National Human Exposure Assessment Survey (NHEXAS)

Arizona Study

Quality Systems and Implementation Plan for Human Exposure Assessment

The University of Arizona
Tucson, Arizona 85721

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Standard Operating Procedure **FDA-Compendium**

Title: Methods for Analysis of Trace Metals in Dietary Samples Using Total Dietary Study Procedures

Source: The University of Arizona

U.S. Environmental Protection Agency
Office of Research and Development
Human Exposure & Atmospheric Sciences Division
Human Exposure Research Branch

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Compendium of Methods for Analysis of Trace Metals in Dietary Samples Using Total Diet Study Procedures

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Standard Operating Procedure for Quality Control for Analysis of NHEXAS Food or Beverage Composites for Trace Elements and Pesticides

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Center for Food Safety and Applied Nutrition
Elemental Research Branch
Washington, DC

and

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1. Scope and Application

To define quality assurance provisions for the performance of trace element and pesticide analytical methods applied to generation of data for duplicate diet food or beverage composite samples submitted under the U.S. Environmental Protection Agency/U.S. Food and Drug Administration interagency agreement "Implementation and Analytical Support for NHEXAS Pilot Studies" (EPA DW75936418-01-0; FDA 224-94-2461).

2. Summary of Method

Quality control samples are subjected to the same analytical conditions as actual samples. The conditions are the same throughout the entire analysis sequence beginning with sample aliquoting and preparation and ending with data reduction. Analytical methods are described in the individual analytical method SOPs. The determinative techniques employed for the analytes are as follows:

Analyte	Determinative Technique
Cd, Cr, Pb, and Ni	graphite furnace atomic absorption spectrometry (GFAAS) [SOP 101]
As and Se	hydride generation atomic absorption spectrometry (HGAAS) [SOP103]
Ba, Cu, Mn, V and Zn	inductively coupled plasma-atomic emission spectrometry (ICPAES) [SOP 102]
As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Se, V, and Zn	inductively coupled plasma-mass spectrometry (ICPMS) (pending equipment receipt and validation)
Pesticides	gas chromatography with ECD, FPD, HECD or TSD detectors [SOPs 202 and 203]
Carbaryl	HPLC with UV/fluorescence detectors [SOP 204]

The estimated method detection limits (MDL) or limits of detection (LOD) for the analytes in food duplicate diet composites are as follows:

Analyte	MDL (mg/kg)
Pb	0.01
Cd	0.002
Cr	0.02
Ni	0.02
As	0.003
Se	0.002
Ba	0.05
Cu	0.02
Mn	0.02
V	0.04
Zn	0.04

Analyte	LOD (mg/kg)
Chlorpyrifos	0.0010
Diazinon	0.0007
Atrazine	0.0017
Malathion	0.0010
Carbaryl	0.0080
Chlordane, cis-	0.0005
Chlordane, trans-	0.0005
Dieldrin	0.0005
Heptachlor	0.0005
p,p'-DDE	0.0007
p,p'-TDE	0.0007
p,p'-DDT	0.0010

3. Definitions [8.1,8.2]

3.1 *Laboratory Sample*—The sample or subsample sent to or received by the laboratory. Laboratory samples will be received from EPA cooperators by FDA Kansas City District Laboratory.

3.2 *Analytical Sample*—The sample, prepared from the laboratory sample, from which analytical portions are removed for analysis. FDA Kansas City District Laboratory will prepare the analytical sample and will retain portions for pesticide analysis and send portions to FDA Elemental Research Branch for trace element analysis, and to EPA and EPA cooperatives.

3.3 *Analytical Portion*—The quantity of material, of proper size for measurement of the concentration of interest, removed from the analytical sample.

3.4 *Analytical Solution*—The solution prepared by dissolving, with or without reaction, the analytical portion in a liquid.

3.5 *Treated Solution*—The analytical solution that has been subjected to reaction or separation procedures prior to measurement of some property.

3.6 *Laboratory Sample (Background for Spike) (LSO)*—The first of two analytical portions of a food or beverage test sample. This portion is analyzed according to the analytical method to establish background concentration(s) and may be analyzed prior to fortification (spiking) with the method analyte(s).

3.7 *Laboratory Spiked Sample (LSF)*—The second of the two analytical portions described under *Laboratory Sample (Background for Spike)*. This portion is subject to fortification (spiking) prior to sample preparation, and the measurement(s) of the final concentration(s) is then made according to the analytical method.

3.8 *Laboratory (Reagent) Blank (LRB)*—An aliquot of reagent water or equivalent neutral reference material treated as an analytical portion in all aspects in the laboratory. This includes addition of all reagents, internal standards, surrogates, labware, apparatus, equipment, solvents, and analyses.

3.9 *Laboratory (Dry) Blank (LDB)*—Exactly the same as the *LRB* except the aliquot of reagent water or equivalent neutral reference material is omitted.

3.10 *Laboratory Fortified Blank (LFM)*—An aliquot of reagent water or equivalent neutral reference material, known to be below detection limits for an analyte(s), to which a known quantity(ies) of method analyte(s) was added. The *LFM* is then treated as a food or beverage test sample in all aspects in the laboratory including addition of all reagents, internal standards, surrogates, glassware, equipment, solvents, and analyses.

3.11 *Laboratory Reference Material (LRM)*—An analytical portion of a food or beverage matrix having a certified value. These materials are usually obtained from the National Institute of Standards and Technology (NIST), the National Research Council of Canada (NRCC), Bureau of Reference Materials of the European Communities (BCR), etc. The *LRM* is treated as a food or beverage analytical sample in all aspects in the

laboratory. This includes addition of all reagents, internal standards, surrogates, labware, apparatus, equipment, solvents, and analyses.

4. Quality Control

The following minimum laboratory quality control samples are to be analyzed with each batch of samples. The number of samples in a batch is defined in the individual analytical method SOP. A batch is a group on analyses using common quality control.

4.1 Trace Elements

4.1.1 At least one Laboratory (Reagent) Blank or Laboratory (Dry) Blank.

4.1.2 One Laboratory Sample (Background for Spike) and corresponding Laboratory Spiked Sample or one Laboratory Fortified Blank. Restrictions on the concentration of analyte fortification of the Laboratory Spiked Sample and Laboratory Fortified Blank are defined in the individual analytical method SOP.

4.1.3 One Laboratory Reference Material. Appropriate Laboratory Reference Materials are defined in the individual analytical method SOP.

4.2 Pesticides

4.2.1 At least one Laboratory (Reagent) Blank

4.2.2 One Laboratory Sample (Background for Spike) and corresponding Laboratory Spiked Sample. Restrictions on the concentration of analyte fortification of the Laboratory Spiked Sample are defined in the individual analytical method SOP.

5. Data Analysis and Calculations

5.1 Spike Calculated Result (percent recovery) of Laboratory Spiked Sample is calculated as follows:

$$R = \frac{C_s - C}{s} \times 100$$

where, R = percent recovery
C_s = fortified sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to sample.

5.2 True Value Recovery of Laboratory Reference Material is calculated as follows:

$$R = \frac{C}{s} \times 100$$

where, R = percent recovery
C = reference material concentration
s = certified value (true value) of analyte for reference material.

6. Method Performance

In the event that the results for a set of quality control samples do not meet expected values, the analysis of all analytical solutions for the batch are routinely repeated once. Unacceptable instrument performance measures will be corrected before the repeat analysis. If the repeated results for the quality control samples are still not acceptable, those results are reported, and sample analysis continued. Unacceptable quality control samples will be reported to the supervisory chemist and reviewed with the chemist to determine the acceptability of the results.

6.1 Trace Elements

6.1.1 Laboratory (Reagent) Blank(s) must be below the Method Detection Limit.

6.1.2 Recovery of Laboratory Spiked Sample must be 70–130% of the expected concentration.

6.1.3 Laboratory Reference Material must be 70-130% of the expected concentration.

6.2 Pesticides

6.2.1 Laboratory (Reagent) Blank(s) must be below the Method Detection Limit.

5.2.2 Recovery of Laboratory Spiked Sample must be 70-130%.

7. Electronic Format for Analytical Results

7.1 Trace Elements

7.1.1 FDA Elemental Research Branch will produce computer text files of the analytical data as per document “Electronic Format for Analytical Results (August 15, 1995)” [appendix E of reference 8.3].

7.1.2 FDA Elemental Research Branch will transfer the computer text files of analytical results to the FDA Project Officer, NHEXAS IAG with the documentation listed in section 5.0 of reference 8.3.

7.1.3 The FDA Project Officer, NHEXAS IAG will send the FDA Elemental Research Branch computer text files of analytical results to NERL-CI as per reference 8.3.

7.2 Pesticides

7.2.1 FDA Kansas City District Laboratory will produce computer text files of the analytical data as per document "Electronic Format for Analytical Results (August 15, 1995) [appendix E of reference 8.3].

7.2.2 FDA Kansas City District Laboratory will transfer the computer text files of analytical results to the FDA Project Officer, NHEXAS IAG with the documentation listed in section 5.0 of reference 8.3.

7.2.3 The FDA Project Officer, NHEXAS IAG will send the FDA Kansas City District Laboratory computer text files of analytical results to NERL-CI as per reference 8.3.

8. References

8.1 Horwitz, W. (1990) *Pure & Appl. Chem.* **62**, 1193-1208.

8.2 Terwilliger, D. T., and Behbehani, A. L. (1994) User Guide for Manual Data Entry Software, version 2.0. U.S. EPA EMSL-Cincinnati Contract No. 68-C0-0001.

8.3 Berry, M., and Melnyk, L. J. (1995) Procedures for NHEXAS Phase I Sample Analysis by Federal Support Laboratories (NHEXAS.N1), Revision No. 0. U.S.EPA NERL-CI.

Standard Operating Procedure for Determination of Cadmium, Chromium, Lead, and Nickel in NHEXAS Food or Beverage Composites by Graphite Furnace Atomic Absorption Spectrometry

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1. Scope and Application

1.1 This test method describes procedures for determining trace concentrations of cadmium, chromium, lead, and nickel (Cd, Cr, Pb, Ni, respectively) in foods utilizing the technique of graphite furnace atomic absorption spectrometry (GFAAS). The test method is applicable to foods which may be solid, semi–solid, or liquid. The test method is not applicable, without special modification, to those foods which have a total lipid content greater than 70% by weight, for example, butter, margarine, mayonnaise, and cooking oils. The test method is applicable to the analysis of food and beverage samples with a trace metal concentration between 1 and 100 μg/kg. Therefore, the need may often arise to dilute or to concentrate test samples prior to their analysis. The measurement technique is limited to Zeeman correction of GFAAS because of high non–specific background absorbance usually present in food test samples.

1.2 This method is applicable to the following analytes:

Analyte	Chemical Abstract Services Registry Number (CASRN)
Cadmium (Cd)	7440-43-9
Chromium (Cr)	7440-47-3
Lead (Pb)	7439-92-1
Nickel (Ni)	7440-02-0

1.3 Achievable method detection limits (MDL) for food and beverage duplicate diets, in μg/kg, for each metal are: 2, 20, 10, and 20 for Cd, Cr, Pb, and Ni, respectively. The MDLs are appropriate for peak area measurement mode. The MDLs are expected to be also a function of food identity, instrumental operating conditions, sample preparation practices, and other analytical features. MDLs are not constants; the example magnitudes given here may substantially differ in use.

1.4 Maximum method imprecision, expressed as relative standard deviation (RSD), is ± 20%. The method RSD is a function, in part, of analyte concentration in the food test sample. The maximum method imprecision RSD is based on samples containing at least twice the MDL concentration level for the analyte. Method recovery is 100 ± 30% for all metal determinations.

2. Summary of Method[1]

2.1 Homogenized food test samples are prepared for trace metal determinations by thermal treatment in a muffle furnace for complete decomposition. Trace metals are then solubilized from residues remaining after test sample decomposition.

2.2 Aliquots of the resulting test solution are injected into a graphite furnace atomizer of an atomic absorption spectrophotometer capable of applying Zeeman correction to signals. The graphite furnace atomizer applies, through automatic program control, thermal steps to dry and calcine dissolved solids in an argon atmosphere. Immediately thereafter the argon flow is interrupted, and solid residues from the test solution are atomized rapidly. GFAAS instrumentation is operated in accordance with manufacturer's instructions for setting operational parameters for standardization and atomic absorption measurements of metals. Background correction is usually required for Cd and Pb signals. Calculation of results is an automatic function of the GFAAS

instrument. The analytical response curve is not expected to be linear, and, therefore, proprietary or other statistical fitting may be chosen to be implemented.

3. Definitions [2,3]

3.1 *Analytical portion*—The quantity of material, of proper size for measurement of the analyte of interest, removed from the analytical sample.

3.2 *Analytical response (standardization) curve*—the graphical or mathematical representation of a function relating the physicochemical response produced from a measurement procedure to a property magnitude of a standard.

3.3 *Analytical sample*—The sample, prepared from the laboratory sample, from which analytical portions are removed for analysis.

3.4 *Analytical solution*—The solution prepared by dissolving, with or without reaction, the analytical portion in a liquid.

3.5 *Calibration or standardization verification*—the action taken to establish the continued validity of calibration (standardization) during a time period between the original calibration (standardization) and required recalibration (restandardization).

3.6 *Characteristic mass*—the mass of analyte which corresponds to a 1% absorption of the radiant energy for an atomic absorption spectrophotometer and derived from an analytical response function defining the conversion for the scale of measurement. Customarily, characteristic mass is the smallest amount of standard that will produce a sufficient response for the analytical technique in the method and, therefore, is a quantitative expression for sensitivity.

3.7 *Instrumental detection limit (IDL)*—The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of ten replicate measurements of an LIB.

3.8 *Laboratory (dry) blank (LDB)*—Exactly the same as the *LRB* except the aliquot of reagent water or equivalent neutral reference material is omitted.

3.9 *Laboratory fortified blank (LFM)*—An aliquot of reagent water or equivalent neutral reference material, known to be below detection limits for an analyte(s), to which a known quantity(ies) of method analyte(s) was added. The *LFM* is then treated as a food or beverage test sample in all aspects in the laboratory including addition of all reagents, internal standards, surrogates, glassware, equipment, solvents, and analyses.

3.10 *Laboratory (instrument) blank (LIB)*—An aliquot of reagent water or other solvent(s), possibly adjusted in pH, analyzed as analytical solution by the instrument. This includes addition of internal standards and surrogates; but excludes all other sample preparation and cleanup.

3.11 *Laboratory reagent blank (LRB)*—an aliquot of laboratory pure water that is treated the same as a sample and is taken through the entire preparation process. The laboratory control blank is used to assess possible contamination for the sample preparation practice.

3.12 *Laboratory reference material (LRM)*—an analytical portion of a material or substance for which one or more properties are sufficiently well established to be used for calibration (standardization) verification of a device, the assessment of a measurement method, or for assigning values to comparable objects.

3.13 *Laboratory sample (background for spike) (LSO)*—The first of two analytical portions of a food or beverage test sample. This portion is analyzed according to the analytical method, to establish background concentrations prior to fortification (spiking) with the method analyte(s).

3.14 *Laboratory spiked sample (LSF)*—The second of the two analytical portions described under LSO. This portion is subject to fortification (spiking) prior to sample preparation, and the measurement(s) of the final concentration(s) are then made according to the analytical method.

3.15 *Matrix*—the principal constituent in a sample useful for identifying salient chemical behavior.

3.16 *Measurement*—a process, not necessarily direct, in which a comparison of a value, extent, or amount of a property for a test object is based on an established magnitude of the same property of an object accepted as a standard.

3.17 *Method detection limit (MDL)*—the mass or concentration of an analyte at which false negative and false positive error rates are specified for a single determination by an analytical method.

3.18 *Practice*—a definitive procedure for performing one or more specified operations or functions that does not produce a test result. [ASTM definition].

3.19 *Quality control sample (QCS)*—any sample used to establish or monitor measurement system performance.

3.20 *Recovery factor*—operationally defined as a measurement result produced from an analytical method for a physical or chemical property of a laboratory reference material with known value and expressed as a ratio (decimal) of that method's measurement result for the property to the property's known or expected value. Recovery assesses the capability of the analytical method of measurement to isolate the property to be measured from other properties not of interest but present and potentially or actually influential on the measurement method. Therefore, recovery characterizes the analytical method's specificity and has a quality of contrast. Recovery is an assessment of the total error (systematic bias and random error) for an analytical method considered in its entirety. As an error factor, recovery may be employed as a correction for test sample results obtained through application of a test method provided that the random error component is small.

3.21 *Sample blank*— a sample analytically prepared in its final test form and determined not

to contain a detectable amount of analyte. The sample blank is used to assess detection limit. The sample blank to which an addition of analyte in known amount has been made is used to assess recovery bias.

3.22 *Standard*—any substance, material, device, item, process, method, design, or accepted philosophical principle for which a property reference value can be established of known accuracy and technical limitation and application which can then serve as a comparison to the same property of another. [Standard connotes consensus and approval].

3.23 *Standardization*—the assignment, by reference to a standard, of a value or scale of measurement to a physicochemical property of a chemical system of defined composition or physical device being employed in a measurement procedure. [Standardization involves preparation of a chemical system or measuring device in a defined condition prior to assignment of measurement value or scale. The canonical condition of the measuring system has a quality of impermanence. For example, an analytical instrument if turned off after standardization and then turned on again will have lost its standardization and intended capacity for measuring properties. In contrast, a calibration is permanent because the settings or indicators of a reading are fixed to the measuring device or chemical system during its manufacture].

3.24 *Test method*—a definitive procedure for the identification, measurement, and evaluation of one or more qualities, characteristics, or properties of a material, product, system, or service that produces a test result. [ASTM definition].

4. Interferences

4.1 Interferences which may affect measurement accuracy have been minimized through the use of appropriate test sample preparation practices, the use of chemical modifier additions to the test sample preparations if required, the use of Zeeman background correction as applicable, and the use of an automatic program which controls temperature stages of drying, calcining, atomizing, and cleaning for the graphite furnace atomizer.

4.2 Test samples prepared in concentrated mineral acids engender difficulties during atomization of metal analytes. These analytical difficulties manifest themselves ultimately in degradation of the symmetry and substantial reduction in the magnitude for the atomization signals of the elemental analytes. The physical materials of the graphite furnace are not resistant towards the high temperature corrosive action of concentrated mineral acid solutions. Continued use of concentrated acids causes release of copious amounts of light–scattering, incandescent, carbon soot and perforation of the tube wall. To prevent deterioration of the graphite furnace components with consequent deleterious effects on the atomization signal, strong acids must be diluted substantially so that final concentrations are less than 5% v/v. Sulfuric (20% v/v), nitric (20–50% v/v), and perchloric (10% v/v) acids render ineffective any attempt at routine analyte measurement.

4.3 For some foods, use of sulfuric acid induced considerable losses of both Cd and Pb. Because losses were not observed in fortified reagent blanks containing sulfuric acid, the causes were attributable to alkaline metal residues that had been converted to fixed sulfates during sample decomposition.

4.4 If present in abundance, the ash from a food tends to perturb atomic absorption measurements for the four trace metals. Instrumental correction for non–specific absorbance and the addition of chemical agents, matrix modifiers, help to remove this perturbation.

4.4.1 For Cd and Pb, an addition of a dilute solution of ammonium dihydrogen phosphate is usually required. Also trace concentrations of Cd and Pb in food test samples are best determined using platform atomization.

4.4.2 For Cr and Ni, proper selection of temperature treatment in the graphite furnace is usually sufficient to control interferences. Wall atomization provides the best conditions for effectively reducing interference effects on trace Cr and Ni by food ash.

4.4.3 Using dilute nitric acid (1–5% v/v) as the principal solvent is beneficial in establishing the most appropriate high temperature phase transformations for all four elements into their oxides.

4.4.4 If chloride is present in significant amount in the test sample solution, the use of palladium modifier is usually effective in countering interference effects. (For Cd and Pb, ammonium dihydrogen phosphate can also eliminate chloride interference to some extent. Palladium offers no special advantages to Cd and Pb determinations, except in the presence of abundant chloride, in food test samples. Usually palladium reduces sensitivity as compared with phosphate modifier). Often magnesium nitrate may be used in concert with palladium for eliminating chloride interference for trace Cr and Ni determinations.

4.4.5 Occasionally, high iron foods may also present similar problems to the determination of all these trace elements. Zeeman correction of the atomization signal eliminates the interference effect of iron. Other background correction systems are less effective in removing the interference effect of iron.

4.5 All atomic absorption instruments, regardless of manufacturer, have an upper limit to reliable background correction. Though strictly not an interference, background correction limits are an additional complication in interpreting analyte atomization signals. Background signals larger than about 1.5 absorbance usually are fruitful sources of measurement problems for any analyte.

5. Safety

The test method involves use of heat sources, chemical fumes, and toxic substances. Exposure should be kept to a minimum.

6. Equipment and Supplies

6.1 This method has been developed and tested for determination of trace metal concentrations in foods with Varian Models SpectrAA 300Z and SpectrAA 800Z. These instruments were selected because of superior technical performance capabilities in conducting trace element measurements in foods after comparisons with other GFAAS instruments. (The practices and procedures described will have to be modified if other GFAAS instrument models are employed). The graphite furnace and magnetic work heads of these instruments are cooled with Neslab CFT 33 refrigerated recirculators. Using potable water service to cool the instruments is not recommended.

6.1.1 Single element hollow cathode lamps serve as the preferred radiation sources to generate atomic resonance line spectra for the four elements.

6.1.2 Argon gas (99.99% pure) is exclusively used for furnace operations to determine Cd, Cr, Pb, and Ni.

6.2 All other apparatus and materials, e.g. muffle furnaces, glass ware, hot plates, analytical balances, drying ovens, desiccators, etc., are usual laboratory appliances. Reusable lab ware are cleansed by scrubbing with detergent, rinsing in tap water, overnight immersion in 10% nitric acid, and finally rinsing with reagent water.

7. Reagents and Standards

7.1 Required reagents are of sufficient purity so that no detectable quantity of trace metal will be present in their final prepared forms as utilized in the analytical method. Preparation and handling of working solutions follow customary practices for dilution of stock solutions. Standard stock solutions are obtained commercially as certified preparations.

