

ELEMENTAL ANALYSIS MANUAL

(FOR FOODS AND RELATED PRODUCTS)

(first revision)

Initial Draft Issued 08/27/82

First Revision Issued 06/15/84

Incomplete Reprinting 05/04/99

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TABLE OF CONTENTS

<u>SECTION</u>	<u>TOPIC</u>	<u>PAGE</u>
I	INTRODUCTION	I-1
II	QUALITY ASSURANCE	II-1
III	CONTAMINATION CONTROL	III-1
IV	ANALYTICAL STANDARDS	IV-1
V	PREANALYSIS SAMPLE PREPARATION	V-1
VI	SAMPLE MINERALIZATION	VI-1
VII	SEPARATION, PRECONCENTRATION, AND SPECIATION	VII-1
VIII	INSTRUMENTATION	VIII-1
IX	DATA TREATMENT	IX-1
X	METHODS	X-1

I INTRODUCTION

BACKGROUND

The need for an Elemental Analysis Manual (EAM) was expressed by FDA analysts for some time. The 1974 and 1975 Metals Workshops and the 1978 and 1980 Elemental Analysis Workshops all resulted in strong recommendations for such a manual. Ten FDA Headquarters and Field chemists initially met and began development of the EAM in the summer of 1981. After considerable review and revision, the initial draft EAM was issued in late summer of 1982 to those FDA laboratories conducting elemental analyses. The latest revision reflects changes identified at the most recent Elemental Analysis Workshop as being necessary to keep the EAM current. The coauthors would like to thank the Executive Director of Regional Operations, the Center for Food Safety and Applied Nutrition, and the Laboratory Directors of the participating laboratories for the allocation of personnel time and funds that have made development of this EAM possible.

A. PURPOSE

This EAM was developed to serve as a ready reference for chemists conducting elemental analyses in FDA laboratories. It brings together in one place a compilation of existing analytical methods commonly used by FDA analysts, as well as comments based on the experiences of these analysts in using the methods included. It is intended to serve as a source of information to help standardize the way elemental analyses are conducted in FDA laboratories. However, it must be stressed that the EAM is intended for internal FDA use only. It is not intended at this time to have any regulatory status, as does the Pesticide Analytical Manual. Thus, in the case of regulatory samples, use of the EAM does not relieve the analyst in any way from following procedures required by the Analyst Operations Manual, compliance program instructions, or other superseding instructions. Like wise, the quality assurance procedures described in the EAM have been shown to be effective in improving the quality of data produced in elemental analyses, but they are meant to supplement, not supersede, local quality assurance instructions in individual laboratories. Because the EAM is a compilation of methods already published or available elsewhere, it should not be quoted directly. When there is a need to reference a method, the original source should be quoted rather than the EAM method number.

B. FORMAT

The EAM is intended to be flexible and easily updated as improvements in analytical methods evolve. Hence, the loose-leaf nature of the EAM will be retained. It is intended that the methods, comments from EAM users, and other contents of the EAM will be reviewed at least annually for necessary changes.

Any analyst using this manual is encouraged to forward comments for improvement or to report any errors found at any time to the Head, Elemental Research Section, Division of Chemical Technology, CFSAN, for consideration during the next annual content review.

C. DEFINITIONS

To ensure conformity with the generally accepted definitions of concentration ranges (1), the following are given:

a) Major - component present in range of >1% to 100% of total by weight. b) Minor - component present in range of >100 ppm to 1%. c) Trace - component present in range of >1 ppm to 100 ppm. d) Ultratrace - component present at <1 ppm.

The American Chemical Society's Subcommittee on Environmental Monitoring and Analysis (2) has defined the Limit of Detection (LOD) as the lowest concentration level that can be determined to be statistically different from the blank. They also define the Limit of Quantitation (LOQ) as the level above which quantitative results may be obtained with a specified degree of confidence. For a blank signal, S_b , and a total signal (sample + blank), S_t , the analyte signal is then the difference, $S_t - S_b$. If s is the standard deviation in the measurements of S_t and S_b , then at the 99% confidence level, $LOD = 3s$ and $LOQ = 10s$.

D. ACKNOWLEDGEMENTS

The following individuals coauthored the initial draft of the EAM: Kenneth Boyer and Stephen Capar (CFSAN, Division of Chemical Technology); Curtis Edwards (EDRO, Division of Field Sciences); Fred Fricke, Duane Satzger, and Karen Wolnik (Elemental Analysis Research Center); Paul Beavin (BLT-DO); Walter Holak (NY-DO); Michael Loges and Jerry McNerney (BUF-DO); Ronald Marts (KAN-DO); and Kenneth Panaro (BOS-DO). The special assistance and guidance of Helen Reynolds, Head of the CFSAN Editorial Staff, during compilation of the initial draft of the EAM is also gratefully acknowledged.

REFERENCES

- (1) Zief, Morris, and James W. Mitchell (1976) Contamination Control in Trace Element Analysis, Wiley, New York, p. 2.
- (2) Keith, L.H., Crummett, W., Deegan, J., Jr., Libby, R.A., Taylor, J.K. & Wentler, G. (1983) Anal. Chem. 55, 2210-2218.

II QUALITY ASSURANCE

INTRODUCTION

The main purpose of the analytical chemist is to determine accurate, reliable results. The analytical result is the final product of many operations, each of which must be monitored to ensure analytical reliability. The process of monitoring each individual operation and all related steps is called quality assurance (QA).

Basically QA should focus on identifying, preventing and correcting defects in the analytical scheme. Each FDA Field laboratory has an internal QA program which is made up of a central (EDRO) program and supplemented locally as necessary.

Each section of this manual incorporates QA practices as normal procedure. This section is designed to supplement inherent QA practices and follows the normal sample analysis process, from sample collection through reporting of results. A brief overview is provided for each operation in the analytical scheme in which salient QA aspects are identified and discussed.

This section also provides general guidelines concerning method validation, the process which evaluates the method performance in the laboratory. Criteria for commonly used methods, for minor modifications to commonly used methods, and for completely new methods are discussed.

A. SAMPLING

The first step in the analytical scheme is the collection of a sample which both reflects the purpose of the collection and is representative of the material sampled. Even with the greatest of analytical expertise, meaningful results cannot be ascertained if the sample is not representative of the parent material.

Normally, FDA samples are collected by inspectional personnel. Specific sample collection instructions are documented in the Inspectors Operations Manual (IOM), Chapter 4, by sample type. Collection information is provided to the analyst via a collection report.

The analyst should review the collection report prior to sample analysis to ensure that the sample reflects the purpose for collection. If no documentation exists, the analyst should check with the collector.

The following are general observations concerning sampling. Unless the sample is otherwise documented, the laboratory should provide specific instructions concerning these observations to the collector.

- The sample collected must be representative of the material being sampled.
- Contamination should be minimized during collection, shipping, and storage, especially when ubiquitous contaminants such as lead and iron are involved.
- The sample should be amply protected or preserved to eliminate possible physical, chemical, or biological changes during collection, shipping and storage.

B. SAMPLE DOCUMENTATION IN THE LABORATORY

Each laboratory should maintain a systematic tracking system in which the sample integrity is documented. This system should include all steps related to sample transfer through the laboratory and final disposition.

Refer to Analyst Operations Manual (AOM) section 910 for sample accountability procedures, AOM section 920 for analyst worksheet documentation procedures, and the Regulatory Procedures Manual, chapter 7-60, for sample disposition procedures.

C. PHYSICAL FACILITY

Generally the physical facility does not fall under QA aspects. The main emphasis of QA is placed on equipment, reagents, methodology, and the analyst. However, consideration of laboratory location and design may eliminate potential QA problems. The section on Contamination Control expresses certain concerns which could be more readily accomplished during construction or upgrading of the laboratory.

QA aspects which relate to the facility concern:

1. Location - avoid or minimize possible contamination from smelters, auto fumes, dust.
 2. Design - place emphasis on laboratory design to avoid internal contamination and interferences.
- avoid construction materials which may contain possible contaminants.

- avoid transference or vibration, e.g., don't locate sample preparation equipment in proximity to very sensitive instrumentation.
- provide areas for micro/macro analysis.
- ensure adequate power accessibility for all instruments.
- ensure adequate ventilation.

D. ANALYSIS

The analysis is most affected by QA. The general scheme of elemental analyses consists of:

- Method selection
- Sample preparation
- Mineralization
- Determination
- Computation and reporting of results

Each part of the analytical scheme will be discussed in more detail in the relevant EAM section. The following identify and discuss salient QA variables related to the analysis.

1. Method Selection

Before the analytical method is selected, the analyst should consider method:

- | | |
|------------------|---|
| a. reliability | - overall dependability |
| b. applicability | - pertinence, practicality, and
Workable range (micro/macro) |
| c. economy | - time, manpower, materials,
equipment required |
| d. simplicity | - level of expertise needed |
| e. safety | - degree of hazard associated with
technique |

In some cases, regulatory provisions require the use of official procedures which may supersede other method selection criteria.

2. Sample Preparation

- a. Definition of what should be analyzed, e.g., edible portion, or other portion to satisfy specific regulatory concerns.
- b. Selection of the most appropriate compositing procedure which provides the most homogenous sample, e.g., blending, milling, and/or dissolution.
- c. Minimizing possible sample preparation contamination sources.

3. Mineralization

- a. Emphasize efficacy and applicability in selecting a mineralization procedure.
- b. Minimize possible contamination sources related to the mineralization apparatus, equipment, reagents, etc.

4. Determination Step

- a. Determinative step selection should be based on:
 - specificity - uniqueness of response
 - accuracy - correctness, absence of bias
 - precision - reproducibility
 - detectability - minimum measurable amount or concentration above background levels
 - sensitivity - response per unit concentration
 - linearity - uniformity of response per unit of concentration
- b. Equipment should be maintained in good working condition. A preventive maintenance program should be developed to ensure reliability and stability of the equipment.

c. The quantitation procedure selected must be applicable to the technique, e.g., the standard additions technique is required for ASV analyses.

d. The standard must be of acceptable quality.

5. Computation and Reporting

a. The computation procedure should be applicable and concisely defined.

b. Data reduction practices should be documented and consistent among all laboratories.

c. Data reporting practices should ensure that all results transcribed from one source to another be adequately verified.

E. ANALYST

One aspect of QA commonly overlooked is the analyst. While most of the QA aspects previously discussed remain constant, the most pronounced variable in the analytical scheme is usually the analyst.

Elemental analysis techniques continue to evolve. New instruments and techniques are constantly being introduced which maximize performance and lower detection limits. These changes have increased the complexity of the analytical operations, and therefore require an increased awareness in areas related to analyst QA. Because of the dynamic state of elemental analyses and their ever increasing complexity, increased emphasis should be placed on:

- Training - via workshops, seminars, specific courses.
- Communication - scientific meeting attendance.
- Awareness - addressing the problem of increased variability with the ability to analyze for lower levels.
- Maintenance of Skills - low level analyses must be performed on a regular basis to maintain analytical expertise.

F. OVERALL MEASURES

The evaluation of the total program is the final measure. The aspects discussed above were primarily concerned with the individual laboratory QA. Conversely, overall or interlaboratory QA should demonstrate the ability of a group of laboratories to achieve comparable performance. Interlaboratory QA is normally measured by two procedures.

- Interlaboratory check samples - The ability of a group of participants to obtain similar results are assessed. The samples sent for analysis should be homogeneous and have an analyte level measurable by the designated analytical method. This procedure allows for identification of problematic areas and ensures that the laboratories' analytical scheme works properly. The data also provide the degree of variability one may expect among various laboratories.
- QA Assessment Visits - national QA specialists review all aspects of the internal laboratory QA program. The expert can point out problems, provide corrective procedures, and contrast your QA program with that of other related laboratories.

G. METHOD VALIDATIONS

Method validation is the process of measuring the performance of a method. This section suggests plausible guidelines for analytical method validations for:

- Methods in common use for everyday operations
- Slight modifications or extensions of present methods
- New methodology or major changes in methods

Techniques that are commonly used to characterize the performance of a method include recoveries, analysis of standard reference materials, replicate sample analyses, and method reagent blank determinations. Perhaps the most commonly used measure for validation is the percent recovery of an analyte added to the sample. The recovery experiment determines the percent (%) of known substance recovered through a procedure. (See the data treatment section for further explanation.) The recovery determination is performed by adding a known amount of analyte (spike) to a portion of the sample and then analyzing both the spiked and unspiked portions using the same analytical method. Another validative means involves analysis of a

well-characterized material, such as an NBS reference material. Replicate sample analyses are used to measure the repeatability of the procedure. Replicate samples can readily point out reliability problems when large differences exist. Determination of the method reagent control blank is necessary to measure method performance. This consists of analyzing all the reagents in the quantities used in the analysis by carrying them through the entire analytical method in the same manner as actual samples being analyzed. The blank determination can indicate both inherent contamination problems and procedure variability.

The following are general guidelines for validating method performance. Factors such as cost, time, applicability, etc. may justify modifications. Analyst experience is a key factor in determining what is applicable.

1. Methods in Common Use:

a. Validation guidelines:

- Recovery - Analyte (spike) - Determine recovery of analyte added to sample for 5% of all sample analyses.
- Standard reference material or well characterized material - analyze for analyte of interest for 5% of all sample analyses.
- Duplicate analysis - analyze 10% of all samples in duplicate.
- Control blanks - Determine for 10% of all sample analyses, or at least 1 per sample run when consolidating samples.

b. Suggested assessment criteria include:

- Recovery - reanalyze all samples in the run if the % recovery and/or standard reference material results are not within 80-120% of the expected values.

NOTE: The minimum spike level should not be less than the limit of quantitation (2).

- Replicate - reject sample run if analyte detected exceeds the maximum allowable % difference for duplicate analyses:

<u>Analyte Level</u>	<u>Maximum Allowance % Difference (MAPD)</u>
Limit of Detection	100
Limit of Quantitation (LOQ)	30
LOQ x 5	15
LOQ x 20	5

MAPD is calculated by:

$$2 \times \frac{(D_1 - D_2)}{D_1 + D_2} \times 100\% = \text{MAPD}$$

D_1 = value for first replicate analysis

D_2 = value for second replicate analysis

- Control Blank - No specific criteria. The method control blank results should be consistent and less than 10% of the desired level of quantitation. Lack of consistency indicates contamination or methodology problems.

2. Method Modifications or Extension

This category includes those methods in which minor changes are made which do not affect the chemistry of the method. These changes normally occur for convenience. In all cases, the changes should be documented and, where possible, compared with results of alternative methods.

a. Validation guidelines:

Incorporate guidelines for methods in common use with the following additions.

- Replicate analyses are mandatory.
- Determination of recovery of added analyte at approximate native level (or at the limit of quantitation if analyte native level is nondetectable) and one at 3x the above level are required.

b. Assessment criteria:

Same as for method in common use.

3. New methodology or major changes to existing methodology

Major change includes those methods in which the chemistry of one or more of the procedural steps are significantly altered, e.g., change in mineralization step.

a. Validation guidelines:

- Initially, i) determine the linear portion of the standard calibration or standard additions wave; ii) ensure a constant control blank; iii) ensure that method, when the entire analytical procedure is carried out, will satisfactorily recover the analyte from fortified (spiked) reagent blanks at (1-10)x the level of interest and at 2x the blank level.
- After ensuring sample homogeneity via duplicate analysis, conduct recovery studies at (1-3)x the native level, or at (1-3)x the quantitation limit if the analyte is not detected. If the variability of the recovery results are reasonable, i.e., within 80-120%, continue.
- Perform comparison test via analysis of a comparable NBS standard reference material or a well characterized food stuff. Ensure reasonable comparison with known values.
- Attempt to confirm accuracy through comparison with results performed by an independent method. Ensure that improvement is realized over existing methodology.
- Method modifications or extensions to other elements or matrices should be validated by a second analyst or lab.

b. Assessment criteria:

The assessment criteria are built into the validation guidelines. The analyst should ensure that the new method results in a significant improvement over existing methodology. When no comparable data exist between the existing method and the new method, analyst expertise must suffice.

NOTE: If the new method warrants collaboration contact the AOAC General Referee for Metals and Other Elements. Refer to the Statistical Manual of the AOAC, Youden, W.J. and Steiner, E.H., 1975, in designing the collaborative study.

REFERENCES

1. Horwitz, W., et al. (1978) "Analytical Food Chemistry," in Quality Assurance Practices for Health Laboratories, S.L. Thorn (ed.), American Public Health Association, Washington, D.C. pp 545-646.
2. Keith, L.H., Crummett, W., Deegan, J., Jr., Libby, R.A., Taylor, J.K. & Wentler, G. (1983) Anal. Chem. 55, 2210-2218.

APPENDIX II-A

The following are QA procedures used by FDA's Elemental Analysis Research Center (EARC) for the determination of Pb and Cd by differential pulse anodic stripping voltammetry. These procedures exemplify the checklist approach to QA by being concise, yet covering all aspects of the analysis.

QUALITY CONTROL PROCEDURE USED IN THE DETERMINATION OF
Pb AND Cd BY DPASV

1. Prior to digestion or analysis, all glassware is subjected to the following cleaning procedure:
 - Rinse - 18 Megohm cm-1 deionized water (x 5)
 - Soak - Hot 30% nitric acid (2 hr)
 - Rinse - 18 Megohm cm-1 deionized water (x 5)
 - Rinse - Redistilled concentrated nitric acid
 - Rinse - 18 Megohm cm-1 deionized water (x 5)
2. All glassware is stored in a clean air environment. All stages of analysis, including digestion, dilution and sample analysis, are carried out in a clean air environment. These clean areas are checked monthly to monitor the level of contaminants, specifically Pb and Cd. These consist of allowing an uncovered blank solution to remain in a clean area for four days. This solution is then analyzed for any increase over the normal daily blank level. There should be no change.
3. DPASV instrumentation is checked daily to verify adequate sensitivity and reproducibility. Sensitivity is checked by standard additions to a blank. Resultant Cd and Pb current values are dependent on voltammetric operating parameters. For example, a 200 + 20 nA stripping peak is observed for a 0.04 ug Cd spike. A 100 + 10 nA stripping peak is observed for a 0.04 ug Pb spike. Reproducibility is checked on these standard additions to ensure duplicate scans on the same solution are within 5%. Drop size reproducibility is checked daily by scanning FUNCTION 1 TST, where the initial potential is continuously applied and current samples are taken at the specified drop time. This should produce a straight line on a blank solution.

4. Calibration of the microliter pipettes used for standard additions are checked bi-weekly. These pipettes should perform within the manufacturer's specified tolerances. The calibration of each pipette is checked gravimetrically by using distilled water and an electronic analytical balance. For example, the 20 ul pipette most frequently used for standard additions should be within + 0.20 ul or 1%.
5. Ten percent of the samples are rerun in duplicate. The percent difference between duplicate analyses is dependent on the analyte level. The table below is a guide to the criteria applied to duplicates.

<u>Analyte Level</u>	<u>% Difference</u>
Limit of Detection	100
Limit of Quantitation (LOQ)	30
2 x LOQ	15
20 x LOQ	5

These criteria are based on precision studies performed at the EARC on samples at these levels. For intermediate values interpolate from the above table.

6. A precision study is done at the EARC on each crop type to ensure reproducibility of the method for each crop type.
7. A recovery on a fortified sample is run with each set of samples (i.e., 1 of 15). In cases where recoveries are not within 80-120%, reanalysis is necessary.
8. Appropriate NBS biological standard reference materials are analyzed for each crop type when available.
9. A reagent blank is run with each set of samples to monitor Pb or Cd contamination (i.e., 1 of 15).
10. Samples are reanalyzed whenever an outlier in a particular crop series is obtained.
11. Maintenance is required on the working and reference electrodes. The VYCOR reference frit must be replaced when the reduction potentials shift outside the acceptable range (Cd, $-.62 \pm .05\text{v}$; Pb, $-.45 \pm 0.05\text{v}$). The working electrode must be cleaned or replaced when FUNCTION I TST does not produce a straight line, or when the capillary will not hold a Hg drop for the duration of an analysis.

APPENDIX II-B

(Copy of Principles of Environmental Analysis, Anal. Cher. 1983, 55,
2210-2218)

III CONTAMINATION CONTROL

INTRODUCTION

For many of our analytical methods the lower limit of reliable quantitation is not determined by the measurement capabilities of the instrument used in the determinative step, but by the variability of the analytical blank. Contamination can come from many sources, including the laboratory air, sample and reagent containers, glassware, reagents, and also the analyst.

This section discusses the control of contamination from the two perspectives of facilities and procedures. Improvement of laboratory facilities can reduce the general level of contamination from the laboratory environment. Following specific procedures can minimize the amount of the remaining contamination that gets into the sample prior to mineralization, or into the solution aliquot that is analyzed in the determinative step.

In setting priorities for implementing an effective contamination control program, it pays to make improvements and procedural changes first that will provide the largest reduction in contamination for the least amount of resources expended. As progressively more stringent contamination measures are taken the time and cost of implementing them usually increase.

One of the best references available on this subject is the book by Zief and Mitchell (1).

A. FACILITIES IMPROVEMENT

The following checklist provides in approximate priority order steps for progressive upgrading of a conventional laboratory to make it suitable for trace elemental analyses. For more complete details see Chapter 3 of "Contamination Control in Trace Element Analysis" by Zief and Mitchell (1).

LABORATORY FACILITIES IMPROVEMENT CHECKLIST

<u>Status</u>	<u>Item</u>
	Replace all (rusted and corroded) hot plates, heating units, ashing ovens, etc., with ceramic-top hot plates and ceramic-lined ovens. Likewise replace rusting ring stands, clamps, racks, etc., with ones that are free from corrosion.

Remove all unnecessary shelving, partitions, furniture and other dust-collecting items from the laboratory.

Install filters on all incoming air sources (air conditioning, heating, ventilation, makeup air, etc.). Filters should be 85% efficient for 0.5-5.0 μm particles and 95% efficient for particles greater than 5.0 μm in diameter.

Install a high purity ion-exchange water purification system capable of producing water with 18 megohm cm^{-1} resistance. Use distilled water as the water source for the ion-exchange system.

Install an exhausting laminar flow high efficiency particulate air (HEPA) filter hood to be used for operations where the analyst would be exposed to acid fumes and other noxious gases.

Install HEPA filter non-exhausting modules approximately 1 m above bench tops where critical (low-level or regulatory) work will be done.

Coat all bench tops with epoxy paint and then cover them with adhesive-backed Teflon or polyethylene sheeting.

Coat all metal drawer handles, metals valve handles, metal fixtures, nameplates, etc., with epoxy paint or replace them with plastic, wherever possible, to minimize metal contamination due to corrosion.

If possible remove all metal gas cylinders from the laboratory to an adjacent laboratory or separate gas cylinder room. Run a Teflon or polyethylene distribution line from each gas source to each laboratory requiring that gas.

Paint walls in elemental analysis area with two-component epoxy paint (one component bisphenol A epoxy ether and a solvent cured by addition of an amine or amide curing agent in a second solvent).

Seal all windows and wall openings with silicone caulking to reduce dust penetration.

Cover floors with one-piece vinyl flooring coved at floor and wall intersections.

B. CONTAMINATION CONTROL DURING SAMPLE PREPARATION AND ANALYSIS

The ability to perform elemental analyses at trace (ug/g) and ultratrace (ng/g) levels is dependent upon the elimination and identification of contaminants. Most samples are not amenable to direct analysis and, therefore, must be treated chemically and/or physically to produce a substance suitable for analysis. Physical methods of treatment are necessary to ensure a homogenous composite since, generally, only a fraction of the total sample is taken for analysis. Routine procedures commonly used in sample homogenization include washing, chopping, freeze-drying, blending and grinding. These procedures must be closely monitored due to potential introduction of contaminants. Chemical methods of treatment include conventional digestion and extraction procedures for separating interfering matrix elements and concentrating the species of interest. Performance of all sample pretreatment with meticulous care will reduce random contamination from the laboratory environment. Systematic contamination is controlled by careful choice of equipment, reagents and cleaning procedures. Procedures must be designed with reduction of contamination in mind in order to provide an acceptable blank level.

The following checklist provides steps that should be taken in order to minimize contamination even without the more expensive equipment previously mentioned:

ANALYSIS CONTAMINATION CONTROL CHECKLIST

<u>Status</u>	<u>Item</u>
	Design or modify equipment to be free from the species undergoing measurement. Use no spatulas or other metal utensils unless they have been coated with Teflon or other suitable polymeric material (e.g., Kynar).
	Remove all metal stopcock leashes from volumetric glassware. Dedicate this glassware entirely to a particular analysis. Polypropylene volumetric ware is preferable to glass because of reduced memory effects.
	In sample preparation, use only equipment made of glass (Pyrex or quartz) or plasticware (knives, strainers, wash tubs, vegetable drainers, sample bags), if possible.

Perform all sample handling with polyethylene or polyvinylchloride gloves. Prepounded gloves are not acceptable.

Where feasible, use food processing equipment which is totally plastic (Cuisinart with plastic blade) or coated with an acceptable polymeric material (Hobart food cutter - Kynar coated). If homogenization requires the use of metal, use equipment incorporating hardened stainless steel blades (Polytron homogenizer, Sears blender). Note that stainless steels are generally composed of the elements listed in Table III-I. Incorporate checks (e.g., blanks, NBS SRM's) into any procedure requiring homogenization to determine the levels of systematic contaminants.

Acid wash all equipment used in sample preparation, storage or analysis prior to use. The extent to which equipment must be washed is determined by the level of elements of interest in comparison to the blank level.

Soak plasticware and sample bottles (LPE or PP) in dilute (10%) HNO₃ for 15 minutes, soak for 15 minutes in distilled deionized water (DDW), and rinse (x3) with DDW. In each case, the analyst must determine the cleaning procedure most applicable for the analysis to be performed. For example, analyses for trace Pb and Fe require more stringent cleaning procedures for laboratory ware, as discussed in (1).

Determine and control the level of trace elements in the water, acids, solvents, buffers, chelating agents and supporting electrolytes used in the analytical determination. High purity acids available for ultratrace work include:

- (a) J.T. Baker ULTREX sulfuric, nitric, perchloric and hydrochloric acids available in sealed 500 mL ampules.
- (b) J.T. Baker "Instra-Analyzed sulfuric, nitric, perchloric and hydrochloric acids.
- (c) G.F. Smith double distilled acids, sulfuric, nitric, perchloric and hydrochloric.

Zief and Mitchell (1) also include a section on purification of reagents.

Regardless of the validity of the analytical method or the capability of the analyst, reliable data cannot be obtained without proper contamination control during preparation and analysis of an analytical sample.

REFERENCES

1. Zief, M., & Mitchell, J.W. (1976) Contamination Control in Trace Element Analysis, Wiley, New York.

Table III-I - Composition of stainless steel*

Mn	1-10%
P	0.04-0.02%
S	0.5-3%
Si	0.5-3%
Cr	12-26%
Ni	1-15%
Mo	0-4%
Zn	0-0.6%
Se	0-15%

*Fe is the major constituent of stainless steel

IV ANALYTICAL STANDARDS

INTRODUCTION

In any analysis, the quality of data can be only as good as that of the standards used in the analysis. The preparation of stock and working solutions of these essential, pure, and highly accurate metal standards is a formidable task. Many variables must be taken into account to arrive at the best standard solutions possible. The best starting materials should be selected, the correct containers chosen, and strict contamination control procedures should be observed. In all cases, good quality assurance should be maintained.

