifications of apparatus are essential elements of the method. Follow AOAC policy on *Equivalency of Products Used in AOAC® Official MethodsSM* (see Appendix A), and describe apparatus and reagents generically in terms of performance and suitability tests.
 - (d) If sampling, test sample preparation, or preparation of a standard curve is crucial or involved, present the information in separate sections.
 - (e) Describe procedural steps under the heading "Determination." Be as explicit as possible in listing details. (Example: volume and number of extractions; order of elution; critical times and temperatures; special spectrophotometric conditions; size of containers, if important; vigorous or gentle shaking or stirring; criteria for judging an end point; suitable stopping places for a lengthy method; etc.)
 - (f) Provide calculations with SI units if applicable.
 - (g) Include any necessary alerts to critical steps, precautions, or warnings.
- (8) **REPORTING RAW DATA:** Provide instructions on how to report the data from the analysis. Give draft data reporting sheet (see Part 7 for example of the reporting form.)
- (9) **ANALYZING RAW DATA:** Indicate how the data will be analyzed. Indicate what method performance statistics will be determined from study design (recovery, RSD_r , RSD_R , S_r , S_R , HORRAT, etc.). See Appendix D of the OMA (Part 6) for additional information.
- (10) **APPENDICES or FIGURES AND TABLES:** Provide examples of the the draft letter to collaborators that would include the information on the study, e.g., type of analysis, equipment and reagents requirements, the intended date of beginning of the study, length of the study, time frame for reporting results of analyses, etc. See Part 7 for the example of a letter to collaborators, solicitation postcard, etc.
- (11) **SUPPORTING DATA:** See Checklist for Protocol Design of Collaborative Studies (Appendices Q and R).

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FORMAT OF A COLLABORATIVE STUDY MANUSCRIPT

Refer to the AOAC website for examples of collaborative studies (www.aoac.org).

- **Title**-Title of manuscript that ends with ":Collaborative Study."
- **Author(s)**- provides authors' full (e.g. no initials) names and contact information.
- **Abstract**-Specific information on the method and study.
- **Introduction**-Information on why collaborative study was conducted, how many collaborators participated in the study, previous work done, and information on compound or process that was studied.
- **Collaborative study**-Information on matrices and number of test samples tested, test sample preparations, instructions for collaborators, etc.
- **Method**-Written in AOAC style.
- **Collaborators' comments**-Any comments and suggestions received from collaborators and information on how they were addressed by the Study Director, e.g., incorporating instructions into the method, etc.
- **Results and Discussion**-Information on type of statistical analyses performed on raw data, reasons for rejecting some of the data, discussion of results with references to tables and figures, discussion of the method performance, etc.
- **Recommendation**-Study Director's recommendation to adopt method First Action.
- **Acknowledgments**-Full names and addresses of all collaborators that participated in the study.
- **References**-All references cited in the text.

Collaborative Study manuscripts and supporting documentation can be directly submitted to AOAC by using our website (www.aoac.org). Examples of collaborative studies for the 10 methods committees are located on our website (www.aoac.org).

FORMAT OF A COLLABORATIVE STUDY MANUSCRIPT

Your manuscript may be in either of two categories: (1) a preliminary or first manuscript in which you give the background of the problem, describe a method that you either devised yourself or selected for study, and present data obtained in your own laboratory (such as optimization studies, ruggedness testing); or (2) a manuscript of a collaborative study, either successful or unsuccessful. If your manuscript does not seem to be in either of these two categories, you may still be able to adapt these guidelines for your particular situation. Alternatively, contact AOAC OMA/PVM department for special instructions.

1. PRELIMINARY OR FIRST MANUSCRIPT (OPTIONAL)

Choose a title. Be explicit and descriptive enough to give an idea of your approach. Include matrix, analyte and technique. For example, *Determination of Ochratoxin A in Baby Food by Immunoaffinity Column Cleanup and HPLC*.

Include your complete name, address, and phone number. Add a footnote identifying the manuscript as your Study Director manuscript. Include the exact title of your study and, if appropriate, the year(s) of poster presentation at the Annual Meeting in the footnote.

Include the following in your introduction:

- a. The purpose of the study.
- b. A brief summary of previously published work directly related to your problem and a statement of how your work is related to previous work. Include pertinent literature references. This may be a "why I did this study and why the proposed method is better than the existing method" statement.
- c. A description of your approach to the problem and a brief statement of whether your work was successful.
- d. A concise but complete explanation of the principles of the method, including the chief chemical reaction or reactions on which it is based, if applicable.
- e. Why the method is important to the scientific community and needed.
- f. If a reference method is used, it should be described.

Write the method in AOAC style, as follows:

- a. Write the method as a series of commands, e.g., Add 10 mL or Stir solution.
- b. List Apparatus and Reagents as separate sections at the beginning of the method in a list format, apart from the procedural steps. Stock items found in every laboratory that don't need special preparation are not listed. Concentrations of reagents and any directions for purification, preparation, and storage and specifications of apparatus are essential elements of the manuscript and must be included. Follow AOAC policy on *Equivalency of Products Used in AOAC® Official MethodsSM* (See Appendix A), and describe apparatus and reagents generically in terms of performance and suitability tests.
- c. If sampling, test sample preparation, or preparation of a standard curve is crucial or involved, present the information in separate sections.
- d. Describe procedural steps under the heading "Determination." Be as explicit as possible in listing details. [Example: volume and number of extractions; order of elution; critical times and temperatures; special spectrophotometric conditions; size of containers, if important; vigorous or gentle shaking or stirring; criteria for judging an end point; suitable stopping places for a lengthy method; etc.]

- e. Provide calculations if applicable.
- f. Include any necessary alerts to critical steps, precautions, or warnings.

Describe your experimental plan, for example, the number and types of commodities studied. Present experimental results, preferably in tables or figures. Tables and figures should have detailed titles or legends.

Discuss or clarify your data wherever appropriate and draw suitable conclusions.

Make a recommendation of some type. For example: "Collaborative study of the method is recommended."

Provide complete and accurate bibliographic references for any literature citations in the manuscript.

Collaborative Study manuscripts, protocols and supporting documentation can be directly submitted to AOAC by using our website (www.aoac.org).