7.2 Matrix modifier[4-7]

7.2.1 Ammonium dihydrogen phosphate: Dissolve 5.00 g monobasic ammonium phosphate in water containing 3.0 mL of nitric acid and then dilute to 1 L with additional water. The white powder dissolves slowly and requires agitation. The modifier is used at this concentration. This modifier is used for Cd and Pb determinations in foods not rich in phosphate.

7.2.2 Magnesium nitrate: Dissolve 105.5 g of magnesium nitrate hexahydrate in water and dilute to 1 L. The white, often caked, crystal dissolves slowly and endothermically. Vigorous agitation is necessary for complete solution in minimal time. The concentration is equivalent to 61 g $Mg(NO_3)_2$/L or 10 g Mg/L. The concentration is normally further diluted to suit requirements. Usually the modifier is used for foods rich in chloride for determinations of all four trace metals. The modifier may be combined with other modifiers for best effectiveness.

7.2.3 Palladium: Dissolve 1.0 g palladium metal, preferably as sponge, in 15 mL of concentrated nitric acid. Moderate warming of the reaction mixture often helps to initiate the dissolution. Dilute to 100 mL with water. The solution color is red–

brown in excess nitric acid. The concentration must be further diluted in analytical applications as required.

7.3 Metal standards. Standard solutions may be purchased or prepared from known purity materials as required. Working standards are prepared by dilution to suitable concentrations.

7.3.1 Cadmium stock solution, 1 mL = 1000 μg Cd: Dissolve 1.000 g Cd metal in 50 mL (1+1) HNO_3 with heating. Dilute to 1 L with water.

7.3.2 Chromium stock solution, 1 mL = 1000 μg Cr: Dissolve 1.923 g CrO_3 in 120 mL (1+5) HNO_3. Dilute to 1 L with water.

7.3.3 Lead stock solution, 1 mL = 1000 μg Pb: Dissolve 1.599 g $Pb(NO_3)_2$ in 20 mL (1+1) HNO_3. Dilute to 1 L with water.

7.3.4 Nickel stock solution, 1 mL = 1000 μg Ni: Dissolve 1.000 g nickel metal in 20 mL hot concentrated HNO_3. Dilute to 1 L with water.

7.4 Nitric acid, concentrated.

7.5 Hydrogen peroxide, 50%[8].

8. Sample Collection, Preservation and Storage

Samples for analysis are kept frozen and stored in plastic containers which have metal– free surfaces prior to implementation of any analytical procedures. Specific details on insuring analytical sample integrity and record keeping are contained in the Storage and Custody SOP (ERB SOP 104).

9. Quality Control

9.1 In part, measurement validation is controlled with a set of quality control samples which may be comprised of at least one each of laboratory reagent blanks (LRB), sample blanks, fortified sample blanks (LFM), or laboratory reference materials (LRM) used as test samples.

9.1.1 Laboratory (Reagent) Blank (LRB)—Analytical results for LRBs are acceptable when the analyte concentration is less than 2.3 x MDL.

9.1.2 Laboratory Fortified Blank (LFM)—Analytical results for LFMs are acceptable when recoveries are 70 to 130% of the expected fortified concentration.

9.1.3 Laboratory Reference Material (LRM)—Analytical results for LRMs are acceptable when true value recoveries are 70 to 130% of the expected concentration.

9.2 In the event that the results for the set of quality control samples do not meet expected values, the analysis of all analytical solutions for the batch are routinely repeated once. Preferably, this action is automatically controlled by the instrument program. Unaccept-

able instrument performance measures will be corrected before the repeat analysis. If the repeated results for the quality control samples are still not acceptable, those results are reported, and the sample analysis continued. Unacceptable quality control samples will be reported to the supervisory chemist and reviewed with the chemist to determine the acceptability of the results.

9.3 New preparations of working standards are compared to previous standards to maintain continuity of analytical sensitivity. Continuity in working standards shall be acceptable provided that the peak area characteristic mass (pg/1%) be not greater than 0.6, 8, 15, and 8 for Cd, Cr, Pb, and Ni, respectively.

9.4 Instrument records of operating parameters and calculated results are kept in electronic or printed format for a period of one year.

9.5 Instruments are serviced by the manufacturer through a maintenance contract to assure that instrumental performance parameters are within original specifications.

10. Calibration and Standardization

10.1 *Spectrophotometer and furnace standardization*—Operating conditions for GFAAS are set according to manufacturer's guidelines. Specific conditions are listed in Table 1. The settings are regarded as parameters. No verification of wavelength and slit accuracy is made. Manufacturer's tolerances are accepted as is.

10.1.1 *Temperature programs for the graphite furnace are often compromise conditions. Foods and their trace elements always display varied and sometimes intractable analytical behaviors.* An attempt is made to achieve similar behaviors for standards and trace analytes in test food sample preparations through an appropriate choice of temperature and time settings for the furnace. Because Cd and Pb both have similar thermal behavior, the two elements may be conveniently classed into the same analytical category. Both Cr and Ni behave alike thermally, and both fundamentally behave differently from Cd and Pb. The volatility of Cd and Pb requires that a platform be used in thermal treatment of residues containing them. The refractory behavior of Cr and Ni requires that residues containing these elements be deposited directly on the furnace wall. Based on sensitivity, Pb is the most difficult element to determine, and Cd is the easiest to determine of the four considered in this test method. Ni is somewhat less difficult to determine than Cr. *It is usually necessary to test the furnace settings for time and temperature to achieve maximum sensitivity.*

10.1.2 Zeeman correction of Cd atomization signals for background is readily achieved (background < 1 absorbance) because of the Cd sensitivity and the temporal separation of the Cd signal from that of the background signal. The background signal often follows the sharp Cd signal. For the Pb signal, no combination of time and temperature is adequate to consistently achieve its separation from the background. Qualitatively, Pb signals are broader than Cd signals. Both signals are symmetrical. Sulfur and phosphorous compounds of alkali and alkaline earth elements, excluding hydrogen, severely disrupt Cd and Pb atomization signals. The molecular absorption band heads for many compounds of sulfur and phosphorous released from the hot carbon furnace surface begin in the ultra

violet wavelength region used for determinations of Cd and Pb. These molecular absorbance bands have spectral structure under Zeeman conditions which cause systematic errors in determining Cd or Pb. Allotropic forms of S and P, CS_2, and reduced oxides of SO_4^{-2} and PO_4^{-3} all affect the solid and gas state phase transformations of Cd and Pb during calcination and atomization. Most foods have sulfur contents which are, for the most part, readily removed during the test sample preparation as a volatile sulfide. The formation of a fixed sulfate as the result of oxidative physicochemical treatment is not usually encountered unless sulfuric acid has been employed in the destruction of foods containing abundant salt. The natural abundance of sulfate in foods is low enough to be inconsequential[9] during the graphite furnace temperature program. On the other hand, many foods contain phosphate or substances degradable to phosphate. Dairy foods are particularly troublesome for determination of Cd and Pb because of their low concentrations in a phosphate–rich matrix. There is little justification in making a problem worse by addition of a phosphate modifier in the graphite furnace to a dairy test food sample. Palladium or platinum modifiers are not useful substitutes for chemical modification in phosphate–rich residues. Also, alternative resonance lines of Cd and Pb are not beneficial for determinations in phosphate–rich residues.

10.1.3 Unlike Cd and Pb, both Cr and Ni interact chemically to some degree with the hot carbon furnace surface or sublimated carbon or volatile oxides of carbon produced during atomization. The atomization signals for both elements are asymmetric with pronounced tailing for Cr more so than for Ni. Because the atomic resonance line for Ni is at a low wavelength in the ultra violet, it suffers from the same effects of background molecular absorbance as for Cd and Pb. The wavelength for the atomic resonance line of Cr is largely unaffected by structured molecular absorbance of background species for which band heads arise in the ultra violet.

10.1.4 The furnace temperature/time program incorporates stages for drying, calcining, atomizing, and cleaning. A careful selection of temperature and duration can be effective in reducing the effects of background and increasing the sensitivity. The most critical settings are for the calcination step. Most volatilization losses for analytes occur during the ramp to and dwell at the calcination temperature. The behavior of Cd, Cr, Ni, and Pb as standards and as trace elements in food decomposition residues can be strikingly different. To insure maximum sensitivity, the temperature program for the graphite furnace should be tested on a standard and on a test food preparation. Adjustments to temperature and time are often required. Usually the instrumental parameter settings controlling temperature/time conditions are a compromise rather than a fine tuning. Nevertheless, it is necessary to at least check the settings. Aging of the tube makes a test check advisable.

10.1.4.1 Injection of test samples and additional solutions into a preheated graphite furnace ("hot injection") is typically employed in order to shorten furnace time between successive atomizations or to limit the spreading of the deposit. For a hot injection onto a platform, the furnace temperature is 130 to 150°C. For a hot injection onto the wall, the furnace temperature is 100 to 120°C. In cases were the test sample has substantial alcohol content, the hot injections are done at about 75 to 85°C. Hot injections are programmed to

deliver the injection volume in times between 16.5 and 23.7 seconds which correspond to instrument rate settings of 6 and 10 respectively. The test sample and any additions are submitted to the action of the drying temperature for at least 15 seconds but no longer than 30 seconds. Of course a cold injection will suffice as well.

10.1.4.2 The temperature is then ramped at a rate of 50C°/s until the calcination stage is reached. Water of crystallization or occluded water may form during the drying stage. Usually the ramp rate is slow enough to prevent decrepitation. Sometimes it is useful to incorporate a pause at about 300°C to insure that most water is removed without residue spattering taking place as a result of crystal breaking. Crackling sounds from the furnace during this time are usually a bad sign. However, entrained water in oil or residual solvent in oil will sometimes superheat and be violently expelled unavoidably with a popping sound.

10.1.4.3 Two controlling factors influence the choice of calcination temperature: phase transformations and atomization temperature. Generally calcination causes losses of the analyte. As long as the losses are reproducible and small, they may be accepted if concordant reduction in background is achieved. Recognition of possible phase transformations aids in proper selection of temperature and duration of the calcination stage to achieve maximum sensitivity. A calcination temperature is chosen to avoid active phase transitions, such as melting or decomposition, in order to establish reproducible sensitivity. Atomization temperatures are chosen relative to the calcination temperature. The maximum temperature ramp rate is desired in changing from the calcination temperature to the atomization temperature in the shortest possible time. Typically the maximum rate of temperature change is 2000C°/s for a graphite furnace. Therefore, the maximum practical calcination temperature should be selected so that the atomization temperature is not more than 2000°C higher and, preferably, so that atomization is achievable in a few tenths of a second.

10.1.4.4 It is sometimes useful to incorporate a "cool–down" step after the calcination stage is finished. A "cool–down" simply means that the furnace temperature is programmed to some lower temperature than an immediately preceding step. Recognition that a substantial amount of background producing residue yet remains after calcination serves as an incentive for using a "cool–down" step. With a maximum ramp rate, the temperature is necessarily slowly raised from the "cool–down" step to the atomization step. The net effect is that residue smoke is ideally dispelled from the furnace before any atomic vapors from a chemically stabilized analyte are atomized. This approach may be of some benefit to high lipid or high carbohydrate test samples which were not prepared through a muffle furnace decomposition and which leave substantial carbonized residues in the furnace following calcination of the mineral components. Also for refractory trace elements requiring a high temperature calcination, a "cool–down" may be appropriate to accommodate a rapid atomization so that the furnace can be maintained at the atomization temperature for a longer time.

10.1.4.5 On initiating atomization, it is useful to have all gas flow through the

furnace stopped. Atomic vapor then will be in the light path of the resonance line for a longer time because diffusional transport will be the only operative mechanism to remove the atomization cloud. Typically the inert gas flow is stopped in the last two seconds of the calcination step. The two second time interval is required to mechanically shut the solenoid gas valve and allow inert gas flow to come to a full stop. The stopped gas flow is continued for the duration of the atomization, 3 to 5 seconds, during which measurements are made with and without magnetic degeneracy of the transient atomic absorbance. After atomization, restoration of gas flow is implemented to sweep out any remaining vapor ("clean–out"). The furnace is then permitted to cool for the next test sample injection cycle.

10.2 *Measurement scale standardization*—Instrumental response, in absorbance, is converted to physically meaningful units of mass or concentration by a scale conversion. Scale definition of response may be performed using standards combined within the test sample, internal standardization mode, or using standards separate from the test samples, external standardization mode. Internal standardization of absorbance is often called method of standard additions. External standardization of absorbance is most often used for GFAAS because of experimental simplicity. Internal standardization is used for test samples which have interferences affecting the analyte absorbance (usually suppression effects rather than enhancement effects, though both occur). GFAAS standardizations are often automated following manufacturer's instructions for use of equipment.

11. Procedure

11.1 Weigh, to the nearest 0.01 g, an analytical portion of 1.00 to 20.00 g well mixed sample in a quartz beaker. Add an amount of nitric acid sufficient to cover the test sample. Magnesium nitrate may also be added in the ratio of 0.2 mL of 50% w/v to 1.00 g sample. The batch of analytical samples and their quality control samples are digested on a hot plate at about 100°C until just dry. Addition of two or three portions of 0.5 to 1.0 mL concentrated hydrogen peroxide (30 or 50%) near the end of the digestion has been beneficial in some cases. The batch is transferred to a programmable muffle furnace to complete the decomposition through step wise temperature stages of 100°C, 300°C, and 475°C in eight hours. The procedure is repeated for the residue without the pause at 300°C until it becomes grayish white. With magnesium nitrate, often a single thermal treatment is sufficient whereas without magnesium nitrate two cycles of thermal treatment are required. The final residue is treated with 5.0 or 10.0 mL of dilute nitric acid (2% v/v), and the resulting test solution is analyzed.

11.2 Determinations of Cd and Pb are performed with platform atomization. The platform may be forked or L'vov style and should be solid pyrolytic graphite. Manufacturer's recommendations for spectrometer's optical parameters are employed. Usually 2 to 5 µL of 0.5% w/v of ammonium dihydrogen phosphate is injected along with 8 µL of test solution onto the furnace platform. Other chemical solutions may be required to control the physicochemical interactions between the analyte and its concomitant components. Peak area measurement mode is employed. Background correction is required. An aqueous based test solution is dried at a suitable temperature between 100 to 150°C for 20 seconds, calcined at 900°C for 8 seconds, atomized for 3 seconds at 2600°C for Pb and 2400°C for Cd under gas stop conditions, and residues removed from the furnace at

2600°C with gas flow fully restored for 3 seconds. External standardization of instrumental response is normally chosen to define the analytical response curve. One of the GFAAS instrument's own algorithms is normally used for automated standardization and calculation of results. However, recourse to other styles of standardization and operating conditions may be required and is discretionary to the analyst's best professional judgement.

11.3 Determinations of chromium and nickel are performed with wall atomization. Pyrolytic graphite coated tubes are recommended. Manufacturer's recommendations for spectrometer's optical parameters are employed. An injection of 8 μL of test solution is deposited on the inner wall of the furnace. Addition of other chemical solutions as modifiers may be required to control one or both of the interactions of analyte with concomitant components and with the graphitic carbon furnace surfaces. Peak area measurement mode is employed. Background correction is required for Ni and is recommended for Cr. An aqueous based test solution is dried at a suitable temperature between 100 and 120°C for 20 seconds, calcined at 1200°C for 8 seconds, atomized for 4 seconds at 2600°C under gas stop condition, and residues removed from the furnace at 2600°C with gas flow fully restored for 3 seconds. External standardization is normally chosen to define the analytical response curve through implementation of one of the GFAAS instrument's algorithms. Standardization and operating conditions are discretionary to the analyst's best professional judgement.

12. Data Analysis and Calculations

Calculations of results are automated. The instrumental algorithms have been tested and are found to be reliable.

12.1 Analytical solution and analytical portion concentrations are calculated by the spectrometer data system.

12.2 LRBs should be reported on the basis of the analytical solution in units of μg/L or ng/L.

12.3 LRMs and food or beverage composites should be reported on the basis of the analytical sample in units of mg/kg or μg/kg.

12.4 Spike calculated result (percent recovery) of laboratory spiked sample is calculated as follows:

$$R = \frac{C_s - C}{s} \times 100\%$$

where,
- R = percent recovery
- C_s = fortified sample concentration (i.e., LSF or LFM)
- C = sample background concentration (i.e., LSO or 0)
- s = concentration equivalent of analyte added to sample.

12.5 True value recovery of LRMs is calculated as follows:

$$R = \frac{C}{s} \times 100\%$$

where, R = percent recovery
C = reference material concentration
s = certified value (true value) of analyte for reference material.

13. Method Performance

The validity of the analytical method was assessed by replicated analyses of laboratory reagent blanks (LRB) and reference materials (LRM). Blanks were used to establish that the analytical conditions were free of contamination. LRMs were used to define limits of systematic bias and detection.

13.1 Detection limits of the analytical method were established for each metal. The method detection limit (MDL) was based on experimental findings for eight independently prepared replicates of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1549 (Non–fat milk powder). The MDLs for each metal were assigned values based on a Type B evaluation of uncertainty as defined and explained in B.N. Taylor and C.E. Kuyatt, *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results,* **NIST Technical Note 1297**, September 1994 Edition (US Government Printing Office, Washington, DC, 1994). Each MDL was applicable for peak area scale. The MDLs were: 2, 20, 10, and 20 μg/kg for Cd, Cr, Pb, and Ni, respectively.

13.2 Method precision was assessed in terms of relative standard deviation (RSD) for replicate analysis of LRMs. The judgement for an acceptable method relative precision for samples containing at least twice the MDL concentration level for each metal was: ± 15%, ± 15%, ± 20%, and ± 10% for Cd, Cr, Pb, and Ni, respectively.

13.3 Analytical specificity of the method was evaluated using different LRMs as test samples. Independently prepared replicates of each of the LRMs were processed through the entire method as analytical batches. A batch consisted of at least eight replicates of LRM and two blank replicates. DORM–1 test samples were processed in two batches. All other LRMs were processed as single batches. Method recovery was acceptable within limits of 70 to 130% for all four metals. Results for metal findings for LRMs from NIST, National Research Council of Canada (NRCC), and the International Atomic Energy Agency (IAEA) are listed in Table 2.

14. Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, recycling is the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15. Waste Management

15.1 Laboratory waste management practices should be conducted consistent with all applicable rules and regulations. Laboratories should protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in section 14.2.

16. References

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Table 1. GFAAS Operating Conditions

			Temperature			
Element	Line[a]	Slit[b]	Calcine[c]	Atomize[c]	Modifier	Sensitivity[d]
Cd	228.8	0.5	900	2400	5–10 μg PO_4	0.6
Cr	357.9	0.2	1200	2600		3
Pb	283.3	0.5	900	2600	5–10 μg PO_4	15
Ni	232.0	0.2	1200	2600		5

[a]Atomic resonance line, nm
[b]Slit width, nm
[c]Graphite furnace temperature, °C
[d]Characteristic mass for peak area, pg/1% integrated absorption

Table 2. Initial Demonstration of Performance—GFAAS Average Analytical Results[a]

	Element			
	Cd	Cr	Pb	Ni
NRCC DORM–1 (dogfish muscle) (n=16)				
Composition, mg/kg	0.086±0.012	3.60±0.40	0.4±0.12	1.20±0.30
Method Result, mg/kg	0.098±0.004	4.53±0.51	0.4±0.05	1.41±0.06
Method Result RSD, %	7.2	11	10	7.6
True Value Recovery[b], %	110	126	100	118
IAEA H–9 (mixed human diet) (n=8)				
Composition, mg/kg	0.0315±0.0045	0.15±0.04	0.11±0.03	0.27±0.05
Method Result, mg/kg	0.0318±0.0014	0.12±0.01	0.11±0.04	0.21±0.01
Method Result RSD, %	12	13	23	4.2
True Value Recovery[b], %	101	80	100	78
NIST RM 8431 (mixed diet) (n=8)				
Composition, mg/kg	0.0418±0.0112	0.102±0.006	nv	0.644±0.151
Method Result, mg/kg	0.0417±0.0027	0.111±0.013	na	0.651±0.026
Method Result RSD, %	11	14	na	4.8
True Value Recovery[b], %	99.8	109	na	101
NIST SRM 1549 (non–fat milk powder) (n=8)				
Composition. mg/kg	0.0005±0.0002	0.0026±0.0007	0.019±0.003	nv
Method Result, mg/kg	0.0007±0.0002	< MDL	0.025±0.006	< MDL
Method Result RSD, %	60	undefined	29	undefined
True Value Recovery[b], %	130	undefined	130	undefined
NIST SRM 1566 (oyster tissue) (n=9)				
Composition, mg/kg	3.5±0.4	0.69±0.27	0.48±0.04	1.03±0.19
Method Result, mg/kg	na	0.71±0.05	na	1.06±0.03
Method Result RSD, %	na	9.8	na	3.2
True Value Recovery[b], %	na	100	na	103
NIST SRM 1572 (citrus leaves) (n=9)				
Composition, mg/kg	0.03±0.01	0.8±0.2	13.3±2.4	0.6±0.3
Method Result, mg/kg	na	0.8±0.03	na	0.5±0.03
Method Result RSD, %	na	3.9	na	7.0
True Value Recovery[b], %	na	100	na	80

[a] nv = no value provided by certifying organization
na = not analyzed
± = uncertainty on the basis of judgement or statistical interval
< MDL = less than method detection limit (no reliable measurable signal observed)

[b] percent recovery of method result based on compositional information provided by reference material's issuing organization

Standard Operating Procedure for Determination of Barium, Copper, Manganese, Vanadium, and Zinc in NHEXAS Food or Beverage Composites by Inductively Coupled Plasma Atomic Emission Spectrometry

U. S Food and Drug Administration

Center for Food Safety and Applied Nutrition
Elemental Research Branch
Washington, DC

Prepared by: _____
Chemist, ERB

Approved by: _____
Chief, ERB

: _____
Director, DPIC

: _____
FDA Project Officer
NHEXAS EPA IAG

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1. Scope and Application

1.1 This method is used to determine trace elements in duplicate diet food and beverage homogenates by microwave digestion/inductively coupled plasma-atomic emission spectrometry (ICP-AES).