A. STANDARD SOLUTIONS PREPARATION

Accurately weigh the standard material (amount needed so that the final concentration of the standard is 1000 ug of metal/ml of solution) into a previously acid-washed and tared plastic weighing dish. Record the weights to four (4) decimal places in a standards log book. Carefully transfer the material to a previously acid-washed one (1) liter volumetric flask using some of the recommended solvent. Then add the remaining solvent directly to the volumetric flask. Wait until the entire standard is in solution before diluting to volume. If necessary, heat gently to effect complete dissolution. Dilute to volume with deionized water. Transfer the standard solution to the recommended acid-washed containers. See Table IV-1 for recommended weights, solvents, and containers to be used.

B. STOCK SOLUTIONS

1. The use of "spec pure" materials, four-nines (99.99%) or better, or solutions made from four-nines material is recommended.
2. Hydrates are generally a poor choice of starting materials since the water content may vary from one lot to another, and most are hygroscopic.
3. Purchase of stock solutions from the public sector is permissible, provided that the starting material used in the preparation is 99.99% or better.

4. In all cases, deionized water is used, preferably water approaching theoretical resistivity of $18 \text{ megohm cm}^{-1}$. Systems are commercially available that can achieve this water purity.
5. Electronic grade or Ultrex acids should be used in the solubilization of the standard when acid is called for.
6. Check the purity of new standard solutions by one or more of the following procedures:
 - a. Analyze the new standard solution against the old standard solution
 - b. Analyze standard solutions made up from two different sources
 - c. Assay by coulometric deposition
 - d. Assay using a proven method based on a primary standard

Analysis of the new standard or standard solution should be within 0.5% of the expected value for the new standard or standard solution to be used. Analysis of a National Bureau of Standards Reference Material (SRM) for the element of interest provides another useful comparison, but should not be used to confirm the purity of a new standard solution, since the uncertainty in the NBS values is greater than 0.5% for most elements in most SRM's.

7. Replacement of stock solution is recommended yearly. For analysis of official samples check analysts must always make a fresh stock solution.
8. Never sample directly from, nor return solution to the stock standard.

C. WORKING STANDARD SOLUTIONS

1. All working standard solutions should be prepared fresh on the day of use.
2. Working standard solutions should be in a concentration range so that the final concentration of the standard solution analyzed is as similar as possible to the concentration of the sample solution being analyzed.

3. Working standard solutions should be acidic enough (2%) to ensure stability (for exceptions see Table IV-1).

D. STANDARD CONTAINERS

1. The best choice for plastic containers is either Teflon (TFE) or conventional polyethylene (CPE).
2. Reuse of containers for the same stock standard solution is permissible.
3. Cleaning Instructions for Standard Solution Containers
 - a. Fill with 1+1 HNO₃ (AR grade)
 - b. Allow to stand one week at room temperature (80°C for Teflon)
 - c. Empty and rinse with purest available deionized water
 - d. Fill with purest available deionized water
 - e. Allow to stand several weeks, changing water periodically to ensure continued cleaning
 - f. Rinse with purest water available and allow to dry in a particle-free and fume-free environment
4. Always acid wash any working standard container after cleaning with 10% nitric acid (reagent grade) and rinse with purest water available; allow to dry in particle-free and fume-free environment.

E. QUALITY ASSURANCE

Good laboratory quality assurance dictates that histories of stock solutions be maintained in one permanent register. All significant data concerning the freshly prepared solution should be entered into this Laboratory Record Book for Metal Stock Standards. This information includes:

- a. Source, lot number and manufacturer's purity
- b. Assayed purity, if applicable
- c. Standard weight and final dilution volume

- d. Final element concentration in appropriate units, e.g., g/liter
- e. Final acid content, if applicable
- f. Comparison data for analyses of old stock standard versus new stock standard, as well as results of any other concentration or purity analyses
- g. Name of preparer and date of preparation
- h. Name of person doing calculation check

F. STANDARD REFERENCE MATERIALS

Table IV-2 lists selected NBS-SRM's that are useful in analyses for foods and related products. The NBS certified and informational values for elements in these SRM's are listed in Table IV-3. For extensive literature values in addition to the NBS values, see NBS publication 260-88 (reference 6).

REFERENCES

1. Miller, W., and DeManna, G. (1980) Preparation of Standards for Plasma Emission Spectroscopy, The Spex Speaker, Year 25, Issue 3, pp. 1-4
2. Ward, A., Jarrell Ash Co. Technical Aid Note 4, Stock Standard Preparation
3. Moody, J.R., and Lindstrom, R.M. (1977) Anal. Chem., 49, 2264
4. National Bureau of Standards Special Publication 492 (1977) "Procedures used at the National Bureau of Standards to Determine Selected Trace Elements in Biological and Botanical Materials," R. Mavrodineanu, (ed.) Appendix XIV, p. 247
5. National Bureau of Standards Certificate of Analysis for the following Standards Reference Materials and Research Materials:

SRM 1566-1571, 1573, 1575, 1577, 1643a, and RM 50.
6. National Bureau of Standards Special Publication 260-88 (1984) "1982 Compilation of Elemental Concentration Data for NBS Biological, Geological, and Environmental Standard Reference Materials," National Bureau of Standards, Washington, D.C.

TABLE IV-1
STOCK STANDARD SOLUTION PREPARATION
Weight to one (1) liter

Element	Recommended Form	Weight (g)	Solvent ^a	Container
ALUMINUM	Al ⁰	1.0000	6M HCl	TFE ^b , CPE ^c
ANTIMONY	Sb ⁰	1.0000	Aqua-Regia	Glass ^d
ARSENIC	As ₂ O ₃	1.3203	e	TFE, CPE
BARIUM	BaCl ₂	1.1516	Water	TFE, CPE
BERYLLIUM	Be ⁰	1.0000	0.5M HCl	TFE, CPE
BISMUTH ^f	Bi ⁰	1.0000	4M HNO ₃	TFE, CPE
BORON	H ₃ BO ₃	5.7195	Water	TFE, CPE
CADMIUM	Cd ⁰	1.0000	4M HNO ₃	TFE, CPE
CADMIUM	CdSO ₄	1.9546	0.3M HCl	TFE, CPE
CALCIUM	CaCO ₃	2.4973	0.5M HNO ₃	TFE, CPE
CERIUM	(NH ₄) ₂ Ce(NO ₃) ₆	3.9125	Water	TFE, CPE
CHROMIUM	Cr ⁰	1.0000	4H HCl	TFE, CPE
COBALT	Co ⁰	1.0000	4M HCl	TFE, CPE
COPPER	Cu ⁰	1.0000	4M HNO ₃	TFE, CPE
GOLD	AuCl ₃	1.5400	Water	TFE, CPE
IRON	Fe ⁰	1.0000	4M HCl	TFE, CPE
IODINE	KI	1.3080	Water	TFE, CPE
LANTHANUM	La ₂ O ₃	1.1728	4M HCl	TFE, CPE
LEAD	Pb ⁰	1.0000	4M HNO ₃	TFE, CPE
LEAD	Pb(NO ₃) ₂	1.5985	0.5M HNO ₃	TFE, CPE
LITHIUM	LiCl	6.1092	Water	TFE, CPE
MAGNESIUM	MgO	1.6581	0.5M HCl	TFE, CPE
MANGANESE	MnO ₂	1.5825	Conc. HCl	Glass
MERCURY	Hg ⁰	1.0000	5M HNO ₃	TFE, CPE
MERCURY	HgCl ₂	1.3540	0.5M HNO ₃	TFE, CPE
MOLYBDENUM	MoO ₃	1.5003	Aqua-Regia	Glass
NICKEL	Ni ⁰	1.0000	4M HCl	TFE, CPE
PHOSPHORUS	(NH ₄) ₂ HPO ₄	4.2600	Water	TFE, CPE
POTASSIUM	KCl	1.9067	Water	TFE, CPE
SCANDIUM	Sc ₂ O ₃	1.5338	4M HCl	TFE, CPE
SELENIUM	Se	1.0000	0.5M HNO ₃	TFE, CPE
SELENIUM	SeO ₂	1.4053	Water	TFE, CPE
SILVER ⁶	Ag ⁰	1.0000	4M HNO ₃	TFE, CPE
SODIUM	NaCl	2.5421	Water	TFE, CPE
TIN	Sn ⁰	1.0000	4M HCl	Glass
TITANIUM	Ti ⁰	1.0000	4M HCl	Glass
ZINC	Zn ⁰	1.0000	4M HNO ₃	TFE, CPE

a-Use 100 ml of solvent to dissolve, then bring to volume with deionized water

b-TFE means preleached tetrafluorethylene (teflon)

c-CPE means preleached conventional polyethylene

d-Glass means preleached borosilicate glass

e-Dissolve in 20 ml of 10% NaOH. After dissolution dilute to 200 ml with deionized water, then neutralize with HCl and bring to volume with deionized water.

f-Boil gently to expel brown fumes then bring to volume

g-Store in brown CPE and keep in the dark

TABLE IV-2
NATIONAL BUREAU OF STANDARDS - STANDARD REFERENCE
MATERIALS RELATED TO FOODS

SRM	NUMBER	YEAR OF ISSUE
Oyster Tissue	SRM #1566	1979
Wheat Flour	SRM #1567	1978
Rice Flour	SRM #1568	1978
Brewers Yeast	SRM #1569	1976
Spinach ¹	SRM #1570	1976
Orchard Leaves ¹	SRM #1571	1977
Tomato Leaves	SRM #1573	1976
Pine Needles	SRM #1575	1976
Bovine Liver ¹ (old)	SRM #1577	1977
Bovine Liver (new)	SRM #1577a	1982
Albacore Tuna	RM #50	1977
Water	SRM #1643a	1980

¹No longer available.

TABLE IV-3 ELEMENTAL CONTENT OF STANDARD REFERENCE MATERIALS
(Content in ug/g, or where noted, WT. %)

ELEMENT	SRM/ NO.	OYSTER/ 1566	WHEAT FLOUR/ 1567	RICE FLOUR/ 1568
Aluminum				
Antimony				
Arsenic		13.4 ± 1.9	(0.006)	0.41 ± 0.05
Barium				
Beryllium				
Bismuth				
Boron				
Bromine		(55)	(9)	(1)
Cadmium		3.5 ± 0.4	0.032 ± .007	0.029 ± .004
Calcium		0.15% ± 0.02	0.019% ± .001	0.014% ± .002
Cerium				
Cesium				
Chlorine		(1.0%)		
Chromium		0.69 ± 0.27		
Cobalt		(0.4)		0.02 ± 0.01
Copper		63.0 ± 3.5	2.0 ± 0.3	2.2 ± 0.3
Europium				
Fluorine		(5.2)		
Gallium				
Indium				
Iodine		(2.8)		
Iron		195 ± 34	18.3 ± 1.0	8.7 ± 0.6
Lanthanum				
Lead		0.48 ± 0.04		
Lithium				
Magnesium		0.128% ± .009		
Manganese		17.5 ± 1.2	8.5 ± 0.5	20.1 ± 0.4
Mercury		0.057 ± .015	0.001 ± .0008	0.0060 ± .0007
Molybdenum		(<0.2)	(0.4)	(1.6)
Nickel		1.03 ± 0.19	(0.18)	(0.16)
Nitrogen				
Phosphorus		(0.81%)		
Potassium		0.969% ± .005	0.136% ± .004	0.112% ± .002
Rubidium		4.45 ± 0.09	(1)	(7)
Scandium				
Selenium		2.1 ± 0.5	1.1 ± 0.2	0.4 ± 0.1
Silicon				
Silver		0.89 ± 0.09		
Sodium		0.51% ± 0.03	8.0 ± 1.5	6.0 ± 1.5
Strontium		10.36 ± 0.56		
Sulfur		(.76%)		
Tellurium			(<0.002)	(<0.002)
Thallium		(<0.005)		
Thorium		(0.1)		
Uranium		0.116 ± .006		
Vanadium		(2.8)		
Zinc		852 ± 14	10.6 ± 1.0	19.4 ± 1.0

NOTES: a - Values in parentheses are non-certified values

b - All values are based on dry weight, except the tuna and water

TABLE IV-3 ELEMENTAL CONTENT OF STANDARD REFERENCE MATERIALS (ctd)
(Content in ug/g, or where noted, WT. %)

ELEMENT	SRM/ NO.	YEAST/ 1569	SPINACH/ 1570	ORCHARD LEAVES/ 1571
Aluminum			870 ± 50	
Antimony			(0.04)	2.9 ± 0.3
Arsenic			0.15 ± 0.05	10 ± 2
Barium				(44)
Beryllium				0.027 ± 0.010
Bismuth				(0.1)
Boron			(30)	33 ± 3
Bromine			(54)	(10)
Cadmium			(1.5)	0.11 ± 0.01
Calcium			1.35% ± 0.03	2.09% ± 0.03
Cerium				
Cesium				(0.04)
Chlorine				(690)
Chromium		2.12 ± 0.05	4.6 ± 0.3	2.6 ± 0.3
Cobalt			(1.5)	(0.2)
Copper			12 ± 2	12 ± 1
Europium			(0.02)	
Fluorine				(4)
Gallium				(0.08)
Indium				
Iodine				(0.17)
Iron			550 ± 20	300 ± 20
Lanthanum			(0.37)	
Lead			1.2 ± 0.2	45 ± 3
Lithium				(0.6)
Magnesium				0.62% ± 0.02
Manganese			165 ± 6	91 ± 4
Mercury			0.030 ± .005	0.155 ± .015
Molybdenum				0.3 ± 0.1
Nickel			(6)	1.3 ± 0.2
Nitrogen			(5.9%)	2.76% ± 0.05
Phosphorus			0.55% ± 0.02	0.21 ± 0.01%
Potassium			3.56% ± 0.03	1.47% ± 0.03
Rubidium			12.2 ± 0.2	12 ± 1
Scandium			(0.16)	
Selenium				0.08 ± 0.01
Silicon				(480)
Silver				
Sodium				82 ± 6
Strontium			87 ± 2	37 ± 1
Sulfur				(1900)
Tellurium				(0.01)
Thallium			(0.03)	
Thorium			0.12 ± 0.03	0.064 ± 0.006
Uranium			0.046 ± .009	0.029 ± 0.005
Vanadium				
Zinc			50 ± 2	25 ± 3

NOTES: a - Values in parentheses are non-certified values

b - All values are based on dry weight, except the tuna and water

TABLE IV-3 ELEMENTAL CONTENT OF STANDARD REFERENCE MATERIALS (ctd)
(Content in ug/g, or where noted, WT. %)

ELEMENT	SRM/ NO.	TOMATO LEAVES/ 1573	PINE NEEDLES/ 1575	BOVINE LIVER/ 1577 ^(c)
Aluminum		(0.12%)	545 ± 30	
Antimony			(0.2)	(5)
Arsenic		0.27 ± 0.05	0.21 ± 0.04	0.055 ± 0.005
Barium				
Beryllium				(.017)
Bismuth				
Boron		(30)		
Bromine		(26)	(9)	
Cadmium		(3)	(<0.5)	0.27 ± 0.04
Calcium		3.00% ± 0.03	0.41% ± 0.02	124 ± 6
Cerium		(1.6)	(0.4)	
Cesium				
Chlorine				(0.27%)
Chromium		4.5 ± 0.5	2.6 ± 0.2	0.088 ± 0.012
Cobalt		(0.6)	(0.1)	(0.18)
Copper		11 ± 1	3.0 ± 0.3	193 ± 10
Europium		(0.04)	(0.006)	
Fluorine				
Gallium				
Indium				(50)
Iodine				(180)
Iron		690 ± 25	200 ± 10	268 ± 8
Lanthanum		(0.9)	(0.2)	
Lead		6.3 ± 0.3	10.8 ± 0.5	0.34 ± 0.08
Lithium				
Magnesium		(0.7%)		604 ± 9
Manganese		238 ± 7	675 ± 15	10.3 ± 1.0
Mercury		(0.1)	0.15 ± 0.05	0.016 ± 0.002
Molybdenum				(3.4)
Nickel			(3.5)	
Nitrogen		(5.0%)	(1.2%)	10.6% ± 0.6
Phosphorus		0.34% ± 0.02	0.12 ± 0.02%	(1.1%)
Potassium		4.46% ± 0.03	0.37% ± 0.02	0.97% ± 0.06
Rubidium		16.5 ± 0.1	11.7 ± 0.1	18.3 ± 1.0
Scandium		(0.13)	(0.03)	
Selenium				1.1 ± 0.1
Silicon				(17) Silver
Sodium				(0.06)
Strontium		44.9 ± 0.2	4.8 ± 0.2	0.243% ± .013
Sulfur				(0.14)
Tellurium				
Thallium		(0.05)	(0.05)	(0.05)
Thorium		0.17 ± 0.03	0.037 ± 0.003	
Uranium		0.061 ± 0.003	0.020 ± 0.004	(0.0008)
Vanadium				
Zinc		62 ± 6		130 ± 13

NOTES: a - Values in parentheses are non-certified values

b - All values are based on dry weight, except the tuna and water

c - "Old" Bovine Liver

TABLE IV-3 ELEMENTAL CONTENT OF STANDARD REFERENCE MATERIALS (ctd)
(Content in ug/g, or where noted, WT. %)

ELEMENT	SRM/ NO.	ALBACORE TUNA/ RM60 ^(d)	WATER/ 1643a	BOVINE LIVER/ 1577a ^(e)
Aluminum				(2)
Antimony				(3)
Arsenic		(3.3 ± 0.4)	76 ± 7	.047 ± 0.006
Barium			46 ± 2	
Beryllium			19 ± 2	
Bismuth				
Boron				
Bromine				(9)
Cadmium			10 ± 1	0.44 ± 0.06
Calcium				120 ± 7
Cerium				
Cesium				
Chlorine				0.28% ± 0.01
Chromium			17 ± 2	
Cobalt			19 ± 2	0.21 ± 0.05
Copper			18 ± 2	158 ± 7
Europium				
Fluorine				
Gallium				
Indium				
Iodine				
Iron			88 ± 4	194 ± 20
Lanthanum				
Lead			27 ± 1	0.135 ± 0.015
Lithium				
Magnesium				600 ± 15
Manganese			31 ± 2	9.9 ± 0.8
Mercury		0.95 ± 0.1 ^(c)	(<0.2)	0.004 ± 0.002
Molybdenum			95 ± 6	3.5 ± 0.5
Nickel			55 ± 3	
Nitrogen			(1.2%)	10.6% ± 0.6
Phosphorus				1.11% ± 0.04
Potassium				0.996% ± 0.007
Rubidium				12.5 ± 0.1
Scandium				
Selenium		(3.6 ± 0.4)	11 ± 1	0.71 ± 0.07
Silicon				
Silver			2.8 ± 3	
Sodium				0.243% ± 0.013
Strontium			239 ± 5	0.138 ± 0.003
Sulfur				0.78% ± 0.01
Tellurium				
Thallium				(.003)
Thorium				
Uranium				0.00071 ± 0.00003
Vanadium			53 ± 3	
Zinc		(13.6 ± 1)	72 ± 4	123 ± 8

NOTES: (a) Values in parentheses are non-certified values (b) All values are based on dry weight, except the tuna and wate (c) As methyl mercury, 0.93 ±0.1 (d) "Research Material" - not SRM (e) "New" Bovine Liver

APPENDIX IV-A

MINIMUM SAMPLE SIZE AND DRYING INSTRUCTIONS

SRM-1566: OYSTER TISSUE

Minimum Sample Size: 250 mg

Instructions for Drying: Before weighing, samples of SRM 1566 should be dried to constant weight by one of the following procedures:

1. Reduced-pressure drying at room temperature for 48 hours over $\text{Mg}(\text{ClO}_4)_2$ in a vacuum desiccator at approximately 1.3×10^4 Pa (100 mm Hg).
2. Vacuum drying at room temperature for 24 hours at a pressure of approximately 30 Pa (0.2 mm Hg) using a cold trap.
3. Freeze drying for 20 hours at a pressure of approximately 30 Pa (0.02 mm Hg).

Homogeneity Assessment: Randomly selected bottles of SRM 1566 were sampled and tested for homogeneity by neutron activation analysis (NAA) and atomic absorption spectrometry (AAS). No excessive inhomogeneity was observed for the elements Na, Cl, V, and Mn determined by NAA, nor for Mg, K, Cu, Zn, and Cd determined by AAS. However, Ca, which was determined by AAS, does exhibit some inhomogeneity--approximately 4% relative standard deviation.

SRM-1567: WHEAT FLOUR

Minimum Sample Size: 400 mg

Instructions for Drying: Except for Se and Hg, elements may be determined in samples which have been dried as follows: Vacuum-dry the material at approximately 25°C for 24 hours at a pressure not greater than 70 Pa (0.5 mm Hg) with a cold trap at a temperature of about -30°C or below. Se and Hg should be determined in undried sample; other elements may be so determined. However, because the Certificate values are reported on a "dry-weight" basis, the elemental concentrations determined on un-dried samples should be adjusted for the moisture content of

the samples. The moisture content, which was approximately 9% when bottled, should be determined on separate samples by either the vacuum-drying procedure described above or drying the sample in air in an oven at 85°C for 24 hours. Both of these procedures yielded the same loss in weight. Samples for analysis should not be oven-dried lest elements be lost by volatilization.

Homogeneity Assessment: A preliminary evaluation of homogeneity was made by instrumental NAA using samples of 150 to 300 mg and counting the activities from radionuclides of Mn, K, Zn, Na, and Br. The homogeneity of other certified elements was evaluated using samples of 400 mg or less with the exception of mercury and calcium for which 500 mg and 1 g, respectively were used. The uncertainties for the concentrations in Table IV-3 include these results.

SRM 1569: RICE FLOUR

Minimum Sample Size: 150 mg

Instructions for Drying: The analytical determinations should be made on samples without drying. The determinations should be corrected to a dry weight basis by heating separate samples at 85°C for 3 hours to determine the weight loss.

Homogeneity Assessment: Material homogeneity was determined by NAA using 150 mg random samples from bottled material representing different locations of the bulk material. The statistical test pattern was proposed by J. Mandel of the NBS Institute for Materials Research.

Precautions: Samples should not be dissolved in open vessels.

SRM 1570: SPINACH

Minimum Sample Size: 300 mg

Instructions for Drying: Samples of this Standard Reference Material must be dried before weighing by either of the following procedures:

1. Drying in air in an oven at 85°C for 2 hours.

2. Lyophilization using cold trap at or below -50°C at a pressure not greater than 30 Pa(0.2 mm Hg) for 24 hours.

NOTE: Drying either in an oven at 105°C or in a vacuum oven at 25°C causes large losses of volatiles other than water and should not be used.

Homogeneity Assessment: Material homogeneity was evaluated by determining ten of the certified elements, Al, Fe, Mn, Zn, Rb, Cu, Cr, As, U, and Hg, on samples of 300 mg or less taken at various locations in the freeze-dried bulk material. The other certified elements, K, Ca, P, Sr, Pb, and Th were determined using sample weights not exceeding one gram. The uncertainties for the concentrations given in Table IV-3 include these results.

SRM 1571: ORCHARD LEAVES

Minimum Sample Size: 250 mg

Instructions for Drying: Before weighing, samples of this Standard Reference Material must be dried by either:

1. Drying in air in an oven at 85°C for at least 4 hours.
2. Lyophilization using a cold trap at or below -50°C at a pressure not greater than 30 Pa(0.2 mm Hg) for at least 24 hours.

NOTE: Drying at 135°C results in large losses and discoloration and should not be used.

Homogeneity Assessment: The homogeneity of this material was established on the premise that the minimum sample size be 250 mg. Assessment of homogeneity was made using analyses for N, K, and Mg. A statistical analysis on the data shows that there is evidence for a small degree of variability between samples with respect to K. The data for the other elements do not reveal such an effect. Statistical design and analysis of data were performed by J. Mandel of the NBS Institute for Materials Research.

SRM 1573: TOMATO LEAVES

Minimum Sample Size: 500 mg

Instructions for Drying: Samples of this Standard Reference Material must be dried before weighing by either of the following procedures:

1. Drying in air in an oven at 85°C for 2 hours.
2. Lyophilization using a cold trap at or below -50°C at a pressure not greater than 30 Pa(0.2 mm Hg) for 24 hours.

NOTE: Drying either in an oven at 150°C or in a vacuum oven at 25°C causes large losses of volatiles other than water and should not be used.

Homogeneity Assessment: Material homogeneity was evaluated by determining nine of the certified elements, P, Fe, Mn, Zn, Rb, Cu, Cr, As, and U on samples of 500 mg or less taken at various locations of the freeze dried bulk material. The other certified elements, K, Ca, Sr, Pb, and Th were determined using sample weights not exceeding one gram. The uncertainties of the concentrations given in Table IV-3 include these results.

SRM 1575: PINE NEEDLES

Minimum Sample Size: 500 mg

Instructions for Drying: Samples of this Standard Reference Material must be dried before weighing by either of the following procedures:

1. Drying in air in an oven at 85°C for 2 hours.
2. Lyophilization using a cold trap at or below -50°C at a pressure not greater than 30 Pa(0.2 mm Hg) for 24 hours.

NOTE: Drying either in an oven at 150°C or in a vacuum oven at 25°C causes large losses of volatiles other than water and should not be used.

Homogeneity Assessment: Material homogeneity was evaluated by determining nine of the certified elements, P, Fe, Mn, Zn, Rb, Cu, Cr, As, and U on samples of 500 mg or less taken at various locations of the freeze dried bulk material. The other certified elements, K, Ca, Sr, Pb, and Th were determined using sample weights not exceeding one gram. The uncertainties of the concentrations given in Table IV-3 include these results.

SRM 1577: BOVINE LIVER

Minimum Sample Size: 250 mg

Instructions for Drying: Lyophilization for at least 24 hours using a cold trap at or below -50°C and a pressure not greater than 30 Pa (0.2 mm Hg).

Homogeneity Assessment: Statistical analysis of the data to demonstrate homogeneity of the sample was performed by H.H. Ku of the Statistical Engineering Section. Homogeneity is based on the data for Na, K, Fe, and Zn, using 28 samples and a minimum sample size of 250 mg. The variation of the entire lot is within ± 5 percent (relative) of the average content for the trace elements.

RM 50: ALBACORE TUNA

Minimum Sample Size: 250 mg

V PREANALYSIS SAMPLE PREPARATION

INTRODUCTION

The accuracy of an analytical determination is only as good as each step carried out to arrive at the results. Sample preparation is the first step in an analysis, and careful consideration must be given to each sample. For elemental analyses it involves two distinct steps: first, selection of the representative sample, i.e., that portion of the lot or product that will be analyzed; and second, compositing and homogenization of the edible portion of the representative sample so that representative subsamples can be withdrawn from the composited sample for analysis. Procedures are described to produce a composite that represents the edible portion of the product, i.e., portion used for food or feed purposes.