2. COLLABORATIVE STUDY MANUSCRIPTS

The following format should be followed when writing collaborative study manuscripts:

Title—Use title from protocol or choose a title based on your study. Be explicit and descriptive enough to give an idea of your approach. Include matrix, analyte and technique. For example, *Cholesterol Analysis in Foods by Direct Saponification-Gas Chromatographic Method: Collaborative Study*. The title of the collaborative study manuscript, method and interlaboratory study results table must be the same.

Author(s)—Include your complete name, affiliation, mailing address, phone number, fax number and email address. Include in the footnote, if appropriate, the year(s) of poster presentation at the Annual Meeting.

Abstract— Provide an abstract of the paper, however, do not send manuscript to publications at this time. Manuscript will be published after OMA Methods Committee's approval for First Action.

Introduction—Include the following in your introduction:

- a. The purpose of the study.
- b. A brief summary of previously published work directly related to your problem and a statement of how your work is related to previous work. Include pertinent literature references. This may be a "why I did this study and why the proposed method is better than the existing method" statement.
- c. A description of your approach to the problem and a brief statement of whether your work was successful.
- d. A concise but complete explanation of the principles of the method, including the chief chemical reaction or reactions on which it is based, if applicable.
- e. Why this method is important to the scientific community and needed.

Collaborative Study—Describe the design of your collaborative study. Include the number of collaborators, number and nature of test samples, special instructions to collaborators, etc.

Method—Write the method in AOAC style (see Format of the AOAC® *Official Methods*SM in Part 8 and example of the method):

- a. Write the method as a series of commands. For example, "add 10 mL," "stir the solution."
- b. List Apparatus and Reagents as separate sections at the beginning of the method in a list format, apart from the procedural steps. Stock items found in every laboratory that need no special preparation

need not be listed. Concentrations of reagents and any directions for purification, preparation, and storage and specifications of apparatus are essential elements of the manuscript and must be included. Follow AOAC policy on *Equivalency of Products Used in AOAC® Official Methods*SM (see Appendix A), and describe apparatus and reagents generically in terms of performance and suitability tests.

- c. If sampling, test sample preparation, or preparation of a standard curve is crucial or involved, present the information in separate sections.
- d. Describe procedural steps under the heading "Determination." Be as explicit as possible in listing details. (Example: volume and number of extractions; order of elution; critical times and temperatures; special spectrophotometric conditions; size of containers, if important; vigorous or gentle shaking or stirring; criteria for judging an end point; suitable stopping places for a lengthy method; etc.)
- e. Provide calculations with SI units if applicable.
- f. Include any necessary alerts to critical steps, precautions, or warnings.

Collaborator Comments—Summarize any of the collaborators' comments or experiences that were significant.

Results and Discussion—Present experimental results, preferably in tables or figures. Tables and figures should have detailed titles or legends. Give all collaborator results. Identify outlier results, by outlier test, not included in the statistical calculations, by footnotes. See *AOAC Guidelines for a Collaborative Study*, Part 6.

Discuss and interpret the collaborators' results where applicable.

Recommendation—Make a recommendation to adopt the method as First Action.

Acknowledgements—Acknowledge the help of the collaborators; list each collaborator by name, address and affiliation. Also acknowledge other help you have received. If you wish to recognize anyone whom you feel has made significant and substantial contributions, you may include them as a co-author.

References—Provide complete and accurate bibliographic references for any literature citations in the manuscript.

Collaborative Study manuscripts, protocols and supporting documentation can be directly submitted to AOAC by using our website (www.aoac.org).

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FORMAT OF AOAC® OFFICIAL METHODSSM

- ***Title***-Includes analyte being determined, type of matrix (matrices), and technique used for analysis.
- ***Applicability statement***-Provides range or limits of determination as well as specific matrices.
- ***Precaution statement***-Makes an analyst aware of hazardous materials used in analysis.
- ***Interlaboratory study results*** -Table that presents performance parameters including matrices tested in a collaborative study, levels of analyte(s), % recovery, RSD_r, RSD_R, S_r, S_R, HORRAT, number of observations, etc (see Part 6 for complete list).
- ***Principle***-Explains mechanism of the analysis.
- ***Apparatus***-Section that lists equipment that requires assembly or that has specifications critical to the method performance. Do not use brand names. Describe equipment in terms of performance characteristics.
- ***Reagents***-Section that describes in terms of performance characteristics.
- ***Preparation of test sample.***
- ***Determination***-Describes the actual analysis.
- ***Calculations***-Section that explains how to calculate final results; presented in a form of equation or description.
- ***Other sections as needed.***

Examples of methods written in AOAC format for each of the 10 methods committees are located on our website (www.aoac.org).

FORMAT OF AOAC® *OFFICIAL METHODS*SM

Introduction

AOAC® *Official Methods*SM are designed to be performed by trained scientists who staff the analytical laboratories of regulatory, industrial, and research institutions concerned with agricultural products, food, drugs, environmental media. Because many of the methods are used to define the legal status of regulated materials, it is essential that directions are uniformly interpreted by both the regulating and regulated laboratories. This attribute leads to the basic AOAC requirements of reporting: clarity, completeness, consistency, and brevity.

The AOAC style used for preparing methods for publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* includes the following essentials:

- (1) Standardized format that follows the order of laboratory operations.
- (2) Use of the imperative mode.
- (3) Cross-references to identical reagents, apparatus, and operations.
- (4) Use of standardized definitions, terminology, and style.
- (5) Use of accepted abbreviations and simplifications.
- (6) Use of SI units
- (7) Methods should be written as complete and self-contained as practical.
- (8) Normality should be referred in terms of Molarity.
- (9) ppm should be changed to mg/kg or mg/L
ppb should be changed to ng/g or ng/mL
ppt should be changed to pg/g or pg/mL

The following publications will be useful in conjunction with the preparation of methods of analysis:

- (1) "Definitions of Terms and Explanatory Notes," in *Official Methods of Analysis of AOAC INTERNATIONAL*. (Includes abbreviations and symbols used in OMA, See Appendix D.)
- (2) *Handbook for Authors of Papers in Journals of the American Chemical Society*, American Chemical Society Publications, 1155 6th St, NW, Washington, DC 20036, USA.
- (3) *Reagent Chemicals*, American Chemical Society Specifications, American Chemical Society Publications, 1155 16th St, NW, Washington, DC 20036, USA.