1.2 This method is applicable to the following analytes:

Analyte	Chemical Abstract Services Registry Number (CASRN)
Barium (Ba)	7440-39-3
Copper (Cu)	7440-50-8
Manganese (Mn)	7439-96-5
Vanadium (V)	7440-62-2
Zinc (Zn)	7440-66-6

1.3 Listed in Table 1 are the wavelengths used for validation of these analytes along with adjacent locations for background correction. Listed in Table 3 are instrument detection limits (IDLs Sect. 3.10) determined using reagent acid, ASTM type I water and ultrasonic nebulization (USN) sample introduction into the plasma.

1.4 Specific instrumental operating conditions are given in Table 2.

2. Summary of Method

2.1 Method description—This method describes a technique for simultaneous multielement analysis of foods. The basis of the method is the measurement of atomic emission by an optical spectrometric technique. Food digest solutions are nebulized and the aerosol that is produced is transported to the plasma torch where desolvation and excitation occur. Characteristic atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma (ICP). The spectra are dispersed by a grating spectrometer, and line intensities are monitored by a photomultiplier tube. Photocurrents from the photomultiplier tube are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the determination of the analytes. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must either be free of spectral interference or adequately corrected to reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

2.2 Analytical sensitivity and specificity—Method detection limits (Sect. 3.10) for the analytes listed are presented in Table 4. These data were calculated by estimation of the standard deviation of laboratory reagent blanks (Sect. 3.12), with at least six replicate measurements.

2.3 Analytical recovery and precision—Recovery of analyte was assessed by analysis of reference materials and fortified food and beverage homogenates. Method recovery was within limits of 70 to 130% for all six elements. Method Precision was assessed as relative standard deviation (RSD) of replicate analyses of reference materials and fortified

food and beverage homogenates. Method precision was within limits of ±20% RSD (Sect. 13).

3. Definitions[1-3]

3.1 *Analytical Sample*—The sample, prepared from the laboratory sample, from which analytical portions are removed for analysis.

3.2 *Analytical Portion*—The quantity of material, of proper size for measurement of the analyte of interest, removed from the analytical sample.

3.3 *Analytical Solution*—The solution prepared by dissolving, with or without reaction, the analytical portion in a liquid.

3.4 *Laboratory Sample (Background for Spike) (LSO)*—The first of two analytical portions of a food or beverage test sample. This portion is analyzed according to the analytical method, to establish background concentrations prior to fortification (spiking) with the method analyte(s).

3.5 *Laboratory Spiked Sample (LSF)*—The second of the two analytical portions described under LSO. This portion is subject to fortification (spiking) prior to sample preparation, and the measurement(s) of the final concentration(s) are then made according to the analytical method.

3.6 *Laboratory (Instrument) Blank (LIB)*—An aliquot of reagent water or other solvent(s), possibly adjusted in pH, analyzed as analytical solution by the instrument. This includes addition of internal standards and surrogates; but excludes all other sample preparation and cleanup.

3.7 *Laboratory Reference Material (LRM)*—An analytical portion of a food or beverage material having a certified value. These materials are usually obtained from the National Institute of Standards and Technology (NIST), the National Research Council of Canada (NRCC), Bureau of Reference Materials of the European Communities (BCR), etc. The *LRM* is treated as a food or beverage analytical sample in all aspects in the laboratory. This includes addition of all reagents, internal standards, surrogates, labware, apparatus, equipment, solvents, and analyses.

3.8 *Instrumental Detection Limit (IDL)*—The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of ten replicate measurements of an LIB signal at the same wavelength.

3.9 *Method Detection Limit (MDL)*—The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero (Sect. 9.1.2).

3.10 *Laboratory Reagent Blank (LRB)* (preparation blank)—An aliquot of reagent water that is treated exactly as an analytical portion including exposure to all glassware, equipment, reagents, and acids that are used with other analytical portions. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents or apparatus (Sects. 7.6.2 and 9.2.1).

3.11 *Calibration Blank*—A volume of ASTM type I water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP instrument (Sect. 7.6.1).

3.12 *Stock Standard Solution*—A concentrated solution containing one analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source (Sect. 7.4). Stock standard solutions are used to prepare calibration solutions and other needed analyte solutions.

3.13 *Calibration Standard (CAL)*—A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration (Sect. 7.5 and Table 3).

3.14 *Instrument Performance Check (IPC) Solution*—A solution of method analytes, used to evaluate the performance of the instrument system with respect to a defined set of method criteria (Sects. 7.7, 9.2.2, and Table 3).

3.15 *Linear Dynamic Range (LDR)*—The concentration range over which the analytical curve remains linear.

4. Interferences

4.1 Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from line emission of high concentration elements, overlap of a spectral line from another element, or unresolved overlap of molecular band spectra.

4.1.1 Background emission and stray light can usually be compensated for by subtracting background emission determined by measurement(s) adjacent to the analyte wavelength peak. Spectral scans of samples or single element solutions in the analyte regions may indicate not only when alternate wavelengths are desirable because of severe spectral interference, but also will show whether the most appropriate estimate of the background emission is provided by an interpolation from measurements on both sides or the other. The location(s) selected for measurement of background intensity will be determined by the complexity of spectrum adjacent to the wavelength peak. The location(s) used for routine measurement must be free of off-line spectral interference (interelement or molecular) or adequately corrected to reflect the same change in background intensity as occurs at the wavelength peak.

4.1.2 Spectral overlap may be avoided by using alternate wavelengths or can be compensated for by equations that correct for interelement contributions, which involves measuring the interfering elements. Extensive information on interferant effects at various wavelengths and resolutions is available in Boumans' Tables and Winge's Atlas.[4, 5] Users may apply interelement correction factors determined on their instruments within tested concentration ranges to compensate (off-line or on-line) for effects of interfering elements. Interelement corrections that constitute a major portion of an emission signal may not yield accurate data. Trace element levels typically found in foods do not cause significant spectral overlap for the elements determined by this method. [4-6]

4.1.3 The interference effects must be evaluated for each individual instrument. To

determine the appropriate location for off-line background correction, the user must scan the area on either side adjacent to the wavelength and record the apparent emission intensity from all other method analytes. The user must determine and document both on-line and off-line spectral interference effect from all method analytes and provide for their automatic correction on all analyses. Tests to determine the spectral interference must be done using analyte concentrations that will adequately describe the interference. Normally, 100 mg/L single element solutions are sufficient.

4.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, or using an appropriate internal standard element.

4.3 Chemical interferences include molecular-compound formation, ionization effects, and solute-vaporization effects. Normally, these effects are not significant with ICP-AES. If observed, they can be minimized by careful selection of operating conditions, buffering the sample, matrix matching, and using method of standard-additions. Chemical interferences are highly dependent on matrix type and specific analyte element determined.

4.4 Memory interferences result when analytes in a previous sample contribute to the signals measured in a current sample. Memory effects can result from sample deposition on the uptake tubing to the nebulizer or from build-up of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to either their LDRs or concentrations ten times those usually encountered. The aspiration time should be the same as a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of two of the method detection limit should be noted. Until the required rinse time is established, this method recommends a rinse period of 60 sec between samples and standards. If a memory interference is suspected, the sample should be reanalyzed after a long rinse period.

5. Safety

5.1 Each chemical used in this method should be regarded as a potential health hazard, and exposure to these compounds should be as low as reasonably achievable. Specifically, concentrated nitric acid is moderately toxic and extremely irritating to skin and mucus membranes. Use in a hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection and latex gloves when working with reagents.

5.2 The inductively coupled plasma should only be viewed with proper eye protection from the ultraviolet emissions.

5.3 Precautions should be taken to minimize potential hazards. Basic good housekeeping

and safety practices such as the use of rubber or plastic gloves and safety glasses are highly recommended.

6. Equipment and Supplies

6.1 Analytical Instrumentation

6.1.1 ARL 3580 ICP-OES is a simultaneous spectrometer system.

6.1.2 ICP torch—ARL quartz torch with side arm.

6.1.2 Argon gas supply—Liquid, high purity grade (99.99%).

6.1.3 A variable speed peristaltic pump is required to deliver both standard and sample solutions to the nebulizer.

6.1.4 Mass flow controller to regulate the argon nebulizer flow rate.

6.1.5 CETAC U-5000AT Ultrasonic Nebulizer.

6.2 Analytical balance: Balance capable of weighing to the nearest 0.0001 g.

6.3 Labware—For the determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area, designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by (l) contributing contaminants through surface desorption or leaching, (2) depleting element concentrations through adsorption processes. All reusable labware (glass, quartz, polyethylene, Teflon, etc.), including the sample container, should be cleaned prior to use. Labware should be soaked overnight and thoroughly washed with laboratory-grade detergent and water, rinsed with water, and soaked for four hours in 10% nitric acid followed by rinsing with water.

6.3.1 Labware—Volumetric flasks, graduated cylinders, 500 mL wash bottles and 50 mL polypropylene conical tubes (Falcon brand or equivalent metal-free plastic).

6.3.2 Calibrated pipettes—10 mL, 5 mL, 1 mL, 100 μL, and 20 μL continuously adjustable digital microliter pipettes.

6.4 Microwave Digestion Equipment

6.4.1 Directions on use of microwave digestion equipment are specific to CEM brand equipment and assumes familiarity with the equipment. Use of this method with other brands of microwave digestion equipment may require modifications to this procedure.

6.4.2 Microwave digestion system with temperature and pressure control from 0 to 200°C and 0 to 600 psi. Microwave power range: 0-100% full power (630 watts ±50 watts), programmable in 1% increments (CEM corporation model MDS-2000 equipped for Heavy Duty Vessels (HDVs) or equivalent).

6.4.3 Microwave digestion vessels (CEM corporation Heavy Duty Vessels (HDVs) or equivalent)—TEM Teflon lined digestion vessels capable of operating up to 200°C and 600 psi and accessories.

6.4.4 Cleaning protocol

6.4.4.1 Disassemble the vessel cap and cover, removing the rupture membrane from the vent fitting. Pressure/temperature control covers need not be disassembled, simply remove the rupture membrane from the vent fitting. Vessel covers, liners and vent fittings should be soaked for at least 2 hours in a solution of liquid laboratory-grade detergent (MICRO brand or equivalent) and hot tap water. Other vessel components can be rinsed with reverse osmosis (RO) or higher quality water (at least 10 MΩ resistivity) and dried in a class 100 clean area. Thermowells should be wiped down with a paper towel and detergent solution. Rinse thermowells and vessel components with warm tap water and then rinse thoroughly with reverse osmosis (RO) or higher quality water (at least 10 MΩ resistivity). Dry in a class 100 clean area.

6.4.4.2 Assemble the cover and cap, inserting a rupture membrane in the vent fitting. Add 10 mL of ACS grade nitric acid to each liner and seal liner and cover in the digestion vessel body. Insert a thermowell into the pressure/temperature control cover and secure with a ferrule nut. Connect the pressure line to the pressure/temperature control cover and place the pressure/temperature control vessel in the carousel. Insert the fiber-optic temperature probe into the thermowell. Place the other vessels in the carousel and begin the microwave CLEAN program (Table 5).

6.4.4.3 After the vessels have cooled to less than 50°C remove them from the oven leaving the control vessel in place. Vent excess pressure from the control vessel by slowly loosening the pressure line. Remove the pressure line and fiber-optic temperature probe from the control vessel, placing the probe in one of the inlet/outlet ports. Turn the two-way valve to the open position and rinse the pressure line with at least 30 mL of water. Return the two-way valve to the neutral position.

6.4.4.4 Place the vessels in an exhausting fume hood and vent the excess pressure by slowly loosening the vent fitting. Using the HDV opening station, remove cap and cover assemblies from vessel bodies. Rinse off covers and liners with water into a waste container. Thoroughly, rinse covers and liners with water. A total of six rinses has been found to work but, the minimum number of rinses required to achieve undetectable method blanks has not been studied. Dry in a class 100 clean area. If the vessels are not used after drying store assembled in a class 100 clean area.

7. Reagents and Standards

7.1 Reagents may contain elemental impurities which might affect analytical data. Only high-purity reagents should be used whenever possible. All acids used for this method must be sufficiently pure to generate laboratory reagent blanks which measure below the

instrumental detection limit. Suitable acids are available from a number of manufacturers or may be prepared by sub-boiling distillation.

7.2 Nitric acid, concentrated (sp.gr. 1.41) (CASRN 7697-37-2) - HNO_3.

7.2.1 Nitric acid, ACS grade.

7.2.2 Nitric acid, double distilled.

7.3 Reagent water—All references to water unless otherwise stated refer to ASTM type I grade water.[7]

7.4 Standard Stock Solutions—1,000 µg/mL concentration standards are purchased from a reputable commercial source.

7.5 Mixed Calibration Standard (CAL) Solutions—Prepare mixed CAL solutions by combining appropriate volumes of the stock standard solutions in volumetric flasks to achieve the concentrations listed in Table 3. Transfer freshly prepared mixed CAL solutions to an acid clean, not previously used except for high purity acids, FEP fluorocarbon or polyethylene bottles for storage. Fresh mixed CAL solutions should be prepared as needed with the realization that concentration can change on aging. The CAL solutions must be initially verified using a quality control sample.

7.6 Blanks—Three types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, a laboratory reagent blank is used to assess possible contamination from the sample preparation procedure and a rinse blank is used to flush the instrument uptake system and nebulizer between standards and samples to reduce memory interferences.

7.6.1 The calibration blank is prepared by acidifying reagent water to the same concentration of acids as used for standards. The calibration blank should be stored in a FEP bottle.

7.6.2 The laboratory reagent blank (LRB) must contain all reagents in the same volumes as used in the processing of the samples. The LRB must be carried through the same entire preparation scheme as the sample as the samples including sample digestion.

7.6.3 The rinse blank is prepared by acidifying reagent water to the same concentration of acid as used in the calibration blank. The rinse blank should be stored in a FEP bottle.

7.7 Instrument Performance Check (IPC) Solution—The IPC solution is used to periodically verify instrument performance during analysis. It should be prepared in the same acid matrix as the calibration standards by combining method analytes at appropriate concentrations. The IPC solution should be prepared from the same standard stock solutions used to prepare the calibration standards and stored in an FEP bottle.

8. Sample Collection, Preservation and Storage

Specific details on insuring analytical sample integrity and record keeping are contained

in the SOP for Storage and Custody of NHEXAS Food or Beverage Composites (ERB SOP 104).

9. Quality Control

9.1 Initial Demonstration of Performance

9.1.1 The initial demonstration of performance is used to characterize instrument and laboratory performance prior to using this method for sample analyses.

9.1.2 Method detection limit (MDL)—MDLs are established for each element by analysis of at least six laboratory reagent blanks. The MDL is calulated as follows:

MDL = (t) X (S)
where:
t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.37 for six replicates].
S = Standard deviation of the replicate analyses.

9.1.3 Method Precision and Recovery—Method precision is assessed as relative standard deviation (RSD) of replicate analytical portions of appropriate LRMs and laboratory spiked samples (LSFs). The LSF matrix should be representative of the matrix to be run. Recovery of analyte is assessed by replicate analytical portions of LRMs and LSFs. See Section 13 for method performance data.

9.2 Assessing Laboratory Performance

9.2.1 Laboratory (Reagent) Blank (LRB)—At least two LRBs must be analyzed with each batch of 19 to 36 analytical portions and one LRB for batches of 18 or fewer analytical portions. LRB values that exceed the MDL indicate laboratory or reagent contamination. When LRB values constitute 10% or more of the analyte level determined for a sample or is 2.2 times the analyte MDL whichever is greater, fresh analytical portions must be prepared and analyzed for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.2.2 Instrument performance check (IPC) solution—For all determinations the laboratory must analyze the IPC solution (Sect. 7.11) immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution immediately following calibration must verify the instrument is within ± 5% of calibration with a relative standard deviation less than 3% from 5 or more replicate integrations. Subsequent analyses of the IPC solution must be within ± 10% of calibration. If the calibration can not be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution fails, sample analysis must be discontinued, the cause determined, corrected and/or the instrument recalibrated. All analytical solutions following the last acceptable IPC solution must be reanalyzed.

9.3 Assessing Analyte Recovery and Data Quality

9.3.1 Laboratory Spiked Sample (LSF) and Laboratory Sample (Background for Spike) (LSO)—At least one LSF and corresponding LSO must be analyzed with each batch of 36 or fewer analytical portions. Analyte fortification of the LSF should be 0.7 mg/kg and 0.1 mg/kg for food and beverage homogenates, respectively or a concentration approximately 100 times their respective MDLs, whichever is greater. The analytical sample chosen for fortification should be alternated between food and beverage homogenates. Recovery of the LSF must be 70–130% of the expected concentration.

9.3.2 Laboratory Reference Material (LRM)—At least one NIST RM 8415 (whole egg powder) must be analyzed with each batch of 36 or fewer analytical portions. The LRM must be 70-130% of the expected concentration with the exception of Ba which is only an informational value and is not certified.

9.4 In the event quality control sample results do not meet expected values, analysis of all analytical solutions for the batch are routinely repeated once. Unacceptable instrument performance measures will be corrected before the analysis is repeated. If repeated results for quality control samples are still not acceptable, those results are reported, and sample analysis is continued. Unacceptable quality control samples will be reported to the supervisory chemist and reviewed with the chemist to determine acceptability of the results.

10. Calibration and Standardization

10.1 Specific wavelengths are listed in Table 1. Other wavelengths may be substituted if they can provide the needed sensitivity and are free from or corrected for spectral interference. Instrument operating conditions are given in Table 2. If the method is used with a make and model spectrometer other than the one specified, operating conditions must be established which, when used, are capable of providing data of acceptable quality to the data user. The analyst should follow instructions provided by the instrument manufacturer unless other conditions provide similar or better performance for a particular analysis. Operating conditions for analytical solution analyses usually vary from 800 to 1500 W forward power, 12 to 20 L/min argon outer gas flow (plasma), 0.0 to 2.0 L/min argon intermediate gas flow (auxiliary), 0.3 to 1.5 L/min argon injector gas flow rate (nebulizer), 10 to 20 mm observation height (viewing), 1.0 to 3.0 mL/min sample uptake rate, and a measurement time near 1 s per wavelength peak for sequential instruments and 15 s per sample for simultaneous instruments.

10.2 Prior to using this method optimize the plasma and USN operating conditions.

10.2.1 Verify the instrument operating conditions are set to those found to be optimum (Table 4). Ignite the plasma and allow the instrument to become thermally stable before beginning. This usually requires at least 30 to 60 minutes of operation.

10.2.2 Optically profile the spectrometer with a 1 μg/mL solution of Mn.

10.2.3 Determine instrumental detection limits before sample analysis to verify instrumental performance.

10.3 Before using the procedure (Section 11) to analyze samples, there must be data available documenting initial demonstration of performance. The required data and procedure is described in Section 9.1 This data must be generated using the same instrument operating conditions and calibration routine (Sect. 11.2) to be used for sample analysis. This data must be kept on file and be available for review by the data user.

11. Procedure

11.1 Sample Preparation

11.1.1 Analytical Portions

11.1.1.1 For the digestion of a beverage homogenate reblend the analytical sample by vigorously shaking and immediately weigh a 10 mL analytical portion with a 10 mL air displacement pipetter into a tared, clean (see Sect. 6.4.3) microwave digestion vessel liner.

11.1.1.2 For the digestion of a food homogenate reblend the analytical sample by vigorously shaking or stirring with a Teflon spatula (depending on consistency) and immediately weigh approximately a 1.5 g analytical portion with an air displacement pipetter or Teflon spatula into a tared, clean (see Sect. 6.4.3) microwave digestion vessel liner.

11.1.1.3 For the digestion of a Laboratory Reference Material weigh approximately a 0.5 g analytical portion of NIST RM 8415(Whole Egg Powder) with a Teflon spatula into a tared, clean (see Sect. 6.4.3) microwave digestion vessel liner.

11.1.2 Move analytical portions to an exhausting fume hood and pipette 9 mL of double distilled nitric acid into each liner, washing down any material on the walls. Slowly slide the liner into the vessel body, while holding the body at a 30 to 45° angle or use the capping station. This is to avoid any splash back of the sample acid mixture. Seal vessels by hand tightening the caps. Insert a thermowell into the thermowell port of the pressure/temperature control vessel and secure with a ferrule nut. Verify vessels are properly sealed by tightening vent fittings and rotating the pressure/ temperature control cap.

NOTE: After repeated uses it may become difficult to insert a thermowell into the thermowell port. This is caused by a swelling of the temperature control stem. Forcing the thermowell into the port can pose a danger if the thermowell breaks. In addition, the thermowell may block the pressure line from accurately sensing the vessel pressure, which can cause an over pressurization of all vessels and a resulting loss of analyte if the vessels vent. The pressure/temperature control cover should be replaced when it becomes difficult to insert the thermowell into the port.

11.1.3 Connect the pressure/temperature control vessel to the pressure line and place

the control vessel in the carousel. Insert the fiber-optic temperature probe into the thermowell and place the rest of the vessels into the carousel. Verify that the pressure line and fiber-optic temperature probe do not tangle when the carousel is rotating, and the two-way valve is in the neutral position. Run the HDV microwave digestion program (Table 5).

11.1.4 After the vessels have cooled to less than 50°C remove them from the oven leaving the control vessel in place. Vent excess pressure from the control vessel by slowly loosening the pressure line. Remove the pressure line and fiber-optic temperature probe from the control vessel, placing the probe in one of the inlet/outlet ports. Turn the two-way valve to the open position and rinse the pressure line with at least 30 mL of water. Return the two-way valve to the neutral position.

11.1.5 Place the vessels in an exhausting fume hood and vent the excess pressure by slowly loosening the vent fittings. Using the HDV opening station remove the cap and cover assembly from the vessel body. Rinse the cover with a wash bottle into a 50 mL centrifuge tube. Wash down the walls of the vessel liner. Pour the contents of the vessel liner into the centrifuge tube. Rinse the liner and transfer the contents to the centrifuge tube a total of three times. Bring the analytical solution to 50 mL total volume in the tube. Begin the vessel cleaning protocol (Sect. 6.4.3).

11.2 Sample Analysis

11.2.1 Prior to daily calibration of the instrument, inspect the sample introduction system including the nebulizer, torch, injector tube and uptake tubing for salt deposits and dirt that would restrict solution flow and affect instrument performance. Clean the system when needed or on a daily basis.