Many samples being analyzed for trace elements have a unique character and require extensive homogenization and contamination control procedures. Therefore, this section is intended to serve as a guideline for preparation of general food classes and some specific foods. As procedures are developed for additional foods they will be added. These procedures may need to be modified depending on sample type and type of contamination to be investigated. For example, if surface contamination of a raw agricultural crop is suspected during shipping and is to be determined, the sample would not be cleaned in the same manner as a plant grown on contaminated soil if the objective is to determine the levels of toxic elements assimilated by the plant.

In many cases, sample preparation for elemental analysis will differ from that required for pesticides and other contaminant analyses. The sample preparation procedures described in the Pesticide Analytical Manual (PAM) are designed to meet the special requirements imposed by pesticide regulations. By contrast there are few regulations for metals and other elements; and most analyses are concerned with measuring the level present to accurately determine the dietary intake. However, whenever possible it is desirable to use the same sample preparation procedure for either elemental and pesticide analyses, provided that the unique requirements for the other analysis are not violated.

Appendix V-A includes Sections 141 and 142, Pesticide Analytical Manual (PAM), Vol. 1, which gives guidelines for preparation and compositing of samples. These procedures may differ from those described in the EAM in that generally more contamination control precautions must be taken for trace and ultratrace elemental analyses. Usually a sample prepared for elemental analysis can also be analyzed for pesticides. However, the converse is not necessarily true, because ubiquitous contamination by pesticides in the laboratory is not generally a problem. Exceptions exist where an elemental analysis sample could not be analyzed for pesticides, for example, raw agricultural crops such as lettuce, cabbage, etc. which cannot be washed for pesticide analysis and may be washed for elemental analysis (depending on the type of contamination). Where EAM and PAM sample preparation procedures differ, the EAM procedure should be followed for elemental analyses. Otherwise the PAM procedure may be followed.

A. RECOMMENDED MATERIALS USED IN SAMPLE PREPARATION:

1. Clean air hoods - class 100
2. Distilled deionized water system (18 megohm cm^{-1})
3. Hobart food cutter (Kynar coated)
4. Homogenizer (Polytron or Dupont)
5. Food processor with plastic or stainless steel blade (Cuisinart)
6. Blender - glass bowl with stainless steel blades (Waring or Sears)
7. Freeze-dryer, flasks, lids
8. Glovebox - plastic
9. Can opener - stainless steel
10. Knives, spatulas, potato peeler, etc., - stainless steel or epoxy-coated stainless steel
11. Sample storage bottles - linear polyethylene or polypropylene
12. Gloves - polyethylene or polyvinylchloride
13. Plasticware - knives, spoons, spatulas, strainers, wash tubs, drainers, vegetable brush, sample bags, vegetable spinner
14. 40 mesh polypropylene sieve

B. GENERAL PROCEDURES

1. All water is distilled deionized water (DDW), specific resistance = 18 megohm cm^{-1} unless otherwise stated.
2. Always wear gloves when handling samples.
3. All hoods are clean air hoods.

4. All equipment is plastic or glass (Pyrex, quartz) unless otherwise stated.
5. Washing sample preparation equipment:
 - a. Glassware (including blender jars and freeze-dry jars) - Wash glassware with DDW and Alconox, rinse with DDW, soak for 15 min in DDW, soak for 15 min in 30% HNO_3 , rinse with DDW, and place in drainer in hood to dry.
Note: Omit acid step when washing blender cutting unit.
 - b. Plasticware (including sample storage bottles) - Rinse with DDW, soak for 15 min in 10% HNO_3 , soak for 15 min in DDW, rinse with DDW, and place in drainer in hood to dry.
6. Sample cleaning and preparation - See section C for specific commodity preparation procedures and reduction to edible portion. Sample cleaning is dictated by type of contamination being investigated (e.g., surface, systemic).
7. Compositing:
 - a. Divide prepared sample into three representative portions: original analysis, check analysis, and reserve portions (2). Store check and reserve portions in freezer to prevent decomposition. With canned foods, keep one half of the sample intact (reserve), and mix the other half well before division (into check and original portions).
Note: A sample is divided at the analyst's discretion where representative portions may be obtained with the least amount of preparation.
 - b. Freeze-dried composite -

If sample is to be freeze-dried before compositing, place remaining portion of sample into weighed freeze-dry jars and record sample weight. Place freeze-dried sample in blender and grind to pass a 40-mesh polypropylene sieve. Transfer sample to plastic storage bottle. Note: When freeze-drying a sample with <10% moisture, water must be added (which

is absorbed by the sample) before shell-freezing. The freezing process ruptures the cellular structure of the sample, which after freeze-drying, facilitates grinding.

- c. Wet composite - Homogenization of foods based on relative % water (3). <25% water - Weigh sample for homogenization into a blender or food processor; add one equal weight of DDW and blend to slurry uniform in appearance if possible. If further homogenization is necessary, transfer this slurry to a homogenizer and grind to a homogeneous state. Additional equal weights of DDW may be necessary. Always add equal weights of DDW to maintain proportionate ratios, i.e., 1:1, 1:2, etc. Record this ratio to account for sample dilution. Transfer sample to plastic storage bottle.

>25% but <50% water - Addition of DDW is at the analyst's discretion. Obtain a homogeneous slurry by using a conventional blender or food processor. If further homogenization is necessary, finish with a homogenizer. Transfer sample to plastic storage bottle.

>50% water - Generally no addition of DDW is necessary for obtaining a homogeneous slurry; however, analyst discretion is implied. Obtain a homogeneous slurry by first using a conventional blender or food processor. If further homogenization is necessary, finish with a homogenizer. Transfer sample to plastic storage bottle.

8. Storage -

- a. Store freeze-dried composites in laboratory cabinets until analysis is complete.
- b. Refrigerate or freeze wet composite until analysis is complete. Samples that are homogenized and stored in this manner will separate physically; restore homogeneous state before sampling.

9. Moisture determination for freeze-dried crops -

- a. Weigh composited crop before and after freeze-drying.

$$\frac{\text{total wet wt.} - \text{freeze-dry wt.}}{\text{total wet wt.}} \times 100\% = \text{moisture lost during freeze-drying}$$

- b. Determine % residual moisture in the final composite by oven drying a portion of the sample to constant wt. Weigh 2-5 g of composite into oven-dried, tared beakers. Determine drying temperatures and times initially by drying 12 samples of each crop to a constant wt. (weighings made at 1 h intervals). The following are examples of drying times and temperatures required:

<u>CROP</u>	<u>TIME</u>	<u>TEMP. °C</u>
Lettuce	4 h	75
Peanuts	4 h	100
Soybeans	7 h	85
Wheat	5 h	85

Note: Determine % residual moisture in corn by Karl Fischer titration. Oven drying measures the total moisture content in soybeans.

- c. Correct the % moisture lost during freeze-drying for the final moisture loss and calculate a conversion factor (final dry wt. to wet wt.). Total dry wt. after F.D. x % Residual Moisture = Total residual moisture.

$$\text{Wet wt. factor} = \frac{\text{Total dry wt.} - \text{Total residual moisture}}{\text{Total wet wt.}}$$

C. SPECIFIC COMMODITY PROCEDURES

1. Homogenization of general classes of canned products: In preparing canned products for analysis, a rule of thumb is to analyze the edible portion unless otherwise specified.

- a. Homogenize means: chop, grind, and blend (blender, food processor, or Polytron) to obtain at least the minimum sample homogeneity required by the analytical method.
 - b. Discard liquid implies: drain the non-edible liquid by partially removing the lid, using a stainless steel can opener, inverting the can and allowing the excess liquid to drain off, retaining the edible portion with the lid.
 - c. All containers requiring special devices (e.g., can openers) for opening, should be opened with stainless steel or plastic devices.
 - d. DDW should be incorporated when necessary to achieve homogeneity.
2. Homogenization of general classes of raw products:
- a. Meats, fish, poultry - (See Appendix V-C for specific food) - Meats - debone and trim excess fat; use a Hobart chopper to homogenize.
 - Fish - follow the PAM procedure (Appendix V-A)
 - Poultry - debone; use Hobart chopper to homogenize
 - b. Dairy products - (See Appendix V-C for specific food)
 - c. Raw agricultural products - (See Appendix V-D)
Specific preparation procedures included were developed at the EARC for determination of background levels of elements in the specific commodity.

REFERENCES:

1. Pesticide Analytical Manual, Vol. 1, Sections 141 and 142, Food and Drug Administration.
2. Federal Food, Drug, and Cosmetic Act, Section 702, paragraph B.
3. Composition of Foods, USDA Agriculture Handbook No. 8, 1963.

Pesticide Analytical Manual Vol. 1
Foods and Feeds

APPENDIX V-A

GENERAL INFORMATION
Section 1.1)

140

SAMPLES

This section gives general guides for preparing and compositing routine samples. It does not provide for handling the unusual sample. Because complete background information on samples is ordinarily unknown, and since residue analysts are usually unaware of what residues are present or of how they were incurred, *no sample should be assumed to be routine.*

A thorough visual examination of the gross sample should always be made before any preparation or compositing is begun. This should be on a sub by sub basis if sample is received in subsample form. A key to proper sample analysis can often be found by observation of the general appearance and odor of the product. Presence of soil, dust, wax, powder or stains; and foreign or off odors should be noted and recorded. When appearance or odor of the sample (or any of its subs) is unusual, the applicability of instructions in 141 and 142 should be carefully weighed before preparation and compositing are begun.

SAMPLE PREPARATION

Where samples are analyzed to determine whether they are in compliance with the Food, Drug, and Cosmetic Act, they must be prepared for analysis according to the procedure specified in the Regulations¹ or in Administrative guidelines which have been established for the residue on the commodity. The various ways to prepare raw agricultural and processed foods are given in 141.1 and 141.2 as an aid to residue analysts in proper choice of how to handle the samples.

The portion of sample taken for analysis must be representative of the gross laboratory sample. It must be carefully handled to prevent loss of residue by volatilization and to prevent concentration of residue through physical separation of product during preparation. Meaningful residue data can only be obtained when integrity of sample is preserved. Haphazard preparation results in data that is useless and often misleading.

Federal Food, Drug, and Cosmetic Act Regulations. Published in the Code of Federal Regulations, 10 CFR, part 180- Protection of Environment, and 21 CFR, part 121, and 21 CFR, part 122 - Food and Drugs.

141.1 Raw Agricultural Commodities. Raw agricultural commodities include, among other things: fresh fruits, whether or not they have been washed, peeled or otherwise treated in their unprocessed natural form; vegetables in their raw or natural state, whether or not they have been stripped of their outer leaves, waxed, or processed into fresh green salads, fruits, nuts, seeds, raw milk, meats, and similar agricultural products.

Various different ways are required for preparing raw agricultural commodities for residue analysis. The various preparations are described as follows:

(1) **Whole Raw Agricultural Commodity.** — Most tolerances have been established on the product in its raw or natural state as shipped in interstate commerce. The whole raw agricultural product is prepared for analysis as in 141.12a.

(2) **Whole Basis.** According to Regulation 40 CFR 180.1(j).—This regulation directs which portion of the commodity is to be discarded and which portion is to be taken for analysis, and is in accordance with how most tolerances were established on these products. Commodities for which preparation has been specified are listed in 141.12b, along with their regulation reference. This preparation is considered "whole basis" preparation for these commodities only.

(3) **Whole Basis According to Specific Tolerance Regulations.** (See 10 CFR 180 Subpart C).—Special preparation for certain commodities is dictated by the individual tolerance regulation. When samples are submitted for a specific tolerance, the tolerance regulation to determine if the portion of the commodity to be analyzed is specified.

(4) **Edible Portion.**—In edible portions of the product are discarded and the edible portion only is analyzed. The edible portion preparation for saponins (commodities are listed in 141.12c. Analysts should use discretion in determining the edible portion of products not listed in that section.

Analytical report must give full description of product as received for analysis and must clearly state the exact portion of food used for analysis.

141.11 Guide to Determining How to Prepare Raw Agricultural Commodities. The criteria for

GENERAL INFORMATION

Suction 141.11

Samr,Jr.

Type

Criteria

Procedure

I-R

Meets *ALL* of the following criteria:

- (a) Collected for multi residue determination.
- (b) No background information or background information does not indicate likelihood of contamination by specific pesticide or industrial chemical.

(OBJECTIVE SAMPLES).

- (c) Commodity has at least one residue tolerance established and that residue is determined by methodology to be USPd.

Consult 141.12b and, if product is listed there, prepare as directed; if not listed, prepare the whole raw agricultural commodity as in 141.12a

II-R

Meets *EVERY* of the following criteria:

- (a) Selectively collected for a particular residue for which a tolerance is established.
- (b) Analysis of a type I-R sample reveals a significant residue which has an established tolerance on the product.

Consult the specific tolerance" regulation listed in 40 CFR 180 Subpart C or 21 CFR 122 for preparation that may be required by regulation.

III-R

Meets *ANY* of the following criteria: (a) No tolerances have been established for any residue on the commodity or the chemicals with established tolerances on the commodity are not determined by methodology used.

- (b) Selectively collected for a particular residue for which no tolerance has been established on that particular commodity (e.g. endrin in melons).
- (c) Collected from an area where a known residue problem exists for a chemical for which no tolerance has been established on the particular commodity.
- (d) Analysis of a sample prepared as in 141.12a or in 141.12b ("whole basis") reveals significant quantity of a

Prepare sample according to the edible portion guide 141.12c.

Pesticide Analytical Manual .. Vol. I
Foods and Feeds

GENERPL INFORMATION
Section 141.12

141.12 Preparation of Raw Agricultural Commodities

141.12a Whole Raw Agricultural Commodity. Remove obviously decomposed leaves, berries, etc. Prepare the whole raw agricultural product. See 141.1(1).

141.12b Whole Raw Agricultural Commodity with Preparation Specified in 40 CFR 180.1(j). Prepare commodities listed in table below according to preparation in column b. See 141.1(2). Preparation given in Editors' Notes is in keeping with current policy.

141.12c Edible Portion. Prepare commodities listed in table below according to preparation in column c. See 141.1(4).

Commodity	Preparation	
	b Specified in 40 CFR 180.1(j)	c Edible portion
Bananas	Remove and discard crown tissue and stalk. 40 CFR 180.1(j)(1) (EditorNote: Several specific tolerance regulations establish separate level for pesticide in pulp.)	Remove and discard peel; examine pulp only.
Corn, sweet	(Editors• Note: Some tolerance regulations specify portion for analysis as "kernels plus cob; husks removed.")	Remove and discard- husks and cob; examine kernels.
Crabs, hard shell	(Editors• Note: Use edible portion guide.)	Examine a homogeneous mixture of meat and fatty materials isolated as described below: Heat crab in boiling water or place in autoclave under flowing steam for one minute if previously frozen, or five minutes if sample has been merely chilled and is possibly still alive. Remove claws and other appendages and pick out meat. Remove back shell. Clean out and discard viscera and gills (easily remove by hand).

Pesticide Analytical Manual - Vol. I
Foods and Feeds

GENERAL INFORMATION
 Section 141.12

Commodity

Preparation

	b Specified in 40 CFR 180.1(j)	c Edible portion
		Include in the edible portion fatty material {yellowish colored) from inside tips of the back shell and any fatty material (yellowish colored) adhering to meat. Break crab in half and remove meat from body cavity excluding shell and other obviously extraneous materials.
Crab, soft shell	(Editors• Note: Use edible portion guide.)	Examine entire crab.
Egg\$	(Editors• Note: Use edible portion guide.)	Discard shells; examine combined yolks and whites.
Fish (raw)	(Editors• Note: Use edible portion guide.)	Remove and discard heads, scales, tails, fins, guts and inedible bones; do not remove skin; fillet to obtain all flesh and skin from head to tail and from top of back to belly on both sides. Where extremely large whole fish are to be analyzed and filleting is impractical, 3 cross-sectional slices from each fish may be taken and combined.

PESTICIDE ANALYTICAL MANUAL - VOLUME 1
Foods and Feeds

GENERAL INFORMATION
Section 141.12

Commodity	Preparation	c Edible portion
h Specified in 40 CFR 180.1(j)		
		Clean, scale and eviscerate fish. Take 1" thick slices, one from behind the pectoral fins, one from half way between first slice and the vent, and one from behind the vent. Remove bones from each slice before combining.
		Rule of edibility supersedes these directions; e.g., catfish skin (inedible) is discarded.
Fruits (general comment)		Remove and discard stems.
Fruits, stone		Remove and discard stones or pits.
Garlic bulbs	Remove and discard roots, stems and outer sheaths (or husks); examine garlic cloves only. 40 CFR 180.1(j)(5)	Same as preparation in 40 CFR 180.1(j)(5)
Langoustes		Remove and discard rind and stone.
Melons	Remove and discard stems. 40 CFR 180.1(j)(4)	Remove and discard rind, stem and seeds; examine edible portion.

PESTICIDE ANALYTICAL MANUAL - VOLUME 1
Foods and Feeds

GENERAL INFORMATION
Section 141.12

Commodity	Preparation	
	^b Specified in 40 CFR 180.1(j}	^c Edible portion
Nuts	Remove and discard shells. 40 CFR 180.1(j)(2}	Same as preparation in in 40 CFR 180.1(j)(2}
Oysters, Clams (raw}	(Editors Note: Use edible portion guide.}	Examine a homogeneous mixture of meats and liquor.
Peanuts	(Editors Note: Use edible portion guide.)	Remove and discard shells.
Pineapple	Remove and discard crowns (leaves at the top of the fruit). 40 CFR 180.1(j)(7)	Remove and discard crown and flowers (outer protective petals}; examine edible portion only.
Pumpkins		Remove and discard rind, stem and seeds; examine edible portion only.
Root crops (general collilient)	(Editors Note: Use edible portion guide.}	Rinse lightly to remove adhering soil.
Root vegetables including tops or with tops	Examine the roots and tops separately. Neither the pesticide residues on the roots nor on the tops shall exceed the tolerance level. except that in the case of carrots the tops shall be removed and discarded before analyzing roots for pesticide residues. 40 CFR 180.1(j)(6}	Same as preparation in 40 CFR 180.1(j)(6)
*** Shrimp (raw}, crawfish and similar shellfish	(Editors Note: Use edible portion guide.}	Remove and discard heads, tails and shells;

PESTICIDE ANALYTICAL MANUAL - VOLUME 1
Foods and FeedsGENERAL INFORMATION
Section 141.12

Commodity

Preparation

	b	c
	Specified in 40 CFR 180.1(j)	Edible portion
Strawberries	Remove and discard caps (hulls). 40 CFR 180.1(j)(3).	Same as preparation in 40 CFR 180.1(j)(3).

PESTICIDE ANALYTICAL MANUAL - VOLUME 1
Foods and Feeds

GENERAL INFORMATION
Section 141.21

141.2 Processed Foods. Processed foods include foods that have been processed, fabricated, or manufactured by cooking, freezing, dehydrating or milling.

The various ways of preparing processed foods for analysis are as follows:

(1) "As is" Product. The food or feed as shipped in interstate commerce is prepared for analysis. Concentrates, dehydrated foods, etc. are analyzed "as is". Do not reconstitute to whole basis before analysis. See 143.12b for reporting results on concentrates and dehydrated products. Prepare low fat dairy products (e.g., skim milk, buttermilk, nonfat dried milk and uncreamed cottage cheese) on an "as is" basis. See 143.12a for reporting results on low fat dairy products.

(2) Specific Product Preparation - Special preparation is specified for certain processed foods in 141.22b.

141.21 Guide to Determining How to Prepare Processed Foods. Determine the type of sample and reasons for analysis. Use the preparation procedure for that type sample.

Sample Type	Criteria	Procedure
I-P	Meets ALL the following criteria: (a) Collected for multi residue determination (b) No background information or background information does not indicate likelihood of contamination by specific pesticide or industrial chemical.	Consult 141.22b and if product is listed there, prepare as directed; if not listed, prepare the "as is" product as received or as introduced into interstate commerce.
Li-P	Meets ANY of the following criteria: (a) Selectively collected for particular residue(s) (b) Selectively collected because of suspected likelihood of particular residue. (c) Collected from an area where a known residue problem exists. (d) Analysis of a type I-P sample reveals a significant residue for which a tolerance is established on the product	Consult the specific tolerance regulations listed in 21 CFR 121 or 21 CFR 122 for preparation that may be required by regulation. If no tolerance is established for the residue in the particular processed food, the analyst must decide, based

PESTICIDE ANALYTICAL MANUAL - VOLUME 1
Foods and Feeds

GENERAL INFORMATION
Section 141.22

141.22 Preparation of Processed Foods

141.22a "As is" Product. Prepare the "as is. food (including concentrates, dehydrated foods, etc.) as received or as when introduced into interstate commerce.

141.22b Certain Commodities with Specified Preparation. Prepare as directed below:

Ginned foods	Examine a homogeneous mixture of can contents; except, drain and discard brine and remove pits and stones.
Cheese	Do not remove or discard natural cheese rind. Do remove and discard waxed or oiled rings. Grind, dice, shred or blend cheese. See 142.22b.
Citrus pulp, Milk, Tomato pomace	Examine produce as received or as when introduced into interstate commerce.
Fish, breaded, raw or cooked	Do not remove breading. Fillet as necessary (as described in 141.12 "fish (raw)") to remove bones and/or tails.
Fish, canned in brine or water	Drain and discard liquid, examine remainder.
Fish, canned in oil, broth or sauce	Examine a homogeneous mixture of can contents.
Fish, frozen	Thaw, drain and discard drainings. Fillet - use entire piece. Whole fish - proceed as in 141.12 "fish (raw)."
Fish, smoked	Proceed as in 141.12 "fish (raw)."
Frog legs	Discard bones; examine edible meat only.
Oysters and Clams, canned or frozen	Examine a homogeneous mixture of meats and liquor.
Shrimp and similar shellfish, breaded	Examine as received.
Shrimp and similar shellfish, canned in brine	Drain and discard brine; examine edible meat.
Shrimp and similar shellfish, frozen	Thaw, drain and discard drainings. Remove and discard heads, tails and shells; examine edible

meat only.

142

SAMPLE COMPOSITING

Composite prepared sample according to guidelines in 142.2 by chopping, grinding, blending, etc. to obtain homogeneous mixture. The relatively small portion (25-100 g) of prepared composite that is taken for analysis must be representative of gross laboratory sample. Routine chopping, grinding, blending, etc. does not always produce a proper homogenate, as is the case with dried hays and some fish samples. When product is not visibly homogeneous, use standard mixing and quartering techniques to insure that portion for analysis is representative. See 142.4 for notes on preparing composites for analysis. Select representative portion of uniformly mixed sample for analysis.

142.1 Portion of Sample for Dithiocarbamate Analysis. Some dithiocarbamate compounds decompose rapidly in presence of slurry of crop material. Cullen (Anal. Chem., 221-224 (1964)) reported that speed is essential as soon as surface of crop is broken and dithiocarbamate is in intimate contact with water, enzymes, and sugars. He noted a rapid decrease in recovery with time of contact in aqueous crop solution and recommended that samples for dithiocarbamate analysis be either analyzed immediately after harvest or frozen for storage.

When dithiocarbamate residues are to be determined, select representative units for dithiocarbamate analysis prior to chopping, grinding or blending sample. Where sample units are small and free flowing (e.g. grains, beans, berries, etc.), mix well and take whole units for analysis; where sample units are large, take wedges from each unit. Analyze immediately or freeze immediately after cutting. An exception to above is where commodity contains free juices (e.g. tomatoes, apples, oranges, etc.) and requires cutting in pieces to fit into apparatus. In such cases, take representative whole units and freeze before cutting. Dice frozen units without allowing them to thaw; mix and take sample for analysis.

142.2 Guidelines for Preparation of Composites.

142.21 Total Sample Compositing and Comminuted. Where practical, comminute and thoroughly mix entire prepared sample. See 142.1 for portion to be removed if sample is to be analyzed for dithiocarbamates.

142.22 Total Sample Compositing and Fraction of Sample Comminuted.

142.22a Product with Small Units. Where sample product consists of small units (e.g., grains, cherries, nuts, dried peas and beans) and it is not practical to prepare and comminute entire sample, mix and quarter down to approximately 4 pounds or 4 quarts. From quartered sample, prepare product as in 141.1 or 141.2 and chop or grind prepared sample to obtain minimum of 1 pound or 1 quart comminuted sample for original analysis. See 142.4(4) for

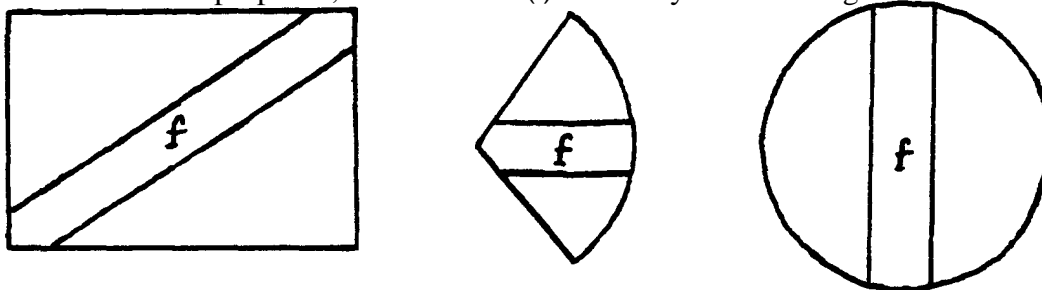
GENERAL INFORMATION

Section 142.22b

Pesticide Analytical Manual Vol. I

Foods and Feeds

142.22b Product of Homogeneous Nature. Where large sample of homogeneous nature must be composited (e.g., butter, cheese), and melting entire sample of butter or dicing, shredding, or blending entire sample of cheese is not practical, prepare sample for original analysis by taking equal portions from each packaged unit. Where large blocks, wedges or wheels of cheese are to be prepared, take fraction (f) for analysis as in diagram below.



Prepare cheese as in 141.22b(2) and composite by dicing, shredding or blending.

142.23 Composite of Individual Subdivisions. Where identity of subdivisions must be maintained for possible additional analysis of individual subs, prepare composite as follows:

- | | |
|--------------------------|--|
| (1) Animal tissue | Grind each sub (meat grinder). Composite 100 g from each sub and grind again. |
| (2) Dairy products | Equal weight from each sub. Grind, dice or blend. |
| (3) Eggs | Half of eggs in each sub. Blend. See 142.4(2). |
| (4) Feed, forage and hay | Quarter each sub down to 200 g (100 g for processed feeds and silage; . Composite 200 (100) g from each sub. Chop or grind to pass 20 mesh. See 142.4(4). |
| (5) Fruits | <p>(a) Large (apples, pears, tomatoes, etc.): sample each unit within sub. Composite an equal weight from each sub. Chop or blend.</p> <p>(b) Small: 200 g from each sub. Chop or blend.</p> |
| (6) Grains | 100 g from each sub after thorough mixing. Grind composite to pass 20 mesh. See 142.4(4). |
| (7) Milk | 100 g (ml) from each sub after thorough mixing. |
| (8) Nuts | Remove and discard shells. Composite equal weight, 100 g or more, of nut meats from each sub. Chop or grind. |
| (9) Pod Vegetables | (Beans, peas, etc., also asparagus) 200 g from each sub after thorough |

Pesticide Analytical Manual - Vol. ■
Foods and Feeds

GENERAL INFORMATION
Section 142.4

- (10) Root Vegetables Sample each unit within sub taking equal weight from each sub. Chop or grind.
- (11) Seeds 100 g from each sub after thorough mixing. Grind composite to pass 20 mesh. See 142.4(4).
- (12) Spices 200 g from each sub after thorough mixing. Grind or chop.
- (13) Stalk Vegetables (Celery, broccoli, etc.). Quarter each stalk in sub lengthwise. Take two opposite quarters from each stalk and composite these quarters by chopping.
- (14) Vegetables (a) Head: quarter each head in sub. Take two opposite quarters from each head and composite these quarters by chopping.