EDITING METHODS IN AOAC STYLE

This editing section is for the method author. Before submitting your method to AOAC INTERNATIONAL, you should do the following:

The language of the method should be concise and completely free from ambiguity (see example of method edited in AOAC style). Conciseness is desirable, both to ensure clarity and to save space. Whenever there is a conflict between clarity and style, clarity is more important. Points that should be considered in editing are listed below:

1. Present Tense and Imperative Mode

Check sentences that do not begin with a verb and change them, if feasible, to the imperative mode (e.g. Pipet 10 mL..., Stir..., etc.). Exceptions are: use of adverb modifier ("Accurately weigh..."), prepositional clause ("For refined sugars, use..."), permissive statements ("Ferric hydroxide may be used..."), and statements in the "Principle" section.

2. Abbreviations

Most abbreviations are the same as those used by Chemical Abstracts. Do not use abbreviations in titles and headings. See the *Definitions of Terms and Explanatory Notes* in Appendix D.

3. Repetition and Redundancy

Eliminate repetition and redundancy as far as possible; use only for emphasis. Do not use "distilled" with water, "concentrated" with common acids, "95%" with alcohol, or "ACS" with reagents covered by ACS specifications. These are understood by definition.

4. Formulas and Chemical Names

In general, use the chemical formula when the compound is easily recognizable. Use the chemical name at the beginning of a sentence (rare) for more complicated organic structures, and where the formula is longer than the name. Follow Chemical Abstracts nomenclature, in general. Check the spelling of all chemical names. Be sure that the correct number of molecules of water of hydration is used.

5. Consistency

Watch for internal contradictions in the text: volumes that do not add up or that exceed the capacity of the container; too abrupt a transition from one operation to another (a line may be omitted); and impractical or impossible numbers (e.g., 100 g NaCl will not dissolve in 100 mL water).

6. Cross-references

All new AOAC methods should be written as complete and self-contained as practical. Do not refer to other AOAC methods. If part of a procedure in an *Official Method*SM is taken from material previously published elsewhere, incorporate those steps in the method rather than referring the analyst to another publication.

7. Definitions

The section "Definition of Terms and Explanatory Notes," *Official Methods of Analysis of AOAC INTERNATIONAL*, is the basic guide to conventions and consistency (see Appendix D).

8. Illustrations

If symbols are used on the figure, include an explanation in the caption or text.

9. Tables

Provide descriptive titles for tables. Explain any obscure headings in a footnote.

10. Bibliographic References

Check all references for accuracy. Use standard Chemical Abstracts abbreviations for *Journal* titles. In general avoid references in method. Cite background references in the "Introduction" or "Discussion" section of the collaborative study manuscript -- not in the method. If part of a procedure in an *Official Method*SM is taken from material previously published elsewhere, incorporate those steps in the method rather than referring the analyst to another publication.

11. Terminology

For names of chemical compounds, use the spelling, hyphenation, and word division given in Chemical Abstracts. Use a national pharmacopeia for names for drugs. Use ISO nomenclature for pesticides and Codex nomenclature for names of food additives and color additives.

12. Safety

All methods must be reviewed against the *Safety Checklist* (see Appendix H) for potential hazards. Authors and editors should become familiar with the general criteria set forth in the introduction of the chapter on *Laboratory Safety* (see Appendix C). They should automatically incorporate cross-references to the safety statement(s), or bring questioned conditions to the attention of the Committee on Safety for resolution.

Decisions regarding inclusion of safety statements should be practical, recognizing that overuse will be self-defeating.

Methods that create toxic, obnoxious or environmentally hazardous fumes and wastes should contain practical directions for disposal.

13. Checking Edited Copy

The author must review a copy of the original version and edited copy to ensure that there has been no change in meaning, to correct typographical errors, and to answer any questions posed by the editor.

14. Proofreading

The author must review the typeset method for accuracy.

GENERAL STYLE GUIDE

The main sections of a method should flow from one operation to the next as they will be performed. Interruptions in operations for preparing reagents, assembling apparatus, and making standard curves must be kept to a minimum. Similar methods in the *Official Methods of Analysis of AOAC INTERNATIONAL* should be examined for guidance.

The main sections of a method and possible major subdivisions in lengthy methods are as follows:

1. Title

The title of the method should state the substance being determined (analyte) in terms of its common name, with chemical name (as used by Chemical Abstracts), trade names, and/or synonyms given in parenthesis. The placement of a method within the chapter is included in the top, left-hand corner of the method. Title includes analyte being determined, type of matrices, and technique used for analysis. For example,

AOAC Official Method 2001.01

Determination of Trans-galactooligosaccharides in Selected Food Products by Ion-Exchange Chromatography

Proposed First Action 2001

2. Applicability Statement

Applicability statements must be definitive, and must state the matrices for which the collaborative study was conducted and approved. This section is also used to include the scope and sensitivity of the method; its applicability to certain types of test samples and its non-applicability, because of interference, solubility, or other reasons, to other types of test samples. Statement about limit of detection and limit of quantitation must be listed here if given and defined appropriately. (Miller, J.C, and Miller, J.W., *Statistics for Analytical Chemistry* First Edition, 1984, reprinted 1986; Ellis Horwood, Publishers, Chichester, England. ISBN 0-85312-662-3. Second Edition, 1987. Third Edition, 1993, Ellis Horwood, Publishers, Prentice Hall, London. ISBN 0-13-030990-7. For example,

(Applicable to the detection of fluoride in peanut butter at concentrations of 0.8-200 mg/kg)

3. Caution Statement

Special safety or operational precautions that are generally applicable throughout the method are also placed into this section. Safety statements or cross-references applicable to a single reagent or operation are inserted at the specific point of applicability (see Appendix I, *Safety Checklist*). Remove the repetitious statement of the need for safe handling of solvents, acids, and alkalis and proper disposal of waste solvents. Make sure you include unusual situations that require specific advice, particularly with regard to disposal and destruction. Do not refer to Appendix B, the safety chapter in OMA. For example,

Caution: Listeria monocytogenes infections can cause fetal death. It is recommended that pregnant women avoid handling this organism. Attention should be given to sterilization of contaminated equipment and media before disposal or reuse.