11.2.2 Configure the instrument to the power and operating conditions listed in Table 2.

11.2.3 The instrument must be allowed to become thermally stable before calibration and analyses. This usually requires at least 30 to 60 minutes of operation. After instrument warmup, perform optical profiling (Sect. 10.).

11.2.4 For initial and daily operation, calibrate the instrument according to the instrument manufacturer's recommended procedure, using the mixed calibration standard solution (Sect. 7.5) and the calibration blank (Sect. 7.6.1). A peristaltic pump must be used to introduce all solutions to the nebulizer. To allow equilibrium to be reached in the plasma, aspirate all solutions for 30 sec after reaching the plasma before beginning integration of the background corrected signal to accumulate data. When possible, use the average value of replicate integration periods of the signal to be correlated to the analyte concentration. Flush the system with rinse blank (Sect. 7.6.3) for a minimum of 60 seconds (Sect. 4.4) between each standard.

11.2.5 Analytical solutions are analyzed in the same operational manner used in the calibration routine with the rinse blank aspirated for a minimum of 60 seconds between all sample and check solutions (Sect. 7.6.3).

11.2.5 During sample analysis, quality control measures described in Section 9 must be followed.

11.2.6 Determined analytical solution analyte concentrations that are 90% or more of the upper limit of the analyte LDR must be diluted with reagent water that has been acidified in the same manner as calibration blank and reanalyzed.

11.2.7 Report data as directed in Section 12.

12. Data Analysis and Calculations

12.1 Analytical solution and analytical portion concentrations are calulated by the spectrometer data system.

12.2 LRBs should be reported on the basis of the analytical solution in units of μg/L or ng/L.

12.3 LRMs and food or beverage composites should be reported on the basis of the analytical sample in units of mg/kg or μg/kg.

12.4 Spike calculated result (percent recovery) of laboratory spiked sample is calculated as follows:

$$R = \frac{C_s - C}{s} \times 100\%$$

where, R = percent recovery
C_s = fortified sample concentration (i.e., LSF)
C = sample background concentration (i.e., LSO or LDX)
s = concentration equivalent of analyte added to sample.

12.5 True value recovery of LRMs is calculated as follows:

$$R = \frac{C}{s} \times 100\%$$

where, R = percent recovery
C = reference material concentration
s = certified value (true value) of analyte for reference material.

13. Method Performance

13.1 Listed in Table 2 are MDLs determined using the procedure outlined in Section 11 and conditions listed in Tables 1 and 3. MDLs for analytical solutions were determined by analyses of six LRBs (best case senerio). MDLs listed for food and beverage homogenates are estimates and were calculated from the solution MDLs and the analytical portion weight and volume used in the method.

13.2 Method precision was assessed as relative standard deviation (RSD) of triplicate analytical portions of National Research Council Canada (NRCC) and National Institute of Standards and Technology (NIST) LRMs and four fortified food and beverage homogenates. Method precision was within limits of ±20% RSD. Findings

are summarized in Table 6.

13.3 Recovery of analyte was assessed by triplicate analytical portions of LRMs and fortified food and beverage homogenates. Method recovery was within limits of 70 to 130% for all six elements.

14. Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, recycling is the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15. Waste Management

15.1 Laboratory waste management practices should be conducted consistent with all applicable rules and regulations. Laboratories should protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in section 14.2.

16. References

1 T.D. Martin, C.A. Brockhoff, J.T. Creed, and EMMC Methods Work Group - Method 200.7, Revision 4.4 (1994)

2 Terwilliger, D. T., and Behbehani, A. L. (1994) User Guide for Manual Data Entry Software, version 2.0. U.S. EPA EMSL-Cincinnati Contract No. 68-C0-0001.

3 Horwitz, W. (1990) *Pure & Appl. Chem.* **62**, 1193-1208.

4 Boumans, P.W.J.M. *Line Coincidence Tables for Inductively Coupled Plasma Atomic Emission Spectrometry*, 2nd edition. Pergamon Press, Oxford, United Kingdom, 1984.

5 Winge, R.K. et al. *Inductively Coupled Plasma-Atomic Emission Spectroscopy: An Atlas of Spectral Information*, Physical Science Data 20. Elsevier Science Publishing, New York, New York, 1985.

6 Jones, J.W. (1988) in *Quantitative Trace Analysis of Biological Materials: Food Samples*, Chapter 20, Elsevier Science Publishing, New York, New York, pp. 353-365.

7 American Society for Testing and Materials. Standard Specification for reagent Water, D1193-77. Annual Book of ASTM Standards, Vol. 11.01. Philadelphia, PA, 1991.

Table 1. Wavelengths with Locations for Background Correction

Analyte	Wavelength(nm) x Order	Location for Bkgd. Correction
Ba	493.41x1	±0.040 nm
Cu	324.75x2	±0.040 nm
Mn	257.61x3	±0.040 nm
V	292.40x2	±0.040 nm
Zn	213.86x2	±0.040 nm

Table 2. Instrument Operating Conditions

ARL-3580 Inductively Coupled Plasma - Optical Emission Spectrometer	
Incident RF power	1200 watts
Reflected RF power	< 10 watts
Viewing height above work coil	15 mm
Injector tube orifice i.d.	1 mm
Argon pressure	> 90 psi
Coolant argon flow rate	12 L/min
Auxiliary (plasma) argon flow rate	1 L/min
Aerosol carrier argon flow rate	0.85 L/min
CETAC U-5000AT Ultrasonic Nebulizer	
Sample uptake rate controlled to	2.5 mL/min
Heating Temperature	140°C
Cooling Temperature	0.5°C

Table 3. Estimated Instrument Detection Limits (IDL), Calibration Standard (CAL) and Instrument Performace Check (IPC) Solution Concentrations.

Analyte	Estimated IDLs µg/L (1)	CAL µg/L	IPC µg/L
Ba	0.2	100	50
Cu	0.3	100	50
Mn	0.08	100	50
V	0.5	100	50
Zn	0.2	100	50

(1) The IDLs were estimated from three times the standard deviation of 11 replicate measurements of the calibration blank. The calculated IDL was rounded upward and reported to a single digit.

Table 4. Method Detection Limits (MDL)

		MDLs	
Analyte	Analytical Solution, µg/L(1)	Foods, mg/kg(2)	Beverages, mg/kg(3)
Ba	0.9	0.03	0.004
Cu	0.3	0.01	0.002
Mn	0.3	0.009	0.001
V	0.7	0.02	0.003
Zn	0.8	0.03	0.004

(1) Samples were processed in Teflon and diluted in 50-mL plastic centrifuge tubes. The calculated MDL was rounded upward and reported to a single digit.
(2) Based on analytical solution determination and a 1.5 g test portion.
(3) Based on analytical solution determination and a 10 g test portion.

Table 5. Microwave Digestion Programs

FILE NAME: CLEAN
STANDARD DIGESTION

stage	(1)	(2)	(3)	(4)	(5)
POWER	100%	0%	0%	0%	0%
PRESSURE	0080	0020	0020	0020	0020
RUN TIME	10:00	00:00	00:00	00:00	00:00
TIME @ P	03:00	00:00	00:00	00:00	00:00
TEMPERATURE	170C	0C	0C	0C	0C
FAN SPEED	100%	100%	100%	100%	100%

FILE NAME: HDV
STANDARD DIGESTION

stage	(1)	(2)	(3)	(4)	(5)
POWER	55%	65%	100%	100%	0%
PRESSURE	0085	0200	0450	0600	0020
RUN TIME	10:00	10:00	10:00	10:00	00:00
TIME @ P	03:00	03:00	03:00	06:00	00:00
TEMPERATURE	130C	150C	180C	200C	0C
FAN SPEED	100%	100%	100%	100%	100%

Table 6. Initial Demonstration of Performance, Average (n=3)

	Element				
	Ba	Cu	Mn	V	Zn
NRCC DORM-1(dogfish muscle)					
Composition, mg/kg(a):	nv	5.22±0.33	1.32±0.26	nv	21.3±1.0
Method Result, mg/kg:	0.24	5.18	1.36	<MDL	21.3
Method RSD, %:	10	1	4		3
True Value Recovery, %:		99	103		100
NRCC TORT-1(lobster hepatopancreas)					
Composition, mg/kg(a):	nv	439±22	23.4±1.0	1.4±0.3	177±10
Method Result, mg/kg:	3.8	396	24.8	1.35	185
Method RSD, %:	4	1	1	14	1
True Value Recovery, %:		90	106	97	105
NIST SRM 1548(total diet)					
Composition, mg/kg(a):	nv	2.6±0.3	5.2±0.4	nv	30.8±1.1
Method Result, mg/kg:	1.1	2.58	5.29	<MDL	32.1
Method RSD, %:	4	2	3		3
True Value Recovery, %:		99	102		104
NIST RM 8415(whole egg)					
Composition, mg/kg(a):	(3)	2.70±0.35	1.78±0.38	0.459±0.081	67.5±7.6
Method Result, mg/kg:	3.21	2.82	1.84	0.51	70.1
Method RSD, %:	2	2	2	3	4
True Value Recovery, %:	107	104	103	111	104
Beverage (231-H11)					
Method Result, mg/kg:	0.122	0.099	0.163	<MDL	0.586
Fortification Level, mg/kg:	0.193	0.193	0.289	0.0193	0.965
Fortification Result, mg/kg:	0.299	0.267	0.463	0.016	1.44
Fortification RSD, %:	1	1	1	9	1
Recovery, % (b):	92	87	104	80	88
Beverage (231-H10)					
Method Result, mg/kg:	0.035	0.016	0.037	<MDL	0.429
Fortification Level, mg/kg:	0.0982	0.0295	0.0982	0.0196	0.982
Fortification Result, mg/kg:	0.119	0.037	0.128	0.018	1.17
Fortification RSD, %:	3	1	0	4	1
Recovery, % (b):	86	73	92	91	75
Food (131-H7)					
Method Result, mg/kg:	0.27	1.01	1.26	<MDL	6.55
Fortification Level, mg/kg:	0.655	1.31	1.97	0.131	6.55
Fortification Result, mg/kg:	0.89	2.15	3.28	0.11	12.5
Fortification RSD, %:	2	3	0	10	2
Recovery, % (b):	94	87	103	82	90
Food (131-H11)					
Method Result, mg/kg:	0.47	0.65	1.61	<MDL	9.10
Fortification Level, mg/kg:	0.674	1.35	2.02	0.135	13.5
Fortification Result, mg/kg:	1.05	1.80	3.59	0.11	20.8
Fortification RSD, %:	3	4	3	8	5
Recovery, % (b):	86	85	98	79	87

(a) nv = no value provided by certifying organization

(b)value calculated using full numerical precision

± = certified value ± 95% tolerance limit, ( ) = informational value

<MDL = less than method detection limit

Standard Operating Procedure for Determination of Arsenic and Selenium in NHEXAS Food or Beverage Composites by Hydride Generation Atomic Absorption Spectrometry

U. S. Food and Drug Administration

Center for Food Safety and Applied Nutrition
Elemental Research Branch
Washington, DC

Prepared by: _____
Chemist, ERB

Approved by: _____
Chief, ERB

_____
Director, DPIC

_____
FDA Project Officer
NHEXAS EPA IAG

TABLE OF CONTENTS

1. Scope and Application

1.1 This method is used to determine trace elements in duplicate diet food and beverage homogenates by hydride generation atomic absorption spectrometry (HG-AAS).

1.2 This method is applicable to the following analytes:

Analyte	Chemical Abstract Services Registry Number (CASRN)
Arsenic (As)	7440-38-2
Selenium (Se)	7782-49-2

1.3 Elements are determined after a microwave assisted nitric acid mineralization - dry ash combination procedure has been performed. The combination preparation procedure permits the quantification of total arsenic and total selenium including refractory organometallic compounds present in some foods and eliminates the need for perchloric acid.

1.4 Instrument operating conditions for the applicable elements are listed in Tables 1 and 2.

2. Summary of Method

2.1 Method description—This method describes a technique for sequential multi-element analysis of foods. The basis of the method is the generation of a volatile analyte hydride compound and the measurement of atomic absorption by an optical spectrometric technique.

2.1.1 An aliquot of a well mixed, homogeneous liquid or solid analytical sample is accurately weighed for preparation. Initial mineralization is performed using a nitric acid microwave digestion. Refractory organometallic compounds present in some foods do not break down completely in a microwave digestion. Some of these compounds do not react with sodium borohydride to release the metal hydride resulting in a low bias.

2.1.2 To insure that all arsenic and selenium is available for hydride generation a measured aliquot of the analytical solution is then subjected to a dry ash procedure. Magnesium nitrate and magnesium oxide are utilized as ashing aids. The ash is dissolved in 50% HCl, heated to reduce Se^{+6} to Se^{+4} and diluted to volume. An aliquot of the analytical solution is diluted and analyzed for selenium. Another aliquot of the analytical solution is treated with hydroxylamine hydrochloride and potassium iodide/ascorbic acid solutions resulting in As^{+5} reduction to As^{+3}. The treated solution is analyzed for arsenic.

2.1.3 The analytes are determined by hydride generation atomic absorption spectroscopy (HG-AAS). An automated flow injection system is utilized to inject standards and test solutions into a 10% HCl matrix stream. Sodium borohydride solution is injected to reduce analytes to their volatile hydrides. A gas/liquid separator is employed to separate the hydride vapors from the liquid matrix. The hydrides are swept by an argon stream into a quartz cell electrothermally heated to 900°C. The high temperature of the cell induces decomposition of the metal hydride compounds resulting in an atom cloud that absorbs element specific emission produced from an electrodeless discharge lamp (EDL).

2.2 Analytical sensitivity—Method detection limits (sect. 3.17) are listed in Table 3. These limits are specific for FDA's Elemental Research Branch laboratory. Detection limits will vary among laboratories depending on the spectrometer, laboratory environment and instrument operating parameters.

2.3 Analytical recovery and precision—Recovery of analyte was assessed by analysis of reference materials and fortified food and beverage homogenates. Method recovery was within limits of 70 to 130% for both elements. Method Precision was assessed as relative standard deviation (RSD) of replicate analyses of reference materials and fortified food and beverage homogenates. Method precision was within limits of ±20% RSD (Sect. 13).

2.4 Dynamic Range—Valid results range from detection limit to the highest concentration calibration standard. Test solutions with absorbance greater than the high standard must be diluted and reanalyzed.

3. Definitions [1-3]

3.1 *Laboratory Sample*—The sample or subsample sent to or received by the laboratory.

3.2 *Analytical Sample*—The sample, prepared from the laboratory sample, from which analytical portions are removed for analysis.

3.3 *Analytical Portion*—The quantity of material, of proper size for measurement of the analyte of interest, removed from the analytical sample.

3.4 *Analytical Solution*—The solution prepared by dissolving, with or without reaction, the analytical portion in a liquid.

3.5 *Treated Solution*—The analytical solution that has been subjected to reaction or separation procedures prior to measurement of some property.

3.6 *Test Solution*—The solution that is injected into the instrument from the autosampler tube or cup. Test solutions can be calibration standards, analytical solutions or reference standards.

3.7 *Dynamic Range (DR)*—The concentration range over which the analytical results are valid. Valid results range from detection limit to the highest calibration standard.

3.8 *Laboratory Sample (Background for Spike) (LSO)*—The first of two analytical portions of a food or beverage test sample. This portion is analyzed according to the analytical method, to establish background concentrations prior to fortification (spiking) with the method analyte(s).

3.9 *Laboratory Spiked Sample (LSF)*—The second of the two analytical portions described under *Laboratory Sample (Background for Spike)*. This portion is subject to fortification (spiking) prior to sample preparation, and the measurement(s) of the final concentration(s) are then made according to the analytical method.

3.10 *Laboratory (Reagent) Blank (LRB)*—An aliquot of reagent water or equivalent neutral

reference material treated as a food or beverage analytical portion including exposure to all glassware, equipment, reagents, and acids that are used with other samples. This includes addition of all reagents, internal standards, labware, apparatus, equipment, and analyses. The LRB is used to determine if method analytes are present in the laboratory environment, the reagents or apparatus.

3.11 *Laboratory Instrument Blank (LIB)*—An aliquot of reagent water, adjusted in pH and other reagents common to test solutions, analyzed as analytical solution by the instrument. This includes addition of internal standards and reagents but excludes all other analytical sample preparation. The LIB is a zero standard and is used to calibrate the instrument.

3.12 *Laboratory Reference Material (LRM)*—An analytical portion of a food or beverage material having a certified value. These materials are usually obtained from the National Institute of Standards and Technology (NIST), the National Research Council of Canada (NRCC), Bureau of Reference Materials of the European Communities (BCR), etc. The *LRM* is treated as a food or beverage analytical sample in all aspects in the laboratory. This includes sample preparation, addition of all reagents, internal standards, labware, apparatus, equipment, and analyses.

3.13 *Quality Control Sample (QCS)*—A solution of method analytes, used to evaluate the performance of the instrument system.

3.14 *Instrument Performance Check Solution* (IPC)—A solution of method analytes of known concentration obtained from a source external to the laboratory and different from the source of the calibration standards. It is used to check either the laboratory or instrument performance.

3.15 *Characteristic Mass* (M_o)—The mass of analyte in pg that will produce an absorbance signal of 0.0044 A.

3.16 *Instrumental Detection Limit (IDL)*—The concentration equivalent to the analyte signal equal to three times the standard deviation of a series of ten replicate measurements of a laboratory instrument blank signal at the same wavelength.

3.17 *Method Detection Limit (MDL)*—The concentration equivalent to the analyte signal equal to *t* times the standard deviation of a series of *n* laboratory reagent blank signals at the same wavelength where *t* is the Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.

3.18 *Stock Standard Solution*—A concentrated solution containing one or more analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source. Stock standard solutions are used to prepare calibration solutions and other needed analyte solutions.

3.19 *Intermediate Standard Solution*—A solution containing one or more analytes prepared in the laboratory by diluting an aliquot of stock standard solutions.

3.20 *Calibration Standard* (CAL)—A solution prepared from the dilution of stock standard or intermediate standard solutions. The CAL solutions are used to calibrate the instru-

ment response with respect to analyte concentration.

4. Interferences

4.1 The analyst must be aware of several types of interferences that can be present with hydride generation atomic absorption analysis. These interferences can be classified into three major divisions; spectral, matrix and memory. Most modern instruments have the capability to graphically display time verses absorbance to aid the analyst in determining if some types of interferences are present.

4.2 Spectral interference is the result of absorption of light by an element or molecule that is not the analyte of interest. This is rare with hydride generation because the analyte is volatilized and separated from the test solution matrix.

4.3 Matrix interferences are caused by components in the test portion matrix that inhibit the formation metal hydride during the reduction step.

4.3.1 A complete mineralization procedure allows analytes, originally present as a refractory organometallic, to react with the borohydride reductant.

4.3.2 Optimized acid/reductant concentration can assist with more complete reduction of analyte to the hydride.

4.3.3 Transition metals that can severely suppress the analyte-hydride generation reaction are not expected to be present at levels that will interfere based on food composition information and the dilution factor built in to the preparation procedure. The nature of a duplicate diet sampling program further dilutes the matrix effect of any one particular type of food.

4.4 Memory effects can lead to false positive signals resulting from test portions containing high levels of analyte.

4.4.1 The flow injection system program must be adjusted to completely remove all traces of analyte during the analysis cycle.

4.4.2 Program adequate rinse time between samples to flush system tubing completely.

4.4.3 Program adequate FIAS prefill and fill time to ensure sample loop is completely flushed and filled.

4.4.4 Check for memory by analyzing the highest CAL standard followed by the LIB. The LIB result must be <MDL. If control limit is not met adjust rinse, prefill and/or fill time parameters and check again.

5. Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Laboratory is responsible for maintaining access to OSHA regulations regarding the safe handling of chemicals specified in this method. Material safety data sheets must be available to personnel involved in chemical analysis.

5.2 Concentrated acids are extremely irritating to skin, eyes and mucus membranes. If eye or skin contact occurs, flush with large volumes of water. Use these reagents in a fume hood.

5.3 Always wear safety glasses, lab coat and gloves. Goggles or a face shield are recommended for handling concentrated acids.

5.4 Electrodeless discharge lamps emit UV radiation. Do not look along the radiation axis of the lamps when energized.

5.5 Quartz furnace operates at 900°C. Be careful not to touch quartz tube or oven parts when in operation. Apparatus remains hot for several minutes after shutdown.

5.6 Ensure that the outlet of the quartz cell flows into the snorkel hood positioned above instrument and that damper valve is open. Arsine and selenium hydride are very toxic.

5.7 It is the responsibility of the user of this method to comply with federal and local disposal and waste regulations. Dispose of hazardous and acidic waste in accordance with the laboratory hazardous waste plan.

6. Equipment and Supplies

6.1 Perkin Elmer 5100PC Atomic Absorption Spectrometer

6.1.1 DEC LPv 433dx computer.

6.1.2 EDL System 2 Power Supply and Driver.

6.1.2.1 Arsenic System 2 EDL.

6.1.2.2 Selenium System 2 EDL.

6.2 Perkin Elmer FIAS 400 Flow Injection System

6.2.1 Computer controlled electrically heated furnace.

6.2.2 Quartz cell, 166mm long, 7mm ID.

6.2.3 Perkin Elmer AS-90 autosampler.

6.2 Compressed Argon gas - 99.9% purity.

6.3 Analytical balance capable of measuring to 0.1 mg.

6.4 Microwave Digestion Equipment

6.4.1 Directions on use of microwave digestion equipment are specific to CEM brand equipment and assumes familiarity with the equipment. Use of this method with other brands of microwave digestion equipment may require modifications to this procedure.

6.4.2 Microwave digestion system with temperature and pressure control from 0 to 200°C and 0 to 600 psi. Microwave power range: 0-100% full power (630 watts ±50 watts), programmable in 1% increments (CEM corporation model MDS-2000 equipped for Heavy Duty Vessels (HDVs) or equivalent).

6.4.3 Microwave digestion vessels (CEM corporation Heavy Duty Vessels (HDVs) or equivalent)—TEM Teflon lined digestion vessels capable of operating up to 200°C and 600 psi and accessories.