 (b) Leafy
 - (1) Leaf cut: mix sub well and select leaves at random until a 200 g portion is obtained. Composite 200 g from each sub and chop entire composite.
 - (2) Field cut (leaves attached to stalk): select bunches at random until 500 g portion is obtained. Composite 500 g from each sub and chop entire composite.

142.3 Portions of sample retained. Select three portions from total sample homogenate (142.21) and identify one as "original analysis", second as "check analysis", and third as "reserve" {for claimant.} Where fraction sample composites (142.22) and individual sub composites (142.23) have been prepared, retain prepared composite and reserve of sample. Seal and store all retained portions of sample in such manner as to prevent decomposition of product and residue. This requires that all products be frozen until findings of original analysis have been verified. The amount of composite retained is governed by extent of analysis required on sample. However, in no case should portions be less than one quart each (or for products of high density, one pound) for original, check, and reserve. Sample size for analysis is given in method.

142.4 Notes on compositing and comminuting.

(1) Using Hobart vertical cutter mixer. The 40 quart Hobart vertical cutter mixer was tested to determine its mixing and chopping ability. Varying quantities of several agricultural products were chopped for varying time intervals and resulting mixtures were checked for distribution and particle size. Based on this study, a minimum of 20 lbs. of compactly formed products (such as potatoes, beets, carrots, etc.) or a minimum of 1/2 bushel of loosely

formed products (such as cabbage, lettuce, greens, etc.) is recommended for

of chopper at least once during operation. (More, C.A., private communication, Food and Drug Adm., June, 1966).

(2) Blending eggs. Blend at low speed for minimum of five (5) min or until sample is homogeneous. Low speed blending will minimize foaming or "Whipping" of sample -

(3) Thawing frozen composites. Thaw frozen composites completely and remix before portion is taken for analysis. Any liquid phase separation that occurs in freezing or thawing must be reincorporated into composite before taking sample for analysis.

* (4) Grinding low moisture products, oilseeds, and other difficult samples. Grind samples to fine mesh (ca 20 mesh) in Ultra Centrifugal Mill (see 111) or equiv. Grind oil seeds first through a large sieve (3-5 mm), then regrind through a fine (<0.5 mm) sieve to minimize drag on the motor. Collect ground material in the 500-800 g capacity collecting pan and thoroughly mix several batches as necessary to provide appropriate sample size from which to take the analytical sample. (Sawyer, L.O., private communication, Food and Drug Admin., Jan., 1977.)

In the absence of a centrifugal mill grind samples through a Wiley mill or equiv., taking care to prevent physical separation of the product in the mill. A stepwise grinding procedure, in which sample is coarsely ground, then quartered down and a smaller portion ground to 20 mesh or smaller, may be necessary with some products. Loss of volatile pesticides can occur during grinding where heat is generated in process. Dry Ice has been used to precool mills before sample is ground.

It may also be advisable to grind materials such as hay through the Wiley mill prior to final grinding through the centrifugal mill.

* (5) Grinding of fish. To prevent the skin of fish from clogging the grinder during the preparation of fish samples, the fish may be frozen prior to grinding. Sample handling must be consistent with the directions given in 141.12c and 141.22b, in terms of the portion of the sample retained for analysis. A distinction must be made between (1) fish frozen by a processor for sale as frozen fish and (2) raw fish sampled by an inspector and frozen for preservation prior to analysis. In the former situation, the sample must be thawed and the drainings discarded, no matter what further handling is required for analytical sample preparation. In the latter case, no drainings should be discarded and fish need be thawed only enough to facilitate preparation of the analytical samples •

Prepare raw fish (or fish sampled raw and frozen by the inspector) as described in 141.12c, then freeze in portions of suitable size for introduction into the grinder.

Thaw frozen fillets and discard drainings as described in 141.22b. Then refreeze in portions of suitable size for introduction into the grinder •

Pesticide Analytical Manual -Vol. I
Foods and Feeds

GENERAL INFORMATION
Section 14: .4

Thaw whole fish frozen for sale as frozen fish and discard drainings, then prepare as described in 141.22b. Refreeze in portions of suitable size for introduction into the grinder. Grind immediately three times in a Hobart Food Cutter (or equiv.) with grinder attachment. {Thompson, T.D., private communication, Food and Drug Admin., Feb., 1976).

APPENDIX V-BPREPARATION OF SOME PROCESSED FOODS

<u>Food Item</u>	<u>Procedure</u>
Canned vegetables ¹	Examine homogeneous mixture of can contents. If liquid is normally consumed, include in sample; otherwise drain liquid before homogenizing.
Canned fruits ¹	Pit fruit if necessary; homogenize liquid medium and fruit.
Juices, frozen concentrate ¹	Do not dilute unless indicated; mix well.
Evaporated ¹ , and nonfat milk	Do not dilute unless indicated; mix well.
Powdered milk	Mix well; do not dilute.
Soups ¹	Do not dilute unless indicated; blend or mix well.
Meats, fish, poultry	Edible medium should be homogenized into sample, i.e., gravies, broths, and sauces are included in homogenate while water is discarded.
Beverages (ready to drink)	Do not dilute; mix well.
Coffee, tea	To extract consumable portion, brew as follows: <ul style="list-style-type: none"> - use DDW with recommended amount of coffee/tea for steeping - brew in acid-washed beaker in clean air environment - allow grounds/leaves to settle and decant brew into an acid-washed plastic bottle

Notes: 1. If canned foods are to be analyzed for Pb, use the homogenization procedure described in method EAM-17, "Sample Preparation Procedure for Determination of Lead in Canned Foods".

APPENDIX V-CPREPARATION OF RAW ANIMAL DERIVED FOODS

<u>Food Item</u>	<u>Procedure</u>
Chuck Roast	Remove bone and trim excess fat ¹
Ground Beef	Mix well
Pork Chop	Remove bone and trim excess fat ¹
Bacon	Remove rind if attached ¹
Chicken	Debone ¹
Fish Fillet	No prep ¹
Liver	No prep ¹
Ham	Remove bone and trim excess fat ¹
Round Steak	Remove bone and trim excess fat ¹
Veal	Remove bone if present and trim excess fat ¹
Lamb	Remove bone if present and trim excess fat ¹
Shrimp	Remove shell and devein ¹
Dairy Products	<p>Dairy products present a unique problem in that they are comprised of water-soluble and water-insoluble constituents, i.e., milkfat. These constituents have a tendency to separate upon storage. This tendency must be acknowledged and the sample homogenized to restore the original state.</p> <p>Milk requires reconstitution of butter fat (cream) if separation has taken place.</p> <p>Cheeses are also unique because they exist in a variety of types and textures. The very manner in which cheese is produced should provide a homogeneous product. Cheese is packed in containers usually of paraffin or plastic, which may or may not promote contamination; therefore analyst discretion is required.</p>

The following general guidelines for sampling/compositing of cheeses are suggested but not limiting upon the analyst (also see the Appendix V-A concerning sampling).

1. Depending upon the reason for analysis, a block of cheese may be sampled directly by:
 - a. Remove wrapper and cut away 1/8" to 1/4" of the cheese which was in contact with the wrapper. A stainless or plastic cutting device is used for this. The subsample may then be taken directly from the sample.
 - b. Several representative sub samples of the parent sample may be taken as in 1.a. and blended with an equal amount of deionized water. This slurry can be further homogenized with a Polytron.
2. Less firm cheeses, i.e., cream cheese, etc, may be sampled in the same way; however, mixing by hand devices may be substituted for the blenders. Analyst discretion is indicated.

Notes: 1. Chop in Hobart chopper to homogenize.

APPENDIX V-DPREPARATION OF SOME RAW AGRICULTURAL PRODUCTS

Food Item	Procedure
Sweet corn	Shuck and remove silk; rinse with DDW; air-dry in hood; slice corn from cob with plastic knife into clean plastic tub; proceed with compositing.
Lettuce	Wash each individual leaf with DDW; spin-dry in vegetable spinner; air-dry in hood; chop in coated food cutter and mix well; proceed with compositing. (Note: Any aqueous portion resulting from excess chopping must be included in the composite.)
Carrots	Scrub with vegetable brush under DDW; cut off ends and damaged portions with plastic knife; air-dry in hood; chop in coated Hobart food cutter and mix well; proceed with compositing.
Onions	Wash under DDW and remove outer skin; remove damaged layers and cut off ends with plastic knife; air-dry in hood; chop in food processor with plastic blade; proceed with compositing.
Potatoes	Scrub with vegetable brush under DDW; peel with coated peeler and remove damaged portions; air-dry in hood; chop in coated Hobart food cutter and mix well; proceed with compositing.
Spinach	Break off stems and remove damaged portions; place leaves in tube of DDW; dip and swirl; repeat with same leaves in two additional tubs of DDW; rinse under DDW and place in drainer; spin-dry in vegetable spinner and air-dry in hoods; chop in coated Hobart food cutter and mix well; proceed with compositing. (Note: Any aqueous portion resulting from excess chopping must be included in the composite.)

Cucumbers	Scrub with vegetable brush under DDW; cut off ends and damaged portions with plastic knife; chop in food processor with plastic blade; proceed with compositing.
Tomatoes	Wash under DDW; remove stem and damaged portions with plastic knife; chop in food processor with plastic blade; proceed with compositing.
Field corn	Remove husks, dry overnight in open plastic bag in drying oven at 50°C; remove corn from cob by hand; wash in plastic tub of DDW to remove dirt, silks; place corn in plastic sieve, rinse and dry in hood; proceed with compositing.
Soybeans	Place in heavy plastic bag; roll bag in hands to remove pods; separate from pods with plastic sieve; shell intact pods by hand; rinse in beaker to remove remaining pods, dirt; place in plastic sieve, rinse and air-dry in hood; place two representative portions of the sample in a plastic bag and store in freezer; homogenize by placing remainder of sample in blender and grinding to pass a 40-mesh sieve; transfer sample to plastic sample bottle.
Peanuts	Wash peanuts under DDW; place in plastic sieve and oven-dry at 50°C; remove shells on a piece of plastic. (Note: If sample is to be freeze-dried before compositing, leave sample on freeze dryer for only 2 or 3 days at which time all DDW added has been removed. This reduces problems with peanut oil during grinding. When grinding peanuts in blender, do not allow the blender to get warm, or the peanut oil problem will return.) Store peanut samples in freezer.

Wheat

Dry in plastic bag in drying oven at 50°C; place wheat in heavy plastic bag; roll bag in hands to separate from chaff; place wheat in beaker and use an air gun to separate the hulls or chaff. (Note: Perform in a glove box to contain dust.) Place wheat in beaker, add DDW and float away remaining hulls or chaff (the wheat should not remain in DDW any longer than necessary); transfer wheat to a plastic sieve, rinse and air-dry in hood; proceed with compositing.

VI SAMPLE MINERALIZATION

INTRODUCTION

In the analysis for an element in an organic matrix, it would be ideal if the analysis could be performed without requiring the destruction of the matrix. This would eliminate contamination from the reagents and apparatus used to destroy the matrix. There are a number of techniques for performing non-destructive analyses, such as neutron activation or X-ray fluorescence. However, the accessibility and/or sensitivity of these techniques in many cases is inadequate for our requirements. For the techniques available to us, almost all of the samples we encounter require some sort of treatment to prepare them for the determinative step. Some samples can be prepared by simply diluting them with water or some other reagent.

Acid hydrolysis, using either HNO_3 or HCl , produces more energy for the partial digestion of more complicated samples. The carbohydrate and fat portion of the samples are not usually destroyed, but analyses have shown that most trace elements are completely extracted into the aqueous phase.

The most common procedure for the complete destruction of organic material is by ashing, either wet or dry. Gorsuch (1) did a thorough study on this subject.

Problems encountered in the mineralizations of organic matrices include:

Contamination: Digestion vessels may introduce contamination either from dirty vessels or from the dissolution of the vessel itself. External contamination (dirty vessels) can be removed by acid cleaning. See Section III for further details. To overcome the problem of contamination by the dissolution of the vessel, consideration should be given to the vessel's composition and to the possible reaction with it by the reagents being used in the digestion. Quartz vessels (pure silica) are recommended for dry ashing at temperatures up to 600°C . Below these temperatures HNO_3 , H_2SO_4 and HCl acids have no action on quartz. However, alkali hydroxides and carbonates will etch quartz. If Si is not one of the elements of interest and does not interfere with the analysis reactions, the dissolution of the container is not of great importance.

Adsorption: In trace and ultratrace analysis there is always the possibility that the element being determined may combine with the wall of the vessel and may not be dissolved by the acids used to dissolve the ash. If the total amount of the element is very small to start with, the small amount that binds with the wall could introduce a significant error.

Coprecipitation: In a high acid solution the formation of a heavy precipitate can occlude ions of low concentration. The classic example is a sample with high Ca content (e.g., milk, bone, etc.) and the formation of insoluble calcium sulfate, causing the loss of Pb. If possible, the formation of precipitates should be avoided.

Volatilization Losses: If the element is in a volatile form or can be converted to a volatile form it can be lost during the oxidation process when the temperature exceeds the vaporization temperature. Mercury is quite volatile in almost any of its forms at the temperatures used for most ashing procedures. Special refluxing conditions are necessary to avoid its loss. Chlorine in the sample, in either the ionic or covalent form, can cause problems with metals such as Pb, Ge, and As. Lead chloride can be lost through sublimation well below its melting point of 501°C. By using an excess of HNO_3 in the original digestion, the chloride can be driven off. Care should be taken when ashing samples containing compounds such as polyvinyl chloride, which contain covalent chlorine. At the high temperature required to ash PVC, chloride ion is released, and may combine with and volatilize metals of interest.

A. PRIMARY ASHING TECHNIQUES

Table VI-1 presents a comparison between wet and dry ashing.

1. Wet Ashing

Wet ashing (digestion) is a procedure used to destroy organic material using oxidizing acids and/or hydrogen peroxide. The common reagents used in the oxidation are HNO_3 , H_2SO_4 , HClO_4 , and H_2O_2 . Each of the oxidizing agents have their own particular advantages and in many cases combinations of the reagents are used to resolve a particular problem.

Nitric acid has a low boiling point of 120°C and thus will have a tendency to boil off before the wet ashing is complete. This can be an advantage in that the excess acid can be boiled off before the determinative step. Nitric acid is often added to samples containing chlorides. The HNO_3 prevents the loss of the volatile metal chlorides by driving off the chlorides as nitrosyl chloride.

Sulfuric acid, which has a considerably higher boiling point of 338°C, is often added to HNO_3 to raise the boiling point of the wet ashing liquid. It also acts as an oxidizing and dehydrating agent. When used by itself, it has a tendency to char the sample and give a reducing condition, and can cause losses by the escape of volatile compounds of As and Se. The high boiling point of H_2SO_4 can cause removal problems at the end of the wet ashing. If the sample has a large amount of Ca (e.g., milk, cheese, etc.) the possible precipitation of insoluble CaSO_4 may also coprecipitate Pb.

Perchloric acid is often used in combination with other acids and is a strong oxidizing agent. Mixtures of HNO_3 , H_2SO_4 and HClO_4 are used in the difficult wet ashing of samples containing fat. Wet ashing with HClO_4 alone and in mixtures is covered extensively by G.F. Smith (2). The hazards involved are covered by G.F. Smith (2) and the Analytical Methods Committee (3). Both articles must be studied by all persons who intend to use HClO_4 . Also, see Appendix VI-A for protocol for use of HClO_4 .

Hydrogen peroxide is another strong oxidizing agent now available in high purity at strengths of 50% or more. An advantage is that the only decomposition product is water.

An excellent and comprehensive review on mineralization procedures is presented by N.T. Crosby (4).

TABLE VI - 1

COMPARISON OF WET AND DRY ASHING

WET ASHING - ADVANTAGES	DRY ASHING - DISADVANTAGES
<p>Low temperature minimizes volatilization losses.</p> <p>Liquid medium minimizes retention onto vessels.</p> <p>Methods generally adaptable to wide variety of sample matrices for many different elements.</p> <p>Requires short time frames.</p> <p>Simple apparatus.</p>	<p>High temperatures promote volatilization losses.</p> <p>Dry conditions promote retention losses.</p> <p>Methods must be tailored to specific sample matrix for specific elements.</p> <p>Requires long time frames.</p> <p>Specialized and expensive equipment for ultra trace analysis.</p>
DRY ASHING - ADVANTAGES	WET ASHING - DISADVANTAGES
<p>Large sample size.</p> <p>Small amounts or no reagents required.</p> <p>Very low blanks possible.</p> <p>Minimal supervision required.</p> <p>Large number of samples handled easily.</p>	<p>Smaller sample size.</p> <p>Large amounts of reagents required.</p> <p>Higher blanks.</p> <p>Constant supervision required.</p> <p>Fewer number of samples possible at a time.</p> <p>Coprecipitation losses.</p> <p>Some elements have volatility problems (As, Hg & Se).</p>

2. Dry Ashing

The term "dry ashing" is applied to a procedure in which organic matter is oxidized through the action of atmospheric O_2 at high temperatures. The objective of dry ashing is to oxidize and volatilize the organic matter while quantitatively retaining the elements of interest in a recoverable form. This must be accomplished without contamination. In the elemental analysis of foods and other biological material, the organic matter itself constitutes the principal source of interference.

Dry ashing involves four main steps: (a) dehydration -- usually at low temperature, i.e., 60°C to 120°C, (b) evaporation of volatile materials including those produced by the thermal cracking or partial oxidation -- usually at temperatures less than 350°C, (c) oxidation of the non-volatile residue until all organic matter is removed -- usually 450°C to 650°C for 4 to 24 hours, and (d) dissolution of the residue into an appropriate medium, usually dilute acid.

The first step, dehydration, is usually carried out in an oven at 60 to 120°C to drive off moisture. Precautions must be taken in this step to avoid losses thru spattering and volatilization. If all moisture is not driven off, spattering losses may occur at the higher temperatures of the next step. Most samples can be dried at 100 to 120°C until dehydrated. Although volatilization is rarely a problem at these temperatures, some samples contain elements in volatile form (e.g., $ZnCl_2$) that may be partially lost. If so, a lower drying temperature of 60 to 70°C may be appropriate with the addition of an ashing aid, as discussed later. The first step is completed when the sample is completely dry or free from moisture.

The second step, volatilization and partial oxidation, is usually carried out under an IR lamp or in a muffle furnace at less than 350°C. In this step, it is important to drive off oils, fats and other volatile and easily oxidized organics so they will not ignite in a later step at higher temperatures. Precautions must be taken in this step to avoid losses thru particle ejection, ignition, and volatilization. If this step proceeds too vigorously, particles of sample may be blown out, carried out with heavy smoke, or foam out of the ashing vessel. Also the sample may ignite and cause the temperature to rise uncontrollably with accompanying losses. Ashing aids that increase the ash bulk help to reduce particle loss and the hazard of ignition as discussed later. The second step is completed when only a small rate of smoke is evolved from the charred sample.

The third step, complete oxidation, is usually carried out in a muffle furnace at 450 to 650°C. Below 450°C many organics are not completely oxidized. Above 650°C many compounds that are otherwise considered non-volatile are lost. The three main problems with this step are losses through incomplete oxidation, volatilization, and adsorption or retention. Incomplete oxidation can cause losses by not freeing an organically bound element or by carbon particles adsorbing trace elements.

Most losses due to volatilization occur during this oxidation step. The majority of the metal volatiles are chlorides. If the metal is in the chloride form in the sample, then treatment with sulfating or nitrating ash aids will usually produce non-volatile forms. Metal chlorides can also be produced during the ashing process, however. Covalent chlorides (e.g., PVC) and some ionic chlorides (e.g., NH_4Cl) break down with heat to produce HCl , which can then react with many metals to form metal chlorides (e.g., PdCl_2 or ZnCl_2) which are volatile. The sulfating and nitrating aids will react with the ionic chlorides and evolve them at a low temperature (step 1), thus preventing volatilization losses. These aids do not work as successfully with covalent chlorides. A temperature of 450 to 500°C greatly reduces the volatilization problems. It is essential to keep the temperature of the furnace and sample at the lowest level consistent with complete oxidation in a reasonable time.

Retention losses refer to losses due to the element of interest being unavailable for dissolution because it remains affixed to the vessel or the ash. Elements with a positive electrode potential (e.g., Cd, Pb) can react with silica to form stable silicates. Elements with a negative electrode potential

(e.g., Cu, Ag, Au) are reduced and diffuse into the silica. These reduced elements can also alloy with Pt vessels. Retention losses increase with increasing temperature, and increase sharply beyond 550°C. Ionic chlorides (e.g., NaCl) promote retention losses in silica by weakening the silica bonds. Also low temperature glasses (phosphosilicates) can be formed which incorporate elements. Elements retained by any of these processes are very difficult to get into solution. The retention problem will be dealt with in greater depth during discussion of step 4. The third step is completed when no carbonaceous material (black particles) remains.

The fourth step, dissolution, is usually accomplished by dissolving the ash into a dilute acid. Frequently the acid solution is heated to assist dissolution. The main problem here is retention, i.e., the element of interest is not completely solubilized. Even though retained elements can be solubilized with extreme measures (e.g., HF), the object is to prevent retention in the first place. Retention is very temperature dependent and is much less of a problem below 500°C. Also ionic chlorides that promote retention can be eliminated by using sulfating or nitrating ashing aids. Ashing aids [e.g., $\text{Mg}(\text{NO}_3)_2$] that increase the bulk of the ash tend to dilute an element and reduce its contact with reactive surfaces. This is especially important for samples with very little ash. Platinum dishes prevent retention of positive electrode potential elements (e.g., Cd, Pb) onto the ashing vessel, although these elements may still react with silica within the sample. Avoid the use of H_3PO_4 because it tends to form phosphosilicate glasses with silica vessels. This step is completed when the sample ash is completely in solution. If the entire ash does not go into solution it is important to assure yourself that the element of interest is not being retained by the insoluble material.

B. ASHING AIDS

An ashing aid is a substance which is added to the sample in order to assist the oxidation process or to enhance the recovery of a particular element. An ashing aid can be added before or during the ashing process.

Ashing aids enable the sample to be modified to suit individual needs. Generally they should be added early in the ashing process and they should be mixed so as to make contact with all parts of the sample. The biggest problem with adding ashing aids is the possible contamination. The larger the amount of added aid, the larger the contamination may be.

1. Wet Ashing

A number of reagents are used with wet ashing procedures as catalysts and/or as oxidizing agents. Some of the more common reagents used are V_2O_5 , NH_4VO_3 , $KMnO_4$. They tend to shorten the time required for the sample to be completely oxidized and minimize analyte losses by completing the oxidization under milder condition. This is especially advantageous for As, Se, and Hg.

2. Dry Ashing

Sulfuric acid dehydrates and sulfates. It can sulfate volatile elements (e.g., chlorides of Pb, Cd, Zn), making them more heat stable and thereby reducing volatility. It also eliminates ionic chlorides through the release of HCl (e.g., $2NaCl + H_2SO_4 \rightarrow 2HCl + Na_2SO_4$) at low temperature. This reduces both retention and volatility losses. However, covalent chlorides are not eliminated as efficiently. The H_2SO_4 will slow down the rate of oxidation, but in doing so, it reduces the risk of ignition.

Nitric acid helps to oxidize and nitrate the sample. It nitrates volatile elements, making them more heat stable and eliminating ionic chlorides in a manner similar to H_2SO_4 . Most nitrates are heat stable but some $Cd(NO_3)_2$ is lost through volatilization, even at $500^\circ C$. If HNO_3 is added too early in the ashing process, deflagration may occur with accompanying losses. Nitric acid should be added when only small amounts of organic matter remain.

Magnesium nitrate helps to oxidize and nitrate the sample much as HNO_3 does. It also eliminates ionic chlorides in a manner similar to H_2SO_4 . Aids such as $Mg(NO_3)_2$ that add bulk to the final ash also help to dilute the sample ash and thereby reduce retention losses.

Potassium sulfate sulfates much as H_2SO_4 does. It also adds bulk to the final ash as $Mg(NO_3)_2$ does. Potassium sulfate does not volatilize chlorides as H_2SO_4 does.

Phosphoric acid enables retention through formation of phosphosilicate glass with silica and generally should be avoided for food analysis.

C. ASHING VESSELS

Wet ashings are usually carried out in small Kjeldahl flasks (e.g., 100 mL) or round-bottom flasks constructed of borosilicate glass. Quartz vessels are also used when borosilicate glass produces unacceptable levels of contamination (e.g., Al, Ti).

Dry ashings are usually carried out in beakers, dishes, or crucibles constructed of quartz or Pt. Borosilicate glass, Vycor, and glazed porcelain are used less often because of contamination problems and the low working temperature of glass. A discussion of the types of construction materials follows.

Vitreous silica vessels are >99.9% SiO_2 . Silica's common impurities are Na, Al, Fe, Mg and Te in trace levels or below. High purity quartz can be as low as 1 ppm in total metal contaminants. Silica resists etching by HNO_3 , H_2SO_4 and HCl up to 1000°C. Phosphoric acid, HF, alkali hydroxides and carbonates attack silica at elevated temperatures. Silica is not suitable for dry ashing samples containing high levels of K, Ca or Mg. Silica forms stable silicates with the oxide of many elements (e.g., Pb, Cd, Zn). Chlorides (e.g., NaCl) at high temperatures weaken the silica structure by replacing some of its oxygen, thereby increasing its reactivity with metal oxides. The previous history of a silica vessel has an effect on its retention. New vessels seem to be the worst retainers.

Platinum is usually alloyed with Ir and contains Cu and Fe as impurities. Platinum resists HCl, HF, molten halides and sulfates. It has a working temperature of up to 1400°C. The fused nitrates, nitrites, cyanides, oxides and hydroxides of Na and K attack Pt. Mixtures of HNO_3 and HCl will damage Pt. It can alloy with readily reducible metals (e.g., Cu, Ag, Au).

Vycor vessels are 95 to 96% SiO_2 and have about 3% B_2O_3 . Its main impurities are Na, K and Al. Vycor has fewer impurities than Pyrex glass but more than quartz. Vycor has about the same properties as quartz.

Borosilicate glass (Pyrex) has a low continuous-use working temperature of 500°C. Its major components are Si, Al, Na, K, Ca, B, and Ba as well as relatively high levels of Mg, Fe, Ti, As, and Sb. Any of its components can be etched out but the main contaminant is Na. Metals (e.g., Pb) can be adsorbed onto the glass and at a later time be desorbed into solution.