4. Interlaboratory Study Results Table

Immediately following the precaution statement, the method must also contain statistical information if the collaborative study provides sufficient information with regard to the reliability of the method. The required statistical study results are S_r , S_R , RSD_r , RSD_R , HORRAT, % Rec, mean, # of labs retained after eliminating outliers, and # of outlier labs removed (see Part 6) and should be presented in a table. For example:

See Table 2001.xxA for Interlaboratory study results that support acceptance of the method.

Table xxx.xx. Interlaboratory Study Results for...

Material
No. of Labs ^{a(b)}
Mean (units)
Recovery
_c
%

Repeatability

Reproducibility

Matrix
Level, (units)

RSD_r (%)
RSD_R (%)
HORRAT^c

5. Principle

The method should include a statement explaining the purpose of various steps during analysis and the basis of unfamiliar or unusual reactions. For example, the method for the alkaline titration of cyanide with silver nitrate was the subject of many letters that pointed out an apparently incorrect stoichiometric factor. An insertion was made in the method to the effect that 1 Ag is equivalent to 2 CN in that reaction. The principle should establish the scope of the method and purpose. For example,

A. Principle

Trans-galactooligosaccharides (TGOS) and lactose are extracted from a test portion with hot phosphate buffer. The extract is treated with β -galactosidase to hydrolyze TGOS and lactose. Both the initial and the treated solution are analyzed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). In the first assay, free galactose and lactose are determined in the initial test solution. In the second assay, the total amount of galactose released from TGOS and lactose is determined in the treated solution. TGOS are calculated from concentrations of lactose and galactose.

6. Apparatus

Ordinary apparatus -- beakers, flasks, funnels, etc. -- which are usually part of the standard equipment of the ordinary analytical laboratory, or which are listed in the catalogs of the larger supply houses, are not generally listed in the apparatus section of a method. Common laboratory equipment such as analytical balances and pH meters do not need to be mentioned in a method.

Apparatus that requires assembly and/or specifications, or which may not be readily available, is listed in this section. Descriptions or specifications are preferred for assembled apparatus, however, line drawings or, in rare instances, photographs may be included for clarity. With drawings, the scale should be indicated and the parts should be labeled or coded. The source of commercially available apparatus used in the collaborative study and *Official Method*SM should be given, with complete (and correct) name, address, city, state, and zip code. A recent letterhead, advertisement, or catalog should always be checked for this information. The annual *Guide to Scientific Instruments* published by Science and the *Laboratory Guide* published by Analytical Chemistry are useful for this purpose. All proprietary equipment must be described in performance language giving suitability tests where possible. Critical parameters must be identified and defined, and system suitability standards must be established for non-proprietary equipment or reagents so that the product can be defined generically and equivalency can be readily determined. When writing a method, the author must refer and comply with the AOAC policy for *Determination of Equivalency of Products Used in Official Methods*SM (see Appendix A).

If the equipment must be operated in accordance with the instructions supplied by the manufacturer, this may be stated, as is frequently the case with flame photometers and spectrophotometers. Checking the reliability and accuracy of instruments is an implied function of the operator as is calibration of weights and volumetric apparatus. Convenient calibration standards for both wavelength and absorbance scales of spectrophotometers are given in *Definitions of Terms and Explanatory Notes* (see Appendix D).

Chromatographic columns:

When several columns must be prepared for a single method, a separate section may be used. For example:

- (a) *Column 1.* (1) *Lower layer*-- Mix 3 g diatomaceous earth and 2 mL citrate buffer; transfer to tube and tamp. (2) *Upper layer* -- Add 0.5 mL aliquot of extract. Add 3 g diatomaceous earth, mix, and transfer to tube. Dry-wash beaker with 1 g diatomaceous earth and add wash to column; tamp and add glass wool pad.
- (b) *Column 2.* -- Mix 3 g diatomaceous earth and 2 mL 1.0M K_2HPO_4 (17.42 g/100 mL); transfer to tube, tamp, and add glass wool.

Gas and liquid chromatography:

Because of the numerous parameters involved in gas or liquid chromatographic equipment, a separate section may be devoted to this type of apparatus, including preparation of columns. The following items should be included (adapted from J. Agric. Food Chem. **17**, 160 (1969)):

- (a) *Apparatus* -- Performance criteria are preferred over specific manufacturer's makes and models. Summarize, if feasible, performance requirements by specifications as in examples below:
 - (1) Monitor performance of gas chromatograph by noting separation of campesterol and sitosterol expressed as peak resolution = $2D/(C+B)$, where D = distance between the 2 peak maxima, C = campesterol peak base width, B = beta-sitosterol peak base width. Peak resolution should be < 1.6 .
 - (2) Optimum conditions for gas chromatographic separation are obtained when peaks for solvent, methyl enanthate standard, and formic acid are completely resolved. Conditions vary to some degree from instrument to instrument and should be experimentally reestablished.
 - (3) Select as operating voltage that voltage at which heptachlor epoxide causes ca 40-50% full-scale recorder deflection. Check linearity of system from 0.2 - 2.0 ng heptachlor epoxide.

Detector type, such as thermal conductivity, flame ionization, electron capture, etc., and associated parameters such as recorder range, detector voltage, and bridge current must be described.

- (b) *Column length and diameter* -- Inside diameter is preferred, but outside diameter and wall thickness, particularly if this is commercial usage, may be indicated. Materials: glass, copper, stainless steel, etc. Packing: weight percent of liquid phase on support. Give mesh size and pre-treatment of support. Supply sources of materials. Capillary: only liquid phase or support-coated liquid phase. Conditioning: if necessary.
- (c) *Conditions* -- Temperatures: injection, column, detector isothermal or programmed (give initial and final temperatures and rate of temperature rise); e.g., "Temperatures ($^{\circ}\text{C}$) - injection 250, column 135, detector 235." Flow rates: carrier gas (mL/min at exit port), other gases; e.g., "Flow rates (mL/min)-N 25, H 25, air 300."
- (d) *Analyte solution* -- Quantity (mL) injected, solvent, if used, retention time (minutes or distance), retention time relative to reference compound and internal standard, if used.
- (e) *Chromatogram* -- Recorder response for specific quantity of standard; resolution in terms of separation of specified compounds; peak measurements and baseline correction; calculation.