6.4.4 Cleaning protocol

6.4.4.1 Disassemble the vessel cap and cover, removing the rupture membrane from the vent fitting. Pressure/temperature control covers need not be disassembled, simply remove the rupture membrane from the vent fitting. Vessel covers, liners and vent fittings should be soaked for at least 2 hours in a solution of liquid laboratory-grade detergent (MICRO brand or equivalent) and hot tap water. Other vessel components can be rinsed with reverse osmosis (RO) or higher quality water (at least 10MΩ resistivity) and dried in a class 100 clean area. Thermowells should be wiped down with a paper towel and detergent solution. Rinse thermowells and vessel components with warm tap water and then rinse thoroughly with reverse osmosis (RO) or higher quality water (at least 10 MΩ resistivity). Dry in a class 100 clean area.

6.4.4.2 Assemble the cover and cap, inserting a rupture membrane in the vent fitting. Add 10 mL of ACS grade nitric acid to each liner and seal liner and cover in the digestion vessel body. Insert a thermowell into the pressure/temperature control cover and secure with a ferrule nut. Connect the pressure line to the pressure/temperature control cover and place the pressure/temperature control vessel in the carousel. Insert the fiber-optic temperature probe into the thermowell. Place the other vessels in the carousel and begin the microwave CLEAN program (Table 7).

6.4.4.3 Remove vessels from oven when cooled to <50°C leaving the control vessel in place. Vent excess pressure from the control vessel by slowly loosening the pressure line. Remove the pressure line and fiber-optic temperature probe from the control vessel, placing the probe in one of the inlet/outlet ports. Turn the two-way valve to the open position and rinse the pressure line with at least 30 mL of water. Return the two-way valve to the neutral position.

6.4.4.4 Place the vessels in an exhausting fume hood and vent the excess pressure by slowly loosening the vent fitting. Using the HDV opening station, remove cap and cover assemblies from vessel bodies. Rinse off covers and liners with water into a waste container. Thoroughly, rinse covers and liners with water. A total of six rinses has been found to work but, the minimum number of rinses required to achieve undetectable method blanks has not been studied. Dry in a class 100 clean area. If the vessels are not used after drying store assembled in a class 100 clean area.

6.5 Gravity convection drying oven with thermostatic control capable of maintaining temperature of 40-180 ±5°C.

6.6 Set of Gilson Pipetman air displacement pipetters capable of delivering volumes ranging 10 μL to 10 mL.

6.6.1 Ranin brand pipet tips for Pipetman pipetters.

6.7 Muffle furnace for dry ash procedure

6.7.1 Microprocessor controlled multi-step capability.

6.7.2 Capable of maintaining up to 450 ±10°C.

6.8 Temperature adjustable hot plate capable of maintaining solutions at 95°C.

6.9 Labware— All reusable labware (glass, polyethylene, PTFE etc.) must be sufficiently clean for trace metals analysis. Cleaning procedure for all labware includes washing in special clean rinsing laboratory detergent, reagent water rinse, soaking in 20% nitric acid and final reagent reagent water rinse.

6.9.1 Griffin beakers, borosilicate glass, 20 mL.

6.9.2 PTFE watch glass, 40 mm diameter.

6.9.3 Polystyrene disposable beakers, 10 mL and 50 mL.

6.9.4 Erlenmeyer flasks, class A volumetric flasks and polyethylene storage bottles.

6.9.5 Falcon brand centrifuge tube; 15 mL polystyrene, 50 mL polypropylene.

7. Reagents and Standards

7.1 Reagents may contain elemental impurities which might affect analytical data. Only high purity reagents should be used. Where not specified use ACS reagent grade. If the purity of a reagent is in question, analyze for contamination.

7.2 Hydrochloric acid, concentrated (sp. gr. 1.18)—HCl. Use trace metals grade.

7.2.1 Hydrochloric acid (50% v/v)—Cautiously add 500 mL concentrated HCl to 400 mL reagent water and dilute to 1000 mL.

7.2.2 Hydrochloric acid (10% v/v)—Cautiously add 100 mL concentrated HCl to 800 mL reagent water and dilute to 1000 mL.

7.3 Nitric acid, concentrated (sp. gr. 1.41)—HNO_3. Use high purity double distilled grade.

7.3.1 Nitric acid (20% v/v)—Cautiously add 200 mL concentrated HNO_3 to 600 mL reagent water and dilute to 1000 mL.

7.3.2 Nitric acid (50% v/v)—Cautiously add 100 mL concentrated HNO_3 to 80 mL reagent water and dilute to 200 mL.

7.4 Reagent water. All references to water in this method refer to ASTM Type I grade water.

7.5 Stock Standard Solutions—Stock standard solutions of analytes may be purchased or prepared from high purity chemicals.

7.5.1 Arsenic solution, 1000 μg As/mL—Use commercially available prepared solution from reputable supplier.

7.5.2 Selenium solution, 1000 μg Se^{+4}/mL—Dissolve 1.4053g SeO_2 (99.999%) in 500 mL 10% HCl. Dilute to 1000 mL. Dry SeO_2 for 1 hour at 105°C before use.

7.6 Intermediate Standard Solution—Make solutions in volumetric flasks by diluting an appropriate volume of stock standard solution with 10% HCl. Store in capped polypropylene centrifuge tube.

7.6.1 10 μg As/mL Intermediate Standard Solution—Dilute 0.5 mL 1000 μg As/mL to 50 mL with 10% HCl. Prepare fresh monthly.

7.6.2 100 ng As/mL Intermediate Standard Solution—Dilute 0.5 mL 10 μg As/mL to 50 mL with 10% HCl. Prepare fresh weekly.

7.6.3 10 μg Se^{+4}/mL Intermediate Standard Solution—Dilute 0.5 mL 1000 μg Se^{+4}/mL to 50 mL with 10% HCl. Prepare fresh monthly.

7.6.4 100 ng Se^{+4}/mL Intermediate Standard Solution—Dilute 0.5 mL 10 μg Se^{+4}/mL to 50 mL with 10% HCl. Prepare fresh weekly.

7.7 Preparation of Calibration Standards—Fresh calibration standards (CAL solution) must be prepared in volumetric flasks daily by diluting an appropriate volume of intermediate standard solution. Store in capped polypropylene centrifuge tube.

7.7.1 Prepare As CAL solutions with concentrations of 0, 0.5, 1, 2, 5 and 10 ng As/mL in clean 50 mL volumetric flasks.

7.7.1.1 Add approximately 20 mL of water to each flask.

7.7.1.2 Add 5 mL concentrated HCl to each flask.

7.7.1.3 Add 10 mL 50% hydroxylamine hydrochloride solution and 2 mL KI/Ascorbic reducing solution.

7.7.1.4 Pipet 0, 0.25, 0.5, 1.0, 2.5, and 5.0 mL of 100 ng As/mL intermediate standard solution into the flasks labeled as calibration blank, 0.5 ng/mL, 1 ng/mL, 2 ng/mL, 5 ng/mL and 10 ng/mL respectively.

7.7.1.5 Dilute to 50 mL with water. Mix thoroughly and transfer to 50 mL capped polypropylene centrifuge tubes. Allow at least 1 hour for reduction of As^{+5} to As^{+3} before using for calibration.

7.7.2 Prepare Se^{+4} CAL solutions with concentrations of 0. 0.5, 1, 2, 5 and 10 ng Se^{+4}/mL in clean 50 mL volumetric flasks.

7.7.2.1 Add approximately 20 mL of water to each flask.

7.7.2.2 Add 5 mL concentrated HCl to each flask.

7.7.2.3 Pipet 0, 0.25, 0.5, 1.0, 2.5, and 5.0 mL of 100 ng Se^{+4}/mL intermediate standard solution into the flasks labeled as calibration blank, 0.5 ng/mL, 1 ng/mL, 2 ng/mL, 5 ng/mL and 10 ng/mL respectively.

7.7.2.4 Dilute to 50 mL with water. Mix thoroughly and transfer to 50 mL capped polypropylene centrifuge tubes.

7.8 Hydroxylamine Hydrochloride—NH_2OH•HCl, ACS reagent grade.

7.8.1 50% (m/v) Hydroxylamine Hydrochloride Solution—Add 100 g NH_2OH•HCl to a 250 mL Erlenmeyer flask. Add water to a predetermined 200 mL mark and a teflon coated stir bar. Place flask on hot plate stirrer and heat gently while stirring till all compound dissolves. Adjust volume if necessary and transfer to a clean polyethylene bottle.

7.9 Potassium Iodide—KI, ACS reagent grade.

7.10 Ascorbic Acid—ACS reagent grade, powder.

7.11 KI/Ascorbic Reducing Solution—25%KI / 20% Ascorbic acid solution (m/v): Add 12.5g KI and 10g Ascorbic acid to 50 mL polypropylene centrifuge tube. Add water to the 45 mL mark, cap and shake until dissolved. Dilute to 50 mL.

7.12 Selenium Dioxide—SeO_2, 99.999% purity.

7.13 Sodium Borohydride—$NaBH_4$ pellets, 98% purity.

7.13.1 Sodium Borohydride Solution—0.2% $NaBH_4$ / 0.05% NaOH (m/v): Add 2.0 g $NaBH_4$, 0.5 g NaOH and 400 mL water to a 500 mL Erlenmeyer flask.

7.13.2 Add teflon stir bar and place on magnetic stirrer. When dissolved, vacuum filter through fine porosity filter paper and dilute to 1000 mL.

7.13.3 Transfer solution to a polyethylene bottle. Make fresh daily.

7.14 Sodium Hydroxide—NaOH, pellets, ACS Reagent grade.

7.15 Magnesium Nitrate—$Mg(NO_3)_2 \cdot 6H_2O$, ACS Reagent grade.

7.16 Magnesium Oxide—MgO, powder, ACS Reagent grade.

7.17 Ashing Aid—2% MgO suspension in 20% $Mg(NO_3)_2 \cdot 6H_2O$ solution (m/v). Add 20 g $Mg(NO_3)_2 \cdot 6H_2O$ and 2 g MgO to a 125 mL wide mouth polyethylene bottle. Add water and dilute to a previously determined 100 mL volume. Shake to dissolve the $Mg(NO_3)_2 \cdot 6H_2O$ (MgO will not dissolve). Shake solution before using to uniformly suspend the MgO.

8. Sample Collection, Preservation and Storage

Samples for analysis are kept frozen and stored in plastic containers which have metal–free surfaces prior to implementation of any analytical procedures. Specific details on insuring analytical sample integrity and record keeping are contained in the Storage and Custody SOP (ERB SOP 104).

9. Quality Control

9.1 This method requires several quality control parameters to insure the quality and reliability of the data. Requirements include initial demonstration of laboratory performance, sample fortification, calibration verification and analysis of LRMs and LRBs.

9.2 A batch consists of 36 or less samples.

9.3 Initial Demonstration of Laboratory Performance—Initial demonstration of laboratory performance is used to characterize instrument performance through the analysis of LRM, fortified and unfortified foods and beverages. Estimates of limits of detection are also determined.

9.3.1 Laboratory Reference Material (LRM)—At least 2 food/biological LRMs must be prepared and analyzed in triplicate using procedures listed in section 11. Recovery based on certified value must be 100±30%. Precision assessed as relative standard deviation (RSD) of the triplicate test portions must be ≤20%.

9.3.2 Fortified Samples—At least 2 food/food homogenates and at least 2 beverage/beverage homogenates must be analyzed in triplicate both unfortified and fortified. Recovery of fortification must be 100±30%. Precision assessed as relative standard deviation (RSD) of the triplicate fortification recovery results must be ≤20%.

9.3.3 Method Detection Limit (MDL)—Estimates of the limits of detection must be determined. Analyze at least 5 laboratory reagent blanks (LRB). The MDL is calculated as follows:

$$MDL = S \times t$$

where:

t = Student's t value appropriate for a 99% confidence level (one sided) and a standard deviation estimate with n-1 degrees of freedom.

S = standard deviation estimate for the set of 5 or more LRB analyses.

9.4 Laboratory Reagent Blank (LRB)

9.4.1 LRB data is used to assess contamination from the laboratory environment. At least one LRB must be prepared and analyzed with each batch of 18 or fewer samples and at two LRBs for batches of 19 to 36 analytical portions.

9.4.2 LRB values that exceed the MDL indicate possible laboratory or reagent contamination.

9.4.3 If LRB value constitutes 10% of the analyte value determined in any of the samples in the associated batch or is > 5 times MDL which ever is greater, then contamination is suspected and the LRB exceeds control limits. The samples in this batch with analyte concentration ≤ 10 times the LRB value must be prepared and analyzed again.

9.5 Laboratory Spiked Sample (LSF)

9.5.1 One sample from each batch must be prepared and analyzed in duplicate. The duplicate portion must be fortified with each analyte. Fortification must be performed prior to microwave digestion.

9.5.2 Calculate percent recovery for each analyte. Acceptable recoveries range 70% - 130% if the amount of analyte added is > 25% of the unfortified level.

9.5.3 If recovery of analyte falls outside of designated range the problem must be investigated to determine if the cause is matrix or solution related.

9.5.3.1 Fortify a portion of unfortified test solution with analyte (Due to limited volume an additional aliquot of sample may have to be prepared) and analyze. Analyte should be recovered within 85% to 115%.

9.5.3.2 If recovery is within acceptable range, the low recovery of analyte in

the LSF may be related to the heterogeneous nature of the sample, an incorrect addition prior to preparation, or the result of precipitation or volatilization loss during preparation.

9.5.3.3 If recovery is outside of this range, a matrix effect should be suspected and the sample should be reanalyzed at a greater dilution.

9.6 Laboratory Reference Material (LRM)—At least one laboratory reference material must be prepared and analyzed as a sample for each batch. The LRM must have a certified value established for the analyte.

9.6.1 Acceptable recovery for each analyte is 70% to 130% of certified value. If recovery is outside of this range, the batch of samples must be prepared and analyzed again.

9.7 Calibration Verification—To insure accuracy the calibration curve must be verified during an analytical run. Immediately following standardization the curve must be verified using an independent standard. Periodically during sample analysis lack of instrumental drift must be verified. All sample analyses must be bracketed by valid calibration verification checks.

9.7.1 Initial Calibration Verification—Immediately following standardization analyze the LIB and IPC. Results for the LIB must be ≤ MDL but ≥ a negative value of MDL. Results for IPC must be 90% to 110% of expected value. If control limits are not met on either the LIB or IPC then diagnose the problem, make necessary adjustments and restandardize the instrument.

9.7.2 Continuing Calibration Verification—To verify lack of instrumental drift analyze the QCS and LIB at a frequency of 10% and at the end of the analytical run. One of the CAL standards can function as the QCS. Results for the LIB must be ≤ MDL but ≥ a negative value of MDL. Results for the QCS must be 90% to 110% of expected value. If the control limits are not met, reanalyze the LIB or QCS or both. If the second analysis confirm the calibration to be outside the limits, sample analysis must be discontinued, cause determined and instrument restandardized. All test solutions following the last acceptable LIB/QCS must be reanalyzed.

9.10 Precision—All test solutions results, including calibration standards, prepared samples and various quality control solutions, shall be based on the mean of 2 replicate injections. The RSD of these replicate injections must be ≤10 RSD for values greater than 10 times MDL. If control limits are not met, reanalyze test solution. If control limits are still not met, diagnose the problem, make necessary adjustments and reanalyze the test solution.

9.11 In the event that the results for the set of quality control samples do not meet expected values, the analysis of all analytical solutions for the batch are routinely repeated once. Preferably, this action is automatically controlled by the instrument program. Unacceptable instrument performance measures will be corrected before the repeat analysis. If the repeated results for the quality control samples are still not acceptable, those results are reported, and the sample analysis continued. Unacceptable quality control samples will be reported to the supervisory chemist and reviewed with the chemist to determine

the acceptability of the results.

9.12 Instrument Logbook—The following must be recorded in a bound notebook for each analytical run:

Date of analysis, Element, Lamp ID, Lamp Energy, M_o, Analyst Initials, and any comments to describe problems and unusual results.

10. Calibration and Standardization

10.1 Specific wavelengths and instrument operating conditions are listed in Tables 1 and 2. These parameters have been optimized for FDA's Elemental Research Branch's instrumentation.

10.2 Linear dynamic range is not applicable to this method because a nonlinear curve fit algorithm is applied to absorbance data. Method dynamic range (DR) over which analytical results are valid extend from detection limit to the highest calibration standard. Test solutions with an absorbance greater than the highest CAL standard must be diluted and reanalyzed.

10.3 For all determinations allow instrument and EDL to warm up for at least 30 minutes.

10.4 Before using this procedure (sect. 11.0) to analyze samples, there must be data available documenting initial demonstration of performance. The required data and procedures are described in section 9.3. This data must be generated using the same instrumental operating conditions and standardization routine to be used for sample analysis.

10.5 Standardize instrument using the LRB and the 5 CAL standards (sect. 7.7).

10.5.1 Use the following concentrations for the 5 CAL solutions: 0.5, 1.0, 2.0, 5.0, and 10.0 ng/mL.

10.5.2 Use the nonlinear, zero intercept curve fit algorithm supplied by manufacturer for converting absorbance values to ng/mL concentration units.

10.5.3 Use mean of two replicate injections for calibration blank and each CAL standard.

11. Procedure

11.1 Analytical Portions

11.1.1 For the digestion of a beverage homogenate reblend the analytical sample by vigorously shaking and immediately weigh a 10 mL analytical portion with a 10 mL air displacement pipetter into a tared, clean (sect. 6.4.3) microwave digestion vessel liner.

11.1.2 For the digestion of a food homogenate reblend the analytical sample by vigor-

ously shaking or stirring with a Teflon spatula (depending on consistency) and immediately weigh approximately a 1.5 g analytical portion with an air displacement pipetter or Teflon spatula into a tared, clean (sect. 6.4.3) microwave digestion vessel liner.

11.1.3 For the digestion of Laboratory Reference Materials weigh approximately a 0.9 g analytical portion of NIST RM 1568A (Rice Flour) with a Teflon spatula into a tared, clean (sect. 6.4.3) microwave digestion vessel liner.

11.2 Microwave Digestion

11.2.1 Move analytical portions to an exhausting fume hood and pipette 9 mL of double distilled nitric acid into each liner, washing down any material on the walls. Slowly slide the liner into the vessel body, while holding the body at a 30 to 45° angle or using the capping station. This is to avoid any splash back of the sample acid mixture. Seal the vessels by hand tightening the caps. Insert a thermowell into the thermowell port of the pressure/temperature control vessel and secure with a ferrule nut. Verify vessels are properly sealed by tightening the vent fittings and rotating the pressure/ temperature control cap.

NOTE: After repeated uses it may become difficult to insert a thermowell into the thermowell port. This is caused by a swelling of the temperature control stem. Forcing the thermowell into the port can pose a danger if the thermowell breaks. In addition, the thermowell may block the pressure line from accurately sensing the vessel pressure, which can cause an over pressurization of all vessels and a resulting loss of analyte if the vessels vent. The pressure/temperature control cover should be replaced when it becomes difficult to insert the thermowell into the port.

11.2.2 Connect the pressure/temperature control vessel to the pressure line and place the control vessel in the carousel. Insert the fiber-optic temperature probe into the thermowell and place the rest of the vessels into the carousel. Verify that the pressure line and fiber-optic temperature probe do not tangle when the carousel is rotating and the two-way valve is in the neutral position. Run the HDV microwave digestion program (Table 7).

11.2.3 Remove vessels from oven when cooled to <50°C leaving the control vessel in place. Vent excess pressure from the control vessel by slowly loosening the pressure line. Remove the pressure line and fiber-optic temperature probe from the control vessel, placing the probe in one of the inlet/outlet ports. Turn the two-way valve to the open position and rinse the pressure line with at least 30 mL of water. Return the two-way valve to the neutral position.

11.2.4 Place the vessels in an exhausting fume hood and vent the excess pressure by slowly loosening the vent fittings. Using the HDV opening station remove the cap and cover assembly from the vessel body. Rinse the cover with a wash bottle into a 50 mL centrifuge tube. Wash down the walls of the vessel liner. Pour the contents of the vessel liner into the centrifuge tube. Rinse the liner and

transfer the contents to the centrifuge tube a total of three times. Bring the analytical solution to 50 mL total volume in the tube. Begin the vessel cleaning protocol (Sect. 6.4.3).

11.3 Hotplate Evaporation Procedure

11.3.1 Pipet 20 mL of microwave digest analytical solution into a clean 20 mL beaker. Add 1 mL ashing aid solution. Place beakers on hot plate and heat on medium. Heat without boiling until 2-3 mL remains.

11.3.2 Remove from hot plate. When cool add 1 mL ashing aid solution replace watch glasses and return to hot plate on medium heat setting. Continue heating until all liquid is evaporated and then for an additional 15 minutes to ensure all liquid is gone.

11.3.3 Use extreme care during the evaporation of the last mL to ensure that bumping does not occur. If material in beaker starts to turn brown, immediately remove beaker from hot plate, cool and add 0.5 mL ashing aid solution. Return to hot plate and continue heating.

11.3.4 Remove beakers from hotplate and place in muffle furnace.

11.4 Muffle Furnace Procedure

11.4.1 Ash samples using the following furnace program:

Step 1:	Ramp to 150°C	1 hour
Step 2:	Hold 150°C	1 hour
Step 3:	Ramp to 450°C	2 hours
Step 4:	Hold 450°C	8 hours
Step 5:	Cool to ambient temperature	

11.4.2 Remove beakers from muffle furnace.

11.5 Ash Dissolution

11.5.1 Ash should be white or slightly off white if dry ash procedure was complete.

11.5.1.1 If ash is slightly yellow or light brown, add 1 mL 50% HNO_3 and 0.5 mL ashing aid solution to dissolve ash. Heat on medium until dry. Be careful not to let solution boil or spatter especially during the final stage of evaporation. Heat on high for 30 minutes. Remove from hotplate and cool.

11.5.2 Add 5 mL 50% HCl to dissolve ash. Cover with PTFE watch glass, place on hotplate and heat on medium.

11.5.2.1 Label a 20 mL beaker “Temp”. Add 5 mL 50% HCl. Cover with PTFE watch class and heat with others. Use this beaker and a thermometer to monitor temperature.