Glazed porcelain has a fused glaze coating on a much more refractory body. Its working temperature is 1100°C. The glaze determines its resistance and nature of contamination. It is usually too contaminated for use in trace and ultra-trace analyses. Lead and Cu are its main impurities.

D. OTHER TECHNIQUES

1. Low Temperature Ashing (LTA)

Low temperature ashing is a process whereby a gas plasma is generated by a high frequency electromagnetic field in oxygen, air or other gas to produce reactive atoms and radicals which react with organic material. Ideally, the reaction products are gaseous and these are pumped away during the ashing, leaving a dry ash, which contains all of the inorganic material in the sample (5).

With LTA complete destruction of organic material generally occurs below 100°C. Because of the relatively low temperature, loss of trace elements by volatilization or by reaction with the vessel is decreased or eliminated.

Low temperature ashing is not widely used at the present time because the instrumentation is expensive and the technique is time consuming and limited to small samples. However, since LTA is essentially a contamination-free technique, it may be more extensively used in the future to meet the demand for analyses at very low levels.

2. Microwave Oven

In a microwave oven, acid mixtures are heated internally by an oscillating electromagnetic field resulting in rapid digestion. The modifications to the commercial oven that are required are a glass jar to protect the oven from corrosion and an efficient exhaust for the elimination of acid fumes. One of the disadvantages of conventional wet ashing is that it requires close and constant operator attention. After the ashing parameters are established, unattended operation is possible with wet digestion being done in an inexpensive commercial microwave oven (6-8). Of course if HClO_4 is used, the normal safety precautions regarding its use must be followed.

3. Fusion

Conventional fusions involve the treatment of the sample with acidic or basic fluxes at fairly high temperatures before elemental determinations. Because of impurities from the crucible, the blank value is seldom sufficiently low or reproducible to permit reliable determination at submicrogram levels. However, one fusion procedure that has been used successfully to digest organic material involves heating the sample with an equimolar mixture of sodium and potassium nitrates before voltammetric determination of trace metals. The nitrate salts, in addition to destroying the organic matter, also become the supporting electrolyte when the residue is dissolved in water or dilute acid.

In practice, the sample is first ashed in a muffle furnace or predigested with HNO_3 to destroy the bulk of the organic material. The nitrate salts are then added and the temperature is gradually raised to about 400°C . As the nitrates melt, decompose, and release oxygen, the oxidation of the residual organic matter proceeds very rapidly. The residue is then dissolved in acid and heated to expel the carbonates and nitrates. A voltammetric determination, such as anodic stripping, is performed after the addition of a buffer, if necessary (8-11).

4. Teflon Decomposition Vessel

Many of the drawbacks of conventional wet ashings can be eliminated by the use of a closed-system Teflon decomposition vessel. If HNO_3 is used, the process is similar to the Carius digestion, which uses a heavy-walled sealed glass tube. A typical design of a Teflon vessel consists of a metal jacket containing a Teflon cup which is closed by a Teflon disk retained in a metal screw cap. There are several commercially available designs capable of withstanding pressures of about 1200 psi.

The procedure for digesting food samples in a 70 ml Teflon vessel is as follows: a sample, not exceeding 0.3 g on a dry weight basis is placed into the vessel, 5 ml HNO_3 is added, and the vessel is closed and placed into an oven at 150°C for 1-2 hours. After cooling, the contents can be transferred to a volumetric flask and diluted to volume for the required determinations. Note that if a smaller vessel is used, the maximum sample size must be proportionally smaller.

Some of the advantages of this procedure are: (a) volatilization losses are eliminated; (b) only a small volume of acid is required; (c) close analyst attention is not required; and (d) contamination problems are minimized. For safety reasons it is important not to overload the vessel. Numerous published applications for Teflon vessels are summarized in Reference (12).

E. SPECIAL CONSIDERATIONS BY ELEMENT

1. Arsenic

Wet ashing with H_2SO_4 , HNO_3 and HClO_4 has been used successfully to digest samples before the determination of As by atomic absorption spectrophotometry using the hydride technique. It is important not to allow the mixture to char because volatility losses of As may occur, especially in the presence of covalent chlorine.

Dry ashing oxidation can generally be carried out successfully in the presence of an ashing-aid, particularly $\text{Mg}(\text{NO}_3)_2$.

2. Cadmium

Wet ashing procedures have been widely used with no difficulty for the decomposition of a variety of samples for the determination of Cd. There are problems, however, when dry ashing procedures are used, resulting in Cd losses at a temperature as low as 500°C . The addition of HNO_3 to speed up the oxidation makes the losses even greater.

3. Calcium

The destruction of organic matter before the determination of Ca is generally a straightforward procedure with no difficulties. Wet or dry ashing procedures can be used. For large amounts of Ca, H_2SO_4 and sulfate ashing aids should be avoided because of low solubility of CaSO_4 .

4. Chromium

The destruction of organic matter prior to the determination of Cr is generally a straightforward procedure. Wet or dry ashing procedures can be used. Losses of Cr by its retention on silica during dry ashing have been reported, as well as losses of Cr during wet ashing by the formation of volatile chromyl chloride.

5. Copper

Wet ashing procedures for Cu have largely been successful. However, losses of Cu have occurred in dry ashing procedures. The only losses of significance are those arising from the retention of Cu on a solid material, either on the ashing vessel itself or the inorganic components in the sample. This type of interaction can be reduced by the use of low temperatures and ashing aids, particularly $\text{Mg}(\text{NO}_3)_2$.

6. Fluorine

Dry ashing at 450-550°C is performed with an alkaline ashing aid, usually $\text{Ca}(\text{OH})_2$ as a F fixative. Losses of F become significant above 550°C. Fluorine in the ionic form can be determined directly by fluoride ion selective electrode.

7. Iron

Hydrochloric acid hydrolytic procedures have been used successfully. Wet or dry ashing may be used when complete destruction of organic matter is required. If H_2SO_4 is used, it should be kept in mind that anhydrous $\text{Fe}_2(\text{SO}_4)_3$ in the ash dissolves very slowly when dilute acids are used to dissolve the ash. Another possible source of difficulty is in samples containing phosphates, due to the formation of complex polyphosphates that tie up the Fe. Dissolving the ash in HCl and evaporating to dryness on a steam bath will hydrolyze the phosphates, allowing the residue to redissolve.

8. Mercury

Due to the volatility of Hg compounds, dry ashing procedures cannot be used and special precautions must be taken with wet ashing. Most methods use HNO_3 and H_2SO_4 acid mixtures under reflux conditions. These mixtures do not digest all the fat.

9. Iodine

Both wet and dry ashing procedures have been used. Because I is volatile, steps must be taken to prevent volatility losses. The use of an alkali ashing aid at the lowest temperature possible is required for dry ashing for I. Temperatures below 450°C are recommended. Wet ashing can be performed in the presence of large amounts of oxidizing agents such as chromic-sulfuric acid or permanganate-sulfuric acid.

10. Potassium

Wet or dry-ashing procedures may be used for K determination, but acid hydrolytic procedures appear to be most easily applied. Potassium losses at ashing temperatures of 450° to 500°C due to reactions with silica or borosilicate glass have been reported. If chlorides are present, H_2SO_4 should be added to convert the volatile chloride to the sulfate. Wet ashing procedures for K give uniformly good results with any of the common acids. The choice of a method depends on the nature of the sample.

11. Manganese

Both wet and dry ashings have been used. Wet ashing procedures for Mn using mixtures of HNO_3 , HClO_4 , H_2SO_4 and/or H_2O_2 have been employed with no difficulties. Dry ashing at temperatures not higher than 500-550°C is advisable. The low temperatures will avoid the problem of reactions with silica vessels.

12. Molybdenum

Wet or dry ashing procedures may be used for Mo determinations. Wet ashing procedures give uniformly good results with any of the commonly used acid mixtures. Dry ashing procedures with and without ashing aids and at temperatures up to 550°C have been found satisfactory. As temperatures are raised above 550°C, however, losses may be due to retention of Mo on silica. The retention is promoted by the presence of chlorides at high temperatures. The use of HNO_3 as an ashing aid may cause losses of Mo.

13. Sodium

Wet or dry ashing procedures can be used for Na determinations. Acid hydrolytic methods and simple water dissolution have also given good results. Wet ashing procedures give uniformly good results with any of the commonly used acid mixtures. Quartz vessels should be used for wet ashing but the high-Na borosilicate vessels may be used if the Na contaminant level is low compared to the level of Na in the sample. Dry-ashing procedures with and without ashing aids and at temperatures up to 550°C have been found satisfactory. Losses of Na due to volatilization have been reported. Volatilization losses can be controlled by treating the sample with H_2SO_4 and keeping the temperature below 550°C. Losses of low level Na can also occur because of retention by silica.

14. Phosphorus

Wet ashing procedures for P give uniformly good results with any of the commonly used acid mixtures. Dry ashing with and without ashing aids and at temperatures up to 550°C have been found satisfactory. For a sample to be successfully dry ashed without an ashing aid, the resultant ash must be alkaline.

15. Lead

Wet ashing procedures for Pb give uniformly good results with any of the commonly used acid mixtures if certain precautions are observed. The presence of H_2SO_4 may result in precipitation losses, because H_2SO_4 forms insoluble compounds with alkaline earth elements, which may coprecipitate Pb. In the presence of large amounts of Ca it is best to avoid H_2SO_4 if possible. Lead contamination in reagents as well as glassware can contribute significantly to errors in trace analyses. Dry ashing procedures with and without ashing aids at temperatures from 450 to 550°C have been used successfully, but problems have been reported in some cases. Losses of Pb due to volatilization and retention have been reported. The presence of covalent as well as ionic chlorine makes Pb losses even greater. Lead losses can also occur due to reaction of lead oxide with silica. Ashing at a temperature not exceeding 500°C with ashing aids generally gives good results.

16. Selenium

Wet ashing procedures are used for Se determinations because losses occur when dry ashing procedures are used. Losses due to Se volatilization have been reported for samples dried at 100°C. Wet ashing procedures give good results if the reaction is carefully controlled and oxidizing conditions are maintained throughout the digestion.

17. Zinc

Wet or dry ashing procedures may be used for Zn determinations. Acid hydrolytic methods have also given good results. Dry ashing procedures with and without ashing aids at temperatures from 450 to 900°C have been used but there are some problems. Losses of Zn due to volatilization and retention have been reported. The presence of chloride appears to cause the problems. In the absence of a high amount of chlorides, good results can be obtained by ashing at or below 500°C. If a large amount of chloride is present, wet ashing procedures should be considered.

REFERENCES

- (1) Gorsuch, T.T. (1970) The Destruction of Organic Matter, Pergamon Press, Oxford, UK
- (2) Smith, G.F. (1965) The Wet Chemical Oxidation of Organic Compositions Employing Perchloric Acid, The G. Frederick Smith Chemical Co, Inc., Columbus, OH 43223
- (3) Anal. Methods Committee (1959) Analyst 84, 214-216
- (4) Crosby, N.T. (1977) Analyst 102, 255-268
- (5) Technical Paper T7320, International Plasma Corporation Hayward, CA 94544
- (6) Abu-Sama, A., Morris, J.S., & Koirtyohann, S.R. (1975) Anal. Chem. 47, 1475-1477
- (7) Barrett, P., Davidowski, Jr., L.J., Panaro, K.W., & Copeland, T.R. (1978) Anal. Chem. 50, 1021-1023
- (8) Bowen, H.J.M. (1968) Anal. Chem. 40, 969-970
- (9) Holak, W. (1977) J. Assoc. Off. Anal. Chem. 60, 239-240
- (10) Holak, W. (1975) J. Assoc. Off. Anal. Chem. 58, 777-780
- (11) Chittleborough, G. & Steel, B.J. (1980) Anal. Chem. Acta 119, 235-241
- (12) "A Literature Study on Applications of Uniseal Decomposition Vessels in Chemical Analysis by AAS and Other Instrumental Methods 1968-1977". Uniseal Decomposition Vessels, LTD, P.O. Box 9463, Haifa, Isreal, IFU 7602

APPENDIX VI-A

The following was taken from the FDA Office of Physical Sciences SAFETY GUIDE 1980 pages 6-12 to 6-14.

PERCHLORIC ACID PROTOCOL

Perchloric acid (HClO_4) has the potential to be a very hazardous substance. Many severe accidents have been reported involving, for example, digestions, extractions, or accidental spills or leaks resulting in contact of HClO_4 with oxidizable organic materials. The chances of accidents such as these can be minimized if certain basic rules are followed. If the answer to any of the following questions is "yes", the procedure should conform to the recommended standards:

1. Is the work with perchloric acid likely to be continuing rather than occasional and infrequent?
2. Will the use of perchloric acid involve the use of heat?
3. Is it intended to use perchloric acid more concentrated than the 72% azeotrope?

The hazards of perchloric acid solutions are described in the Manufacturing Chemists' Association Chemical Safety Data Sheet SD-11 as follows:

1. Perchloric acid is a strong acid, and contact with the skin, eyes, or respiratory tract will produce severe burns.
2. Perchloric acid is a colorless, fuming, oily liquid. When cold its properties are those of a strong acid, but when hot, the concentrated acid acts as a strong oxidizing agent.
3. Aqueous perchloric acid can cause violent explosions if misused, or when in concentrations greater than the normal commercial strength (72%).
4. Anhydrous perchloric acid is unstable even at room temperature and ultimately decomposes spontaneously with violent explosion. Contact with oxidizable material can cause immediate explosion.

The following are listed among the causes of fires and explosions involving perchloric acid.

1. The instability of aqueous or of pure anhydrous perchloric acid under various conditions.
2. The dehydration of aqueous perchloric acid by contact with dehydrating agents such as concentrated sulfuric acid, phosphorus pentoxide or acetic anhydride.
3. The reaction of perchloric acid with other substances to form unstable materials (for example, with hydroxylic compounds to form the very unstable esters, with metals or metal salts, such as Ag^+ , Sb^{+3} or Bi^{+3} , to form extremely dangerous metal perchlorates or with strong reducing agents).

Combustible materials, such as sawdust, excelsior, wood, paper, burlap bags, cotton waste, rags, grease, oil and most organic compounds, contaminated with perchloric acid solutions are highly flammable and dangerous. Such materials may explode on heating, in contact with flame, by impact or friction, or may ignite spontaneously.

Recommendations for the Safe Handling of Perchloric Acid

Several organizations have drawn up recommendations for the safe handling of perchloric acid, among them the Association of Official Analytical Chemists, the Factory Mutual Engineering Division, and the Association of Casualty and Surety Companies. The recommendations from these and other sources are combined and summarized below.

Building Design and Equipment

Building design should be such that HClO_4 cannot easily come into contact with readily oxidizable materials such as wood or waxed floors (concrete, masonry, or tile is recommended).

Benches, cabinets and shelves should be of HClO_4 -resistent materials and be non-absorbent.

Total wash-down perchloric acid hoods are preferred. Where this is not possible, a good vacuum scrubber system will avoid contamination of regular hoods used for HClO_4 work.

HClO₄ Storage

The amount of HClO₄ stored in the lab should be the minimum and in no case more than two (2) 7-lb bottles. The bottle of acid should be stored in a fume hood in a vessel which will not react with HClO₄ and will hold all the HClO₄ in case of breakage (glass preferred).

Acid Handling

1. Whenever any operation involving perchloric acid is contemplated, prior clearance should be obtained from the Bureau Safety Officer.
2. Use goggles for eye protection whenever the acid is handled and shields or barriers where required to guard against the possibility of an incident due to failure of the apparatus or other causes.
3. Always transfer acid over a sink to catch any spills and afford a ready means of disposal.
4. In wet combustions with perchloric acid, treat the sample first with HNO₃ to destroy easily oxidizable matter. Nitric acid should be added before the perchloric acid and definitely before evaporation to fumes. The effect of HNO₃ is to moderate the reaction by oxidizing the more reactive components at lower temperatures. It is also acceptable to digest small amounts of low-fat organic material (0.5 g or less of sample) with a 1:5 mixture of HClO₄:HNO₃ (vol:vol) at room temperature for 18-24 hours before heating and final digestion. Never allow perchloric acid solutions to evaporate to dryness.
5. Any procedure involving heating of the HClO₄ must be conducted in a ventilated hood.
6. No organic materials should be stored in the perchloric acid hood.
7. Do not allow HClO₄ to come in contact with strong dehydrating agents (concentrated H₂SO₄, anhydrous P₂O₅, etc.) except where H₂SO₄ is required as part of a validated analytical procedure. Wherever possible, an excess of water should be present to prevent an accumulation of unstable organic perchlorates and to moderate temperature rises.

8. Perchloric acid should be used only in well documented analytical procedures from well recognized analytical sources. Check procedures with a minimum quantity of sample to ensure compatibility with the perchloric acid system.

Acid Disposal

SPILLS. Perchloric acid spilled on the floor or bench top presents a hazard. It should not be mopped up, nor should dry combustibles be used to soak up the acid. The spilled acid should first be neutralized and then soaked up with Vermiculite absorbent. The contaminated absorbent must be kept wet to prevent combustion upon drying. It should be placed in a plastic bag and sealed and the Safety Office called for disposal. If the spill can be rinsed down a chemical drain, neutralization of the wetted area is recommended followed by additional rinsing.

DISPOSAL. A small amount of perchloric acid can be dumped into a sink and flushed down with at least 20 times its volume of water.

VII SEPARATION, PRECONCENTRATION, AND SPECIATION

Before attempting to solve an analytical problem, the analytical chemist must answer several questions:

- What type of information is needed from the analysis?
- What method/instrument is best suited to provide this information?
- What is the sample matrix and will it interfere with the determination?
- Is specific information needed on the form of the analyte in the analytical samples?
- What upfront chemistry must be performed in order to obtain this information?
- Can it be done?

Generally, the sample matrix dictates the methodology/instrumentation that is best suited for the analysis. For example, suppose a sample containing high levels of alkaline earth elements is to be analyzed for lead after mineralization. Analysis of the sample ash solution may be extremely difficult to perform directly by atomic absorption/emission spectrometry, whereas analysis by polarographic techniques would be relatively easy. If in addition to the matrix problem, this same sample contained only an ultratrace concentration of lead, a preconcentration step may be necessary. This could be accomplished electrochemically by using stripping voltammetry, where the analyte is preconcentrated in the mercury drop. On the other hand, the spectroscopic determination would require that some additional chemistry be performed. Chelation/extraction, ion-exchange resins, and chelating ion-exchange resins (all described briefly below) can be used to eliminate chemical interferences and to achieve an increase in concentration.

Sample mineralization followed by analysis using either of the above instrumental techniques provides information on total analyte present in the original sample. When specific information is needed for toxicological reasons on the oxidation state of the analyte, or on its chemical form (oxidation state and ligands), a more challenging problem is posed. Determining

the oxidation state of a metal ion and/or identifying the ligands bound to it are generally referred to as "speciation". Sample mineralization techniques can no longer be used since they will most assuredly change the form of the analyte. The same problem

can arise in many acid/base extractions, where the redox properties of the solvent system must be considered. Some extractions coupled with chromatography (GC, HPLC) and followed by spectroscopic, electrochemical, or mass spectrometric detection have shown potential in this area. However, methodology for most elements in matrices other than aqueous solutions does not presently exist (1,2). A great deal of future research effort is expected to be concentrated in the area of speciation.

A. CHELATION/EXTRACTION

The most commonly used interference elimination technique is formation of a metal chelate which may be subsequently concentrated by extraction into a non-polar solvent (3-6). For example, if an aqueous solution contains two metal ions, one which interferes with the determination of the other, and that solution is equilibrated with an organic solvent containing a chelate which complexes only one of the metal ions, the metal ions can be separated. Commonly used chelating agents included dithiocarbamates, diketones, diphenylthiocarbazone, and quinolin-8-ol. Acetyl acetone is an example of a chelating agent which can also be the organic phase. When a smaller volume of organic solvent than aqueous solvent is used, a concentration increase can be achieved.

B. ION-EXCHANGE RESINS

Ion-exchange resins can be used for the removal of interfering cations and anions, and for the concentration of trace amounts of analyte from large volumes of sample (6,7). A strongly basic anion exchanger is used in the separation of anions of acidic, basic and neutral salts. A strongly acidic cation exchanger is used for concentration and separation of cations from the salts of strong and weak bases for determination.

C. CHELATING ION-EXCHANGE RESINS

Chelating ion-exchange resins have increased specificity and decreased capacity as compared to conventional ion-exchangers. These consist of chelating ligands such as dithiocarbamate or iminodiacetate (Chelex 100) attached to a solid support (8,9). Polydithiocarbamate resin has been used to separate transition elements of interest from alkali and alkaline earth elements (10,11). Chelating ion-exchange resins might also play a role in speciation (12).

REFERENCES

- (1) Kirkland, J.J. (1971) Modern Practice of Liquid Chromatography, Wiley-Interscience, New York, NY
- (2) Lawrence, J.F. (1982) Liquid Chromatography in Environmental Analysis, Hamano Press, Inc., Clifton, NJ
- (3) Stary, J. (1964) The Solvent Extraction of Metal Chelates, MacMillan, New York, NY
- (4) Morrison, G.H., & Freiser, H. (1957) Solvent Extractions in Analytical Chemistry, John Wiley & Sons, Inc., New York, NY
- (5) Zolotov, Y.A. (1970) Extraction of Chelate Compounds, Ann Arbor-Humphrey Science Publishers, Ann Arbor, MI
- (6) Komarek, J. & Sommer, L. (1982) Talanta 29, 159-166
- (7) Samuelson, O. (1963) Ion Exchange Separations in Analytical Chemistry, John Wiley & Sons, Inc., New York, NY
- (8) Biechler, D.G. (1965) Anal. Chem. 37, 1054-1055
- (9) Baetz, R.A., & Kenner, C.T. (1973) J. Agric. Food Chem. 21, 436-440
- (10) Barnes, R.M. & Genna, J.S. (1979) Anal. Chem. 51, 1065-1070
- (11) Jones, J.W., Capar, S.G., & O'Haver, T.C. (1982) Analyst 107, 353-377
- (12) Miyazaki, A., & Barnes, R.M. (1981) Anal. Chem. 53, 299-304

VIII INSTRUMENTATION

INTRODUCTION

This section provides a general discussion of the types of analytical instruments being used by the FDA for elemental analyses. In addition and where applicable, some examples of periodic diagnostic routines designed to ensure consistent and reliable instrument performance are also included for use with each type of instrument.

A. ATOMIC ABSORPTION SPECTROSCOPY (AAS)

Atomic absorption (1-3) is the process that occurs when a ground state atom absorbs energy in the form of light at a specific wavelength and is elevated to an excited state. The amount of light energy absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of the analyte present in known standards can be used to determine unknown concentrations by measuring the amount of light they absorb. Instrument readouts can be calibrated to display concentrations directly.

The basic instrumentation for AAS requires a primary light source, an atom source, a monochromator to isolate the specific wavelength of light to be used, a detector to measure the light accurately, electronics to treat the signal, and a data logging device to show the results. The light source used is either a hollow cathode lamp or an electrodeless discharge lamp.

The atom source used must produce free atoms of analyte material from the sample. The source of energy for free atom production is heat, most commonly in the form of an air-acetylene or nitrous oxide-acetylene flame or a graphite furnace. The sample is introduced as an aerosol into the flame, or as a liquid or solid into the graphite furnace. The flame burner head or furnace is aligned so that the spectrophotometer light passes through the flame or graphite furnace where the atomic absorption takes place.

Other sampling systems included hydride generation (4) for elements such as arsenic and selenium and the flameless mercury technique.

B. ATOMIC EMISSION SPECTROSCOPY (AES)

Atomic emission (2) is a process whereby the light emitted by excited atoms or ions is measured. The emission occurs when sufficient thermal or electrical energy is available to excite a free atom or ion to an unstable energy state. Light is emitted when the atom or ion returns to its stable configuration or the ground state. The wavelengths of light emitted are specific to the elements which are present in the sample.

The instrumentation used for AES is very similar to that used for AAS with the basic difference being that no primary light source is used for AES. The most critical component for AES instruments is the atomization source. The earliest energy sources for excitation were simple flames. Later, electrothermal sources such as arc/spark systems were used, particularly for analyzing solid samples. These sources are extremely useful for doing qualitative and quantitative work with solid samples, but are expensive, difficult to use, and have limited applications. Air-acetylene and nitrous oxide-acetylene flames were also used, but often lacked sufficient thermal energy to be truly effective sources. Consequently, until recently AES has not enjoyed the universal popularity of AAS during the past 15 years.

1. Inductively Coupled Plasma (ICAP)

A recent source used for AES is the inductively coupled argon plasma (5,6) which has eliminated many of the problems associated with past emission sources and has caused a dramatic increase in emission spectroscopy utilization. An ICAP is maintained by the interaction of a radio frequency field and an ionized argon gas. The plasma is reported to reach temperatures as high as 10,000°K, with useful temperatures between 5500 and 8000°K. These temperatures allow complete atomization of elements, thus minimizing chemical interference effects.

The plasma is formed by a tangential stream of argon gas flowing between the outer two quartz tubes of the torch assembly. Radio frequency power is applied through the coil, and an oscillating magnetic field is formed. The plasma is created when the argon is made conductive by exposure to the electron seeding discharge of a tesla coil. Inside the induced magnetic field electrons and ions are forced to flow in a closed annular path. As they meet resistance to their flow, heating takes place and additional ionization occurs. The process occurs almost instantaneously, and the plasma expands to its full dimensions.

In addition, the plasma has a doughnut shape. The sample is injected as an aerosol through the center of the doughnut. This phenomenon has the following advantages: It confines the sample to a narrow region, it provides an optically thin emission source and a chemically inert atmosphere, and it minimizes interactions which can interfere with an analysis. Argon is used as the carrier gas for the sample.

2. Direct Current Plasma (DCP)

A direct current plasma jet (7) is a flowing-gas-stabilized electrical discharge (normally inert gases are used), which is maintained by a core consisting of a continuous direct current arc. It possesses jet dynamic characteristics around and beyond the electrodes, which produce a plasma of low background luminosity and high temperature, suitable for atomic emission.

C. POLAROGRAPHY

Polarography (8-11) is an electrochemical method of analysis based on the measurement of the current flow resulting from the electrolysis of a solution at a polarizable microelectrode, as a function of an applied voltage. The polarogram obtained by this measurement provides qualitative and quantitative information on electro-reducible and electro-oxidizable substances. The normal concentration range for substances being analyzed is from 10^{-2} to 10^{-5} molar.

1. Direct current polarography (DC)

In direct current/polarography, the microelectrode is a dropping mercury electrode (DME) consisting of small reproducible drops of mercury flowing from the orifice of a capillary tube connected to a mercury reservoir. A saturated calomel electrode (SCE) with a large surface area is the most commonly used reference electrode. As the voltage applied to the cell increases, only a very small residual current flows until the substance under assay undergoes reduction or oxidation. Then the current increases, at first gradually, then almost linearly with voltage, then levels off and gradually reaches a limiting value. On the initial rising portion of the polarographic wave, the increased flow of current results in a decrease in the concentration of the electro-active species at the electrode surface. As the voltage and current increase, the concentration of the reactive species decreases further to a minimal value at the electrode surface. The current is then limited by the rate at which the reacting species can diffuse from the bulk of the

solution to the surface of the microelectrode. The final current rise is caused by the reaction of supporting electrolyte. This large concentration of electrolyte is inert within the potential range used in the analysis, and limits the amount of reactive species that reaches the electrode by electrical migration, thus assuring that the limiting current is diffusion-controlled.