For example,

B. Apparatus and Materials

(a) *High performance anion-exchange chromatograph (HPAEC)*. — Liquid chromatograph gradient pump, de-gas module, microinjection valve, pulsed electrochemical detector (1.0-mm diameter gold working electrode and a pH-Ag|AgCl combination reference electrode; the titanium body of the cell serving as the counter electrode) working in pulsed amperometric detection mode (PAD), automated sampler (Dionex Corporation, P.O. Box 3603, Sunnyvale, CA 94088-3603, USA, USA +1-408-737-0700, Fax: +1-408-730-9403, or equivalent) and data integrator (Shimadzu, 1 Nishinokyo Kuwabaracho, Nakagyou-ku, Kyoto 604-8511, Japan, Phone: +81-75-823-1111, Fax: +81-75-823-1361, or equivalent.)

HPAEC conditions — Column temperature, constant $\pm 0.5^\circ\text{C}$ between 20-30 $^\circ\text{C}$, preferably $20 \pm 0.5^\circ\text{C}$; flow rate, 1.0 mL/min; injection volume, 20 μL ; detector sensitivity, analog range 1-3 μC . See Table 2001.01B for eluent gradient and Table 2001.01C for detector time program. Parameters may be varied in order to optimize chromatography.

Table 2001.01B for eluent gradient and Table 2001.01C for detector time program. Parameters may be varied in order to optimize chromatography.

Table 2001.01B. Eluent gradient for HPAEC-PAD analysis

Time (min)	(% Mobile phase)		
	A	B	C
0.00	95	5	0
20.10	95	5	0
35.00	0	100	0
36.00	0	100	0
36.10	0	0	100
46.00	0	0	100
46.10	95	5	0
61.00	95	5	0

Table 2001.01C. Detector program for HPAEC-PAD analysis

Time (s)	Potential (V)	Integration
0.00	0.05	
0.20	0.05	Begin
0.40	0.05	End
0.41	0.75	
0.60	0.75	
0.61	-0.15	
1.00	-0.15	

(b) *Column*. — CarboPac PA-1 Pellicular anion exchange resin, 250 x 4 mm id with 50 x 4 mm id guard column of same resin composed of sulfonated ethylvinylbenzene-divinylbenzene particles agglomerated with 350 μm of Micro Bead quaternary amine-functionalized latex, or equivalent.

(c) *pH-meter*. — Temperature compensated, standardized with pH 4.0 and 7.0 buffer solutions.

(d) *Plastic vials*. — 50 mL, with screw caps, resistant to temperatures up to 100 $^\circ\text{C}$.

(e) *Water baths*. — With shaker, maintaining 60 \pm 2 $^\circ\text{C}$ and 80 \pm 2 $^\circ\text{C}$.

7. Reagents

Do not list common reagents that would ordinarily be expected to be available in a well equipped analytical laboratory. As in the apparatus section, describe reagents in terms of critical performance characteristics, rather than brand names.

Reagents without specifications are automatically reagent grade, conforming to the specifications of the American Chemical Society (ACS) when such specifications exist. If there are no ACS specifications, the best available grade is understood. Designate Reference Standard reagents available from NIST, BCR, USP, etc., as such, e.g., "USP Reference Standard Digitoxin."

The "Reagents" section is also used for materials requiring directions for preparation, purification, or standardization and must be included. Standard compounds will often need specifications or a source of supply (see Apparatus section 6 for designation of suppliers).

List reagents in whatever appears to be the most convenient sequence: alphabetically, order of use in method, or systematically: pure compounds, standard solutions, solutions of approximate strength, and indicators.

For completeness, note the following points: indication of stability, particularly of solutions; designation of anhydrous or number of moles of water of hydration for hydratable compounds (particularly important in buffer solutions and media); alternative use of a homologous alcohol, or special denatured alcohol, for pure alcohol (see "*Definitions of Terms and Explanatory Notes*", Appendix D).

Strength of solutions:

- (1) Common acids and ammonia are always the concentrated reagents, unless otherwise specified.
- (2) Express dilutions as molarity (0.03 M), or by a parenthetical expression [HCl (3 + 2)] where the first number always refers to the volume of reagent and the second to the volume of diluting solvent (which in most cases is understood to be water). For multiple mixtures, use the form: alcohol+acetate+ether (3 + 4 + 1).
- (3) Express reagent concentrations in terms of weight/volume percent, unless otherwise specified. An "x%" solution means that x g of material is dissolved in water or other solvent and diluted to 100 mL. However, avoid use of percent in the case of liquids with a density appreciably different from that of water (e.g., sulfuric acid). Because of possible ambiguity, use form (2) above with liquids or specify whether weight or volume is meant, e.g., (w/w), (w/v), or (v/v). Note that when a compound like hydrochloric acid, sulfuric acid, alcohol, etc., is used in a non-reagent sense (i.e., the material being determined), it means the compound itself, unless otherwise specified [e.g., formalin = 37% (w/w) HCHO in water].
- (4) Although "alcohol" is understood to be the 95% azeotrope, express all other dilutions in terms of the amount of the actual compound present as prepared by the following dilution rule:

An "x%" alcohol solution is prepared by diluting x mL 95% alcohol to 95 mL with water. When anhydrous alcohol is meant, specify as such.