11.5.3 Continue to heating until a temperature of at least 90°C is reached. Maintain

temperature for 20 minutes to insures Se^{+6} is reduced to Se^{+4} (required oxidation state for hydride generation).

11.5.4 Remove beakers from hotplate. Rinse contents with reagent water into a 15 mL polystyrene centrifuge tube. Dilute to 10 mL mark. Label tube with sample ID, date and initials.

11.6 Arsenic Treated Solution Preparation

11.6.1 Pipet 5 mL of solution from section 11.5.4 into a 15 mL polystyrene centrifuge tube. Label tube with sample ID, "As", date and initials.

11.6.2 Add 2 mL 50% hydroxylamine hydrochloride (sec 7.8.1) solution and 0.4 mL KI/Ascorbic reducing solution (sec 7.11).

11.6.3 Dilute to 10 mL. Wait at least 1 hour before analysis to insure reduction As^{+5} to As^{+3}.

11.6.4 Analyze within 1 week.

11.7 Selenium Treated Solution Preparation

11.7.1 Dilute the 5 mL solution left in tube from section 11.5.4 to 10 mL with reagent water. Add "Se" to the label. Solution is ready for analysis.

11.8 Instrument Setup:

11.8.1 Turn on instrument, FIAS, EDL, printer and computer. Open valve on argon tank. Set regulator to 50 psi.

11.8.2 Inspect condition of quartz cell windows. Clean with ethanol if any dirt or deposits can be seen.

11.8.3 FIAS Initialization

11.8.3.1 Install new membrane in cap of gas liquid separator. Inspect pump tubes for wear. Replace if necessary. Clamp tubes in place with pump head. Place carrier and reductant reagent lines into beaker of water.

11.8.3.2 Set pumps 1 and 2 to 100 and 120 speed respectively. Start pumps and adjust for smooth flow. Remove autosampler rinse station reservoir and rinse with water. Replace and fill with 10% HCl.

11.8.3.3 Adjust tension on the pump 2 waste line to insure proper removal of liquid from gas-liquid separator. Insufficient pumping rate can cause liquid to back up through the membrane and into the quartz cell. Sensitivity can decrease if liquid removal rate is too slow.

11.8.3.4 Place "carrier" reagent line into bottle containing 10% HCl solution. Wait a few minutes and then place "reductant" reagent line into bottle containing 0.2% $NaBH_4$ / 0.05% NaOH solution sec 7.13.1). Adjust pump 2 waste line again if necessary.

11.8.3.5 Clean autosampler capillary tip and adjust autosampler arm for correct alignment with respect to the sampler tubes. Check for bubbles in tubing while pumping water. Bubbles indicates a leak. Check and replace all applicable fittings to stop leak.

11.8.3.6 Turn on quartz furnace. Set temperature to 900°C. Do not use system until temperature stabilizes.

11.8.4 Spectrometer Initialization

11.8.4.1 Allow EDL to warm up for 30 minutes. Align EDL for maximum energy output. Set detector gain. Record EDL energy in instrument logbook.

11.8.4.2 Use the instrumental parameters listed in Tables 1 and 2.

11.9 Sample Analysis

11.9.1 Prior to daily calibration of the instrument inspect the furnace, FIAS pumps, pump tubing, multiport valve and autosampler probe for any change in the system that would affect instrument performance.

11.9.2 Configure instrument according to parameters listed in section Tables 1 and 2. Program instrument to perform 2 replicate analyses for each test solution. Use the mean of the 2 absorbances in calculations. Absorbance values and determined concentrations on all solutions must be displayed on computer screen for immediate review by the analyst and be available as hard copy for documentation.

11.9.3 Enter test solution identification information in ID/Weight Parameter file. Enter file name for data archiving. Load autosampler with CAL standards and test solutions.

11.9.4 Sensitivity and Stability—After instrument has warmed up for at least 30 minutes but before standardization instrument sensitivity and stability must be demonstrated.

11.9.4.1 Instrument sensitivity must be demonstrated by analyzing the 5 ng/mL CAL standard and calculating the characteristic mass, M_o. Record M_o value in instrument logbook. The M_o must be within 20% of the mean value for the given set of conditions.

11.9.4.2 Instrument stability must be demonstrated by analyzing the 1

ng/mL CAL standard a minimum of 5 times. The resulting relative standard deviation (RSD) of absorbance signals must be < 5%. If RSD > 5%, determine and correct problem before standardization.

11.9.5 Perform standardization (sect. 10).

11.9.6 Analyze quality control solutions and test solutions.

11.9.6.1 Compare average absorbances to the standard curve. Multiply the concentration of analyte in the test solutions by the dilution factor applicable to the preparation procedure to obtain the concentration in the sample.

11.9.8.2 Typical analytical sequence for analytical run is listed in Table 6.

11.10 Instrument Shutdown

11.10.1 After last test solution has been analyzed, place reductant line in beaker of water. Turn off cell heater. Flush instrument for 5 minutes.

11.10.2 Remove autosampler rinse station reservoir and fill with water. Place carrier line in beaker of water. Flush instrument for 5 minutes while switching multiport injection valve between fill and inject positions.

11.10.4 Raise autosampler tip. Remove reductant and carrier lines from water and pump air through system while switching multiport injection valve between fill and inject positions till all liquid is removed from system.

11.10.5 Shut off pumps and release tension on pump tubes. Turn off EDL power supply, spectrometer, FIAS and computer.

12. Data Analysis and Calculations

12.1 Print data on hard copy printout as it is being generated to enable immediate review. Electronically record data on to computer file to enable electronic transfer.

12.1.1 Program instrument to convert absorbance readings to concentration units based on calibration curve.

12.1.2 Print and record both absorbance data and concentration results.

12.2 Sample data must be reported in units of mg/kg wet weight.

12.2.1 For food composite samples calculate mg/kg as follows:

$$\text{Sample Conc. mg/kg} = \frac{C \times V}{}$$

$$\frac{1000 \times M}{}$$

where: C = Concentration of analyte in test solution (ng/mL)
V = Final volume of microwave digest analytical solution (usually 50 mL, sect. 11.2.4)
M = Mass of analytical portion (g)
1000 = Conversion factor from ng/g to mg/kg

Note that the dilution factor of the analytical solution is unchanged through the dry ash procedure and the arsenic and selenium treated solution preparation.

12.2.2 Do not report analyte data below estimated MDL or an adjusted MDL because of additional dilutions required to complete analysis.

12.3 Laboratory Reagent Blank (LRB) should be reported on the basis of the analytical test solution in units of ng/mL.

12.4 Spike Calculated Result (percent recovery) of Laboratory Spiked Sample (LSF) is calculated as follows:

$$R = \frac{C_s - C}{s} \times 100$$

where, R = percent recovery
C_s = fortified sample concentration (LSF)
C^s = sample background concentration (LSO)
s = concentration equivalent of analyte added to sample.

12.5 Laboratory Reference Material (LRM) should be reported on the basis of the analytical portion in units of mg/kg using the calculations as per samples (section 12.2).

12.5.1 True Value Recovery of Laboratory Reference Material (LRM) is calculated as follows:

$$R = \frac{C}{s} \times 100$$

where R = percent recovery
C = reference material concentration (LRM)
s = certified value (true value) of analyte for reference material.

12.6 Quality Control Sample (QCS) and Instrument Performance Check (IPC) Recovery are

calculated as follows:

$$R = \frac{C}{s} \times 100$$

where R = percent recovery
C = QCS or IPC concentration
s = Expected concentration of analyte for QCS or IPC.

13. Method Performance

13.1 Instrument operating conditions used for testing of the method are listed in Tables 1 and 2. Listed in Table 3 are MDLs determined using the procedure outlined in sections 9.3.3 and 11. MDLs for analytical solutions were determined by analyses of five LRBs. MDLs listed for food and beverage homogenates are estimates and were calculated from the solution MDLs and the analytical portion weight and volume used in the method.

13.2 Accuracy and precision of the method has been evaluated using laboratory reference materials and fortified duplicate diet food and beverage homogenates.

13.2.1 Accuracy and recovery were assessed by the analysis of 4 food standard reference materials and fortified duplicate diet food and beverage composites. Reference materials consisted of NRCC DORM-1 (dogfish muscle), NRCC TORT-1 (lobster hepatopancreas), NIST SRM 1548 (total diet), and NIST RM 8415 (whole egg powder). Samples were prepared and analyzed in triplicate using the procedure described in section 11. Method recovery was within limits of 70 to 130% for both elements. Findings are summarized in Tables 4 and 5.

13.2.2 Method precision was assessed as relative standard deviation (RSD) of triplicate analytical portions of National Research Council Canada (NRCC) and National Institute of Standards and Technology (NIST) LRMs and four fortified food and beverage homogenates. Method precision was within limits of ±20% RSD. Findings are summarized in Tables 4 and 5.

14. Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Whenever feasible, use pollution prevention techniques to address waste generation.

14.2 Buy chemicals and reagents based on projected demand to minimize disposal of old chemicals in the future. Prepare only the amount of reagents and standards as needed.

14.3 For information about pollution prevention that may be applicable to laboratories and

research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15. Waste Management

15.1 Laboratory waste management practices be conducted consistent with all applicable rules and regulations. Laboratories should protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C.

15.2 Comply with FDA's Center for Food Safety and Applied Nutrition Hazardous Waste Management Plan.

16. References

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Table 1. Spectrometer Parameters

	Arsenic	Selenium
EDL Current	300 ma	210 ma
Wavelength	193.7 nm	196.0 nm
Slit Width	0.7 nm	2.0 nm
Integration	15 sec	15 sec
Mode	Peak Height	Peak Height
Peak Smoothing	5 points	5 points
Replicates	2	2
Calibration	Nonlinear	Nonlinear
Intercept	Forced Zero	Forced Zero

Table 2. FIAS Parameters

	Arsenic	Selenium
Cell Temp.	900°C	900°C
Pump 1	100 rpm	100 rpm
Pump 2	120 rpm	120 rpm
Sample Loop	500 μL	500 μL
Reaction Coil	110 mm	110 mm
Stripper Coil	1000 mm	1000 mm
Argon Flow	80 mL/min	100 mL/min
Wash	8 sec	8 sec
Prefill	5 sec	5 sec
Fill	9 sec	9 sec
Autosamp. Probe	0.5 mm	0.5 mm

Table 3. Method Detection Limits [1]

	As (mg/kg)	Se (mg/kg)
Food[2]	0.003	0.002
Beverage[3]	0.0005	0.0003

(1) Calculated MDL was rounded upward and reported to a single digit.
(2) Based on analytical solution determination and a 1.5 g test portion.
(3) Based on analytical solution determination and a 10 g test portion.

Table 4. Initial Demonstration of Performance—Reference Material Average (n=3) Analytical Results [1]

Reference Material	Element	
	As	Se
NRCC DORM-1 (dogfish muscle)		
Composition, mg/kg:	17.7±2.1	1.62±0.12
Method Result, mg/kg:	17.6	1.64
Method Result RSD, %:	0.8	5
True Value Recovery[2], %:	99	101
NRCC TORT-1 (lobster hepatopancreas)		
Composition, mg/kg:	24.6±2.2	6.88±0.47
Method Result, mg/kg:	25.9	5.81
Method Result RSD, %:	1.9	1.4
True Value Recovery[2], %:	105	84
NIST SRM 1548 (total diet)		
Composition, mg/kg:	nv	0.245±0.005
Method Result, mg/kg:	0.111	0.270
Method Result RSD, %:	6.7	12
True Value Recovery[2], %:	undefined	110
NIST RM 8415 (whole egg powder)		
Composition, mg/kg:	(0.01)	1.39±0.17
Method Result, mg/kg:	0.014	1.50
Method Result RSD, %:	49	4
True Value Recovery[2], %:	undefined	108

(1) nv = no value provided by certifying organization
± = certified value ± 95% tolerance limit
( ) = informational value
< MDL = less than method detection limit (no reliable measurable signal observed)

(2) Percent recovery of method result based on compositional information provided by reference material's issuing organization. Value calculated using full numerical precision

Table 5. Initial Demonstration of Performance—Food and Beverage Homogenate Average (n=3) Analytical Results

Food Material	Element	
	As	Se
Beverage Homogenate (231-H11)		
Method Result, mg/kg:	< MDL	0.0061
Fortification Level, mg/kg:	0.0290	0.0290
Fortification Result, mg/kg:	0.0288	0.0343
Fortification Result RSD, %:	3	6
Fortification Recovery, %[1]:	99	97
Beverage Homogenate (231-H10)		
Method Result, mg/kg:	< MDL	0.0062
Fortification Level, mg/kg:	0.0295	0.0295
Fortification Result, mg/kg:	0.0309	0.0348
Fortification Result RSD, %:	13	2
Fortification Recovery, %[1]:	105	97
Food Homogenate (131-H7)		
Method Result, mg/kg:	0.004	0.073
Fortification Level, mg/kg:	0.206	0.206
Fortification Result, mg/kg:	0.221	0.269
Fortification Result RSD, %:	6	3
Fortification Recovery, %[1]	105	95
Food Homogenate (131-H11)		
Method Result, mg/kg:	0.014	0.089
Fortification Level, mg/kg:	0.212	0.212
Fortification Result, mg/kg:	0.229	0.295
Fortification Result RSD, %:	11	7
Fortification Recovery, %[1]:	101	97

(1) value calculated using full numerical precision

Table 6. Typical Analytical Sequence

Auto-sampler Cup #	Solution	QC Criteria
	sensitivity check	
	stability check	
	calibration standards	
	calibration blank	nonlinear algorithm
1.	lab. instr. blank (LIB)	≤ IDL
2.	Instr. perf. check (IPC)	90% - 110%
3.	lab. reagent blank	< 5 x MDL
4.	laboratory reference material	70% - 130% recovery
5.	sample 1 (LSO)	absorbance < high cal. std.
6.	sample 1 fortification (LSF)	70% - 130% recovery
7.	sample 2	absorbance < high cal. std
8.	sample 3	absorbance < high cal. std.
9.	sample 4	absorbance < high cal. std.
10.	sample 5	absorbance < high cal. std.
11.	sample 6	absorbance < high cal. std.
12	sample 7	absorbance < high cal. std.
13.	lab. instr. blank (LIB)	≤ IDL
14.	qual. control. samp (QCS)	90% - 110%
13.	sample 8	absorbance < high cal. std.
14.	sample 9	absorbance < high cal. std.
15.	sample 10	absorbance < high cal. std.
16.	sample 11	absorbance < high cal. std.
17.	sample 12	absorbance < high cal. std.
18.	sample 13	absorbance < high cal. std.
19.	sample 14	absorbance < high cal. std.
20.	sample 15	absorbance < high cal. std.
21.	sample 16	absorbance < high cal. std.
22.	sample 17	absorbance < high cal. std.
23.	lab. instr. blank (LIB)	≤ IDL
24.	qual. control. samp. (QCS)	90% - 110%
25.	sample 18	absorbance < high cal. std.
26.	sample 19	absorbance < high cal. std.
27.	lab. instr. blank (LIB)	≤ IDL
28.	qual. control. samp. (QCS)	90% - 110%

Table 7. Microwave Digestion Programs

FILE NAME: CLEAN
STANDARD DIGESTION

stage	(1)	(2)	(3)	(4)	(5)
POWER	100%	0%	0%	0%	0%
PRESSURE	0080	0020	0020	0020	0020
RUN TIME	10:00	00:00	00:00	00:00	00:00
TIME @ P	03:00	00:00	00:00	00:00	00:00
TEMPERATURE	170°C	0°C	0°C	0°C	0°C
FAN SPEED	100%	100%	100%	100%	100%

FILE NAME: HDV
STANDARD DIGESTION

stage	(1)	(2)	(3)	(4)	(5)
POWER	55%	65%	100%	100%	0%
PRESSURE	0085	0200	0450	0600	0020
RUN TIME	10:00	10:00	10:00	10:00	00:00
TIME @ P	03:00	03:00	03:00	06:00	00:00
TEMPERATURE	130°C	150°C	180°C	200°C	0°C
FAN SPEED	100%	100%	100%	100%	100%

Standard Operating Procedure for Storage and Custody of NHEXAS Food or Beverage Composites

U. S Food and Drug Administration

Center for Food Safety and Applied Nutrition
Elemental Research Branch
Washington, DC

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1. Scope and Application

This SOP provides storage and custody procedures for the National Human Exposure Assessment Survey (NHEXAS) analytical samples received by ERB from FDA Kansas City District Laboratory (FDA KAN-DO).

2. Summary of Method

One aliquot of each analytical sample is shipped frozen in 50 mL polypropylene conical tubes from FDA KAN-DO. The analytical sample is stored in a -30°C freezer upon receipt at FDA ERB. Receipt of an analytical sample is recorded on the chain of custody sheet accompanying the sample and in the ERB NHEXAS Sample Log computer file. An entry is made in the chain of custody sheet when the sample is destroyed. After the sample is destroyed the original chain of custody sheet is returned to the consortia through the Environmental Protection Agency's National Exposure Research Laboratory, Cincinnati, Ohio (EPA NERL-CI).

3. Definitions

3.1 *Laboratory Sample*—The sample or subsample sent to or received by the laboratory. The laboratory sample is collected by a NHEXAS consortium member and sent to FDA KAN-DO.

3.2 *Analytical Sample*—The sample, prepared from the laboratory sample, from which analytical portions are removed for analysis. The analytical sample is prepared by FDA KAN-DO and sent to FDA ERB for analysis.

3.3 *Analytical Portion*—The quantity of material, of proper size for measurement of the analyte of interest, removed from the analytical sample.

3.4 ERB NHEXAS Sample Log—Data base file on a personal computer containing the following information: Client Identification, Client Sample Descriptor, Laboratory Sample ID, Sample Description, Collection Date, Received Date, Sample Status, Laboratory Status, and Brief Status (see Table 1).

4. Safety

Gloves should be used to prevent frostbite burns when removing analytical sample tubes from the freezer.

5. Equipment and Supplies

5.1 Freezers—Three Forma Scientific, Model 3672 medical freezers equipped with alarms which sound when the freezers deviate from the set temperature range.

6. Sample Collection, Preservation and Storage

Analytical samples will be stored in room 4832 or 4840 in a freezer immediately after receipt from FDA KAN-DO.

7. Quality Control

7.1 Freezers maintain a temperature range of -30 ±3°C. However, the temperature may deviate from this range temporarily when samples are stored or removed.

7.2 Freezer temperatures are checked once a week and recorded in a log attached to the units to verify they operate in the desired temperature range.

8. Procedure

8.1 Receipt of samples are recorded on the sample chain of custody sheets. The chain of custody sheets are kept on file in room 4840 for future entries.

8.2 Receipt of samples is also recorded in the ERB NHEXAS Sample Log.

8.3 60 days after transfer of analytical results to EPA the samples are destroyed. Sample destruction is recorded on the sample chain of custody sheet and the sheet is returned to the appropriate consortium through EPA NERL-CI.

Table 1. Example ERB NHEXAS Sample Log

Client	Client Sample Descriptor	Laboratory Sample ID	Sample Description	Collection Date	Received Date-CFSAN	Sample Status	Laboratory Status	Brief Status
RTI	DD0001ZA	DD0001ZA	FOOD/BEVERAGE HOMOGENATE	95 07 26	95 08 10	Waiting for 60 days (July 9, 1996).	May 9, 1996: Results sent to EPA. ICPMS Batch 1.	Analyzed
RTI	DD0005ZA	DD0005ZA	FOOD/BEVERAGE HOMOGENATE	95 07 26	95 08 10	Waiting for 60 days (July 9, 1996).	May 9, 1996: Results sent to EPA. ICPMS Batch 1.	Analyzed
RTI	DD0006ZA	DD0006ZA	FOOD/BEVERAGE HOMOGENATE	95 07 26	95 08 10	Waiting for 60 days (July 9, 1996).	May 9, 1996: Results sent to EPA. ICPMS Batch 1.	Analyzed
RTI	DD0003ZA	DD0003ZA	FOOD/BEVERAGE HOMOGENATE	95 07 27	95 08 10	Waiting for 60 days (July 9, 1996).	May 9, 1996: Results sent to EPA. ICPMS Batch 1.	Analyzed

Standard Operating Procedure for Determination of Trace Elements in NHEXAS Food Composites by Inductively Coupled Plasma Mass Spectrometry

U. S Food and Drug Administration

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1. Scope and Application

1.1 This method is used to determine trace elements in duplicate diet food and beverage homogenates by microwave digestion/inductively coupled plasma-mass spectrometry (ICP-MS).

1.2 This method is applicable to the following analytes:

Analyte	Chemical Abstract Services Registry Number (CASRN)
Arsenic (As)	7440-38-2
Barium (Ba)	7440-39-3
Cadmium (Cd)	7440-43-9
Chromium (Cr)	7440-47-3
Copper (Cu)	7440-50-8
Lead (Pb)	7439-92-1
Manganese (Mn)	7439-96-5
Nickel (Ni)	7440-02-0
Selenium (Se)	7782-49-2
Vanadium (V)	7440-62-2
Zinc (Zn)	7440-66-6

1.3 Listed in Table 1 are the isotopes used for validation of these analytes. Listed in Table 3 are method detection limits (MDLs Sect. 3.10) determined using laboratory digestion blanks and Meinhard concentric nebulization sample introduction into the plasma.

1.4 Specific instrumental operating conditions are given in Tables 1 and 2.

2. Summary of Method [1]

2.1 Method description—This method describes multielement analysis of foods by ICP-MS. Food digest solutions are nebulized and the aerosol that is produced is transported to a radiofrequency plasma where energy transfer processes cause desolvation, atomization, and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a high resolution magnetic sector mass spectrometer having a resolution capability of 10,000 Resolving Power. The ions transmitted through the flight path are counted by a continuous dynode electron multiplier or Faraday detector and the ion information processed by a data handling system. Interferences resulting from isobaric elements polyatomic ions derived from the plasma gas, reagents or sample matrix are removed by selecting the appropriate resolution. Instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix must be corrected for by the use of internal standardization.