Since, in the case of the DME, the electrode surface is being constantly renewed in a cyclic fashion, the current increases from a small value as the drop begins to form to a maximum value as the drop falls. By the use of a suitable recorder to measure the current, the characteristic saw-tooth record is obtained. The limiting current is the sum of the residual and the diffusion currents. The residual current is subtracted from the limiting current to give the wave height.

The half-wave potential ($E_{1/2}$) occurs at the point on the polarogram one-half the distance between the residual current and the limiting current plateau. This potential is characteristic of the electro-active species and is largely independent of its concentration or the capillary used to obtain the wave. It is dependent upon the solution composition and may change with variations in the pH or in the solvent system or with the addition of complexing agents. The half-wave potential thus serves as a criterion for the qualitative identification of a substance.

The potential of the DME is equal to the applied voltage versus the reference electrode after correction for the iR drop (that potential needed to pass a current, i , through the solution with resistance R). It is especially important to make this correction for nonaqueous solutions, which ordinarily possess high resistance, if an accurate potential for the DME is needed. Correction of the half-wave potential is not required for quantitative analysis. Unless otherwise indicated, it is to be understood that potentials represent measurements made against the SCE.

2. Pulse polarography

In conventional dc polarography, the current is measured continuously as potential is applied as a linear ramp. This current has two components. One is the diffusion (faradaic) current which is produced by the substance undergoing reduction or oxidation at the working electrode. It is directly proportional the concentration of this substance. The second component is the capacitive current (charging of the electrochemical double layer). The changes in these currents as the mercury drop varies in size produce the oscillations present in typical dc polarograms.

In normal pulse polarography, a potential pulse is applied to the mercury electrode near the end of the drop life, with the drop being held at the initial potential during growth period. Each succeeding drop has a slightly higher pulse applied to it, with the rate of increase being determined by the selected scan rate. The current is measured at the end of the pulse where the capacitive current is nearly zero, and thus primarily faradaic current is measured. In addition, since the pulse is applied for only a short duration, the diffusion layer is not depleted as extensively as in dc polarography and larger current levels are obtained for equivalent concentrations. Concentrations as low as 10^{-6} M can be measured, providing approximately a ten-fold increase in sensitivity over that with dc polarography. Limiting current values are more easily measured, since the waves are free from oscillations.

Differential pulse polarography is a technique whereby a fixed-height pulse applied at the end of the life of each drop is superimposed on a linear increasing dc ramp. Current flow is measured just before application of the pulse and again at the end of the pulse. The difference between these two currents is measured and presented to the recorder. Such a differential signal provides a curve approximating the derivative of the polarographic wave, and gives a peak presentation. The peak potential is equivalent to $E_{1/2} - \frac{1}{2}E$, where E is the pulse height. The peak height is directly proportional to the concentration at constant scan rates and constant pulse heights. This technique is especially sensitive (levels of 10^{-7} M may be determined) and affords improved resolution between closely spaced waves.

3. Anodic stripping voltammetry (ASV)

Anodic stripping voltammetry is an electrochemical technique whereby trace amounts of substances in solution are concentrated (reduced) onto an electrode and then stripped (oxidized) back into solution by scanning the applied voltage anodically. The measurement of the current flow as a function of this voltage and scanning rate provides qualitative and quantitative information on such substances. The concentration step permits analyses at 10^{-7} M to 10^{-9} M levels.

Basic instrumentation includes a voltage ramp generator; current-measuring circuitry; a cell with working, reference, and counter electrodes; a recorder or other read-out device. Instruments having dc or pulse-polarographic capabilities are generally quite adequate for stripping applications. The working electrode commonly used is the hanging mercury drop electrode (HMDE), although the mercury thin-film electrode (MTFE) has

acquired acceptance. Analysis for metals whose oxidation potentials are more positive than mercury, such as silver, platinum, gold, and mercury itself, requires use of solid electrodes such as platinum, gold, or carbon. A saturated calomel electrode or a silver-silver chloride electrode serves as the reference electrode except in analysis for mercury or silver. A platinum wire is commonly employed as the counter electrode.

Test specimens containing suitable electrolyte are pipetted into the cell. Dissolved oxygen is removed by bubbling nitrogen through the cell for 5 to 10 minutes.

Generally, an electrolysis potential equivalent to 200 to 300 mV more negative than the half-wave potential of the species to be analyzed for is applied (the optimum electrolysis potential should be determined experimentally) with stirring for 1 to 10 minutes. For reproducible results, constant conditions must be maintained (i.e., deposition time, stirring rate, temperature, specimen volume, and drop size if HMDE is used).

After deposition, the stirring is discontinued and the solution and electrode are allowed to equilibrate for a short period. The potential is then scanned anodically (10 mV/second or greater in dc-ASV and 5 mV/second in differential pulse-ASV). As in polarography, the limiting current is proportional to concentration of the species (wave height in dc and pulse: peak height in differential pulse), while the half-wave potential (dc, pulse) or peak potential (differential pulse) identifies the species. It is imperative that the choice of supporting electrolyte be made carefully in order to obtain satisfactory behavior. Quantitation is usually achieved by a standard addition or calibration technique.

This technique is appropriate for trace-metal analysis, but has limited use in organic determinations, since many of these reactions are irreversible. In analyzing for substances such as chloride, cathodic stripping voltammetry may be used. The technique is the same as anodic stripping voltammetry, except that the substance is deposited anodically and then stripped by a cathodic voltage scan.

D. POTENTIOMETRY

Ion Selective Electrodes (ISE) consist of a semi-permeable membrane which responds mainly to a single ion. When the membrane is in contact with a solution of that ion, an electrode potential develops across the membrane. This potential, which depends on the level of the free ion in solution, is measured against a constant reference potential with a digital pH/mv meter

Figure VIII-1. Example of calculation using Gran's Plot paper.

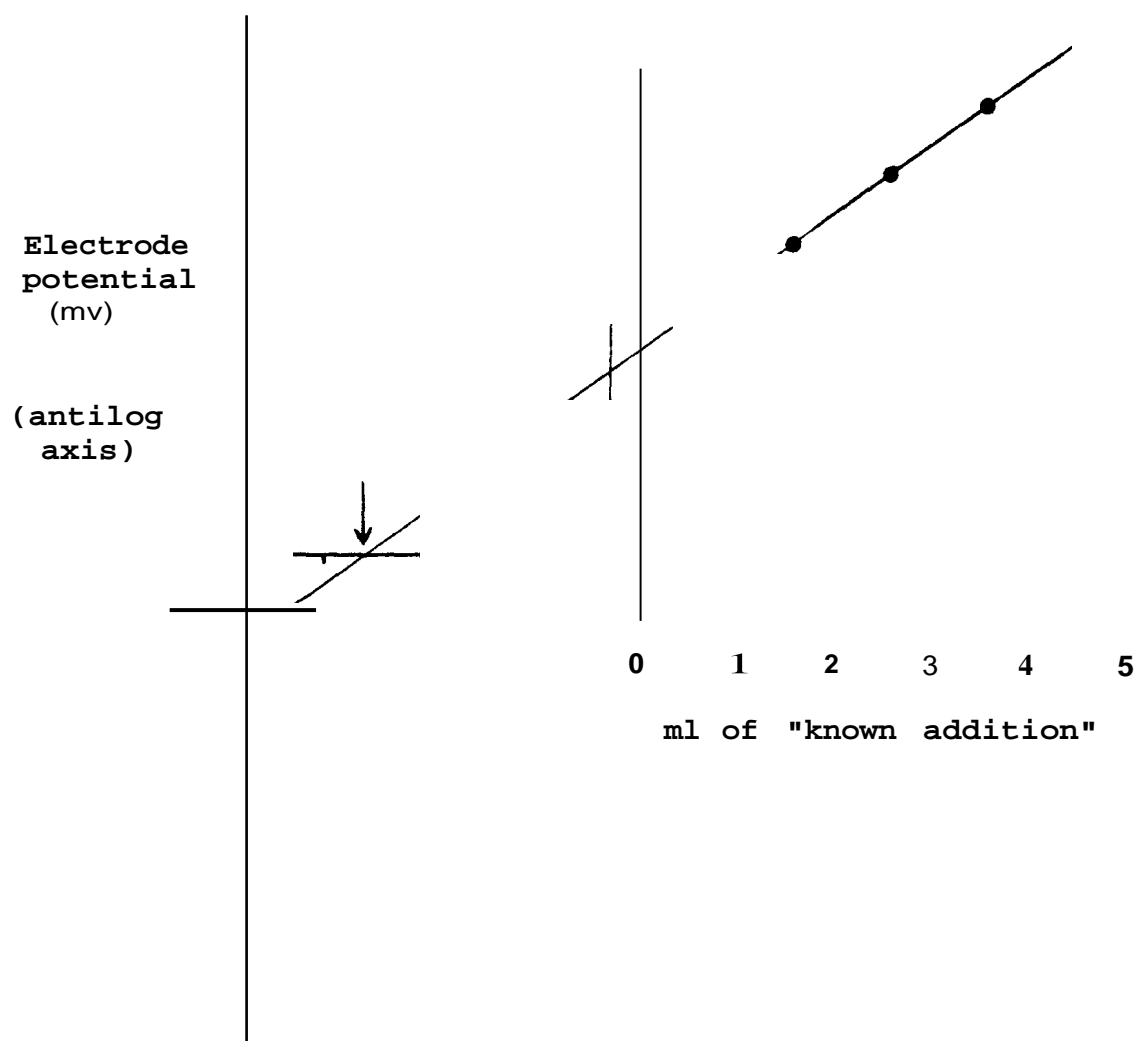


Table VIII - 1
Common Interferences with Ion Specific electrodes(12)

electrode	interferences	notes
ammonia (NH ₃) (ammonium) (NH ₄ ⁺)	volatile amines	measures organic N after Kjeldahl digestion, NO ₃ ⁻ after reduction to NH ₃
bromide (Br ⁻)	max level: S=10 ⁻⁷ M; 2 x 10 ⁻⁷ I ⁻ ; 8 x 10 ⁻⁸ CN ⁻ , high levels of Cl ⁻ and NH ₄ ⁺	
cadmium (Cd ²⁺)	max level: Ag ⁺ , Hg ²⁺ , Cu ²⁺ 10 ⁻⁷ M; high levels of lead and ferric ion interfere	can be used for chelometric indicator titrations for metal ions, e.g. Zn ²⁺ , Ni ²⁺
calcium (Ca ²⁺)	max level*(M) 0.2 Na ⁺ ; 4 x 10 ⁻³ Hg ²⁺ , H ⁺ ; 6 x 10 ⁻⁴ Sr ²⁺ ; 2 x 10 ⁻⁴ Fe ³⁺ ; 4 x 10 ⁻² Cu ²⁺ ; at 10 ⁻³ M Ca ²⁺ 5 x 10 ⁻⁴ Ni ²⁺ ; 0.2 NH ₄ ⁺ ; 0.3 Tris ⁺ , Li ⁺ ; 0.4 K ⁺ ; 0.7 Ba ²⁺ ; 1.1 Zn ²⁺ ; 1.2 Mg ²⁺	
carbon dioxide (CO ₂) (carbonate) (CO ₃ ²⁻)	volatile weak acids	
chloride (Cl ⁻)	max level*(M) 6 x 10 ⁻⁸ ClO ₂ ⁻ ; 8 x 10 ⁻⁸ I ⁻ ; 3 x 10 ⁻⁸ NO ₂ ⁻ , SO ₄ ²⁻ ; at 10 ⁻³ M Cl ⁻ 4 x 10 ⁻⁵ Br ⁻ ; 10 ⁻⁶ OH ⁻ ; 4 x 10 ⁻⁶ OAc ⁻ ; 4 x 10 ⁻⁶ HCO ₃ ⁻ ; 7 x 10 ⁻⁶ F ⁻ ; max level**(M) 10 ⁻⁷ M S ²⁻ ; 3 x 10 ⁻⁸ Br ⁻ ; 5 x 10 ⁻¹⁰ I ⁻ ; 2 x 10 ⁻¹⁰ eN ⁻ ; NO ₂ ⁻ , SO ₄ ²⁻ , HCO ₃ ⁻ do not interfere same as 94-17	biological fluids only preferred electrode for Cl ⁻
chlorine (Cl ₂)	same as for conventional iodimetric titration (strong oxidizing agents)	iodide reagent is added to sample measures total residual chlorine
cupric (Cu ²⁺)	max level: S ²⁻ , Ag ⁺ , Hg ²⁺ 10 ⁻⁷ M; high levels of chloride, bromide, ferric, and cadmium interfere	can be used for chelometric indicator titrations for metal ions, e.g., Zn ²⁺ , Ni ²⁺
cyanide (CN ⁻)	max level**M: 10 ⁻⁷ S ²⁻ ; 10 ⁻⁸ I ⁻ ; 5 Br ⁻ ; 10 ¹ Cl ⁻	electrode life limited at high cyanide concentrations
fluoride (F ⁻)	max level M: <10 ⁻⁶ OH ⁻	can be used to measure aluminum and phosphate by titration
fluoroborate (BF ₄ ⁻)	max level *(M) 2 x 10 ⁻² NO ₂ ⁻ ; 0.2 Br ⁻ , OAc ⁻ , HCO ₃ ⁻ , F ⁻ , Cl ⁻ , OH ⁻ , SO ₄ ²⁻ = at 10 ⁻¹ M BF ₄ ⁻	measures boron after conversion to fluoroborate
iodide(I ⁻)	max level: S= 10 ⁻⁷ M	can be used to measure mercury
lead (Pb ²⁺)	max level: Ag ⁺ , Hg ²⁺ , Cu ²⁺ 10 ⁻⁷ M; high levels of cadmium and ferric ion interfere	can also be used to measure sulfate by titration with lead
nitrate (NO ₃ ⁻)	max level *(M) 10 ⁻² ClO ₂ ⁻ ; 6 x 10 ⁻⁸ I ⁻ ; 6 x 10 ⁻⁸ ClO ₂ ⁻ ; 2 x 10 ⁻⁸ eN ⁻ ; 8 x 10 ⁻⁸ Br ⁻ , at 10 ⁻¹ M NO ₃ ⁻ NO ₂ ⁻ ; 4 x 10 ⁻⁴ HS ⁻ ; 2 x 10 ⁻² CO ₃ ²⁻ ; 3 x 10 ⁻⁴ HCO ₃ ⁻ ; 4 x 10 ⁻⁴ Cl ⁻ ; 0.1 PO ₄ ³⁻ ; HPO ₄ ²⁻ ; H ₂ PO ₄ ⁻ ; 0.3 OAc ⁻ ; 0.7 F ⁻ ; 1.0 SO ₄ ²⁻	Ag ₂ SO ₄ may be added to samples with high chloride levels
nitrogen oxide (NOx) (nitrite)(NO ₂ ⁻)	max level *(M) 3 x 10 ⁻⁴ M CO ₂ , volatile weak acids interfere	measures NO and NO ₂ in air after scrubbing
oxygen		
perchlorate (ClO ₄ ⁻)	max level *(M) 2 x 10 ⁻⁴ I ⁻ ; 5 x 10 ⁻⁴ NO ₂ ⁻ ; 4 x 10 ⁻² Br ⁻ ; at 10 ⁻¹ M ClO ₄ ⁻ sodium interferes in basic solution	not be used in acid fluoride solutions
potassium (K ⁺)	max level *(M) 3 x 10 ⁻⁸ Cs ⁺ ; 6 x 10 ⁻⁴ NH ₄ ⁺ ; 10 ⁻⁴ H ⁺ ; 10 ⁰ Ag ⁺ , Tris ⁺ ; at 10 ⁻¹ M K ⁺ 2.0 Li ⁺ , Na ⁺	
redox	not applicable	
silver/sulfide (Ag ₂ S=)	max level: Hg ²⁺ <10 ⁻⁷ M	can be used to measure cyanide by indicator method
sodium (Na ⁺)	max level *(M) 3 x 10 ⁻⁷ Ag ⁺ ; 10 ⁻⁸ H ⁺ ; 5 x 10 ⁻⁸ Li ⁺ ; 6 x 10 ⁻⁴ Cs ⁺ ; 0.1 K ⁺ ; at 10 ⁻¹ M Na ⁺ 0.2 N(C ₂ H ₅) ₃ ; 0.5 Tl ⁺ same as 94-11	10 ⁻⁸ lower limit possible with proper pH adjustment
thiocyanate (SCN ⁻)	max level**(M) 10 ⁻⁴ H ⁺ , 3 x 10 ⁻⁸ Br ⁻ , 2 x 10 ⁻⁴ Cl ⁻ ; 1.3 x 10 ⁻⁴ NH ₄ ⁺ , 10 ⁻⁵ S ₂ O ₈ ²⁻ ; 7 x 10 ⁻⁸ CN ⁻ ; 10 ⁻¹ M I ⁻ , S ²⁻	
water hardness	max level *(M) at 3 x 10 ⁻⁴ Na ⁺ ; 3 x 10 ⁻⁸ Cu ²⁺ , Zn ²⁺ ; 6 x 10 ⁻⁸ Fe ²⁺ ;	for potable water only, for any other a

or a Specific Ion meter. The electrode responds only to free ions and will not respond to ions bound to complexing agents.

An "Ionic Strength Adjustor" (ISA) is a solution of high ionic strength which is used to dilute samples and standards. The ISA "swamps out" differences in ionic strengths between solutions. When the ionic strength is constant, the ion activity is directly proportional to the concentration.

ISE's are easily checked for performance, as a 10-fold change in concentration will give a 59 mv change for a monovalent ion and a 28.5 mv change for a divalent ion. If interferences are present or if the electrode needs renewal, a smaller response will be obtained. Table VIII-1 lists some common interferences for each electrode.

Quantitation by the standard additions technique uses a 10% volume-corrected Gran's Plot paper. Gran's Plot paper is a semi-antilog paper. Electrode potentials in millivolts are plotted on the vertical antilogarithmic axis and added amounts are on the horizontal linear axis. The paper corrects for up to a 10% dilution factor. The best straight line of the points is extrapolated to the base line to obtain the concentration in the original solution. (See Figure VIII-1.) The newer pH/mv meters with microprocessors will give the concentration in the digital display.

Most electrodes can be stored in air or in a standardizing solution. The analyst should refer to the manufacturer's instructions for specific electrodes (12).

E. NEUTRON ACTIVATION ANALYSIS (NAA)

Neutron activation analysis (13,14) is a non-destructive, multielement analytical technique that is applied to a wide variety of samples at FDA. The FDA maintains an NAA laboratory in the nuclear research reactor building at the National Bureau of Standards in Gaithersburg, Maryland.

1. Principles of NAA

Samples are placed in a field of neutrons in the reactor core. Neutrons react with stable isotopes in the sample to produce new isotopes, many of which are radioactive and decay by giving off gamma rays which can be detected. Observed energies of the gamma rays identify the elements being measured. The number of gamma rays detected is proportional to the amounts of the particular elements being determined.

Table VIII-2 Elements Determined by Neutron Activation Analysis.

Al	Cr	Ir	Rb	Ti
As	Cs	K	S	V
Au	Cu	La	Sb	Y
Ba	Dy	Lu	Sc	Yb
Br	Eu	Mg	Se	Zn
Ca	F	Mn	Sm	Zr
Cd	Fe	Mo	Sn	
Ce	Hf	Na	Ta	
Cl	Hg	Nd	Tb	
Co	I	Ni	Th	

2. Procedures and Concerns Peculiar to NAA

a. Irradiation Approval

Before any material can be placed into the reactor core for irradiation, advance approval must be obtained. Restrictions depend upon the physical and chemical composition of the material and vary greatly. In general, restrictions are much more stringent for liquids (due to their greater volatility) than for solids. While most sample types are approved for some type of irradiation procedure, it is not uncommon to need to gain additional approval for specific analyses.

b. Sample Packaging

All samples must be properly contained in order to be safely irradiated. Care is taken to prevent the escape of radioactive material which would contaminate the irradiation facilities at the reactor. Sample containment vessels used by the FDA NAA lab are made of either polyethylene or quartz and are heat sealed. Usually these containers must remain with the samples and therefore contribute to analytical blanks and limit sensitivities. For this reason, great effort is directed toward the cleaning of these containers and reduction of blank values.

c. Precision and Accuracy

Precision and accuracy for each element depend primarily on sample matrix. Analysis parameters are adjusted to provide optimal conditions, but spectral interferences, background and blanks are the real limiting factors. Analytical errors typically range between 1 and 20% for those elements routinely determined.

3. List of Elements

Table VIII-2 lists those elements which can be determined nondestructively by the FDA activation analysis lab. Detection limits are largely determined by the sample matrix; therefore, many of these elements cannot be determined in certain matrices.

F. CHROMATOGRAPHY

Chromatography is defined (15) as a procedure by which substances encountered in various products are separated by a dynamic differential migration process in a system consisting of two or more phases, one of which moves continuously in a given direction and in which the individual substances exhibit different mobilities by reason of differences in adsorption,

partition, solubility, vapor pressure, molecular size, or ionic charge. The individual substances thus obtained can be identified or determined by analytical methods.

The general chromatographic technique requires that a solute undergo distribution between two phases, one of them fixed (stationary phase), the other moving (mobile phase). It is the mobile phase that transfers the solute through the medium until it eventually emerges separated from other solutes that are eluted earlier or later. Generally, the solute is transported through the separation medium by means of a flowing stream of a liquid or a gaseous solvent known as the "eluant." The stationary phase may act through adsorption, as in the case of adsorbants such as activated alumina, silica gel, and ion-exchange resins, or it may act by dissolving the solute, thus partitioning the latter between the stationary and mobile phases. In the latter process, a liquid coating held on an inert support serves as the stationary phase. Partitioning is the predominant mechanism of separation in gas-liquid chromatography, paper chromatography, and forms of column chromatography designated as liquid-liquid chromatography. In practice, separations frequently result from a combination of adsorption and partitioning effects.

The types of chromatography useful in qualitative and quantitative analysis are Column, Gas, Paper, Thin-layer, and Pressurized Liquid Chromatography. Paper and thin-layer chromatography are ordinarily more useful for purposes of identification, because of their convenience and simplicity. Column chromatography offers a wider choice of stationary phases and is useful for the separation of individual compounds, in quantity, from mixtures. Both gas chromatography and pressurized liquid chromatography require more elaborate apparatus and normally provide high-resolution methods that will identify and quantitate very small amounts of material.

1. Pressurized Liquid Chromatography

Advances in column technology, high-pressure pumping systems, and detectors have transformed liquid column chromatography into a high-speed, high-efficiency technique of separation. This technique is sometimes referred to as HPLC, which is alternatively expressed as either high-performance liquid chromatography or high-pressure liquid chromatography. The column technology is based upon the use of small-bore (2- to 5-mm ID) columns and small-particle (3- to 50- μ m) packings that allow fast equilibrium between mobile and stationary phases. This small-particle column technology requires high-pressure pumping systems capable of delivering the mobile phase at high

pressure, as much as 300 atmospheres, to achieve flow rates of several ml per minute. Since it is often necessary to use small amounts of analyte (usually less than 20 pg) with the column packings, sensitive detectors are needed. With this column technology, liquid chromatography can give high-speed separations comparable in many cases to those achieved by gas chromatography, with the advantage that nonvolatile or thermally unstable materials can be chromatographed without decomposition or the necessity of making volatile derivatives.

The principal reasons for the advances made in liquid chromatography have been the advent of high-efficiency column packings and of sensitive detectors that are effective in the presence of the mobile solvents used. One type of stationary phase support used in these packings consists of micro-particles 30 μm to 50 μm in diameter, having a solid center and a thin porous crust. Some of these pellicular support materials can be pre-activated to give them adsorptive properties, while others can be covered with a thin film of stationary phase for partition or ion-exchange separations. The stationary phase can be either a liquid or a polymer, either coated or chemically bonded to the surface of the support in a thin film that reduced mass transfer resistances so that fast equilibrium between mobile and stationary phases can be attained. A liquid stationary phase must be largely immiscible in the mobile phase solvent; it is usually necessary to pre-saturate the mobile phase solvent with the stationary phase liquid to prevent stripping of the stationary phase from the column. Polymer stationary phases coated on the support are more durable. Stationary phases that have been chemically bonded to the support provide greater convenience for use with a variety of solvents and at elevated temperatures.

Other, smaller-diameter packing materials of 3- to 10- μm diameter are almost completely porous and give much more efficient separations than the 30- μm to 50- μm particle packings. The particles can also be made adsorptive or covered with a stationary phase. It is essential that these packings be slurry-packed in order to obtain high-efficiency columns, in contrast to the 30- μm to 50- μm particles which can be dry-packed.

The three forms of high-performance liquid chromatography most often used are ion-exchange, partition, and adsorption. Ion-exchange chromatography is used mainly for separation of water-soluble ionic or ionizable materials of molecular weight less than 1500. The stationary phases of ion-exchange chromatography are usually synthetic organic resins having different active groups present. Cation-exchange resins contain negatively charged active sites and are used to separate basic substances such as amines, while anion-exchange resins have positively

charged active sites, which will attract substances such as those carrying phosphate, sulfonate, or carboxylate groups. These water-soluble ionic or ionizable compounds are attracted to the resins, and differences in affinity bring about the chromatographic separation. The pH of the mobile phase, temperature, ion type, ionic concentration, and organic modifiers affect the attraction of the solute, and these variables can be adjusted to obtain the desired degree of separation.

In partition chromatography, mobile and stationary phases of different polarity are used. If the mobile phase is polar and the stationary phase nonpolar, as in a reverse-phase system, then nonpolar, hydrocarbon-solution compounds of molecular weight less than 1000, such as metal chelates, can be separated by their affinity for the stationary phase. Modification of the polar mobile phase solvent with a less polar solvent causes a decrease in affinity and the retention of the compounds on the column. If the mobile phase is nonpolar and the stationary phase polar, then polar material can be chromatographed. The nonpolar mobile phase can then be modified with a more polar solvent to decrease retention and change the separation.

Apparatus - The liquid chromatograph consists basically of a pumping system, analyte injection device, chromatographic column, detector, amplifier, and recorder. The high-pressure pumping system delivers the mobile phase solvent from the solvent reservoir to the column through high-pressure tubing and fittings, such as a calibrated loop that is loaded with the test specimen and transferred by the valve system to the flowing stream of mobile phase.

The detectors commonly used include the ultraviolet photometer, the differential refractometer, and the fluorometer. The low-pressure mercury ultraviolet photometer is the most common and stable detector, but its use is limited to the detection of materials that absorb radiation at a wavelength of 254 nm. Its limit of sensitivity to compounds that absorb ultraviolet light strongly may be about 1 ng. Compounds that do not absorb light at 254 nm appreciably may often be converted to suitable derivatives that absorb at this wavelength, thereby increasing the range of applicability of the single-wavelength detector. The introduction of detectors capable of operating at additional wavelengths has broadened the scope of ultraviolet detection.