Standard solutions:

Indicate the material first. If dilutions are required, designate them as stock (if storable), intermediate, and working solutions. Indicate concentration immediately following the title. Note these examples:

- (a) *Manganese standard solution, 50 mg/mL*-- Dissolve 50.0 g pure Mn metal powder in 20 mL 0.1 M H₂SO₄ and dilute to 1 L with water.
- (b) *Riboflavin standard solutions*-- (1) *Stock solution, 100 mg/mL*-- Dissolve 50 mg USP Reference Standard Riboflavin, previously dried and stored in dark in desiccator over P₂O₅, in 0.02 M acetic acid to make 500 mL. Store under toluene at ca 10°. (2) *Intermediate solution, 1.0 mg/mL*-- Dilute 100 mL stock solution to 1 L with 0.02 M acetic acid. Store under toluene at ca 10°. (3) *Working solution 1, 1.0 mg/mL*-- Dilute 10 mL intermediate solution to 100 mL with water. Prepare fresh for each assay. (4) *Working solution 2, 0.1 mg/mL*-- Dilute 10 mL intermediate solution to 1 L with water. Prepare fresh for each assay.

When a number of components are measured in the same method and a separate standard solution must be prepared for each, or if the preparation of the standard solutions occupies a fairly large portion of the reagent section, a separate

section on "Standard Solutions" may be used. Multiple component standard solutions could be written as follows:

Methyl 9,10-dibromostearate (DBS) and methyl 9,10,12,13-tetrabromostearate (TBS) standard solutions -- Pipet 3, 5, and 10 mL (6, 10, and 20 mg) DBS standard solution into 3 separate dry conical flasks and add 3, 5, and 10 mL (3, 5, and 10 mg) methyl pentadecanoate (MPD) standard solution to each. Similarly prepare TBS-MPD solutions. Treat each solution as follows: Evaporate solvent with N at 40°C. Add 25 mL 1% Na in methanol and 12 mL anhydrous benzene, and reflux 1 h. Cool, and transfer to 125 mL separator containing 50 mL water. Acidify with 1.0 M H₂SO₄ and extract with three 30 mL portions of ether, using first 30 mL to rinse flask. Combine ether extracts in second separator, wash with two 10 mL portions of water, dry over anhydrous Na₂SO₄, filter, and evaporate solvent on rotary evaporator at 40°C. Dissolve residue in 3 mL ether.

For natural product reference standards, e.g., mycotoxins, supply the following information: the source of the compound and the method of isolation, the method of purification and criteria of purity, stability data under conditions of dispensing and use, and the method for checking concentration and purity.

Buffers:

Indicate the nature and pH of the solution. Always indicate the hydration state of the salts used, e.g.:

Phosphate buffer solution, pH 8-- Dissolve 16.73 g anhydrous K₂HPO₄ and 0.523 g anhydrous K₂HPO₄ in water and dilute to 1 L.

Water:

"Water" unqualified refers to distilled water, except where it does not mix with the determination, as in a water bath. If deionized water may be used, insert a statement to that effect in the "Reagents" section or in an introductory or parenthetical statement at the beginning of the method or determination.

Purification:

When solids are to be purified by recrystallization, specify the preferred solvent, temperature, and initial solid-to-volume ratio. When liquids are purified by distillation, specify the characteristics of the starting material and the boiling point range of the desired fraction, and state the fraction of the first distillate and residue that should be discarded.

If pre-purified material is available, indicate the source and any physical constants that are important in checking purity.

Media:

Microbiological media are often not well characterized chemically. A particular effort should be made to obtain the exact formula prepared and used by the originator or user. Media designated by an investigator's name may often be found to have several different compositions. When acceptable, use the formulas already given in *Official Methods of Analysis of AOAC INTERNATIONAL*, under disinfectants, antibiotics (in feeds), or microbiological methods. Prepared or dehydrated media may be used, provided directions for preparation by the analyst from the basic ingredients are incorporated.

In the media statement, specify the hydration state of the salts used and the pH of the medium (before or after sterilization), and how to dispense and sterilize.

In a series of methods using the same buffers and media, or with minor modifications, use a combined "Reagents and Media" section for all the methods as is done for the microbiological methods in feeds.

For example,

C. Reagents

- (a) *Phosphate buffer.* — 0.2 M, pH 6.0. Dissolve 22.0 g KH_2PO_4 and 6.0 g $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ in water and dilute to 1 L. Sterilize 30 minutes at 120°C in the autoclave.
- (b) *Hydrochloric acid.* — 1 M. Dilute 8.3 mL HCl to 1 L with water.
- (c) *Sodium hydroxide solution.* — 50%, carbonate-free, density 1.54 kg/L. To 100 g NaOH, containing $\pm 1\%$ Na_2CO_3 , add 100 mL water. Stopper and swirl until solution is complete. Let stand until Na_2CO_3 has settled, leaving a clear liquid (about 10 days). Keep tightly closed when not in use.
- (d) *Sodium hydroxide solution.* — 1 M. Dilute 54 mL NaOH solution, c, to 1 L with CO_2 -free water.

Apparatus and Reagents:

If a method contains either a single apparatus entry or a single reagent entry, they may be combined into a joint section titled "Apparatus and Reagents" (or "Reagents and Apparatus").

8. Preparation of Calibration Curve

If a calibration curve is prepared by conducting a series of standards throughout the entire determination, place this section at the beginning or end of the method with directions to treat the specified standards as in the determination.

If the calibration curve is prepared at only the final determinative step, place the section before the determination as a special section. With this arrangement, most of the conditions of the final measurement can be placed here and need not be repeated for the determination. Some of the points to be checked, with special reference to spectrophotometry, are:

- (1) Preparation of a series of standard solutions in the concentration range of interest;
- (2) Volumes of reagents to be added and the order, waiting periods, temperatures, and dilution to final volume, if necessary;
- (3) Optimum instrument settings for gain, slit width, response, speed, drum drive settings, cell length, etc., or instructions on how to obtain these settings;
- (4) Directions for preparation of positive and negative control solutions, blanks, and reference solutions;
- (5) Spectral range of interest;
- (6) Procedure for making background corrections;
- (7) Plotting of absorbance (A) or transmittance (T) against concentration or absolute quantities; and
- (8) Frequency that the calibration curve should be repeated.

9. Preparation of Test Sample

This section contains the directions for preparing a homogeneous analytical sample; dissolving, dispersing, or diluting the test portion; and preparing a solution that is ready for separations or determinations. It may also contain several alternatives in accordance with the nature of the starting material or in the concentration of the active ingredient.

For example,

D. Preparation of Test Sample

Homogenize liquid laboratory samples immediately before analysis. Cut or shatter hard materials to pass through a 1 mm 2 sieve (No 18).

When a method is applicable to a wide range of component concentrations where different test portion weights and dilutions are required to bring the final concentrations into the measurable range, a table of weights, volumes, dilutions, and dilution factors applicable to specific concentrations or ranges are convenient. This section need not be included in short methods.

Significant figures:

Maintain consistency in the degree of accuracy required in various measurement steps. There is no need to weigh a test portion to five significant figures in a spectrophotometric method where the final absorbance measurement yields data with only three significant figures.

The phrase, "Accurately weigh approx. x [test portion] and record to the nearest y units" means that the weight taken should be $\pm 10\%$ of that specified but that the weight should be known to the number of significant figures commensurate with the other determinative steps in the method.

In general, a measurement is understood to utilize the full degree of sensitivity of the instrument, e.g., 0.1 mg for an analytical balance; 0.01 mL for a buret or pipet. The statement, "accurately weigh" or "pipet," needs no further qualification, i.e., "accurately weigh about 5 g (to 5 significant figures)" or "pipet 10 mL" means 5.xxxx g or 10.00 mL, respectively. Alternative expressions, usually for emphasis, that may be used are: "Weigh 5.000 g" or "Transfer 5.00 mL [from buret]." Do not use the redundant forms, "accurately weigh 5.000 g..." and "Pipet 10.00 mL," unless the exact specified quantity is required.

"Recommendations for Preparation of test samples for Collaborative Study Manuscript of Microbiological Methods", *J. Assoc. Off. Anal. Chem.* **70**, 931-937 (1987).

"Biological/Pass-Fail Task Force Report", *JAOAC*, **70**, 348-349 (1987).

"Validation of modern methods in food microbiology by AOAC INTERNATIONAL collaborative study", *Food Control*, **7**, 19-29 (1996)

"Three validation programs for methods used in the microbiological analysis of foods", *Trends in Food Science and Technology*, **7**, 147-151 (1996)

10. Determination

If a method is fairly straightforward or consists only of a single major step, place all operations directly under the statistical parameters of the method. If the method is complex, however, divide the determinative step into several parts which may be characterized by the type of operation performed. The chapters on metals, natural poisons, and pesticide residues contain numerous examples of special section headings that, if properly selected, give an outline of the method.

All critical control points must be identified. Indicate when a determination may be interrupted overnight. Even more important, indicate when a determination may not be interrupted. Indicate limitations of determinative steps that appear to be precise but in practical terms are not. Examples: "Dry to constant weight" should indicate the weight deviation which is considered as constant, e.g., ± 1.0 mg or ± 0.5 mg; "Color is stable" should indicate for how long; "Read color after 5 min" should indicate the time range which is satisfactory. If the method is empirical, emphasize this fact so that the analyst will make a deliberate effort to maintain constant conditions from test sample to test sample. If steps in various parts of the method are the same, use identical wording throughout the method; do not use synonyms. For example,

E. Determination of Lactose and Galactose

(1) *Preparation of test solutions for HPAEC-PAD analysis.*— Dilute the centrifuged filtrates from F with 3% acetonitrile, g, so that galactose and lactose contents are within the working standards concentration range. It may be necessary to use 3 different dilution factors, depending on the nature of the test materials. Guide values and denotation for dilution factors can be found in Table 2001.01D.

Table 2001.01D Guide values for dilutions for HPAEC-PAD analysis

Laboratory sample category	M ₂ (g)	D ₁	D ₂	D ₃
Yogurt drink (4-7% TGOS)	2.5	25	250	100
Fruit syrup (14-18% TGOS)	1.5	10	300	100
Custard (4-7% TGOS)	2.0	4	200	100
Orange juice (4-7% TGOS)	2.5	3	125	30
Biscuits (7-10% TGOS)	2.0	6	200	60
Cereal (4-7% TGOS)	1.0	5	200	200

D₁ = Dilution factor of galactose in assay A₁

D₂ = Dilution factor of galactose in assay A₂

D₃ = Dilution factor of lactose in assay A₁

(2) *Determination.*— Use the same type of integration for the test solutions and for the standard working solutions by choosing the same peak width, threshold settings, and other integration parameters. Carefully control the baseline selection by extending the baseline next to the peak. Use peak area for quantification.

First run the 4 calibration standards of each sugar to establish linearity. Then repeat the 4 standards. Between every two sets of standards, run 9 test solutions (e.g., WS₁, WS₂, WS₃, WS₄, initial test solution 1A₁ (dilution factor D₁), initial test solution 1A₁ (dilution factor D₃), treated solution 1A₂, initial test solution 2A₁ (dilution factor D₁), initial test solution 2A₁ (D₃), treated solution 2A₂, initial test solution 3A₁ (D₁), initial test solution 3A₁ (D₃), treated solution 3A₂, WS₁, WS₂, WS₃, WS₄, initial test solution 4A₁ (D₁), initial test solution 4A₁ (D₃), treated solution 4A₂, etc.). Continue this process until all test solutions have been analyzed. Use average response factor from the standards bracketing the test solutions to calculate sugar concentration for each test solution.

(3) *Possible interferences.*— Galactose is the most important parameter for the assessment of TGOS content in the product. Inaccuracies in the determination of galactose after enzymatic hydrolysis of test samples high in lactose like cereal and powdered milk based products, can influence the measurement of galactose liberated from TGOS. Also non-selective hydrolysis of alpha-galactans (e.g. carob powder) by β-galactosidase may occur, leading to erratic results. Use fresh enzyme preparations only.

11. Calculations

Calculations are included in a method for convenience to avoid the need for looking up factors and deriving equations, particularly when a series of multiple dilutions or aliquots are used at various steps in the method. In most cases, editors will transform equations into the single-line form. Particular care must be taken to ensure that there is no ambiguity with regard to the entries in the numerator and the denominator. The following equation, although mathematically clear, is often used incorrectly:

$$y = a/b \times c$$

Mathematically this equation means: $y = a/(b \times c)$. Authors usually intend it to mean:

$$y = (a/b) \times c, \text{ or } (a \times c)/b$$

Write an equation so that the interpretation can be followed easily. Use a separate line for each equation, unless the

equation is small and can be incorporated into the paragraph. If the equation is complex, add a simplified final equation in which all of the numerical factors have been combined into a single defined constant.

When the equation includes initial test portion weights or specified aliquots, errors are often made by incorrectly placing modified weights and volumes in the numerator or denominator. Therefore, the preferred form should be similar to the following:

$$\text{Component} = (\text{A of component/g test portion}) \times (\text{total vol./mL aliquot}) \times (\text{g std} \times \% \text{ purity/A of std})$$

If numerous or variable dilutions have been made, the volume relationships may be combined into a single dilution factor which may be mathematically defined in a separate equation for simple calculation. A dilution factor is the final volume to which a solution is diluted, V_f , divided by the volume of the original solution, V_o , contained in the final dilution:

$$F = \frac{V_f}{V_o}$$

For serial dilutions:

$$F = \frac{(V_f)}{(V_o)} \times \frac{(V_{f1})}{(V_{f1})} \times \dots$$

As a rule, use symbols in equations, defining them in a separate entry. Use A only for absorbance, W for weight, and V for volume. To differentiate unknown and standard, use the symbol alone for the unknown (A) and the symbol followed by a prime mark for the standard (A'). Where several components are involved, differentiate the measured values by subscript symbols. Letters of the alphabet are best, especially if they can be related to the component being measured, e.g., use A_p for absorbance of the phenobarbital component and A_p' for that of its standard. Where 2 components have the same initial letter, use numbers or other letters. Do not italicize (underline) subscripts, because these are often difficult to read.

For example,

F. Calculations

Calculate the initial (free) galactose, G_b , and (initial = final) lactose, L_b , contents in the buffered extracts assay A₁ and total (final) galactose content, G_t , of the hydrolyzed solution A₂ in g/100g product of test sample using the following formula:

$$G_b = [C_{Gb} \times D_1 \times (M_9 - M_7)] / (F \times M_8 \times 100)$$

where C_{Gb} = mg galactose/kg in initial test solution A₁ and F is a factor calculated as:

$$F = (M_2 \times 100) / (M_3 - M_1)$$

$$G_t = [C_{Gt} \times D_2 \times (M_6 - M_4)] / (F \times M_5 \times 100)$$

where C_{Gt} = mg galactose/kg treated solution in assay A₂.

$$L_b = [C_{Lb} \times D_3 \times (M_9 - M_7)] / (F \times M_8 \times 100)$$

where C_{Lb} = mg lactose/kg initial test solution in assay A₁.

Calculate galactose released from TGOS (G_g) (g/100g test sample):

$$G_g = G_t - G_b - G_l$$

where G_l = galactose released from lactose = $L_b/1.9$.

Calculate TGOS content (g/100g test sample):

$$TGOS = k \times G_g$$

where $k = (180 + 162n) / (180n)$. n is the average number of galactose moieties in the TGOS molecules. For example, if $n = 2$, k is 1.4.

12. Notes

In general, avoid use of notes at the end of the method. Incorporate their content at the appropriate place in the method. If the note is applicable to the entire method, place it at the beginning of the method directly under the statistical performance parameters, e.g., "(Rinse all apparatus with dilute nitric acid)." Use the Principle section to establish the scope of the method and the modifications necessary for products outside the scope. If modifications have been tested, place them in separate sections or subsections. Place special precautions, preparations, treatments, storage conditions, stability, sources of supply, tests, purifications, specifications, and similar materials pertaining to apparatus and reagents in their specific sections. If the Determination section includes special treatments for interfering materials or elimination of certain steps in special cases, handle these as parenthetical statements, special sections, or subsections. Place comments about when the method may or may not be interrupted in the Determination section.

Use parentheses sparingly and never for essential information. Commas are often a satisfactory substitute. Avoid use of the double and triple parentheses, except in formulas where they may be essential.

13. Automated Methods

Because of the many special parameters and requirements involved in automated analysis, follow the guidelines given below:

- (1) *Principle* -- Indicate, in performance language if possible, the products to which the method applies; give applicable range and statistical information on accuracy and precision. Give reactions involved,

determinative technique, and any interferences.

- (2) *Apparatus* -- List individual components of the automatic analyzer under a single heading when they are part of the basic unit and give instructions for any modifications. List other items separately. Submit a labeled flow diagram and separate diagrams of any specially constructed pieces of equipment. Describe equipment generically, in terms of performance, so that equivalent equipment can be determined.
- (3) *Reagents* -- Give concentrations of reagents and any necessary directions for purification, preparation, storage, etc. State how long a standard solution or reagent may be kept before a fresh one must be prepared, if this point is critical. Do not list stock items usually found in the laboratory that need no special preparation. Indicate the amount of reagent used for each series of analyses.
- (4) *Analytical system* -- Refer to the flow diagram and describe air and solution stream flows. Show pump tube rates in mL/min of analytes, reagents, standard solutions, and air, and specify the pump tube material. Give inner diameters of critical interconnecting tubing and any other information that needs to be specified.
- (5) *Start-up and shut-down of system* -- Give steps for starting and stopping the system, and the procedure for leaving the system in satisfactory condition for the next series. Indicate troubles that may develop and the means to correct them, e.g., irregular baseline, irregular peaks, poor reproducibility.
- (6) *Maintenance* -- Indicate how to clean and maintain the equipment.
- (7) *Checks and calibration* -- Indicate how to check for proper operation of the system, including checks for contamination, recoveries, linearity, drift, and carryover. Give settings for various controls (or condition to be maintained, e.g., temperature) and approximate readings that should be obtained for range of concentration to be measured.
- (8) *Preparation of calibration curve* -- If applicable, give directions, including instructions for handling the blank.
- (9) *Isolation of analyte* -- Indicate how to prepare test solutions from original materials to be analyzed by the method. Give general directions for the applicable commodities, not just for the particular commodities used in the collaborative study.
- (10) *Determination* -- State how many and in what order standards and analytes are to be analyzed and whether replication is necessary. Indicate how to draw the baseline and report results. Provide formulas for calculation if applicable.