2.2 Analytical sensitivity and specificity—Method detection limits (Sect. 3.10) for the analytes listed are presented in Table 4. These data were calculated by estimation of the standard deviation of laboratory digestion blanks (Sect. 3.12), with twelve replicate measurements.

2.3 Analytical recovery and precision—Recovery of analyte was assessed by analysis of reference material and fortified food homogenates. Method recovery was within limits of 70 to 130% for all eleven elements. Method Precision was assessed as relative standard

deviation (RSD) of replicate analyses of reference material and fortified food homogenates. Method precision was within limits of ±30% RSD (Sect. 13).

3. Definitions[1-4]

3.1 *Analytical Sample*—The sample, prepared from the laboratory sample, from which analytical portions are removed for analysis.

3.2 *Analytical Portion*—The quantity of material, of proper size for measurement of the analyte of interest, removed from the analytical sample.

3.3 *Analytical Solution*—The solution prepared by dissolving, with or without reaction, the analytical portion in a liquid.

3.4 *Laboratory Sample (Background for Spike) (LSO)*—The first of two analytical portions of a food or beverage test sample. This portion is analyzed according to the analytical method, to establish background concentrations prior to fortification (spiking) with the method analyte(s).

3.5 *Laboratory Spiked Sample (LSF)*—The second of the two analytical portions described under LSO. This portion is subject to fortification (spiking) prior to sample preparation, and the measurement(s) of the final concentration(s) are then made according to the analytical method.

3.6 *Internal Standard (IS)*—Pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes that are components of the same solution. The internal standard must be an analyte that is not a sample component (Sect. 7.5).

3.7 *Laboratory Reference Material (LRM)*—An analytical portion of a food or beverage material having a certified value. These materials are usually obtained from the National Institute of Standards and Technology (NIST), the National Research Council of Canada (NRCC), Bureau of Reference Materials of the European Communities (BCR), etc. The *LRM* is treated as a food or beverage analytical sample in all aspects in the laboratory. This includes addition of all reagents, internal standards, surrogates, labware, apparatus, equipment, solvents, and analyses.

3.8 *Method Detection Limit (MDL)*—The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero (Sect. 9.1.2).

3.9 *Laboratory Digestion Blank (LDB)* (preparation blank)—An aliquot of reagent water that is treated exactly as an analytical portion including exposure to all glassware, equipment, reagents, and acids that are used with other analytical portions. The LDB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents or apparatus (Sects. 7.6.2 and 9.2.1).

3.10 *Calibration Blank*—A volume of ASTM type I water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP-MS instrument (Sect. 7.6.1).

3.11 *Stock Standard Solution*—A concentrated solution containing one analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source (Sect. 7.4). Stock standard solutions are used to prepare calibration solutions and other needed analyte solutions.

3.12 *Calibration Standard (CAL)*—A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration (Sect. 7.5 and Table 3).

3.13 *Linear Dynamic Range (LDR)*—The concentration range over which the analytical curve remains linear.

4. Interferences

4.1 Isobaric interferences may cause inaccuracies, if they are not resolved, in the determination of trace elements by ICP-MS.

4.1.1 Isobaric elemental interferences - are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by the method have at least one isotope which can be resolved from potential isobaric elemental interferences.

4.1.2 Isobaric polyatomic interferences - Are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. All elements determined by the method have at least one isotope which can be resolved from potential isobaric polyatomic interferences.

4.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. Internal standard elements will be used to compensate for these possible effects.

4.3 Memory interferences result when analytes in a previous sample contribute to the signals measured in a current sample. Memory effects can result from sample deposition on the uptake tubing to the nebulizer or from build-up of sample material on the sampler and skimmer cones, plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to either their LDRs or concentrations ten times those usually encountered. The aspiration time should be the same as a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of two of the method detection limit should be noted. Until the required rinse time is established, this method recommends a rinse period of 5 min between samples and standards. If a memory interference is suspected, the sample should be reanalyzed after a long rinse period.

5. Safety

5.1 Each chemical used in this method should be regarded as a potential health hazard, and exposure to these compounds should be as low as reasonably achievable. Specifically, concentrated nitric acid is moderately toxic and extremely irritating to skin and mucus membranes. Use in a hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection and latex gloves when working with reagents.

5.2 The inductively coupled plasma should only be viewed with proper eye protection from the ultraviolet emissions.

5.3 Precautions should be taken to minimize potential hazards. Basic good housekeeping and safety practices such as the use of rubber or plastic gloves and safety glasses are highly recommended.

6. Equipment and Supplies

6.1 Analytical Instrumentation

6.1.1 VG Plasma Trace II is a high resolution mass spectrometer system.

6.1.2 Argon gas supply—Liquid, high purity grade (99.99%).

6.1.3 A variable speed peristaltic pump is required to deliver both standard and sample solutions to the nebulizer.

6.1.4 Mass flow controller to regulate the argon nebulizer flow rate.

6.1.5 Meinhard concentric Nebulizer (Model # TR-30-A3).

6.2 Analytical balance: Balance capable of weighing to the nearest 0.0001 g.

6.3 Labware—For the determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area, designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by (l) contributing contaminants through surface desorption or leaching, (2) depleting element concentrations through adsorption processes. All labware (quartz, polyethylene, Teflon, etc.), including the sample container, should be cleaned prior to use. Labware should be thoroughly washed with laboratory-grade detergent and water, rinsed with water, and soaked for a minimum of four hours in 10% nitric acid followed by rinsing with water.

6.3.1 Labware—500 mL wash bottles and 250 mL high density polyethylene (HDPE) wide mouth sample bottles.

6.3.2 Calibrated pipettes—10 mL, 5 mL, 1 mL, and 100 µL continuously adjustable digital microliter pipettes.

6.4 Microwave Digestion Equipment

6.4.1 Directions on use of microwave digestion equipment are specific to CEM brand equipment and assumes familiarity with the equipment. Use of this method with other brands of microwave digestion equipment may require modifications to this procedure.

6.4.2 Microwave digestion system with temperature and pressure control from 0 to 200°C and 0 to 600 psi. Microwave power range: 0-100% full power (630 watts ±50 watts), programmable in 1% increments (CEM corporation model MDS-2000 equipped for Heavy Duty Vessels (HDVs) or equivalent).

6.4.3 Microwave digestion vessels (CEM corporation Heavy Duty Vessels (HDVs) or equivalent)—TEM Teflon lined digestion vessels capable of operating up to 200°C and 600 psi and accessories.

6.4.4 Cleaning protocol

6.4.4.1 Disassemble the vessel cap and cover, removing the rupture membrane from the vent fitting. Pressure/temperature control covers need not be disassembled, simply remove the rupture membrane from the vent fitting. Vessel covers, liners and vent fittings should be soaked for at least 2 hours in a solution of liquid laboratory-grade detergent (MICRO brand or equivalent) and hot tap water. Other vessel components can be rinsed with reverse osmosis (RO) or higher quality water (at least 10 MΩ resistivity) and dried in a class 100 clean area. Thermowells should be wiped down with a paper towel and detergent solution. Rinse thermowells and vessel components with warm tap water and then rinse thoroughly with reverse osmosis (RO) or higher quality water (at least 10 MΩ resistivity). Dry in a class 100 clean area.

6.4.4.2 Assemble the cover and cap, inserting a rupture membrane in the vent fitting. Add 10 mL of Trace Metals grade nitric acid to each liner and seal liner and cover in the digestion vessel body. Insert a thermowell into the pressure/temperature control cover and secure with a ferrule nut. Connect the pressure line to the pressure/temperature control cover and place the pressure/temperature control vessel in the carousel. Insert the fiber-optic temperature probe into the thermowell. Place the other vessels in the carousel and begin the microwave CLEAN program (Table 4).

6.4.4.3 After the vessels have cooled to less than 120°C remove them from the oven leaving the control vessel in place. Vent excess pressure from the control vessel, toward the fan, by slowly loosening the pressure line. Remove the pressure line and fiber-optic temperature probe from the control vessel, placing the probe in one of the inlet/outlet ports. Turn the two-way valve to the open position and rinse the pressure line with at least 30 mL of water. Return the two-way valve to the neutral position.

6.4.4.4 Place the vessels in an exhausting fume hood and vent the excess pressure by slowly loosening the vent fitting. Using the HDV opening station, remove cap and cover assemblies from vessel bodies. Rinse off covers and

liners with water into a waste container. Thoroughly, rinse covers and liners with water. A total of six rinses has been found to work but, the minimum number of rinses required to achieve undetectable method blanks has not been studied. Shake water off vessels, reassemble, and store until needed.

7. Reagents and Standards

7.1 Reagents may contain elemental impurities which might affect analytical data. Only high-purity reagents should be used whenever possible. Suitable acids are available from a number of manufacturers or may be prepared by sub-boiling distillation.

7.2 Nitric acid, concentrated (sp.gr. 1.41) (CASRN 7697-37-2) - HNO_3.

7.2.1 Nitric acid, Trace Metals grade.

7.2.2 Nitric acid, double distilled.

7.3 Reagent water—All references to water unless otherwise stated refer to ASTM type I grade water.[5]

7.4 Standard Stock Solutions—1,000 μg/mL and 10,000 μg/mL concentration standards are purchased from a reputable commercial source. The density of standard stock solutions should be determined by measuring the mass of a known volume, so that standards can be prepared on a weight basis.

7.5 Internal Standards (IS) Stock Solution—Prepare the mixed IS solution by combining appropriate weights of the stock standard solutions of scandium, yttrium, indium, and bismuth in a tared 250 mL HDPE sample bottle to achieve a concentration of 2.5 μg/g in a total of 250g of solution. The standard should be stored in an FEP bottle. The IS stock solution is added to samples, calibration blanks and standards to achieve a concentration of 4,000 ng/kg.

7.6 Calibration Standard (CAL) Solutions—Prepare mixed CAL solutions by combining appropriate volumes of the stock standard solutions in volumetric flasks to achieve the concentrations listed in Table 3. Add the internal standards stock solution. Transfer freshly prepared mixed CAL solutions to an acid cleaned, FEP fluorocarbon or HDPE bottle for storage. Fresh mixed CAL solutions should be prepared as needed with the realization that concentration can change on aging. The CAL solutions must be initially verified using a quality control sample.

7.7 Blanks—Three types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, a laboratory digestion blank is used to assess possible contamination from the sample preparation procedure and a rinse blank is used to flush the instrument uptake system and nebulizer between standards and samples to reduce memory interferences.

7.7.1 The calibration blank is prepared by acidifying reagent water and adding the internal standard stock solution to the same concentration of acids and elements as used for standards. The calibration blank should be stored in a FEP bottle.

7.7.2 The laboratory digestion blank (LDB) must contain all reagents in the same volumes as used in the processing of the samples. The LDB must be carried through the same entire preparation scheme as the sample as the samples including sample digestion.

7.7.3 The rinse blank is prepared by acidifying reagent water to the same concentration of acid as used in the calibration blank. The rinse blank should be stored in a FEP bottle.

8. Sample Collection, Preservation and Storage

Specific details on insuring analytical sample integrity and record keeping are contained in the SOP for Storage and Custody of NHEXAS Food or Beverage Composites (ERB SOP 104).

9. Quality Control

9.1 Initial Demonstration of Performance

9.1.1 The initial demonstration of performance is used to characterize instrument and laboratory performance prior to using this method for sample analyses.

9.1.2 Method detection limit (MDL)—MDLs are established for each element by analysis of twelve laboratory digestion blanks. The Rule of the Huge Error was applied and 10 to 12 results were used to calculate the MDLs.[6] The MDL is calculated as follows:

$$MDL = (t) \times (S)$$

where:

t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 2.82 for ten replicates].
S = Standard deviation of the replicate analyses.

9.1.3 Method Precision and Recovery—Method precision is assessed as relative standard deviation (RSD) of replicate analytical portions of appropriate LRMs and laboratory spiked samples (LSFs). The LSF matrix should be representative of the matrix to be run. Recovery of analyte is assessed by replicate analytical portions of LRMs and LSFs. See Section 13 for method performance data.

9.2 Assessing Laboratory Performance

9.2.1 Laboratory Digestion Blank (LDB)—At least one LDB for batches of 20 or fewer analytical portions. LDB values that exceed the MDL indicate laboratory or reagent contamination. When LDB values constitute 10% or more of the analyte level determined for a sample or is 2.2 times the analyte MDL whichever is greater, fresh analytical portions must be prepared and analyzed for the affected analytes after the source of contamination has been corrected and acceptable LDB values have been obtained.

9.2.2 Instrument performance check—For all determinations the laboratory must analyze the CAL solution (Sect. 7.5) after every sixth sample (or more frequently, if required) and at the end of the sample run.

9.3 Assessing Analyte Recovery and Data Quality

9.3.1 Laboratory Spiked Sample (LSF) and Laboratory Sample (Background for Spike) (LSO)—At least one LSF and corresponding LSO must be analyzed with each batch of 20 or fewer analytical portions. Analyte fortification of the LSF should be 1 to 2 times the native level or a concentration approximately 10 to 100 times their respective MDLs, whichever is greater. Recovery of the LSF must be 70–130% of the expected concentration.

9.3.2 Laboratory Reference Material (LRM)—At least one NIST RM 8415 (whole egg powder) must be analyzed with each batch of 20 or fewer analytical portions. The LRM must be 70-130% of the expected concentration with the exception of Ni, As, Ba, and Cd which are not certified.

9.4 In the event quality control sample results do not meet expected values, analysis of all analytical solutions for the batch are routinely repeated once. Unacceptable instrument performance measures will be corrected before the analysis is repeated. If repeated results for quality control samples are still not acceptable, those results are reported, and sample analysis is continued. Unacceptable quality control samples will be reported to the supervisory chemist and reviewed with the chemist to determine acceptability of the results.

10. Calibration and Standardization

10.1 Acquisition parameters and Instrument operating conditions are given in Tables 1 and 2.

10.2 Prior to using this method optimize the plasma and Meinhard nebulizer operating conditions.

10.2.1 Verify the instrument operating conditions are set to those found to be optimum (Table 2). Ignite the plasma and allow the instrument to become thermally stable before beginning. This requires 60 minutes of operation.

10.2.2 Determine instrumental sensitivity with indium at the lowest and highest resolution before sample analysis to verify instrumental performance.

10.2.3 Verify the mass calibration for each isotope.

10.3 Internal Standardization—Internal standardization must be used in all analyses to correct for instrument drift and physical interferences. The four internal standard elements used are: Scandium, Yttrium, Indium, and bismuth. The internal standardization standard contains 2.5 μg/g of each element in 2% nitric acid and is added to the calibration blank and standard, and test solutions to yield the same concentration of internal standard elements.

11. Procedure

11.1 Sample Preparation

11.1.1 Clean microwave digestion vessels (Sect. 6.4.3).

11.1.2 Analytical Portions

11.1.2.1 For the digestion of a food homogenate reblend the analytical sample by vigorously shaking or stirring with a Teflon spatula (depending on consistency) and immediately weigh approximately a 1 to 7 g analytical portion (depending on consistency) with an air displacement pipetter or Teflon spatula into a tared, clean (see Sect. 6.4.3) microwave digestion vessel liner.

11.1.2.2 For the digestion of a Laboratory Reference Material weigh approximately a 0.5 g analytical portion of NIST RM 8415(Whole Egg Powder) with a Teflon spatula into a tared, clean (see Sect. 6.4.3) microwave digestion vessel liner.

11.1.3 Move analytical portions to a hepafiltered exhausting fume hood and pipette 9 mL of double distilled nitric acid into each liner, washing down any material on the walls. Slowly slide the liner into the vessel body, while holding the body at a 30 to 45° angle or use the capping station. This is to avoid any splash back of the sample acid mixture. Seal vessels by hand tightening the caps. Insert a thermowell into the thermowell port of the pressure/temperature control vessel and secure with a ferrule nut. Verify vessels are properly sealed by tightening vent fittings and rotating the pressure/ temperature control cap.

NOTE: After repeated uses it may become difficult to insert a thermowell into the thermowell port. This may be caused by trapped water in the fitting or a swelling of the temperature control stem. Disassemble the control stem and allow to dry if water is present. Reassemble the control cover and insert the thermowell. The pressure/temperature control cover should be replaced if it is still difficult to insert the thermowell into the port. Forcing the thermowell into the port can pose a danger if the thermowell breaks. In addition, the thermowell may block the pressure line from accurately sensing the vessel pressure, which can cause an over pressurization of all vessels and a possible loss of analyte if the vessels vent.

11.1.4 Connect the pressure/temperature control vessel to the pressure line and place the control vessel in the carousel. Insert the fiber-optic temperature probe into the thermowell and place the rest of the vessels into the carousel. Verify that the pressure line and fiber-optic temperature probe do not tangle when the carousel is rotating, and the two-way valve is in the neutral position. Run the HDV microwave digestion program (Table 4).

11.1.5 After the vessels have cooled to less than 120°C remove them from the oven leaving the control vessel in place. Vent excess pressure from the control vessel

by slowly loosening the pressure line. Remove the pressure line and fiber-optic temperature probe from the control vessel, placing the probe in one of the inlet/outlet ports. Turn the two-way valve to the open position and rinse the pressure line with at least 30 mL of water. Return the two-way valve to the neutral position.

11.1.6 Place the vessels in an exhausting fume hood and vent the excess pressure by slowly loosening the vent fittings. Place the vessels in a hepafiltered exhausting fume hood. Using the HDV opening station remove the cap and cover assembly from the vessel body. Transfer the digest to a 250mL HDPE sample bottle. Pour the contents of the vessel liner into the sample bottle. Rinse the liner and transfer the contents to the sample bottle a total of three times. Bring the analytical solution to approximately 250 mL total volume and add the internal standard.

11.2 Sample Analysis

11.2.1 Prior to daily calibration of the instrument, inspect the sample introduction system including the nebulizer, torch, injector tube and uptake tubing for salt deposits and dirt that would restrict solution flow and affect instrument performance. Clean the system when needed or on a daily basis.

11.2.2 Configure the instrument to the power and operating conditions listed in Table 2.

11.2.3 The instrument must be allowed to become thermally stable before calibration and analyses. This requires 60 minutes of operation. After instrument warm-up, perform the sensitivity and mass calibration checks (Sect. 10.).

11.2.4 For initial and daily operation, calibrate the instrument according to the instrument manufacturer's recommended procedure, using the mixed calibration standard solution (Sect. 7.5) and the calibration blank (Sect. 7.6.1). A peristaltic pump must be used to introduce all solutions to the nebulizer. To allow equilibrium to be reached in the plasma, aspirate all solutions for 30 sec after reaching the plasma before beginning integration of the signal to accumulate data. Flush the system with rinse blank (Sect. 7.6.3) for a minimum of 300 seconds (Sect. 4.4) between each solution.

11.2.5 Analytical solutions are analyzed in the same operational manner used in the calibration routine with the rinse blank aspirated for a minimum of 300 seconds between all sample and check solutions (Sect. 7.6.3).

11.2.5 During sample analysis, quality control measures described in Section 9 must be followed.

11.2.6 Determined analytical solution analyte concentrations that are greater than the calibration standard must be diluted with reagent water that has been acidified in the same manner as calibration blank and reanalyzed.

11.2.7 Report data as directed in Section 12.

12. Data Analysis and Calculations

12.1 Analytical solution and analytical portion concentrations are calculated using Microsoft Excel from intensity data.

12.2 LDBs are reported on the basis of the analytical solution times a dilution factor of 1 g in 0.25kg in units of μg/kg.

12.3 LRMs and food or beverage composites are reported on the basis of the analytical sample in units of μg/kg.

12.4 Spike calculated result (percent recovery) of laboratory spiked sample is calculated as follows:

$$R = \frac{C_s - C}{s} \times 100\%$$

where, R = percent recovery
C_s = fortified sample concentration (i.e., LSF)
C = sample background concentration (i.e., LSO or LDX)
s = concentration equivalent of analyte added to sample.

12.5 True value recovery of LRMs is calculated as follows:

$$R = \frac{C}{s} \times 100\%$$

where, R = percent recovery
C = reference material concentration
s = certified value (true value) of analyte for reference material.

13. Method Performance

13.1 Listed in Table 3 are MDLs determined using the procedure outlined in Section 11 and conditions listed in Tables 1 and 2. MDLs for analytical solutions were determined by analyses of twelve LDBs. MDLs listed are estimates for food homogenates and were calculated from the solution MDLs and the analytical portion weight and volume used in the method.

13.2 Method precision was assessed as relative standard deviation (RSD) of triplicate analytical portions of National Institute of Standards and Technology (NIST) LRM and three fortified homogenates. Method precision was within limits of ±30% RSD, with the exception of arsenic in NIST RM 8415(whole egg) which is not a certified element. Findings are summarized in Table 5.

13.3 Recovery of analyte was assessed by triplicate analytical portions of LRMs and fortified homogenates. Method recovery was within limits of 70 to 130%, with the exception of arsenic in NIST RM 8415(whole egg) which is not a certified element.

14. References

1 Long, S.E. and Martin, T.D. (1991) - Method 200.8, Revision 4.4.

2 Martin, T.D., Brockhoff, C.A., Creed, J.T,. and EMMC Methods Work Group (1994) - Method 200.7, Revision 4.4.

3 Terwilliger, D. T. and Behbehani, A. L. (1994) User Guide for Manual Data Entry Software, version 2.0. U.S. EPA EMSL-Cincinnati Contract No. 68-C0-0001.

4 Horwitz, W. (1990) *Pure & Appl. Chem.* **62**, 1193-1208.

5 American Society for Testing and Materials. Standard Specification for Reagent Water, D1193-77. Annual Book of ASTM Standards, Vol. 11.01. Philadelphia, PA, 1991.

6 Taylor, J. K. (1987) *Quality Assurance of Chemical Measurements*, CRC Press,Inc., Boca Raton, Florida, pp. 34-35.

Table 1. Acquisition Parameters

Isotope	Detector	Dwell Time(ms)	Points/ Peak Width	Peak Widths	Scans	Resolution
Sc 45	Multiplier	100	20	3.0	1	4000
V 51	Multiplier	100	20	3.0	1	4000
Cr 52	Multiplier	100	20	4.0	1	4000
Mn 55	Multiplier	20	100	3.0	1	4000
Ni 60	Multiplier	100	20	4.0	1	4000
Cu 63	Multiplier	20	100	4.0	1	4000
Zn 66	Multiplier	20	100	4.0	1	4000
As 75	Multiplier	500	20	3.0	1	8500
Se 78	Multiplier	500	40	3.5	1	9500
Y 89	Multiplier	100	20	3.0	1	400
Cd 111	Multiplier	500	20	3.0	1	400
In 115	Multiplier	100	20	3.0	1	400
Ba 137	Multiplier	100	20	3.0	1	400
Pb 208	Multiplier	100	20	3.0	1	400
Bi 209	Multiplier	100	20	3.0	1	400

Table 2. Instrument Operating Conditions

ARL-3580 Inductively Coupled Plasma - Optical Emission Spectrometer	
Incident RF power	1300 watts
Reflected RF power	< 10 watts
Argon pressure	> 80 psi
Coolant argon flow rate	13 L/min
Auxiliary (plasma) argon flow rate	0.7 L/min
Aerosol carrier argon flow rate	1.00 L/min
Sample uptake rate controlled to	0.7 mL/min

Table 3. Method Detection Limits (MDL) and Calibration Standard (CAL)

Analyte	MDLs Solution, ng/kg(1)	CAL, ng/kg	Foods, µg/kg(2)
V 51	4	1,000	1.0
Cr 52	100	1,000	25
Mn 55	5	50,000	1.3
Ni 60	20	2,000	5
Cu 63	20	10,000	5
Zn 66	70	100,000	20
As 75	6	1,000	1.5
Se 78	20	2,000	5
Cd 111	5	500	1.3
Ba 137	300	10,000	75
Pb 208	5	500	1.3

(1) Samples were processed in Teflon and diluted in 250-mL HDPE sample bottles. The calculated MDL was rounded upward and reported to a single digit.
(2) Based on analytical solution determination and a 1 g test portion.

Table 4. Microwave Digestion Programs

FILE NAME: CLEAN
STANDARD DIGESTION

stage	(1)	(2)	(3)	(4)	(5)
POWER	100%	0%	0%	0%	0%
PRESSURE	0600	0020	0020	0020	0020
RUN TIME	20:00	20:00	00:00	00:00	00:00
TIME @ P	06:00	00:00	00:00	00:00	00:00
TEMPERATURE	200C	0C	0C	0C	0C
FAN SPEED	100%	100%	100%	100%	100%

FILE NAME: HDV
STANDARD DIGESTION

stage	(1)	(2)	(3)	(4)	(5)
POWER	55%	65%	100%	100%	0%
PRESSURE	0085	0200	0450	0600	0020
RUN TIME	10:00	10:00	10:00	10:00	20:00
TIME @ P	03:00	03:00	03:00	06:00	00:00
TEMPERATURE	130C	150C	180C	200C	0C
FAN SPEED	100%	100%	100%	100%	100%

Table 5. Initial Demonstration of Performance, Average (n=3)

	Element					
	V	Cr	Mn	Ni	Cu	Zn
NIST RM 8415(whole egg)						
Composition, µg/kg(a):	459±81	370±180	1780±380	nv	2700±350	67500±7600
Method Result, µg/kg:	475	319	1674	71.1	2718	58735
Method RSD, %:	4	3	5	20	8	3
True Value Recovery, %:	103	86	94	nv	101	87
DD7002Z						
Method Result, µg/kg:	4.64	23.2	1137	53.1	392	3059
Fortification Level, µg/kg:	97.6	97.7	3103	195	1459	9743
Fortification Result, µg/kg:	98.0	110	3792	211	1764	10797
Fortification RSD, %:	10	12	8	8	10	8
Recovery, % (b):	96	91	89	85	95	84
4-51ddsDM						
Method Result, µg/kg:	45.2	39.3	5594	221	1188	9414
Fortification Level, µg/kg:	101	101	3217	202	1513	1010
Fortification Result, µg/kg:	151	150	9038	430	2685	18921
Fortification RSD, %:	10	3	10	7	7	4
Recovery, % (b):	103	107	103	102	99	97
1700002						
Method Result, µg/kg:	25.5	31.1	4091	127	756	7025
Fortification Level, µg/kg:	99.1	99.2	3152	198	1482	9898
Fortification Result, µg/kg:	144	132	7807	336	2341	16584
Fortification RSD, %:	6	4	9	5	7	6
Recovery, % (b):	116	101	108	103	105	98

(a) nv = no value provided by certifying organization
(b)value calculated using full numerical precision
± = certified value ± 95% tolerance limit, ( ) = informational value

Table 5. Initial Demonstration of Performance continued, Average (n=3)

	Element				
	As	Se	Cd	Ba	Pb
NIST RM 8415(whole egg)					
Composition, µg/kg(a):	(10)	1390±170	(5)	(3000)	61±.12
Method Result, µg/kg:	23.6	1565	4.37	3244	45.9
Method RSD, %:	40	10	13	2	8
True Value Recovery, %:	236	113	90	108	75
DD7002Z					
Method Result, µg/kg:	8.1	47.4	14.3	404	5.3
Fortification Level, µg/kg:	9.5	194	48.5	981	39.0
Fortification Result, µg/kg:	18.0	237	58.3	1450	40.0
Fortification RSD, %:	12	16	6	7	10
Recovery, % (b):	103	98	93	105	90
4-51ddsDM					
Method Result, µg/kg:	5.1	18.7	43.0	971	12.7
Fortification Level, µg/kg:	9.8	201	50.3	1018	40.5
Fortification Result, µg/kg:	14.9	229	84.7	1948	49.6
Fortification RSD, %:	8	24	5	4	1
Recovery, % (b):	101	106	91	98	93
1700002					
Method Result, µg/kg:	7.0	195	13.1	1194	5.16
Fortification Level, µg/kg:	9.6	197	49.2	997	39.6
Fortification Result, µg/kg:	18.3	402	58.4	2278	39.9
Fortification RSD, %:	13	6	1	1	1
Recovery, % (b):	110	103	94	104	89

(a) nv = no value provided by certifying organization
(b)value calculated using full numerical precision
± = certified value ± 95% tolerance limit, ( ) = informational value

Standard Operating Procedure for Sample Preparation in NHEXAS Food or Beverage Composites by Homogenization

U.S. Food and Drug Administration
Kansas City District Laboratory
Lenexa, KS

PREPARED BY :
NHEXAS Sample Coordinator (HFR-SW360)

APPROVED BY :
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:
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:
FDA Project Officer, NHEXAS EPA IAG

TABLE OF CONTENTS

1. SCOPE AND APPLICATION

The purpose of this Standard Operating Procedure (SOP) is to set forth the operational procedures used to receive, prepare, ship, store and dispose the samples for the National Human Exposure Assessment Survey (NHEXAS). The analysts assigned to these functions, the supervisory chemist for this part of the NHEXAS Study and the laboratory director will assure adherence to the following SOP. All revisions to this SOP must receive the concurrence of the supervisory chemist, the Kansas City District Laboratory Director and the FDA Project Officer.

2. SUMMARY OF METHOD

The Food and Drug Administration Kansas City District Office Laboratory (FDA KAN-DO) sample preparation team receives NHEXAS consortia parent sample shipments, verifies the shipped items, stores parent samples and assures their integrity, composites parent samples according to this SOP, aliquots the daughter sample according to this SOP, determines moisture content for the analyzing laboratories, prepares chain of custody forms for the daughter samples, ships the daughter samples to the respective consortium, EPA and CFSAN metals analytical laboratory, and maintains KAN-DO pesticide daughter samples.

3. DEFINITIONS

3.1 *NHEXAS:* National Human Exposure Assessment Survey

3.2 *SOP:* Standard Operating Procedure

3.3 *Parent Sample:* The sample or subsample sent to or received by the sample preparation site. The parent sample is prepared by a NHEXAS consortium member and sent to FDA KAN-DO.

3.4 *Daughter Sample:* The sample which results from compositing the parent sample Analytical portions removed for analysis or archival are referred to as daughter samples. The daughter sample is initiated by FDA KAN-DO and sent to the appropriate analyzing laboratory or archival location.

3.5 *Chain of Custody (COC) Form:* The certificate accompanying each parent and daughter sample which documents the successive conveyances of the sample.

4. SAFETY AND HAZARDOUS WASTE

4.1 Take precautions to minimize potential hazards. Basic good housekeeping and safety practices should always be followed.

4.2 Ensure the floors in the sample preparation room are dry and free of any substance that could cause the floor to be slippery.

4.3 Turn off sample preparation equipment before disassembling for cleaning purposes.

4.4 Place broken glass into specially marked containers.

4.5 Know the location of the nearest first aid kit.

4.6 Identify the location of fire extinguishers in the vicinity of the sample preparation room and know how to use them properly.

4.7 Do not use any medications which can cause drowsiness while operating the sample preparation equipment.

4.8 Use the proper step stools to grasp items out of reach.

5. EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Blenders
Quart glass jar blender: Waring Model # 33AM26

Gallon stainless steel jar blender: Waring Model # 34BL22

5.1.2 Food Processors
Six quart commercial food processor: Robot Coupe Model R6N

Ten quart commercial food processor: Robot Coupe Model R10

5.1.3 Sample preparation equipment such as bowls, spoons, knives, utensils are stainless steel.

5.1.4 Polytron
High speed processor: Polytron power controller, Kinematica Model PCU-1, Brinkman Instruments, Westbury, NY 11590 or equivalent.

Polytron generator, titanium probe, Brinkman Model PT 10/35 or equivalent.

5.1.5 Walk-In Refrigerator/Freezer
Penn Refrigerator/Freezer, Penn Refrigeration Service Corp., Wilkes-Barre, PA

Temperature Recorders
Weksler Instruments Corporation
Freeport, NY

5.1.6 Moisture Analyzer
Moisture/Solids Analyzer, Model AVC-80 CEM Corporation, Matthews, NC 28106.

5.1.7 Laboratory Carts, stainless steel or rigid plastic.

5.1.8 Analytical Balance
Mettler PS-15 Analytical Balance, Hightstown, NJ

5.2 Supplies

5.2.1 Texwipe Towels, Texwipe Company, Bluewipe, cat. no. TX512, or equivalent.

5.2.2 Dish Towels, lint free

5.2.3 Disposable 50 mL plastic centrifuge tubes, Cole Parmer Inst. Co. Cat. No. M-06334-30 or equivalent.

5.2.4 Clear, glass, 4 oz Qorpak Bottles, 2 dozen per box, Fisher Scientific Co. Cat. No. 033207C or equivalent.

5.2.5 Clear, colorless, glass pint Mason jars with two piece screw caps. Note: lids are put on jars with the rubber seal side up for pesticides analysis. The rubber seal does not come in contact with the jar's contents.

5.2.6 Deionized water, 18 megohm/cm or better, Model No. HR-3015 DX, system designed by Atlantic Filter Corporation, West Palm Beach, FL or equivalent.

5.2.7 Micro Cleaner, Scientific Products, cat. no. C6286-5, or equivalent.

6. SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1 Sample Collection: Samples are packed with freezer cool packs and shipped directly to KAN-DO via overnight carriers for sample preparation. The Sample Preparation Team (SPT) verifies parent sample receipt by inspection assuring shipment contents coincide with Chain of Custody (COC) documents and/or issuing memorandum. The SPT inspects the condition of the samples and reports compromised samples, i.e., broken or leaking containers, to the originating consortium.

6.2 Sample Custody and Identification

6.2.1 Chain of Custody (COC) Forms: The SPT will document all appropriate information on the COC forms for all of the parent and daughter samples. The consortia will furnish the necessary chain of custody forms at the time of sample shipment. If COC forms for the daughter samples are not furnished by the consortia at the time of shipment, the SPT will prepare daughter COC forms (blank forms to be provided by consortia) for homogenized composite aliquots. The parent sample COC forms will be returned by the SPT to the consortia through NERL-Cl. The SPT will provide NERL-Cl with complete information sufficient to track daughter samples, i.e., date collected, date shipped, sample identification, matrix type and analyses to be conducted (metals, pesticides, etc.).

6.2.2 Identification: Each consortium will supply identification labels in duplicate at the time of shipment for each daughter sample produced and distributed by the SPT. A daughter sample's identification label will be placed on its container. The duplicate labels will be placed on the accompanying daughter COC. The identification labels will be secured, if necessary, by placing clear tape over the label.

6.3 Sample Storage: Prior to sample homogenization, the samples are stored in the freezer at -10°F. During sample preparation, samples will be stored in the refrigerator portion of the Penn Refrigerator/Freezer. After the daughter samples are prepared, the samples will be stored in the freezer portion of the Penn Refrigerator/Freezer. The Penn Refrigerator/Freezer is locked and secured. Only the SPT and supervisors have access to the refrigerator/freezer.

6.4 Aliquot Preparation and Distribution Schemes

6.4.1 RTI Consortium - Research Triangle Institute and Environmental and Occupational Health Sciences Institute (EOHSI)

Base Study - Metals only

Organization	Purpose	Aliquot	
FDA/CFSAN	Metals	(3)	50mL plastic centrifuge tubes
RTI	Archive	(2)	50mL plastic centrifuge tubes
		(1)	125mL glass jar
EPA/NERL-Cin (send to RTI)	Archive	(1)	50mL plastic centrifuge tube
		(1)	125mL glass jar

Pesticide subset (Separate from Base Study) - Pesticides only

Organization	Purpose	Aliquot	
FDA/KC	Pesticides	(1)	125mL glass jar
EPA/NHEERL-RTP	Pesticides/PAH	(2)	125mL glass jars
RTI	Archive	(2)	50mL plastic centrifuge tubes
		(1)	125mL glass jar
EPA/NERL-Cin (send to RTI)	Archive	(1)	50mL plastic centrifuge tube
		(1)	125mL glass jar

6.4.2 AZ Consortium - University of Arizona, Illinois Institute of Technology, and Battelle Memorial Institute

Organization	Purpose	Aliquot	
FDA/CFSAN	Metals	(3)	50mL plastic centrifuge tubes
FDA/KC	Pesticides	(1)	125mL glass jar
AZ-Solid Food Only	Metals	(3)	50mL plastic centrifuge tubes

6.4.3 HAR Consortium - Harvard School of Public Health (HSPH), Johns Hopkins University, and Westat.

Organization	Purpose	Aliquot	
FDA/CFSAN	Metals	(3)	50mL plastic centrifuge tubes
FDA/KC	Pesticides	(1)	125mL glass jar
HSPH	Archive	(2)	125mL glass jars
EPA/NHEERL-RTP	PAH	(1)	125mL glass jar
EPA/NERL-Cin	Archive	(1)	50mL plastic centrifuge tube
		(1)	125mL glass jar

6.4.4 If sample size prohibits preparation of all aliquots, priority for aliquot distribution shall be in the order listed above.

7. SAMPLE PREPARATION PROCEDURE

7.1 Cleaning Procedures: The following sequence is carried out prior to preparation of the first item and between preparation of each subsequent item.

7.1.1 Rinse all equipment (blenders, choppers, food processors, stainless steel utensils) with hot water using the commercial sprayer.

7.1.2 Rinse with deionized water and dry thoroughly with a lint free dish towel.

7.2 Sample Homogenization

7.2.1 Blend the parent samples until homogenous to create the daughter sample.

7.2.1.1 In the event that multiple containers are received as one sample, ensure that the sample from all containers is included in the homogenized sample.

7.2.1.2 For the RTI consortium, samples may be identified as 4day and daily samples. Daily samples are prepared by combining portions of the 4 homogenized samples. These portions must be combined in the same weight ratio as was measured for the 4 daily samples as recorded on the accompanying parent COC form. Aliquots of the combined

4-day composite will be removed and distributed for analysis and archival. See Attachment A for applicable RTI Daily Homogenization Worksheet.

7.2.2 Divide parent sample into daughter samples as outlined in Sections 6.4.1, 6.4.2 and 6.4.3 of this SOP.

7.2.3 Place approximately 50 gm and 125 gm (i.e. full is needed) of the prepared daughter sample into the designated container, i.e. a 50mL plastic centrifuge tube or 125mL glass jar.

7.2.4 Place the identification label on each daughter sample.

7.2.5 Complete the COC form for the parent and daughter samples.

7.3 Moisture Determination: Take 2-3 grams of each prepared item before freezing and determine the moisture content (See Moisture Determination SOP, KCX-2). Moisture content analysis is determined on a CEM AVC-80 Moisture Analyzer. The moisture content results will be included on the daughter COCs which accompany the daughter sample shipment.

7.4 Shipment

Send the prepared daughter samples, COC forms, and shipment cover memo to the appropriate consortium at the following addresses.

7.4.1 FDA/CFSAN [Metals]:
Mr. Scott Dolan
Food and Drug Administration
200 C Street, SW
FB#8, Room 4832
Washington D.C. 20204
202/205-4290

7.4.2 RTI and EPA/NERL-Cin [Archival]:
Mr. Kent Thomas
Dreyfus Laboratory
Research Triangle Institute
3040 Cornwallis Road
Research Triangle Park, NC 27709
919/990-8395

7.4.3 EPA/NHEERL-RTP [Polynuclear aromatic hydrocarbons (PAH)/pesticides]:
Mr. Randy Watts
EPA/US Human Studies Facility
Mail Drop 58C
104 Mason Farm Road
Chapel Hill, NC 27599-7315
919/966-0649

7.4.4 EPA/NERL-Cl (aliquot of HAR samples only)
Ms. Lisa Jo Melnyk
USEPA/NERL-Cl (MS 525)
26 W Martin Luther King Drive
Cincinnati, OH 45268
513/569-7494

7.4.5 HSPH [Archival]:
Mr. Bryan Burnette
Rollins School of Public Health
Emory University
Department of Environmental and Occupational Health
1518 Clifton Road, N.E.
Atlanta, GA 30322
404/727-9259

7.4.6 AZ [Metals]:
Mr. Seumas Rogan
Health and Environmental Studies
University of Arizona
1435 North Fremont Ave
Room 128
Tucson, AZ 85719
502/626-4226

7.5 Maintenance of KAN-DO Pesticide Daughter Samples

7.5.1 Store and secure the KAN-DO pesticide daughter samples in the manner prescribed in Section 6.3.

7.5.2 Analysts are required to make entries in sample COC forms each time they change the storage conditions or manipulate a sample. Entries are not required when samples are only examined and the examination is conducted in a few minutes.

8. QUALITY ASSURANCE

8.1 Semiannually, the SPT will prepare an equipment quality control sample. The quality control sample will be representative of the daughter samples. To prepare the quality control sample, blend the parent sample until homogeneous to produce the daughter sample. Remove a 500 gram portion and note the sample as "Time 0." Continue blending the item for 2, 4 and 6 minute intervals. Collect a sample from each time interval. Label as appropriate. Repeat the above procedure using the blenders and polytron. Forward a portion of the samples to appropriate analytical laboratories to determine if sample preparation activities contribute to the analytical findings. If significant contribution is determined, notify the sample preparation supervisor.

8.2 The major goal of contamination control is to assure the reduction of potential contamination sources. To assure contamination control, the Sample Preparation Team adheres to the following procedures. See "Contamination Control" SOP KCM TD G1 for more details.

8.2.1 Wash and rinse hands with copious amounts of deionized water after handling anything which could collect dust or fine filth.

8.2.2 Ensure that food processor equipment containing Aluminum, which contacts the samples, is coated with inert materials such as teflon to reduce elemental contribution.

8.2.3 Rinse the outside and under the lip of the sample container thoroughly with deionized water before opening.

8.2.4 Wipe down the sample preparation room regularly with damp, lint free dish towels. This includes surfaces of carts, bench tops, cabinets, sinks, freezer/refrigerator storage shelves, chairs and instruments.

8.2.5 Run the deionized water tap 2 to 3 minutes before using each morning or any time it is unused for more than an hour. Rinse deionized water outlet with deionized water prior to use.

8.2.6 Use only Texwipe Towels and lint free dish towels. Do not use aluminum foil and paper towels in the sample preparation room.

9. REFERENCES

9.1 Hazardous Waste Management Program, FDA Kansas City District, memorandum dated February 8, 1991.

9.2 Contamination Control SOP, KCM TD G1

9.3 Moisture Analysis SOP, KCX 2

ATTACHMENT A

RTI DAILY SAMPLE HOMOGENIZATION WORKSHEET

Use this worksheet to determine the amount of sample required from each daily sample to maintain the same proportion for the 4-day composite as for the original set of daily samples.

EXAMPLE:

Sample #	Wt (g) Note 1	Spl_x Proportion Note 2	Proportional Subset Note 3
DD0032A	1000	0.142	114
DD0032B	2000	0.285	229
DD0032C	1500	0.214	171
DD0032D	2500	0.357	286
Total Wt			

Note 1: Record the weight from the accompanying parent COC form.

Note 2: Spl_x Proportion = Wt_x/Total Wt

For Sample # DD0032A: Spl_A Proportion = 1000g/7000g = 0.142
This means that Sample # DD0032A will provide 14.2% of the total 4-day homogenized sample.

Note 3: Proportional Subset = Spl_x Proportion x 800g
The proportional subset is the number of grams required to set aside from each daily sample for the 4-day homogenization to maintain the original proportion for the daily samples. 800g is the minimum 4-day sample size required for complete homogenization.

For Sample # DD0032A: 0.142 x 800g = 114g
114g is the number of grams to be set aside for the 4-day homogenization from Sample # DD0032A.

RTI DAILY SAMPLE HOMOGENIZATION WORKSHEET

DATE:

Use this worksheet to determine the amount of sample required from each daily sample to maintain the same proportion for the 4-day composite as for the original set of daily samples.

Sample #	Wt (g) Note 1	Spl_x Proportion Note 2	Proportional Subset Note 3
A			
B			
C			
D			
Total Wt			

Sample #	Wt (g) Note 1	Spl_x Proportion Note 2	Proportional Subset Note 3
A			
B			
C			
D			
Total Wt			

Note 1: Record the weight from the accompanying parent COC form.

Note 2: Spl_x Proportion = Wt_x/Total Wt

Note 3: Proportional Subset = Spl_x Proportion x 800g
The proportional subset is the number of grams required to set aside from each daily sample for the 4-day homogenization to maintain the original proportion for the daily samples. 800g is the minimum 4-day sample size required for complete homogenization.