The differential refractometer detects differences between the refractive indexes of the pure solvent and of a solution of the chromatographed test substance in the solvent. While more generally applicable, it is a less sensitive detector having a lower limit of about 1 pg and is responsive to small changes in solvent composition, flow rate, and temperature, so that a reference column and flow of mobile phase may be required to give a satisfactory base-line.

The fluorometer is a sensitive detector for compounds that are inherently fluorescent or that can be converted to fluorescent derivatives either by chemical transformation of the compound or by coupling with fluorescent reagents at specific functional groups.

An electrochemical detector employing carbon-paste electrodes mounted in a thin-film cell of very small volume may advantageously be employed to measure very small amounts (1 ng) of easily oxidized compounds. Easily reducible compounds may be measured by detectors employing mercury film or mercury-drop electrodes.

2. Gas Chromatography

In gas chromatography, the moving phase is a gas. The stationary phase is usually a liquid but may be a solid or a combination of solid and liquid.

In gas-liquid chromatography (GLC), the stationary liquid phase is immobilized as a thin film on a finely divided, inert solid support, such as chromatographic siliceous earth, crushed firebrick, glass beads, or even the inner wall of a small-diameter tube. If the tube is filled with liquid-covered, finely divided solid, it is called a packed column. If the inner wall of a small-diameter tube is coated with the liquid, it is called an open tubular or capillary column. If the inner wall of the open tubular column is treated so as to deposit a porous or irregular support on its surface before coating with the liquid phase, it is called a support coated open tubular (SCOT) column. In gas-solid chromatography (GSC), the identical situation holds except that the liquid phase is absent and the solid is an active adsorbant, such as alumina, silica gel, or carbon. In either case, the mobile phase continuously moves over the stationary phase.

When a vaporized substance is introduced into the gas stream at the head of the column, it is swept into the column and undergoes distribution between the gas and liquid or solid phases. In fact, the distribution process reaches a dynamic equilibration, but much of it is adequately described by an extension of the mathematical treatment of the stepwise process of countercurrent distribution. The behavior of a solute in such a partition process is conveniently defined by a dimensionless partition ratio, k' , called the capacity factor, which may be defined alternatively in terms of the relative amounts, or relative residence times, of the substance in the respective phases:

$$k' = \frac{\text{amount of substance in stationary phase}}{\text{amount of substance in gas phase}}$$

$$= \frac{\text{time in stationary phase}}{\text{time in gas phase}}$$

The gas phase simply serves to move the substance down the column between excursions into the stationary phase, and all substances spend the same time in the gas phase in any particular column. The value of the capacity factor, and, therefore, the time in a gas-liquid chromatographic column, depend upon the following considerations: (a) the specific solute; (b) the specific liquid phase; (c) the amount of liquid phase; (d) the temperature; and (e) the gas flow rate. Therefore, a partition ratio exists for each column, solute, and temperature, and in order to reproduce the behavior of a particular solute, almost every experimental factor must be carefully reproduced.

Apparatus - The basic apparatus required for gas chromatography is relatively simple. The carrier gas, usually available in compressed form in a cylinder fitted with a suitable pressure-reducing valve, is conducted into a flow meter, which is used to reproduce the particular flow found to be satisfactory for the resolution of a particular mixture. Helium, nitrogen, and other inert gases are suitable carriers. The actual carrier gas used is often determined by the characteristics of the detector being used. Since solutes to be chromatographed must be in the vapor phase, the injection port is heated to a temperature high enough to ensure rapid vaporization but not high enough to cause thermal degradation. Most test specimens are injected by syringe through a silicone rubber septum in the injection port.

Detectors commonly used for gas chromatography include those that depend upon thermal conductivity, flame-ionization, alkali flame-ionization, electron-capture, and conductivity. Helium, because of its high thermal conductivity, is the carrier gas of choice for use with a thermal conductivity detector. The thermal conductivity detector is applicable to all organic compounds but has a lower sensitivity and lower dynamic range than some other detectors. The flame ionization detector is sensitive to all carbon compounds and has a wide dynamic range. Nitrogen, by virtue of its higher viscosity, reduces zone spreading in the gas phase and may yield higher efficiencies than helium, but the lower viscosity of helium leads to higher carrier gas flow rates at optimum efficiencies and, therefore, to shorter elution times and faster analyses. The alkali flame-ionization detector contains an alkali-metal salt or a glass element containing rubidium or other metal that results in the suppression of the response to carbon, thereby increasing the relative response to nitrogen, sulfur, and phosphorus several fold. It is, therefore, a selective detector which shows little response to hydrocarbons. The electron-capture detector is also selective, showing little response to hydrocarbons and extremely high response to some compounds, such as those containing halogens or some ketones. Depending on the mode of operation, nitrogen or argon containing a small percentage of methane is used as the carrier gas for electron-capture detection. The electron-capture detector is one of the most sensitive detectors available for those compounds to which it responds. The conductivity detection system includes a heated reaction chamber in which compounds are reacted with a reagent gas such as oxygen or hydrogen that converts some compounds to electrically conductive species such as hydrochloric acid or ammonia while simultaneously removing carbon. The conductive species is then trapped in an electrolyte, and the observed change in electrical conductivity is continuously monitored. The conductivity detector can be made selectively responsive to halogens, sulfur, nitrogen, or phosphorus, and provides very high sensitivity.

Columns should be of glass unless otherwise specified. Columns of various dimensions are used, but normally they are 0.6 m to 1.8 m in length and 2 mm to 4 mm in internal diameter. Low-capacity columns, having about 5% (w/w) or less of liquid phase on the solid support, are preferred for analytical use. High-capacity columns, such as those having 20% (w/w) liquid loadings, may be used for large test specimens and for the determination of low molecular weight compounds such as water. The desired capacity influences the choice of solid supports.

Support materials are available in various mesh-size ranges, with 80- to 100- to 120-mesh being the most commonly used with 2- to 4-mm diameter columns. The support material should be as inert as possible.

Liquid phases are drawn from a wide range of chemical classes, such as polyethylene glycols, high molecular weight esters and amides, hydrocarbons, and silicone gums and fluids (polysiloxanes substituted normally by methyl, phenyl, nitrile, vinyl, or fluoroalkyl groups or mixtures of these).

Since gas chromatography is primarily a separation technique, it cannot be used to identify compounds without comparison to reference standards. For qualitative analysis, the retention time for a peak of a known specimen of the compound must be determined. When a peak appears at that same time under the same experimental conditions, the probability of correct identification is quite high. Alternatively, the individual components may be collected in a cold trap as they emerge from the column for independent analysis by other techniques, such as mass spectrometry or infrared absorption spectrometry.

System Suitability Tests - It is necessary to test packed, conditioned columns prior to acceptance of them for assay use. The peaks should be symmetric. Carrier gas flow and temperature should be adjusted for optimum results and assay time. A calibration curve should be prepared for the intended analyte at selected concentrations and operating conditions. Adequate blanks should be tested to preclude interferences. Normal variations in equipment and materials may require adjustment of the experimental conditions to obtain acceptable operation.

To ascertain the effectiveness of the final operating system, it is subjected to a suitability test prior to use. The essence of such a test is the concept that the electronics, the equipment, the specimens, and the analytical operations constitute a single analytical system, which is amenable to an overall test of system function. Specific data are collected from replicate injections of the assay preparation or standard preparation. These are matched to specified maximum and minimum values, such as efficiency, internal precision, resolution, retention time, nature of the calibration curve, response, and recovery.

REFERENCES

- (1) Rubeska, I., & Moldan, B. (1969) Atomic Absorption Spectrophotometry, 2nd Impr., The Chemical Rubber Co., Cleveland, OH 44128
- (2) The Guide to Techniques and Applications of Atomic Spectroscopy (1980) No. L-655, Perkin-Elmer Corp., Norwalk, CT
- (3) Van Zoon, J.C. (1980) Analytical Atomic Absorption Spectroscopy, Academic Press, Inc., New York, NY 10003
- (4) Robbins, W.B., & Caruso, J.A. (1979) Anal. Chem. 51, 889A-899A
- (5) Fassel, V.A. (1978) Science 202, 183-191
- (6) Jones, J.W., & Boyer, K.W. (1978) in Applic. ICP Emission Spect., Barnes, R., Ed., Franklin Press, 83-106
- (7) DC Plasma Emission - An Analytical Technique (1980) Technical Report No. 307, Spectrometrics, Inc., Andover, MA
- (8) Flato, J.B. (1972) Anal. Chem. 44, 75A-87A
- (9) Copeland, T.R., & Skogerboe, R.K. (1974) Anal. Chem. 46, 1257A-1268A
- (10) Gajan, R.J. (1978) FDA By-Lines 9, 113-130
- (11) The United States Pharmacopeia (1980), 20th rev., 938-946, and 969-971
- (12) Analytical Methods Guide (1973) 6th ed., Orion Research Inc., Cambridge, MA
- (13) Coleman, R.F., & Pierce, T.B. (1967) Analyst 92, 1-19
- (14) Bowen, H.J.M. (1980) CRC Critical Reviews in Anal. Chem., December, 127-184
- (15) Bond, A.M. & Wallace, G.G. (1983) J. Liquid Chrom. 6, 1799-1822.

APPENDIX VIII-A

Atomic Absorption Total System Check
(Example for illustration purposes only)

The sensitivity, calibration linearity, and precision, using copper as the test element, will be determined at least every three months as a total system check. Establish analytical conditions for the analyte as described by manufacturer or as previously determined as optimum.

Ascertain that the lamp is operating properly. Replace lamp if the working curve becomes nonlinear or the optimum analytical sensitivity falls to 80% of the normal value.

For conventional flame atomic absorption measurements, ensure that flame conditions are appropriate for each analyte. Prepare a series of calibration standards designed to cover the expected linear operating range. For elements such as the alkali or alkaline earth elements which exhibit substantial ionization interferences, prepare calibration curves in the presence of ionization buffers. Establish the calibration curve by plotting observed absorbance values versus concentration. For consistency, all calibration standards should be prepared in aqueous solution. The instrument should be "zeroed" while aspirating distilled, deionized water. From the calibration curve, calculate the slope and use it as a measure of the analyte sensitivity. Determine the measurement precision (C.V.) for ten replications of a standard solution at the mid concentration of the calibration curve. Record all results on data information forms. (see Exhibit VIII-A).

EXHIBIT VIII-A

Atomic Absorption Total System Check
(Example for illustration purposes only)

Recommended Monitor's Report

Instrument: Atomic Absorption Spectrophotometer

Model No.

Testing Procedures:

1. Record absorbance values of copper at five different concentration levels to cover analytical range (0 + 4 stds.).
2. Make ten replicate absorbance measurements of one standard solution in mid-concentration range.

Test Results:

Cu Conc.	Absorbance
(pg/ml)	

1. Plot and submit calibration curve and calculate coefficient of determination, which should be 0.995 or better.
2. From calibration curve calculate sensitivity, i.e, slope.
Slope = . A/conc. Should be 80% or better of
Instrument Sensitivity which is .
3. Calculate RSD (see Data Treatment Section) of the 10
replicates, should be 5% or less. RSD = %.

(OVER)

Conclusion:

Operating satisfactorily.

Needs repairs, memo showing action to be taken is attached.

Monitor

Supervisor

Date

Date

Monitor's time hr.

Calculation checked by

APPENDIX VIII-B

Atomic Emission Total System Check
(Example for illustration purposes only)

The linearity, precision, and limits of detection in pure, single element solutions will be determined annually. The calibration curves are defined over at least three decades, from the detection limit to 1000 times the detection limit. NBS Standard Reference Materials and EPA water standards should be analyzed periodically. Need for alignment of optical components will be determined by obtaining slit profiles on each element (at 10 pg/ml). Computer peripheral equipment should be periodically certified as error free by running standard diagnostic tests.

APPENDIX VIII-C

Anodic Stripping Voltammetry Total System Check
(Example for illustration purposes only)

The purpose in this test is to ensure that the electrode system is functioning properly. A calibration curve for Pb is established in purified acetate buffer over two concentration decades. A standard addition calibration curve is obtained by appropriate microliter additions of 1-10 ug/ml standards to 10 ml of analyte-free acetate buffer. Measurements are made under previously established standard conditions. Calibration curves should be linear over at least two decades. Precision estimates should be made at mid-concentration by replications of the same cell solution, using a fresh Hg drop for each measurement. For elements or compounds other than those mentioned above, similar checks should be performed in appropriate buffers under previously established optimum conditions. Record all results on data information forms. (see Exhibit VIII-B).

EXHIBIT VIII-B

Anodic Stripping Voltammetry Total System Check
(Example for illustration purposes only)

Recommended Monitor's Report

Instrument: Polarographic Analyzer

Model No.

Testing Procedures:

1. Record anodic stripping voltammograms of Pb covering the concentration range. 0-1 pg/cell.
2. Make ten replicate measurements of the standard solution in mid-concentration range.

Test Results:

Pb Conc.	Peak Height
----------	-------------

(pg/cell)	(uA)
-----------	------

0	
---	--

0.10	
------	--

0.25	
------	--

0.50	
------	--

0.75	
------	--

1.00	
------	--

1. Plot and submit calibration curve and calculate coefficient of determination, which should be 0.995 or better.
2. From calibration curve calculate sensitivity, (i.e., slope).
Slope = uA/conc.
3. Calculate RSD (see on Data Treatment Section) of the 10 replicates, should be 5% or less. RSD = %.

(OVER)

Conclusion:

Operating satisfactorily.

Needs repairs, memo showing action to be taken is attached.

Monitor

Supervisor

Date

Date

Monitor's time hr.

Calculations checked by

APPENDIX VIII-D InstrumentSelection by Element

The following instrumental techniques are commonly used for the determination of the elements listed.

1. As - AAS, (hydride)
2. Ca - AAS, flame emission
3. Cd - AAS, polarography, ASV
4. Cr - AAS, DPP
5. Cu - AAS, ASV
6. F - Ion selective electrode
7. Fe - AAS
8. Hg - AAS, (flameless)
9. I - Ion selective electrode
10. K - AAS, flame emission
11. Mn - AAS
12. Mo - AAS, (N₂O - C₂H₂ flame)
13. Na - AAS, flame emission
14. P - ICP, DCP
15. Pb - AAS, ASV
16. Se - AAS, (hydride)
17. Zn - AAS, ASV, DPP

AAS - Atomic absorption spectrophotometry

DPP - Differential pulse polarography

ASV - Anodic stripping voltammetry

ICP - Inductively coupled plasma

DCP - Direct current plasma

All elements listed, except I and F can be determined by ICP and DCP.

IX DATA TREATMENT

INTRODUCTION

The purpose of the Data Treatment Section is to provide a source for data manipulation procedures frequently used in trace element analyses at FDA. The quantitation procedures outlined in this section will provide consistency to the FDA laboratory calculations.

A. DEFINITION OF TERMS

1. Standard reagent blank - solution which contains the same concentration of reagents used in preparing calibration standards except for added analyte.

2. Sample reagent blank - solution which has gone through the same entire mineralization/dilution procedure as has the sample and includes all added reagents.

3. Replicate analyses - separate analyses of a number of portions of a single sample.

4. Multiple measurements - a number of response measurements made on a single solution.

B. USEFUL STATISTICS

1. Mean - the sum of a set of measurements divided by the number of measurements in the set.

$$\bar{y} = \frac{\sum y_i}{n} = \frac{y_1 + y_2 + \dots + y_n}{n}$$

2. Median - a. Odd number of measurements - the middle measurement when the measurements are arranged in order of magnitude.

b. Even number of measurements - the mean of the two middle observations when the measurements are arranged in order of magnitude.

3. Range - the difference between the largest and smallest measurements.

4. Variance - the sum of the squared deviations of the measurements from their mean, divided by (n-1) where n is the number of measurements.

$$s^2 = \frac{\sum (y_i - \bar{y})^2}{n-1}$$

5. Standard deviation - the positive square root of the variance.

$$S = \sqrt{s^2}$$

6. Standard Error (S.E.) - the standard deviation of a sampling distribution of some statistic (usually \bar{y})

$$S.E. = \frac{S}{\sqrt{n}}$$

where S is the standard deviation and n is the number of measurements. (S can be interpreted as the measure gotten if one repeatedly selected samples of size n from the population, calculated a \bar{y} value for each set of n measurements, and finally calculated the standard deviation of the \bar{y} values.)

7. Relative Standard Deviation (RSD) - the standard deviation of a set of measurements divided by the mean of these measurements. Usually expressed as a percent. (Also called the coefficient of variation.)

$$\%R.S.D = \frac{S}{\bar{y}} \times 100$$

8. 95% confidence interval (C.I.) of a set of n measurements - the limits around the measured mean within which the mean value for an infinite number of measurements can be expected to be found with 0.95 level of probability.

$$95\%C.I. = \bar{y} \pm t(S/\sqrt{n})$$

where t can be obtained from Table IX-1. One can say that 95% of all such similarly constructed confidence bands would encompass the true answer. It does not mean that 95% of each set of readings will be in that confidence band. It can also be said that there is a 95% probability the true answer lies between those limits.

Table IX-1

The t-statistic for 95% Confidence Interval^a

<u>n</u>	<u>t</u>	<u>n</u>	<u>t</u>
2	12.706	19	2.101
3	4.303	20	2.093
4	3.182	21	2.086
5	2.776	22	2.080
6	2.571	23	2.074
7	2.447	24	2.069
8	2.365	25	2.064
9	2.306	26	2.060
10	2.262	27	2.056
11	2.228	28	2.052
12	2.201	29	2.048
13	2.179	30	2.045
14	2.160	31	2.042
15	2.145	41	2.021
16	2.131	61	2.000
17	2.120	120	1.980
18	2.110		1.960

^aTwo-sided interval, $t_{1-\alpha/2} = t_{1-0.05/2} = t_{0.975}$

9. Coefficient of determination (C.D.) - the square of the correlation coefficient.

$$C.D. = r^2 = \frac{(S_{xy})^2}{(S_{xx})(S_{yy})}$$

$$S_{xy} = \frac{2}{n} \sum (x)(y)$$

where:

$$S_{xy} = \sum xy -$$

$$S_{xx} = \frac{2}{n} \sum x^2 -$$

$$S_{xx} = \sum x^2 -$$

$$S_{yy} = \frac{2}{n} \sum y^2 -$$

$$S_{yy} = \sum y^2 -$$

The more closely x and y are linearly related, the more the variability in the y-values can be explained by variability in the x-values and the closer r^2 will be to 1.

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

$$\%R = [(C_f - C_u)/C_a] \times 100$$

Where C_f = concentration of analyte measured in fortified sample;

C_u = concentration of analyte measured in unfortified sample;

C_a = concentration of analyte added in fortified sample.

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

Added analyte concentration should be no less than concentration of analyte in unfortified sample (8). Sum of concentration of added analyte plus analyte present before fortification should be in same range as analyte concentration sought in actual samples. Addition of analyte must not cause measuring instrument to exceed linear dynamic range of standard curve. Both fortified and unfortified samples must be treated identically during analysis to minimize experimental bias.

Table IX - 2^a**BASIC WORKSHEET FOR ALL TYPES OF LINEAR RELATIONSHIPS** X denotes _____ Y denotes _____ $\Sigma X =$ _____ $\Sigma Y =$ _____ $\bar{X} =$ _____ $\bar{Y} =$ _____Number of points: $n =$ _____Step (1) $\Sigma XY =$ _____(2) $(\Sigma X)(\Sigma Y)/n =$ _____(3) $S_{xy} =$ Step (1) - Step (2)(4) $\Sigma X^2 =$ _____(7) $\Sigma Y^2 =$ _____(5) $(\Sigma X)^2/n =$ _____(8) $(\Sigma Y)^2/n =$ _____(6) $S_{xx} =$ Step (4) - Step (5)(9) $S_{yy} =$ Step (7) - Step (8)(10) $b_1 = \frac{S_{xy}}{S_{xx}} =$ Step (3) \div Step (6)(14) $\frac{(S_{xy})^2}{S_{xx}} =$ _____(11) $\bar{Y} =$ _____(15) $(n - 2) s_Y^2 =$ Step (9) - Step (14)(12) $b_1 \bar{X} =$ _____(16) $s_Y^2 =$ Step (15) \div (n - 2)(13) $b_0 = \bar{Y} - b_1 \bar{X} =$ Step (11) - Step (12) $s_Y =$ _____

Equation of the line:

$$Y = b_0 + b_1 X$$

$$s_{b_1} =$$

$$s_{b_0} =$$

Estimated variance of the slope:

$$s_{b_1}^2 = \frac{s_Y^2}{S_{xx}} =$$
 Step (16) \div Step (6)

Estimated variance of intercept:

$$s_{b_0}^2 = s_Y^2 \left\{ \frac{1}{n} + \frac{\bar{X}^2}{S_{xx}} \right\} =$$

^aTaken from reference(2) p. 5-10.

C. LINEAR REGRESSION

Linear regression computer programs are readily available on large computer systems as well as on hand held calculators.

It is recommended that these programs be used to calculate the slope and intercept of linear analytical curves for AAS and DPASV. If the computer programs are not available the calculation procedure in Table IX-2 may be used. For our present purposes it is only necessary to calculate through Step 13 of Table IX-2. In this table, b_1 is the slope and b_0 is the intercept. When performing the linear regression calculation the following rules are to be applied:

1. The linear regression slope and intercept (i.e., regression coefficients) are computed using the standard concentrations for the X values and their respective responses for the Y values. The linear regression assumes that only the Y values contain measurement error. The converse being that the X values (i.e., the concentration levels of the analytical standard) are accurate and without error.

2. It is not assumed that the regression line has intercept = 0. Do not use linear regression techniques which will provide the best line while forcing the intercept to equal 0. Since the AAS standard reagent blank may contain detectable levels of analyte, it is improper to assume an intercept of 0. Also, for the DPASV standard addition calculation described later we use the intercept to determine the level of analyte for the sample.

3. If multiple measurements are made on a particular standard solution (or standard addition), then all the measurements should be used for the linear regression calculation and not their mean. Multiple measurements provide information on the measurement error. When they are incorporated into the linear regression calculation, a better indication of the data to estimate the true linear relationship is provided.

If using a linear regression computer program then it must be verified that it has the above attributes.

D. SAMPLE QUANTITATION BY CALIBRATION CURVE - AAS

The slope and intercept are computed from the standard solution data using linear regression. In this calculation the standard reagent blank response(s) is included with a corresponding x-value of 0, i.e., do not subtract the standard reagent blank response from the standard response measurements. The standard reagent blank response is a valid data point for the

linear regression calculation. The intercept of the linear regression calculation will represent the best estimate of the level of the analyte present in the standard reagent blank (but not the sample reagent blank to be discussed later). Even though the individual standard solution measurements are incorporated into the linear regression calculation only the mean of these responses is used to compute the concentration of the analyte. At this time, no estimate of the error associated with the sample measurement is computed. Therefore, multiple sample measurements are used only to estimate the true sample response. The following calculations are performed for quantitation of the analyte present in the sample. The result of each step should be reported.

1. Calculate sample reagent blank result by dividing its response by the slope of the linear regression line.
2. Calculate sample result(s) by dividing its response by the slope of the linear regression line.
3. Calculate net sample result(s) by subtracting the sample reagent blank result from the sample result.
4. Calculate final sample result(s) by multiplying the net sample result by its proper dilution factor.

Since the intercept of the linear regression line is a "best estimate" of the standard reagent blank response, it is not to be used in the calculation of the sample or sample reagent blank results. Instead, the response vs concentration linear relationship is assumed to pass through the origin (as absorption spectroscopy predicts).

AAS EXAMPLE CALCULATION

Standards

<u>pg Zn/mL</u>	<u>Absorbance</u>
0	0.002
0.25	0.0284, 0.0292
0.50	0.0574, 0.0576
0.75	0.0840, 0.0840
1.00	0.1096, 0.1104
1.25	0.1336, 0.1330

Slope = $0.1055A / (\text{pg/mL})$

Intercept = 0.0035A (Corresponds to
0.033 pg Zn/mL
but is not subtracted
from the standard curve
data points)

Sample

Reagent Blank Response = 0.0020A

Sample Response = 0.0961A

Reagent Blank Result = $\frac{0.002A}{0.1055A/(pg/mL)}$
 = 0.019 pg Zn/mL

Sample Result = $\frac{0.0961A}{0.1055A/(pg/mL)}$
 = 0.911 pg Zn/mL

Sample Result Corrected for Sample Reagent Blank

Net Sample Result = 0.911 pg Zn/mL - 0.019 pg Zn/mL
 = 0.892 pg Zn/mL

With a dilution factor of 1000 and a sample weight of 2.75g the final sample result is:

Final Sample Result = $\frac{0.892 \text{ pg Zn/mL} \times 1000}{2.75g}$
 = 324 pg Zn/g

E. SAMPLE QUANTITATION BY STANDARD ADDITION - DPASV

The slope and intercept are computed for each sample and sample reagent blank from the standard addition data. The unspiked response(s) is included with a corresponding x-value of 0. The following calculations are performed for the quantitation of the analyte present in the sample. The result of each step should be reported.

1. Calculate sample reagent blank result(s) by dividing its intercept by its slope.
2. Calculate sample result(s) by dividing its intercept by its slope.
3. Calculate net sample result(s) by subtracting the mean sample reagent blank result from the sample result.
4. Calculate final sample result(s) by multiplying the net sample result by its proper dilution factor.

The linear regression intercept is used to calculate both the reagent blank and sample results because it provides the best estimate of the unspiked level based on the standard addition data.

DPASV EXAMPLE CALCULATION

Sample Reagent Blank

<u>pg Pb</u> <u>Added to Cell</u>	<u>pAmp.</u>
0	0.0153
0.005	0.0315
0.015	0.0669
0.025	0.0984

$$\text{Slope} = 3.3488 \text{ pAmp/pg}$$

$$\text{Intercept} = 0.01535 \text{ pAmp}$$

$$\begin{aligned} \text{pg Pb in cell} &= 0.01535 \text{ pAmp} / 3.3488 \text{ (pAmp/pg)} \\ &= 0.005 \text{ pg Pb} \end{aligned}$$

Sample

<u>pg Pb</u> <u>Added to Cell</u>	<u>pAmp</u>
0	0.2933
0.1	0.5846
0.2	0.9417
0.3	1.2992

$$\text{Slope} = 3.3742 \text{ pAmp/pg}$$

$$\text{Intercept} = 0.2735 \text{ pAmp}$$

$$\begin{aligned} \text{pg Pb in cell} &= 0.2735 \text{ pAmp} / 3.3742 \text{ (pAmp/pg)} \\ &= 0.081 \text{ pg Pb} \end{aligned}$$

Sample Result Corrected for Sample Reagent Blank

$$\begin{aligned} \text{Net Sample Result} &= 0.081 \text{ pg Pb} - 0.005 \text{ pg Pb} \\ &= 0.076 \text{ pg Pb} \end{aligned}$$

With a dilution factor of 10 and a sample weight of 5.00g the final sample result is:

$$\text{Final Sample Result} = \frac{0.076 \text{ pg Pb} \times 10}{5.00\text{g}}$$

$$= 0.152 \text{ pg Pb/g}$$

REFERENCES

- (1) Mendenhall, William, & Ott, Lyman (1976) Understanding Statistics, 2nd Ed., Duxburg Press, Belmont, CA
- (2) Natrella, Mary Gibbons (1963) Experimental Statistics Handbook 91, Reprinted, U.S. Dept. of Commerce
- (3) Anal. Chem. (1982) 54, 157
- (4) Agresti, Alan, & Agresti, Barbara F. (1979) Statistical Methods for the Social Sciences, Dellen Publishing Company, San Francisco, CA
- (5) Ostle, Bernard (1963) Statistics in Research, 2nd Ed., The Iowa State University Press, Ames, Iowa
- (6) Youden, W.J., & Steiner, E.H. (1975) Statistical Manual of the AOAC, AOAC, Arlington, VA
- (7) AOAC, Changes in Methods, 3rd Supplement to 13th Edition, Official Methods of Analysis, (1982) J. Assoc. Off. Anal. Chem. 65, 450
- (8) Provost, L.P., & Elder, R.S. (1983) American Laboratory, 15, 57-63

X Methods

INTRODUCTION

The analytical method included in this section are all currently being used by FDA analysts. Only methods that have been validated by at least two analysts are included. Depending upon the degree of validation that each method has undergone, it is assigned to one of the following three validation classifications:

Class 1: A method which has been successfully collaboratively studied by six or more laboratories, has been published in a reviewed scientific journal, and has been issued as an acceptable method by the AOAC, ASTM, International Standards Organization, or other similar standard methods issuing organization.

Class 2: A method, which has been validated by analysts from two or more laboratories, and has been published or accepted for publication in a reviewed scientific journal.

Class 3: A method which has been independently validated, as written, by a second analyst (whether working in the same or different laboratory as the analyst who developed the method), but which has not otherwise been validated or published in a reviewed scientific journal.

Each method included in the manual is assigned an EAM Method Number. Part A of this section provides an index listing of the methods. This index includes the EAM number, list of elements that can be determined using the method, the validation classification, and the instrumentation required. Part B of this section provides an alphabetical listing of elements along with the EAM numbers of those methods capable of determining each element. Part C of this section contains each EAM method and an information sheet for each EAM method to aid in selecting the most appropriate method for the problem at hand.

A. INDEX OF METHODS AND PROCEDURES

<u>EAM NO.</u>	<u>ELEMENTS</u>	<u>CLASS</u>	<u>INSTRUMENTATION REQUIRED</u>
1	As, Se, Sb Te	2	AAS Hydride Generator
2	Se	2	AAS Hydride Generator
3	As, Cd, Cr, Cu, Pb, Ni, Se, Zn	1	AAS(As,Cu,Se,Zn) ASV(Pb,Cd,Cu) DPP (Cr, Ni) Hydride Generator (As,Se)
4	Cd, Pb	1	ASV
5	Cd, Pb	3	Flameless AAS
6	Cd, Pb, Cu Zn	2	DPASV(Cd,Pb,Cu) CSDPV(Zn) AAS(Cu,Zn)
7	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	3	ICAP
8	Method Deleted		
9	I	3	UV-VIS Spectro- photometer autoanalyzer
10	Hg	1	AAS
11	Hg	1	AAS Teflon decomposition vessel
12	Hg as methyl Hg	2	GC HPLC/AAS
13	Cd, Pb	1	AAS

14	Be, Ca, Cd Cr, Cu, Fe, K, Mg, Mn Mo, Ni, P Sr, Ti, Zn	3	ICAP
15	Al, As, Ca Cd, Co, Cu Fe, K, Mg, Mn, Mo, Ni P, Pb, Se, Sb, V, Zn	3	ICAP Hydride Generator AAS(As,Se,Sb)
16	Method Deleted		
17	Pb	2	ASV, AAS Polytron Homo- genizer
18	Pb, Cd, F Al, Cr, Cu Fe, Mn, Mo Ni, Ti, Zn	2	ASV(Pb,Cd) ICAP Fluoride Selective Electrode(F)
19	Pb	3	AAS
20	Method Deleted		

B. CROSS REFERENCE BETWEEN ELEMENTS AND METHODS

<u>ELEMENT</u>	<u>APPLICABLE EAM METHODS</u>
Al	15, 18
As	1, 3, 15
Be	14
Ca	7, 14, 15
Cd	3, 4, 5, 6, 13, 14, 15, 18
Co	15
Cr	3, 14, 18
Cu	3, 6, 7, 14, 15, 18
F	18
Fe	7, 14, 15, 18
Hg	10, 11,
Hg as methyl Hg	12
I	9
K	7, 14, 15
Mg	7, 14, 15
Mn	7, 14, 15, 18
Mo	14, 15, 18
Na	7
Ni	3, 14, 15, 18
P	7, 14, 15
Pb	3, 4, 5, 6, 13, 15, 17 18, 19

X-6

Sb	1, 15
Se	1, 2, 3, 15
Sr	14
Te	1
Ti	14, 18
V	15
Zn	3, 6, 7, 14, 15, 18

EAM METHODS

The EAM methods themselves are contained from here through the end of the manual. Each method is preceded by a cover sheet which gives the original reference for the method, as well as closely related supplemental references and additional references that are useful to consult when the method is used. The applicable elements and sample types, method class, estimated quantitation limits, as well as notes, precautions and analyst's comments based on FDA analyst's experiences in using the method are also included.

As each method becomes obsolete or is found to be not applicable or unreliable, it will be deleted from the EAM. If a suitable replacement method is available, it will be included in the EAM in place of the deleted method. As methods for additional analytes become available, they will also be added to the EAM.

Each method and its supplemental references should be inserted behind the appropriate cover sheet. At the individual analyst's option, the "additional useful references" may also be inserted behind the cover sheet.

TITLE: SEQUENTIAL DETERMINATION OF ARSENIC, SELENIUM, ANTIMONY,
AND TELLURIUM IN FOODS VIA RAPID HYDRIDE EVOLUTION AND
ATOMIC ABSORPTION SPECTROMETRY
John A. Fiorino, John W. Jones, and Stephen G. Capar
Anal. Chem. (1976) 48, 120-126.

Supplements:

- A. FDA HYDRIDE GENERATOR INSTRUCTION MANUAL:
Determination of As, Se, Sb, and Te in Foods by AAS
Stephen G. Capar and John W. Jones
FDA LIB 1900 (1976) 27 pp.

Other Useful References:

1. SUBSTITUTE HYDRIDE GENERATOR APPARATUS:
Stephen G. Capar and Walter Holak
FDA LIB 1900A (1978) 12 pp.
2. A SIMPLIFIED HYDRIDE GENERATOR:
Larry W. Elliott
FDA LIB 1900B (1980) 5 pp.

APPLICABLE ELEMENTS: As, Se, Sb, Te

CLASS: 2

APPLICABLE SAMPLE TYPES: All Foods.
Whole blood

ESTIMATED QUANTITATION LIMITS: (based on 5g sample)
As and Se - 0.025 ppm
Sb and Te - 0.05 ppm

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . Digestion uses perchloric acid. Use caution when digesting high fat or sugar content foods.
- . During digestion if charring starts to occur, cool digest in cold water bath prior to addition of nitric acid.
- . Charring of sample can cause significant loss of As and Se.
- . Special apparatus (semiautomated hydride generator) required.
- . Use of references 1 or 2 increases quantitation limits by a factor of 4; 0.1 ppm for As and Se. Sb and Te have not been studied using these references but no problems expected.

TITLE: DETERMINATION OF TRACE AMOUNTS OF SELENIUM IN CORN,
LETTUCE, POTATOES, SOYBEANS AND WHEAT BY HYDRIDE
GENERATION/CONDENSATION AND FLAME ATOMIC ADSORPTION
SPECTROMETRY
Mark H. Hahn, Roy W. Kuennen, Joseph A. Caruso and
Fred L. Fricke
J. Agric. Food Chem. (1981) 29, 792-796.

APPLICABLE ELEMENTS: Se

CLASS: 2

APPLICABLE SAMPLE TYPES: Raw Agricultural Crops

ESTIMATED QUANTITATION LIMITS: 1 ng/g (dry wt.)

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . With the addition of vanadium, strong oxidizing conditions exist which require slow initial heating. Peanuts, for example, must be allowed to remain in the digestion solution overnight before heat is applied. Some peanut samples ignite at room temperature - peanut samples in the digestion solution should, therefore, be kept in a perchloric acid hood.

TITLE: ANALYSIS OF FOODS FOR LEAD, CADMIUM, COPPER, ZINC,
ARSENIC, AND SELENIUM USING CLOSED SYSTEM SAMPLE
DIGESTION: COLLABORATIVE STUDY
Walter Holak
J. Assoc. Off. Anal. Chem. (1980) 63, 485-495.

Supplements:

A. DETERMINATION OF COPPER, NICKEL, AND CHROMIUM
IN FOODS:
Walter Holak
J. Assoc. Off. Chem. (1983) 66, 620-624

APPLICABLE ELEMENTS: As, Cd, Cu, Pb, Se, Zn

CLASS: 1

APPLICABLE SAMPLE TYPES: All Foods.
Selected drugs.

ESTIMATED QUANTITATION LIMITS: (for 1.0g wet weight sample)

Pb - 0.1 ppm	As - 0.05 ppm	Zn - 0.5 ppm
Cd - 0.01 ppm	Se - 0.05 ppm	

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . DO NOT EXCEED 0.3g sample size (on dry weight basis).
- . Method was not validated for copper.
- . Because of the small sample size contamination control procedures become even more important.
- . Do not use muffle furnace to heat digestion vessel because of inadequate temperature control.
- . Blood has been analyzed for boron using this digestion procedure.

TITLE: DETERMINATION OF LEAD AND CADMIUM IN FOODS BY ANODIC STRIPPING VOLTAMMETRY: COLLABORATIVE STUDY
Stephen G. Capar, Raymond J. Gajan, Elizabeth Madsar,
Richard H. Albert, Marion Sanders, and John Zyren
J. Assoc. Off. Anal. Chem. (1982) 65, 978-986.

Supplements:

A. DETERMINATION OF LEAD AND CADMIUM IN FOODS BY
ANODIC STRIPPING VOLTAMMETRY: DEVELOPMENT OF
METHOD:
Raymond J. Gajan, Stephen G. Capar, Cheryl A. Subjoc,
and Marion Sanders
J. Assoc. Off. Anal. Chem. (1982) 65, 970-977.

APPLICABLE ELEMENTS: Cd, Pb

CLASS: 1

APPLICABLE SAMPLE TYPES: Collaborated for green beans, infant
formula, baby beef, mackrel, cereal,
applesauce.

Accepted as Official First Action (1981) by AOAC for all
foods except fats and oils.

ESTIMATED QUANTITATION LIMITS: (based on 10g sample)

Cd - 5 ppb	(quantitation limits
Pb - 10 ppb	blank dependent)

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . Check background level of Pb and Cd in reagents prior to use.
- . To avoid oven temperature overshoot at final ashing temperature set furnace at 450° for 1 hour after loading samples in furnace, then set temperature at final ashing temperature.
- . There is a danger of deflagration when heating (on the hotplate) to dryness the ashed sample residue with 2 ml of HNO₃, if an excessive amount of carbon is present. The method will handle only small amounts of carbon residue in this step. A glow in the ash or the evolution of large amounts of smoke indicate deflagration and the probable loss of Cd and/or Pb. If this occurs, the analysis should be repeated with a smaller sample portion, or the sample should be left in the furnace at 500°C for a longer time to reduce the amount of carbon residue before addition of 2 ml of HNO₃.

TITLE: QUANTITATIVE DETERMINATION OF LEAD AND CADMIUM IN TOTAL
DIET MARKET BASKETS BY FLAMELESS ATOMIC ABSORPTION
Barbara E. Young and Kent C. Faul
FDA LIB 2403 (1981) 13 pp.

APPLICABLE ELEMENTS: Cd, Pb

CLASS: 3

APPLICABLE SAMPLE TYPES: All foods.

ESTIMATED QUANTITATION LIMITS: (based on 5g sample)

Pb - 20 ppb

Cd - 4 ppb

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . Method is instrument specific.
- . Experience with method limited to one laboratory.

TITLE: DRY ASH-VOLTAMMETRIC DETERMINATION OF CADMIUM, COPPER,
LEAD, AND ZINC IN FOODS
John W. Jones, Raymond J. Gajan, Kenneth W. Boyer, and
John A. Fiorino
J. Assoc. Off. Anal. Chem. (1977) 60, 826-832.

Supplements:

- A. DRY ASH-VOLTAMMETRIC DETERMINATION OF CADMIUM,
COPPER, LEAD AND ZINC IN FOODS:
John W. Jones, Raymond J. Gajan and Kenneth W. Boyer
FDA LIB 1996 (1976) 8 pp.
- B. DETERMINATION OF BACKGROUND LEVELS OF LEAD AND
CADMIUM IN RAW AGRICULTURAL CROPS USING DIFFERENTIAL
PULSE ANODIC STRIPPING VOLTAMMETRY:
R. Duane Satzger, Charles S. Clow, Evelyn Bonnin,
and Fred L. Fricke
J. Assoc. Off. Anal. Chem. (1982) 65, 987-991

APPLICABLE ELEMENTS: Cd, Pb, Cu, Zn

CLASS: 2

APPLICABLE SAMPLE TYPES: All foods.

ESTIMATED QUANTITATION LIMITS: (for 10g sample)

Cd - 10 ppb	Cu - 20 ppb
Pb - 20 ppb	Zn - 50 ppb

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . Sulfuric acid will degrade ashing furnace. Less acid than specified has been shown to be satisfactory when using quartz beakers by some laboratories.
- . Samples must be moisture-free prior to ashing to avoid spattering or foaming.
- . Cu and Zn form intermetallic complexes.

TITLE: RAPID HEATED BLOCK DIGESTION OF TOTAL DIET SAMPLES
FOR THE DETERMINATION OF Na, K, P, Ca, Cu, Fe, Mg,
Mn, AND Zn BY ICP EMISSION SPECTROMETRY
Ronald Marts
FDA LIB (to be issued)

APPLICABLE ELEMENTS: Na, K, P, Ca, Cu, Fe, Mg, Mn, Zn

CLASS: 3

APPLICABLE SAMPLE TYPES: All foods.

ESTIMATED QUANTITATION LIMITS:

(to be added when LIB is issued)

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . This method is currently being used at the Total Diet Laboratory for analysis of Total Diet samples for the indicated elements. The method will be added to the EAM as soon as the LIB describing the method is issued.

TITLE: The method which previously occupied this space has been deleted from the EAM. This space is reserved for a future method.

APPLICABLE ELEMENTS:

CLASS:

APPLICABLE SAMPLE TYPES:

ESTIMATED QUANTITATION LIMITS:

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

TITLE: SEMI-AUTOMATED METHOD FOR THE DETERMINATION OF IODINE IN
TOTAL DIET MARKET BASKETS
Ronald G. Luchtefeld
FDA LIB 1678 (1974) 8 pp.

Other useful references:

1. NEUTRON ACTIVATION ANALYSIS OF TOTAL DIET FOOD
COMPOSITES FOR IODINE
Massimo Allegrini, Kenneth W. Boyer, and
James T. Tanner
J. Assoc. Off. Anal. Chem. (1981) 64, 1111-1115.

APPLICABLE ELEMENTS: I

CLASS: 3

APPLICABLE SAMPLE TYPES: All foods.

ESTIMATED QUANTITATION LIMITS:

0.06 ppm

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . Experience limited to one laboratory.
- . Reliability of method is questionable, but method is best currently available. Reference 1 contains NAA comparison results.

TITLE: MERCURY-FLAMELESS ATOMIC ABSORPTION METHOD - OFFICIAL
FINAL ACTION
OFFICIAL METHODS OF ANALYSIS (1980) 13th Ed.
AOAC, Arlington, VA 25.110-25.114.

Supplements:

- A. DETERMINATION OF MERCURY IN FISH BY FLAMELESS ATOMIC
ABSORPTION: A COLLABORATIVE STUDY
R.K. Munns and D.C. Holland
J. Assoc. Off. Anal. Chem. (1971) 54, 202-205.
- B. RAPID DIGESTION AND FLAMELESS ATOMIC ABSORPTION
SPECTROSCOPY OF MERCURY IN FISH: COLLABORATIVE
STUDY
Robert K. Munns and David C. Holland
J. Assoc. Off. Anal. Chem. (1977) 60, 833-837.
- C. A $\text{HNO}_3\text{-H}_2\text{SO}_4$ AND/OR $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2\text{-HNO}_3$
SOLUBILIZATION PROCEDURE FOR DETERMINING TOTAL
MERCURY IN FOODS BY THE AOAC METHOD
Ronald W. Marts and John J. Blaha
FDA LIB No. 2708 (1983) 11 pp.

APPLICABLE ELEMENTS: Hg

CLASS: 1

APPLICABLE SAMPLE TYPES: Collaborated for fish.

Validated for all foods via Total Diet Studies Program.

ESTIMATED QUANTITATION LIMITS: (based on 5g sample)

0.02 ppm

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . During digestion an antifoaming agent (e.g., 1-4 drops of octanol per flask) has been used to reduce foaming.
- . During reaction with stannous chloride, addition of a drop of antifoaming agent will prevent excess foaming.
- . If fat remains in the sample digest, ensure that the sample aliquot for analysis does not contain fat (may cause interference).
- . Greater than 100% recoveries have been frequently noted with this method.

TITLE: MERCURY - ALTERNATE DIGESTION METHOD FOR SEAFOOD
Official Method of Analysis (1980) 13th Ed., AOAC,
Arlington, VA 25.115, 25.116, 25.110-25.112.

Supplements:

- A. SIMPLE, RAPID DIGESTION TECHNIQUE FOR THE
DETERMINATION OF MERCURY IN SEAFOOD BY FLAMELESS
ATOMIC ABSORPTION SPECTROSCOPY
Benjamin Krinitz and Walter Holak
J. Assoc. Off. Anal. Chem. (1974) 57, 568-569.
- B. SIMPLE, RAPID DIGESTION TECHNIQUE FOR THE
DETERMINATION OF MERCURY IN FISH BY FLAMELESS
ATOMIC ABSORPTION
Walter Holak, Benjamin Krinitz, and John C. Williams
J. Assoc. Off. Anal. Chem. (1972) 55, 741-742.

APPLICABLE ELEMENTS: Hg

CLASS: 1

APPLICABLE SAMPLE TYPES: Collaborated for seafood (shrimp, tuna)

ESTIMATED QUANTITATION LIMITS: (based on 1g sample)

0.10 ppm

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

TITLE: ELECTRON CAPTURE GAS-LIQUID CHROMATOGRAPHIC DETERMINATION
OF METHYL MERCURY IN FISH AND SHELLFISH: COLLABORATIVE
STUDY

Susan C. Hight and Stephen G. Capar
JAOAC (1983) 66, 1121-1128.

Supplement:

A. DETERMINATION OF METHYL MERCURY IN FISH BY
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY
Walter Holak
Analyst (1982) 107, 1457-1461

Other Useful References:

1. A SIMPLIFIED METHOD FOR THE GAS-LIQUID
CHROMATOGRAPHIC DETERMINATION OF METHYL MERCURY
IN FISH AND SHELLFISH
James O. Watts, Kenneth W. Boyer, Anthony Cortez,
and Edgar R. Elkins, Jr.
J. Assoc. Off. Anal. Chem. (1976) 59, 1226-1233.
2. GAS CHROMATOGRAPHIC DETERMINATION OF METHYL AND ETHYL
MERCURY: "PASSIVATION" OF THE CHROMATOGRAPHIC COLUMN
James E. O'Reilly
J. Chromatogr. (1982) 238, 433-444.

APPLICABLE ELEMENTS: methyl bound Hg

CLASS: 2

APPLICABLE SAMPLE TYPES: Fish and Shellfish

ESTIMATED QUANTITATION LIMITS: (based on 5g sample)

(methyl bound) Hg - 0.1 ppm Hg
(ethyl bound) Hg - 0.1 ppm Hg

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . This method uses benzene which is a suspected carcinogen.
- . GC column packing must not be closer than 1.5 cm from
threaded ends of heated injection and detection parts.

TITLE: CADMIUM AND LEAD IN EARTHENWARE - OFFICIAL FINAL
ACTION - AOAC - ASTM METHOD
Official Methods of Analysis (1980) 13th Ed., AOAC,
Arlington, VA 25.031-25.034.

Changes in Methods: 25. Metals and Other Elements
J. Assoc. Off. Anal. Chem. (1981) 64, 510.

Other Useful References:

1. COLLABORATIVE STUDY OF AN ATOMIC ABSORPTION METHOD
FOR THE DETERMINATION OF LEAD AND CADMIUM EXTRACTED
FROM GLAZED CERAMIC SURFACES
Benjamin Krinitz and Vincent Franco
J. Assoc. Off. Anal. Chem. (1973) 56, 869-875.
2. COLLABORATIVE STUDY OF EFFECT OF LIGHT ON CADMIUM AND LEAD
LEACHING FROM CERAMIC GLAZES
Benjamin Krinitz and Walter Holak
J. Assoc. Off. Anal. Chem. (1976) 59, 158-161.

APPLICABLE ELEMENTS: Cd, Pb

CLASS: 1

APPLICABLE SAMPLE TYPES: Earthenware.
Metalware.

ESTIMATED QUANTITATION LIMITS:

Cd - 0.05 ppm in solution analyzed
Pb - 0.5 ppm in solution analyzed

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

. Extraction of Cd is light dependent.

TITLE: DETERMINATION OF TOXIC AND NUTRITIONAL ELEMENTS IN RAW
AGRICULTURAL CROPS USING ICAP SPECTROSCOPY
Karen A. Wolnik, Roy W. Kuennen and Fred F. Fricke
Dev. At. Plasma Spectrochem. Anal., Barnes, Ed.,
Hyden & Sons, Inc.
Philadelphia, PA (1982) 685-696.

APPLICABLE ELEMENTS: Be, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo,
Ni, P, Sr, Ti, Zn

CLASS: 3

APPLICABLE SAMPLE TYPES: Raw agricultural crops

ESTIMATED QUANTITATION LIMITS: See Table 3

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . The $\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$ wet ash requires constant analyst attention.

TITLE: CRITICAL EVALUATION OF A MULTI-ELEMENT SCHEME USING
PLASMA EMISSION AND HYDRIDE EVOLUTION ATOMIC ABSORPTION
SPECTROMETRY FOR ANALYSIS OF PLANT AND ANIMAL TISSUES
John W. Jones, Stephen G. Capar and T.C. O'Haver
Analyst (1982) 107, 353-377.

Supplements:

- A. DETERMINATION OF HEAVY METALS IN FOODS
Richard A. Baetz and Charles T. Kenner
J. Agr. Food Chem. (1973) 21, 436-440.
- B. DETERMINATION OF TRACE METALS IN FOODS USING
CHELATING ION EXCHANGE CONCENTRATION
Richard A. Baetz and Charles T. Kenner
J. Agr. Food Chem. (1975) 23, 41-44.

APPLICABLE ELEMENTS: Al, As, Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Mo,
Ni, P, Pb, Se, Sb, V, Zn

CLASS: 3

APPLICABLE SAMPLE TYPES: Most foods, most biological matrices,
sludges

ESTIMATED QUANTITATION LIMITS: Reference Table V of publication
for absolute quantitation limits.

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . Requires Teflon labware.
- . Can achieve "clean" separation of many trace elements
from salt matrix.
- . Also good "cleanup" for graphite furnace AAS.

EAM -

TITLE: The method which previously occupied this space has been
deleted from the EAM. This space is reserved for a
future method.

APPLICABLE ELEMENTS:

CLASS:

APPLICABLE SAMPLE TYPES:

ESTIMATED QUANTITATION LIMITS:

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

TITLE: SAMPLE HOMOGENIZATION PROCEDURE FOR DETERMINATION OF LEAD
IN CANNED FOODS

John W. Jones and Kenneth W. Boyer

J. Assoc. Off. Anal. Chem. (1979) 62, 122-128.

Supplement:

A. SAMPLING PROCEDURE AND DETERMINATION OF LEAD IN
CANNED FOODS

Ronald F. Suddendorf, Steven K. Wright, and
Kenneth W. Boyer

J. Assoc. Off. Anal. Chem. (1981) 64, 657-660.

APPLICABLE ELEMENTS: Pb in canned foods.

CLASS: 2

APPLICABLE SAMPLE TYPES: Canned foods in metal cans.

ESTIMATED QUANTITATION LIMITS: Not applicable.

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . Ensure sufficient liquid to prevent burnout of homogenizer bearings.
- . Wear adequate sound protection to prevent ear damage during homogenization with polytron homogenizer.
- . Thoroughly clean homogenizer immediately after use.
- . The following pertains to Supplement A: If perchloric acid digestion is used, a precipitate may form when adjusting the pH with ammonium hydroxide. An additional 1 ml of ammonium hydroxide must be added past the bromocresol green end point to prevent formation of precipitate. No precipitate is formed when using the nitric-sulfuric acid-hydrogen peroxide digestion. This digestion is probably the method of choice.

TITLE: LEAD, FLUORIDE, AND OTHER ELEMENTS IN BONEMEAL
SUPPLEMENTS
Stephen G. Capar and John H. Gould
J. Assoc. Off. Anal. Chem. (1979) 62, 1054-1061.

Supplement:

A. DETERMINATION OF LEAD IN BONEMEAL BY DIFFERENTIAL
PULSE ANODIC STRIPPING VOLTAMMETRY USING A
HYDROCHLORIC ACID SOLUBILIZATION
R. Duane Satzger, Roy W. Kuennen, and
Fred L. Fricke
J. Assoc. Off. Anal. Chem. (1983) 66, 985-988.

APPLICABLE ELEMENTS: Pb, Cd, F, Al, Cr, Cu, Fe, Mn, Mo, Ni,
Ti, Zn

CLASS: 2

APPLICABLE SAMPLE TYPES: Bonemeal and bonemeal mineral
supplements.

ESTIMATED QUANTITATION LIMITS: Various - see method paper.

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

- . Method uses perchloric acid.
- . Vitamin supplements containing bonemeal may have
interferences on Pb and Cd by ASV.

TITLE: DETERMINATION OF LEAD EXTRACTED FROM ELECTRIC TEA KETTLES
FDA LIB 2170 (1978) 3 pp.

APPLICABLE ELEMENTS: Pb

CLASS: 3

APPLICABLE SAMPLE TYPES: Tea kettles.

ESTIMATED QUANTITATION LIMITS: 0.1 ug/ml of leach solution.

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS:

TITLE: The method which previously occupied this space has been deleted from the EAM. This space is reserved for a future method.

APPLICABLE ELEMENTS:

CLASS:

APPLICABLE SAMPLE TYPES:

ESTIMATED QUANTITATION LIMITS:

NOTES, PRECAUTIONS, AND ANALYST'S COMMENTS: