U.S. Department of Health & Human Services

U.S. Food and Drug Administration

# Elemental Analysis Manual
## for Food and Related Products

U.S. FOOD & DRUG ADMINISTRATION

# Elemental Analysis Manual

# 4.7 Inductively Coupled Plasma-Mass Spectrometric Determination of Arsenic, Cadmium, Chromium, Lead, Mercury, and Other Elements in Food Using Microwave Assisted Digestion

Version 1.3 (April 2025)

**Current Validation Status:**

| | |
|---|---|
| MULTI-LAB VALIDATION: | YES |
| SINGLE LAB ANALYTE EXTENSION VALIDATION: | YES |

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GLOSSARY

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## 4.7.1 SCOPE AND APPLICATION

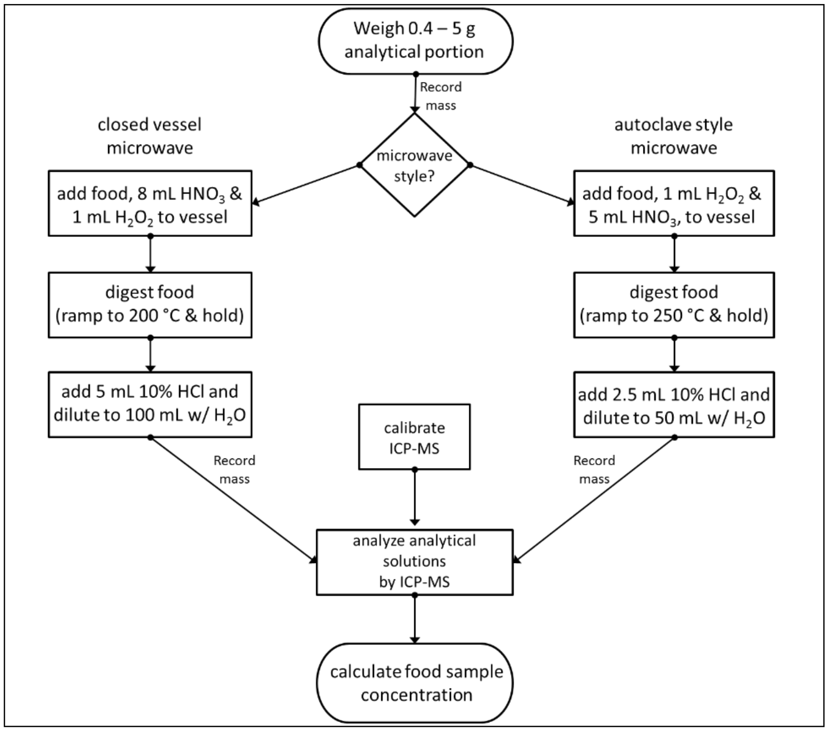
This method describes procedures for determining total acid-extractable concentrations of arsenic, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, selenium, strontium, thallium, tin, uranium, vanadium, and zinc in food by microwave assisted acid decomposition and inductively coupled plasma-mass spectrometry (ICP-MS). Non-food matrices may be analyzed by these procedures if performance is verified in the matrix of interest, at the concentration levels of interest. This method is not validated for non-food matrices such as mineral dietary supplements or cosmetics.

This method should only be used by analysts familiar with trace element analysis and ICP-MS. The analyst must be trained in the interpretation of spectral and matrix interferences and procedures for their correction.

## 4.7.2 SUMMARY OF METHOD

An analytical portion of food is decomposed in acid inside a high-pressure digestion vessel using microwave heating.[1, 2] The analytical solution is analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS). Elemental concentrations are quantified using external calibration and quality controls are incorporated to ensure data quality. 4.7 Figure 1 shows the method procedures.

**4.7 Figure 1. Procedure flow chart**



Typical analytical limits were calculated per §3.2 and are listed in 4.7 Tables 1a and 1b but will vary depending on the specific instrumentation, dilution factor and blank quality. Significantly lower LODs and LOQs have been achieved for several target analytes for different matrices and larger sample masses. Achieving the lowest limits requires meticulous attention to operating conditions and the highest level of quality control for each set of analyses.

**4.7 Table 1a. Nominal Analytical Limits for Multi-Laboratory Validated Elements**

| Element | Symbol | $\text{MBK}_\text{L}$ (µg/kg) | $\text{MBK}_\text{C}$ (µg/kg) | ASDL[a] (µg/kg) | ASQL[a] (µg/kg) | LOD[b] (µg/kg) | LOQ[b] (µg/kg) |
|---|---|---|---|---|---|---|---|
| Chromium | $^{52}$Cr | 0.022 | 0.055 | 0.054 | 0.49 | 5.4 | 49 |
| Manganese | $^{55}$Mn | 0.0091 | 0.023 | 0.023 | 0.21 | 2.3 | 21 |
| Nickel | $^{60}$Ni | 0.021 | 0.059 | 0.064 | 0.58 | 6.4 | 58 |
| Copper | $^{65}$Cu | 0.022 | 0.058 | 0.060 | 0.55 | 6.0 | 55 |
| Zinc | $^{66}$Zn | 0.038 | 0.26 | 0.37 | 3.4 | 37 | 340 |
| Arsenic | $^{75}$As | 0.0034 | 0.011 | 0.013 | 0.12 | 1.3 | 12 |
| Selenium | $^{78}$Se | 0.015 | 0.059 | 0.073 | 0.66 | 7.3 | 66 |
| Molybdenum | $^{95}$Mo | 0.014 | 0.045 | 0.052 | 0.47 | 5.2 | 47 |
| Cadmium | $^{111}$Cd | 0.00064 | 0.0031 | 0.0041 | 0.037 | 0.41 | 3.7 |
| Mercury | $^{201}$Hg | 0.0017 | 0.0069 | 0.0086 | 0.078 | 0.86 | 7.8 |
| Lead | $^{\text{sum}}$Pb | 0.0046 | 0.012 | 0.012 | 0.11 | 1.2 | 10.9 |

[a] Based upon method blanks measured during the single lab validation over 1 year; n = 143 (see §3.2)

[b] Based upon 0.5 g analytical portion and 50 g analytical solution (DF ≈ 100x)

**4.7 Table 1b. Nominal Analytical Limits for Single Laboratory Validated Elements**

| Element | Symbol | $\text{MBK}_\text{L}$ (µg/kg) | $\text{MBK}_\text{C}$ (µg/kg) | ASDL[a] (µg/kg) | ASQL[a] (µg/kg) | LOD[b] (µg/kg) | LOQ[b] (µg/kg) |
|---|---|---|---|---|---|---|---|
| Vanadium | $^{51}$V | 0.00075 | 0.026 | 0.042 | 0.37 | 4.2 | 37 |
| Cobalt | $^{59}$Co | 0.00019 | 0.012 | 0.020 | 0.18 | 2.0 | 18 |
| Strontium | $^{88}$Sr | 0.0013 | 0.067 | 0.110 | 0.99 | 11 | 99 |
| Tin | $^{118}$Sn | 0.0036 | 0.21 | 0.35 | 3.14 | 35 | 310 |
| Thallium | $^{205}$Tl | 0.000048 | 0.0014 | 0.0023 | 0.021 | 0.23 | 2.1 |
| Uranium | $^{238}$U | 0.0000098 | 0.00029 | 0.00048 | 0.0043 | 0.048 | 0.43 |

[a] Based on a single lab validation (n = 30) (see §3.2 and 4.7.10)

[b] Based upon 0.5 g analytical portion and 50 g analytical solution (DF ≈ 100x)

## 4.7.3 EQUIPMENT AND SUPPLIES

*Disclaimer: The use of trade names in this method constitutes neither endorsement nor recommendation by the U. S. Food and Drug Administration. Equivalent performance may be achievable using apparatus and materials other than those cited here.*

(1) Inductively coupled plasma-mass spectrometer (ICP-MS)—Capable of scanning mass-to-charge (m/z) range 5 – 240 amu with a minimum resolution of 0.9 amu at 10% peak height. Must have collision/reaction cell that can be pressurized with helium and kinetic energy discrimination for polyatomic interference attenuation. Method was developed on Agilent™ models 7500ce, 7700x, 7900, 8800, and 8900 and directions are specific to Agilent brand equipment. The method was also single lab validated using a Perkin Elmer NexION 350D and Thermo iCAP-Q. Use of the method with other brands of instruments or models may require procedural modifications. Any such modifications must be validated according to FDA guidelines and method quality control elements (§4.7.6) must pass.[6]

(2) Microwave digestion system—Requires temperature control to at least 200 °C and pressures ≥ 300 psi (~ 20 bar) with appropriate safety features to prevent over-pressurization of vessels. Microwave must have multi-step programming with ramp to temperature capability. Digestion vessels must be PFA, TFM Teflon® lined, or quartz. Directions on use of microwave digestion equipment are specific to CEM™ or Milestone™. Method was developed using CEM MARS Xpress™ and Milestone UltraWAVE™ and UltraCLAVE™ III systems.

(3) Labware—All laboratory ware must be sufficiently clean for trace metals analysis. The recommended cleaning procedure for all laboratory ware includes washing with clean-rinsing laboratory detergent such as Micro-90, reagent water rinse, soaking in 10% nitric acid, and final reagent water rinse. Glass should not be used because of possible contamination. Labware can be tested for contamination before using a particular lot with 1% nitric acid. Virgin (non-recycled) Teflon® FEP, PFA, PP, LDPE, or HDPE are recommended materials. Non-metal spatulas should be used for sampling food portions.

(4) Gloves—Use powder free vinyl or nitrile. Do not use powdered or latex gloves because of possible contamination. Gloves intended for clean rooms and are free from metals contamination are suggested.

(5) Analytical balance—Capable of measuring to 0.1 mg.

(6) Top loading balance—Capable of measuring to 0.01 g.

(7) Micropipettes—Air displacement micropipettes with metal free colorless disposable plastic tips. Do not use colored tips due to possible contamination. If applicable, remove metal tip ejector to avoid potential contamination.

(8) Clean air hood/canopy—Class 100 polypropylene metal free hoods/canopies are recommended for sample handling.

(9) Peristaltic pump tubing—Recommended sample and internal standard (ISTD) peristaltic pump tubing is black:black (0.76 mm inner diameter). At 0.1 rev/s (6 RPM), approximately 200 μL/min sample and 200 μL/min ISTD are delivered to the nebulizer (see 4.7 Figure 2).

   a. The 1:1 sample-to-ISTD ratio dilutes the sample 2x inside the mixing tee so that digests can be diluted to 50 g directly into an autosampler vial.

   b. A 1:1 sample-to-ISTD ratio also ensures both sample and ISTD pump tubing stretch at the same rate over and reduces instrumental drift.

(10) A ~16:1 sample-to-ISTD ratio has been previously used in “Draft Method for Analysis of Foods for As, Cd, Cr, Hg, and Pb by ICP-MS CFSAN/ORS/DBC/CHCB April 25, 2011.” Other pump tubing sizes are acceptable, <u>but all QCs must pass</u> to show adequate performance.

   a. Note: EAM 4.7 was validated with 1:1 sample to ISTD mixing. Solution concentrations and matrices listed assume 1:1 sample-to-ISTD.

   b. For 16:1 sample-to-ISTD ratio (Agilent default), the sample pump tubing is white:white (1.02 mm i.d.) and ISTD is orange:blue (0.25 mm i.d.).

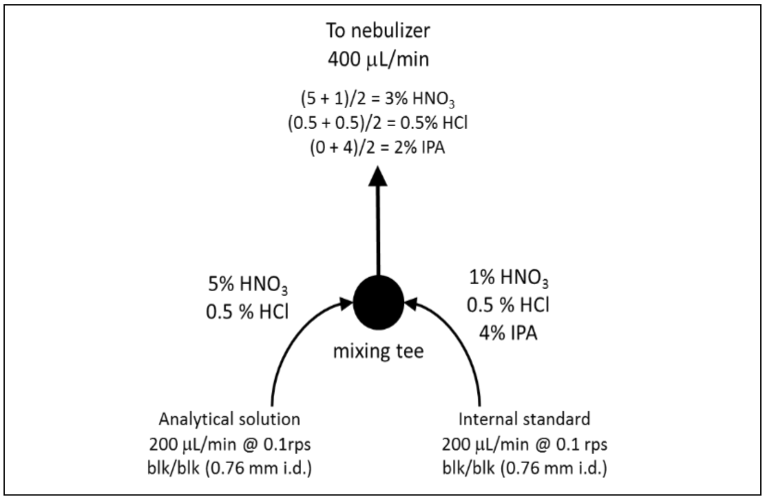
   c. If opting for sample-to-ISTD other than 1:1, the following adjustments must be made:

i. Assume 50% acid consumption during digestion and matrix match standards to analytical solutions.

ii. Make corresponding adjustments in ISTD and isopropanol concentrations.

(11) Drain tubing—Recommended drain tubing is yellow:blue (1.52 mm i.d.) <u>or larger</u> which drains > 650 µL/min from the spray chamber. Smaller drain tubing will cause spray chamber flooding.

**4.7 Figure 2. Recommended peristaltic pump tubing connections**



## 4.7.4 REAGENTS AND STANDARDS

Always use high purity or trace metals grade reagents. Blank levels will be < ASQL if using best laboratory practices and high purity reagents.

*Safety Notes: Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagents.*

*Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that can cause severe eye and skin damage. If acids or bases contact the body, quickly wash the affected area with copious quantities of water for at least 15 minutes and seek medical attention.*

**Reagents**

(1) Reagent water—Water meeting specifications for ASTM Type-I water[3].

(2) Argon supply—High purity (99.99%) argon.

(3) Helium for collision cell—Ultra high purity (99.999%).

(4) High purity nitric acid—Concentrated (67-70%, sp. Gr. 1.42), double distilled. The trade name for double distilled grade will vary by manufacturer.

(5) High purity hydrochloric acid—Concentrated (30-35%, sp. Gr. 1.18), double distilled.

(6) High purity isopropanol—Electronic grade or equivalent.

(7) Nitric acid (for cleaning)—Concentrated (sp gr 1.42), trace metals grade.

(8) Hydrogen peroxide—Concentrated (30%), high purity or trace metals grade.

**Solutions**

(1) Hydrochloric acid 10% (v/v)—Dilute 200 mL (236 g) high purity HCl to 2000 mL with reagent water.

> **Recommended Procedure**: Prepare solution in an empty bottle originally used for concentrated hydrochloric acid. Dilute gravimetrically on a top loading balance with a capacity of a least 2500 g. Tare bottle. Fill with approximately 1000 mL reagent water. Note mass. Add approximately 200 g acid while pouring slowly from the stock bottle. Add the remaining acid from a Teflon® squeeze bottle to enable fine control of acid addition. The total mass of concentrated hydrochloric acid added should be 236 g (200 mL * 1.18 g/mL = 236 g). Add reagent water until a total solution mass of ~2036 g is reached (1800 g water + 236 g HCl). Cap bottle and mix.

(2) Diluent and rinse solution 5% $HNO_3$ & 0.5% HCl (v/v)—Dilute 100 mL $HNO_3$ (142 g) and 10 mL (11.8 g) HCl to 2000 mL with reagent water.

> **Recommended Procedure**: Use an empty bottle originally used for concentrated hydrochloric or nitric acid. Dilute gravimetrically on a top loading balance with a capacity of a least 2500 g if making 2 L of solution. Tare bottle. Fill with approximately 1000 g reagent water. Note mass. Add 11.8 g (10 mL) high purity HCl (double distilled, 30-35%). Swirl to mix. Add 142 g (100 mL) high purity $HNO_3$ (double distilled, 67-70%). Dilute with reagent water to 2 L or ~2044 g. It is recommended to add concentrated acids either with a high purity bottle top acid dispenser or Teflon® PFA squeeze bottle.

(3) Internal standard solution (ISTD)—Multi-element solution prepared by diluting an appropriate volume of stock standard. ISTD matrix is 1% $HNO_3$, 0.5% HCl and 4% isopropanol. The presence of isopropanol will help equalize arsenic and selenium sensitivities due to residual carbon post digestion.[4] The ISTD dilution factor is 1:1 if the autosampler and internal standard peristaltic pump tubes are equal inner diameter. The analytical solution pumped into the nebulizer will be approximately 2% isopropanol.

a. ISTD solution may be prepared volumetrically. The exact concentration is not as important as maintaining the same concentration over an analytical sequence.

b. ISTD elements and suggested concentrations: 20 ng Ge/g, 2.5 ng Rh/g, 5 ng Ir/g, and 2.5 ng Bi/g. These concentrations are only suggestions. Labs may alter at their discretion if QC passes.

c. 0.1% (v/v) Triton X-100 added to the ISTD may help stabilize signal. Triton X-100 is permitted but labs must ensure that ASDL, ASQL, LOD and LOQs are also established with this ISTD mixture, and all QC must pass.

d. If using default Agilent tubing (1.02 mm i.d. sample white/white and 0.25 mm i.d. orange/blue), then increase IPA and ISTD elemental concentrations approximately 8x. The exact concentration is not important.

(4) Recommended tuning solution—2 µg/L Li, Co, Y, Ce, and Tl solution in 5% $HNO_3$ – 0.5% HCl and 4% isopropanol used to tune ICP-MS.

The method suggests sample tubing and ISTD tubing to be equal diameter, diluting tune solution by 2×. Therefore, tune solution should be 2 µg/L so that 1 µg/L is aspirated into the ICP (see 4.7 Figure 2).

### Calibration Standard Solutions

(1) Analyte stock standard solutions—Commercially prepared, single-element, traceable standard solutions in acid matrices prepared specifically for plasma mass spectrometric analysis should be used. Custom made multi-element solutions may be economically viable when one considers the time savings they provide.

a. Standards can be purchased on a mass/mass basis to eliminate density correction factors. If standards are mass/volume then perform a density correction (refer to EAM §3.4.4 for gravimetric standard solution preparation).

b. Use standard solutions prior to expiration. Solutions may slowly become more concentrated due to transpiration, the loss of water vapor through the bottle material, and evaporation while the bottle is uncapped.

c. Example of a multi-element custom standard:

- Hg: 1 µg/g
- As, Cr, Cd, Ni, Mo, Se, Tl, Pb, Co, Sn, U & V: 10 µg/g
- Mn, Sr: 50 µg/g
- Cu, Zn: 100 µg/g

(2) Intermediate standard solutions—Dilute stock standards with 5% $HNO_3$ – 0.5% HCl diluent. Store in Teflon® FEP, PP or HDPE bottles. Single-element standards may be combined in the same solution to prepare multi-element calibration standard solutions.

a. All calibration standards shall be prepared on a mass/mass basis (refer to EAM §3.4.4 for gravimetric preparation). Standard certificates of analyses often provide density information.

b. It is recommended to make an intermediate standard by gravimetrically diluting the multi-element custom standard mix by 100x.

(3) Standard solutions—Dilute intermediate standard with 5% $HNO_3$ – 0.5% HCl to prepare multi-element working standards. Store in Teflon® FEP, PP, or HDPE bottles.

a. Hg concentration should be kept low to minimize memory effects (carryover) and washout times. **The high standard (level 5 in table 2) is optional**, but it is needed if analytical solution concentrations are higher than the level 4 standard as might be the case with predatory fish samples. Preferably, the analyst may further dilute the sample so that the analytical solution concentration falls within the calibration range. Longer washout times may be needed if there is Hg carryover. A rinse solution containing Au (~1 ppm Au) can be placed after samples containing high Hg to assist washout.

b. High concentrations of Mn, Sr, Cu and Zn are often present in foods compared to As, Cr, Cd, Tl, Pb, Ni, Mo, Se, Hg, Co, Sn, U, & V. Some foods are fortified in Se and will have high Se levels. The food label will often list Se (or a Se containing compound) as an ingredient if this is the case.

c. Table 2 is an example of standard concentrations. These may be changed if QC passes and analytical solution concentrations fall within the calibration range.

**4.7 Table 2. Example of calibration curve standard concentrations**

| Analyte | Level 1 (ng/g) | Level 2 (ng/g) | Level 3 (ng/g) | Level 4 (ng/g) | *Level 5 (ng/g) Optional* |
|---|---|---|---|---|---|
| Hg | 0 | 0.01* | 0.1 | 1.0 | *2.5* |
| As | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Cr | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Cd | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Ni | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Mo | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Se | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Tl | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Pb | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Co | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Sn | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| U | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| V | 0 | 0.1 | 1.0 | 10.0 | *25.0* |
| Sr | 0 | 0.5 | 5.0 | 50.0 | *125.0* |
| Mn | 0 | 0.5 | 5.0 | 50.0 | *125.0* |
| Cu | 0 | 1.0 | 10.0 | 100.0 | *250.0* |
| Zn | 0 | 1.0 | 10.0 | 100.0 | *250.0* |

* This level Hg may be less than instrument LOD.

(4) Standard blank—5% $HNO_3$–0.5% HCl.

### 4.7.5 DIGESTION PROCEDURE

Terms and definitions:

(1) A “digestion batch” is defined as digests undergoing the same digestion program at the same time. For example, a CEM MARS Xpress digestion batch will have up to 40 vessels in a digestion batch.

(2) An “analytical sequence” is comprised of the analytical solutions analyzed during a single sequence following instrument tuning and optimization and with one calibration. An analytical sequence may contain solutions from more than one digestion batch.

Perform the following operations in a clean environment to reduce contamination. Use an exhausting fume hood when working with nitric acid. See §2.3.1 for additional information on performing microwave digestions.

Food preparation and homogenization procedures are found in §2.1 through §2.2.2. Elements of interest (e.g., Cr, Ni, Mo, Co, V, and Fe) may leach from stainless steel and contaminate foods, especially when foods are acidic or tough to grind. Care should be taken to prevent leaching of these elements during contact with metallic (stainless steel) equipment. Replace stainless steel grinding components with titanium or tungsten carbide when possible. Cryogrinding is often useful for sticky and gummy foods that do not grind well in a typical knife mill blender.

*Considerations of acid concentration in analytical solutions: Nitric acid is lost or consumed during digestion by reaction with an organic sample, high temperature decomposition and venting as a vapor. HCl is added immediately after digestion to stabilize Hg.*[2] *Assuming 50% acid consumption during digestion, the final matrix concentration is 4-5%* $HNO_3$ *and 0.5% HCl. The ISTD matrix is 1%* $HNO_3$ *and 0.5% HCl. Analytical solution and ISTD are combined in a 1:1 ratio inside a mixing tee so that the final matrix aspirated into the spray chamber is approximately 3%* $HNO_3$ *and 0.5% HCl with 2% isopropanol (see 4.7 Figure 2). Mixing internal standard at a 1:1 ratio narrows the range of acid concentrations and total dissolved solids introduced into the plasma.*

The following digestion procedures describe the use of two types of microwave digestion systems including 1) conventional closed or self-venting vessels and 2) autoclave style digestion systems. Other types of microwave digestion systems can be used but must utilize similar acid combinations as aligned with manufacturers best practices and must result in a total acid-extractable concentrations of the target elements from the matrices. All quality assurance procedures remain in place regardless of microwave digestion system design.

### Digestion procedure using conventional closed or self-venting vessels

(1) Add a few drops of reagent grade deionized water to each vessel prior to taring to pre-wet the analytical portion.

(2) A minimum of 2 MBKs must be included in each digestion batch to verify the absence of contamination that may arise from the vessels. Place MBKs in random vessels.

  a. Use the same mass of reagent water for method blanks (MBK) and optional fortified method blanks (FMB) as used for samples (e.g., 0.5 g sample weight would also use 0.5 g DI $H_2O$ for MBK).

  b. Match the MBK mass to the sample mass when possible. If multiple sample masses are used in a single batch, choose the higher mass for MBKs.

(3) Weigh analytical portion into clean vessel liner and record analytical portion mass to the nearest 0.1 mg.

  a. For samples of unknown composition limit the dry-mass equivalent of food to no more than 0.5 g. If maximum pressure attained for this unknown is less than the vessel limit, then a greater mass may be analyzed.

  b. Use less than 0.5 g for samples high in salt or fat.

  c. Use an analytical portion mass of 2 – 2.5 g for high water content foods such as pureed baby foods.

  d. Use an analytical portion mass of 5 g for ready to drink beverages and liquids. An analytical portion of 5 g should not be exceeded even if calculations based on the food's energy indicate that a larger portion could be taken.

  e. Add 1 g of reagent water with dry foods and certified reference materials (CRMs) to help control exothermic reactions.

(4) Add 8 mL (~11 g) of high purity nitric acid to each vessel liner, washing down any material on the walls. A bottle top acid dispenser is suggested. Acid should be added drop wise until it can be established that the sample will not react violently. If foaming or reaction with the acid is observed, let the vessels sit uncovered in a clean hood until reaction subsides. If a clean hood is unavailable, place caps on vessels without pressing down fully or, if so equipped, cap vessels but loosen the pressure relief nut (with the safety membrane) to allow pressure to escape. If, however, it appears that excessive foaming would result in the sample-acid mixture expanding out of the vessel then cap the vessel and tighten to appropriate torque to prevent loss of sample or acid.

(5) Add approximately 1 mL high purity 30% $H_2O_2$ to each vessel. It may be necessary to pre-digest for more than 20 minutes before adding $H_2O_2$ if samples foam excessively.

(6) 2 mL $H_2O_2$ may further eliminate excess dissolved carbon, reduce its corresponding interference $ArC^+$ at m/z 52, and reduce matrix effects. If using 2 mL $H_2O_2$, ensure ASDL/ASQL remain appropriate for analysis.

(7) Seal vessels, apply correct torque to cap (tighten pressure relief nuts if equipped) and run the digestion program in 4.7 Table 3.

**4.7 Table 3. Closed vessel style microwave digestion program**

*Digestion Programs for CEM MARS XPress™ with 40- Position Carousel with Ramp to Temperature Feature*

Power is applied for the Ramp Time minutes or until Control Pressure or Control Temperature is met. If Control Pressure or Control Temperature are met before end of Ramp Time then program proceeds to Hold Time.

| | Digestion |
|---|---|
| Maximum Power | 100% (1600W[a]) |
| Ramp Time (min) | 25 |
| Hold Time (min) | 15 |
| Control Temperature (°C) | 200 |

[a] Other power level microwaves are acceptable.

(8) 2 cycle microwave digestion programs are allowed, but ASDL/ASQL must not change, and all QC must pass.

(9) After vessels have cooled to less than 50 °C, move to an exhausting clean hood and vent excess pressure slowly. Quantitatively transfer digests to a clean container and dilute digestion solution to approximately 50 g with reagent water followed by 0.5 mL (0.6 g) high purity HCl (or 5 mL of 10% HCl solution). Add more reagent water for a final volume of 100 mL and record final analytical solution mass. The mass of a 100 mL 5% $HNO_3$ – 0.5% HCl analytical solution is approximately 102 g. Gravimetric dilution is required.

**Digestion procedure using microwave autoclave style digestion systems**

(1) Add a few drops of reagent grade deionized water to each vessel prior to taring to pre-wet the analytical portion.

(2) A minimum of 2 MBKs must be included in each digestion batch to verify the absence of contamination that may arise from the vessels. Place MBKs in random vessels.

  a. Use the same mass of reagent water for method blanks (MBK) and optional fortified method blanks (FMB) as used for samples (e.g., 0.5 g sample weight would also use 0.5 g DI $H_2O$ for MBK).

  b. Match the MBK mass to the sample mass when possible. If multiple sample masses are used in a single batch, choose the higher mass for MBKs.

(3) Weigh analytical portion into clean vessel liner and record analytical portion mass to the nearest 0.1 mg.

  a. For samples of unknown composition limit the dry-mass equivalent of food to no more than 0.5 g.

  b. Use less than 0.5 g for samples high in salt or fat.

  c. Use an analytical portion mass of 2 – 2.5 g for high water content foods such as pureed baby foods.

  d. Use an analytical portion mass of 5 g for ready to drink beverages and liquids. An analytical portion of 5 g should not be exceeded even if calculations based on the food's energy indicate that a larger portion could be taken.

  e. Add 1 g of reagent water with dry foods and CRMs to help control exothermic reactions.

(4) Add 5 mL (~7 g) of high purity nitric acid to each vessel, washing down any material on the walls. Acid should be added drop wise until it can be established that the sample will not react violently. If foaming or reaction with the acid is observed, let the vessels sit uncovered in a clean hood until reaction subsides.

  a. If it appears that excessive foaming would result in the sample-acid mixture expanding out of the vessel, then the closed vessel system should be used for this food.

(5) Add approximately 1 mL high purity 30% $H_2O_2$ to each vessel. It may be necessary to pre-digest for more than 20 minutes before adding $H_2O_2$ if samples foam excessively.

(6) After the $HNO_3$ and $H_2O_2$ additions, the analytical portion should be completely wetted and well-mixed. Soft agitation in an ultrasonic bath may be useful to assist mixing.

a. A small amount of reagent water may also be added to completely wet the sample and wash material from the vessel walls.

(7) Fill reaction chamber PFTE liner with manufacturer's recommended base load. Replace the base load after each digestion cycle.

(8) Cap each vessel and place vessels into vessel rack.

(9) Place vessel rack into microwave, turn on chiller, close reaction chamber, pressurize chamber with 40 bar $N_2$ or Ar and begin microwave program (4.7 Table 4).

> *Note: Quartz or TFM vessels are recommended. Ensure that samples are completely wetted by the acid.*

**4.7 Table 4. Autoclave Style Microwave Digestion Program**

Digestion Programs for Milestone UltraWAVE™ with 15-Position Rack with Ramp to Temperature Feature

| Step | Time | Status | T1 | T2 | Pressure | Power |
|---|---|---|---|---|---|---|
| 1 | 00:30:00 | Ramp | 250 °C | 60 °C | 160 bar | 1500 W |
| 2 | 00:15:00 | Hold | 250 °C | 60 °C | 160 bar | 1500 W |

[a] Program adapted from Milestone application note UW-17 (fresh food or feed sample)

(10) After the digestion finishes, allow chamber temperature (T1) to cool to 60 °C and release pressure no faster than 6 bar/min. Effervescence and vessel cap displacement may occur at higher pressure release rates resulting in sample loss.

(11) Move cooled and depressurized vessels to an exhausting clean hood. Quantitatively transfer each digest to a clean container and dilute digestion solution to approximately 25 g with reagent water followed by 0.25 mL (0.3 g) high purity HCl (or 2.5 mL of 10% HCl solution). Dilute with reagent water to approximately 50 g. Record weight of final analytical solution to the nearest 0.01 g. Gravimetric dilution is required.

(12) Assuming 50% oxidative acid consumption, the final matrix composition is 5% $HNO_3$ and 0.5% HCl.

### 4.7.6 METHOD QUALITY CONTROL

Failure of any of the QC elements described below to meet performance criteria requires reanalysis of samples analyzed prior to the loss of method control measures. A single element's QC failure does not automatically fail other elements. For example, if Zn QC fails but As QC passes, it is acceptable to report As results. If QC fails, consider whether high calories (e.g., fat/oil) may be an issue requiring test portion mass to be lowered or additional analytical solution dilution.

The following items are common causes of erroneous results and the associated QC items follow:

- Vessel **contamination** may be identified when analyzing duplicate analytical portions. When RPD of two duplicate analytical portions exceeds 20% and concentrations > LOQ, it is possible that one of the analytical portions has been contaminated. Analyzing duplicate portions will not indicate if the bulk composite is contaminated, or if the sample is heterogeneous and needs further homogenization.

- Inadequate **spectral interference** mitigation is identified through measurement of multiple isotopes. Where possible, EAM 4.7 calls for measurement of two or more isotopes. Elemental concentrations calculated from each isotope must agree to within 20% RPD when concentrations > LOQ. If they do not, this suggests a spectral interference at one of the isotope m/z and should be further investigated and remedied.
- **Matrix effects** may cause unwanted enhancement or depression of sample signal. Internal standards have been chosen to compensate for matrix effects but are not 100% assured. Fortified analytical portion and fortified analytical solution QC failure can indicate a matrix effect. FAPs and FASs do not correct for or indicate spectral interferences (or indicate a lack thereof).

The following is the <u>minimum</u> number of quality control samples:

- 2 method blanks (MBKs) per digestion batch
    - Minimum of 2 MBKs and concentration of both MBKs are ≤ $\text{MBK}_\text{C}$. If 3 or more MBKs are analyzed then at least two-thirds of MBKs are ≤ $\text{MBK}_\text{C}$ (§3.6). MBKs exceeding $\text{MBK}_\text{C}$ should be uncommon, i.e., 5% frequency of MBKs analyzed.
    - If a failure occurs due to contamination, the source of contamination should be investigated and remedied. One of the most common contamination issues is spot (microwave vessel) contamination. When spot contamination is suspected, wash vessels and run a digestion with vessels as MBKs. Place vessels back in the same rotor position (or label vessels to keep track). Analyze MBKs and find possibly contaminated vessels. Repeat and/or remove contaminated vessels from use.
    - If a failure occurs due to polyatomic interference, increase helium flow rate and/or energy discrimination and reanalyze the entire sequence. If a failure is still present, vessels should be thoroughly cleaned, and new analytical portions must be digested.
- 1 stability check per analytical sequence
    - Demonstrate instrument stability by analyzing a midrange multi-element standard containing the analytes (*e.g.,* Continuing Calibration Verification solution (CCV)). Relative standard deviation (RSD) of ion signals must be ≤10%. If RSD > 10%, determine and correct problem before standardization. Stability problems are usually related to sample introduction.
- 1 certified reference material (CRM) per digestion batch

  *Match reference material matrix as closely as possible to the food matrix. In-house RMs are acceptable if no CRM is available and/or the in-house RM is well characterized.*
    - RM % true value recovery: 80 – 120% when concentrations > LOQ or within concentration uncertainty (converted to percent relative uncertainty) supplied on certificate, whichever is greater.
    - If acceptable values are not obtained, the analytical solution may be reanalyzed once. If acceptability is still not met, recalibrate and reanalyze the entire analytical sequence and/or prepare and digest new analytical portions.
- Duplicate analytical portions are required for each food sample

  - RPD < 20% for replicate analytical portions when concentration > LOQ (§3.4.5)
  - If RPD < 20% is not achieved, reanalyze replicate analytical solutions once. If acceptable RPD is still not achieved, the source of imprecision should be investigated and remedied. The entire analytical sequence may need to be reanalyzed and/or new analytical portions be digested.
- 1 fortified analytical portion (FAP) per sample type
  - FAP % recovery can fail due to inappropriate fortification levels. It is recommended to a) analyze the food once as a 'test' sample and then fortifying at the proper levels in a subsequent digestion (report only the second analysis result) or b) fortify duplicate portions at 'low' and 'high' levels (e.g., fortify one portion at ~100 ng/g and a separate analytical portion at ~500 ng/g).
  - FAP preparation: It is recommended that the concentration added by fortification into the digestion vessel with the analytical portion is at the level of interest, or 50-300% of the native elemental concentration, whichever is greater. If a level of interest is not defined, it is recommended to spike at a low level standard analytical solution concentration – (i.e., using 4.7 Table 2 as an example, spike at level 3 calibrant concentration).
  - FAP % marginal recovery: 80 – 120%
  - If acceptable recovery is not obtained, ensure spike level is appropriate and reanalyze analytical solution once. If FAP fails again, reanalyze samples that followed the last acceptable FAP. If FAP fails again, prepare and digest new analytical portions.
- 1 fortified analytical solution (FAS) per sample type (Optional)
  - FAS preparation: Spike 50-300% of the analytical solution concentration. If the native concentration range is unknown, spike at a low level standard analytical solution concentration – (i.e., using 4.7 Table 2 as an example, spike at level 3 calibrant concentration).
    - If FAS fails due to inappropriate fortification levels, then a new FAS solution can be made.
  - FAS % marginal recovery: 90 – 110%
  - If acceptable recovery is not obtained, ensure spike level is appropriate and reanalyze analytical solution once. If FAS fails again, reanalyze samples that followed the last acceptable FAS. If FAS fails again, prepare a new FAS solution.
- Initial calibration verification (ICV)—Dilute an appropriate volume of stock ICV solution gravimetrically with 5% $HNO_3$–0.5% HCl so analyte concentration will be at the approximate midpoint of the calibration curve. ICV and calibration standard solutions should be prepared from different stock solutions (second source).
- Continuing calibration verification (CCV)—Use a mid-level standard.

## 4.7.7 DETERMINATION PROCEDURE

Method parameters are listed in 4.7 Table 5. Internal standards help compensate for matrix effects and instrumental drift.

**4.7 Table 5. ICP-MS Isotopes and Measurement Durations**

| Element | Monitored isotopes | Recommended ISTD | Recommended reporting isotope | Minimum integration time (sec) | Analysis Mode |
|---|---|---|---|---|---|
| Vanadium | $^{51}$V | $^{103}$Rh | $^{51}$V | 0.3 | Helium |
| Chromium | $^{52, 53}$Cr | $^{103}$Rh | $^{52}$Cr | 0.3 | Helium |
| Manganese | $^{55}$Mn | $^{103}$Rh | $^{55}$Mn | 0.1 | Helium |
| Cobalt | $^{59}$Co | $^{103}$Rh | $^{59}$Co | 0.3 | Helium |
| Nickel | $^{60, 62}$Ni | $^{103}$Rh | $^{60}$Ni | 0.3 | Helium |
| Copper | $^{63, 65}$Cu | $^{103}$Rh | $^{65}$Cu | 0.1 | Helium |
| Zinc | $^{66, 68}$Zn | $^{103}$Rh | $^{66}$Zn | 0.1 | Helium |
| Arsenic | $^{75}$As | $^{74}$Ge | $^{75}$As | 0.5 | Helium |
| Selenium | $^{78, 82}$Se | $^{103}$Rh | $^{78}$Se | 0.3 | Helium |
| Strontium | $^{88}$Sr | $^{103}$Rh | $^{88}$Sr | 0.1 | Helium |
| Molybdenum | $^{95, 98}$Mo | $^{103}$Rh | $^{95}$Mo | 0.1 | Helium |
| Cadmium | $^{111, 114}$Cd | $^{103}$Rh | $^{111}$Cd | 0.3 | Helium |
| Tin | $^{118, 120}$Sn | $^{103}$Rh | $^{118}$Sn | 0.3 | Helium |
| Thallium | $^{203, 205}$Tl | $^{209}$Bi | $^{205}$Tl | 0.1 | Helium |
| Lead | $^{206, 207, 208}$Pb | $^{209}$Bi | Sum isotopes | 0.1 | Helium |
| Mercury | $^{201, 202}$Hg | $^{193}$Ir | $^{201}$Hg | 0.5 | Helium |
| Uranium | $^{238}$U | $^{209}$Bi | $^{238}$U | 0.1 | Helium |
| Neodymium | $^{146}$Nd | — |  | 0.1 | Helium |
| Samarium | $^{147}$Sm | — |  | 0.1 | Helium |
| Gadolinium | $^{155}$Gd | — |  | 0.1 | Helium |
| Dysprosium | $^{163}$Dy | — |  | 0.1 | Helium |
| Germanium | $^{74}$Ge | — |  | 0.1 | Helium |
| Rhodium | $^{103}$Rh | — |  | 0.1 | Helium |
| Iridium | $^{193}$Ir | — |  | 0.1 | Helium |
| Bismuth | $^{209}$Bi | — |  | 0.1 | Helium |

### Instrument setup

(1) See §3.6.4 for additional details on ICP-MS.

(2) Perform manufacturer recommended or laboratory start-up and tuning procedures.

(3) Program data acquisition method as shown in 4.7 Table 5.

a. Elements that will not be reported may be removed to save time. Ensure that proper internal standard isotopes are still measured.

b. Use spectrum helium mode and kinetic energy discrimination (Agilent specific nomenclature – other manufacturers will have different names).

c. Reaction gases are not allowed.

d. Program the autosampler probe to go to the rinse station for at least 10 seconds after analyzing an analytical solution and then to a rinse bottle. Multiple (3) rinse bottles are recommended. The rinse time must be great enough so that a standard blank solution analyzed after the highest standard results in all analytes <ASQL.

e. Allow sufficient stabilization time after solution uptake. Typically 30 seconds after solution arrives to nebulizer is enough time for signals to stabilize.

f. An “intelligent rinse” or “smart rinse” feature may be used if so equipped. Analyte levels must return to within 10% RPD of the average CCB before moving to the next analytical solution.

g. Use 3 points per peak and at least 3 replicates for integration. Use the mean of the integrations for reporting.

(4) Optimize instrument

a. Tune instrument according to the guidelines in the manufacturer’s tuning guide. The instrument must exceed minimum manufacturer specifications.

*Note: During tuning, the internal standard tubing is placed in reagent water.*

b. HCl is added to the tuning solution to create chloride-based interferences that would be found in food samples high in salt.

c. Use at least 3 (typically 3 – 4) volts energy discrimination (difference between octopole and quadrupole biases) and at least 3 (typically 3 – 5) mL/min He flow rate. Higher He flow rate is allowable and recommended when analyzing ultra-trace concentrations (< 10 ng/g) of isotopes that suffer from severe polyatomic interferences such as $^{52}$Cr.

e. Keep a record of instrument parameters such as sample gas flow rate, sensitivity, oxide formation, doubly charged ratio, and stability (count rate %RSD).

(5) Set up method interference correction equations. These equations can also be edited after data has been acquired.

a. 3 Pb isotopes are summed to account for isotopic variations between standards and samples. Use the method edit function to sum Pb isotopes.

   i. 208: (206)*1 + (207)*1 + (208)*1

b. $^{74}$Ge has an isobaric interference with $^{74}$Se, which is especially problematic in a selenium fortified dietary supplement or other selenium fortified food. Use $^{72}$Ge or $^{103}$Rh for products with selenium fortification declared on the label.

c. $^{78}$Se suffers an isobaric interference with $^{78}$Kr. The Kr isotope is minor abundance but is occasionally found as an argon impurity. Analysts may use a mathematical correction for the Kr overlap of Se. Correction factors are calculated from the natural abundance of Kr and do not incorporate mass bias factors.

    i. 78: (78)*1 - (83)*0.031
    ii. 82: (82)*1 - (83)*1.008

d. Interference equations for double charged Nd and Sm must be applied to $^{75}$As if Nd or Sm is present at concentrations high enough to cause a signal greater than the detection limit at m/z 75 due to $Nd^{++}$ or $Sm^{++}$

    i. If interference from Nd and/or Sm is suspected from the pre-analysis scan, analyze 10 ng/g Nd and Sm single-element solutions separately.

    ii. Calculate the Nd and Sm correction factors from the ratio of the $Nd^{++}$ and $Sm^{++}$ signals at m/z 75 to the $^{146}Nd^{+}$ and $^{147}Sm^{+}$ signals at their nominal mass.

    iii. Enter these correction factors on $^{75}$As using the appropriate software function.

e. Interference equations for double charged Gd and Dy must be applied to $^{78}$Se if Gd or Dy is present at concentrations high enough to cause a signal greater than the detection limit at m/z 78 due to $Gd^{++}$ or $Dy^{++}$

    i. If interference from Gd and/or Dy is suspected from the pre-analysis scan, analyze 10 ng/g Gd and Dy single-element solutions separately.

    ii. Calculate the Gd and Dy correction factors from the ratio of the $Gd^{++}$ and $Dy^{++}$ signals at m/z 78 to the $^{155}Gd^{+}$ and $^{163}Dy^{+}$ signals at their nominal mass.

    iii. Enter these correction factors on $^{78}$Se using the appropriate software function.

f. Appropriate correction equations for doubly charged rare earth elements (REEs, see table 6) must be applied for all isotopes monitored, not only for the examples listed here ($^{75}$As and $^{78}$Se). e.g. when $^{82}$Se is monitored, a correction factor for $^{164}Dy^{++}$ must be used.

## 4.7 Table 6. Possible interferences

| m/z | Element | Polyatomic Interferences | Elemental Interferences |
|---|---|---|---|
| **Analyte Isotopes** | | | |
| 51 | V | $^{35}Cl^{16}O$ | |
| 52 | Cr | $^{35}Cl^{16}OH$, $^{40}Ar^{12}C$, $^{36}Ar^{16}O$, $^{37}Cl^{15}N$ $^{34}S^{18}O$ | $^{104}Pd^{++}$, $^{104}Ru^{++}$ |
| 53 | | $^{37}Cl^{16}O$, $^{38}Ar^{15}N$, $^{38}Ar^{14}NH$, $^{36}Ar^{16}OH$, $^{40}Ar^{13}C$ | $^{106}Pd^{++}$, $^{106}Cd^{++}$ |
| 55 | Mn | $^{40}Ar^{15}N$, $^{40}Ar^{14}NH$, $^{39}K^{16}O$, $^{38}Ar^{16}OH$ | $^{110}Cd^{++}$ |
| 59 | Co | $^{43}Ca^{16}O$, $^{23}Na^{36}Ar$ | |
| 60 | Ni | $^{44}Ca^{16}O$, $^{23}Na^{37}Cl$, $^{43}Ca^{16}OH$ | $^{120}Sn^{++}$, $^{120}Te^{++}$ |
| 62 | | $^{46}Ti^{16}O$, $^{23}Na^{39}K$, $^{46}Ca^{16}O$ | $^{124}Te^{++}$, $^{124}Sn^{++}$, $^{124}Xe^{++}$ |
| 63 | Cu | $^{31}P^{16}O_2$, $^{40}Ar^{23}Na$, $^{47}Ti^{16}O$, $^{23}Na^{40}Ca$, $^{46}Ca^{16}OH$ | $^{126}Te^{++}$, $^{126}Xe^{++}$ |
| 65 | | $^{49}Ti^{16}O$, $^{32}S^{16}O_2H$, $^{40}Ar^{25}Mg$, $^{40}Ca^{16}OH$, $^{36}Ar^{14}N_2H$ | $^{130}Te^{++}$, $^{130}Xe^{++}$, $^{130}Ba^{++}$ |
| 66 | Zn | $^{50}Ti^{16}O$, $^{34}S^{16}O_2$, $^{3}S^{16}O_2{}^{1}H$,$^{32}S^{16}O^{18}O$,$^{32}S^{17}O_2$ | $^{132}Xe^{++}$, $^{132}Ba^{++}$ |
| 68 | | $^{36}S^{16}O_2{}^{+}$, $^{34}S^{16}O^{18}O^{+}$, $^{40}Ar^{14}N_2{}^{+}$, $^{35}Cl^{16}O^{17}O^{+}$, $^{34}S_2$ | $^{136}Ba^{++}$, $^{136}Xe^{++}$, $^{136}Ce^{++}$ |
| 75 | As | $^{40}Ar^{35}Cl$, $^{59}Co^{16}O$, $^{36}Ar^{38}ArH$, $^{38}Ar^{37}Cl$, $^{36}Ar^{39}K$ | $^{150}Sm^{++}$, $^{150}Nd^{++}$ |
| 78 | Se | $^{38}Ar^{40}Ar$, $^{62}Ni^{16}O$ | $^{78}Kr$, $^{156}Gd^{++}$, $^{156}Dy^{++}$ |
| 82 | | $^{40}Ar_2H_2$, $^{66}Zn^{16}O$ | $^{82}Kr$, $^{164}Dy^{++}$, $^{164}Er^{++}$ |
| 88 | Sr | $^{48}Ca^{40}Ar$, $^{72}Ge^{16}O$ | |
| 95 | Mo | $^{79}Br^{16}O$ | |
| 98 | | $^{82}Kr^{16}O$, $^{82}Se^{16}O$ | $^{98}Ru$ |
| 111 | Cd | $^{95}Mo^{16}O$, $^{94}Zr^{16}OH$, $^{39}K_2$, $^{16}O_2H$ | |
| 114 | | $^{98}Mo^{16}O$, $^{98}Ru^{16}O$ | $^{114}Sn$ |
| 118 | Sn | $^{78}Se^{40}Ar$ | |
| 120 | | | |
| 201 | Hg | | |
| 202 | | $^{186}W^{16}O$ | |
| 203 | Tl | $^{187}Re^{16}O$, $^{186}W^{16}OH$ | |
| 205 | | | |
| 206 | Pb | $^{190}Pt^{16}O$ | |
| 207 | | $^{191}Ir^{16}O$ | |
| 208 | | $^{192}Pt^{16}O$ | |
| 238 | U | $^{198}Pt^{40}Ar$, $^{202}Hg^{36}Ar$ | |
| **ISTD Isotopes** | | | |
| 74 | Ge | $^{34}S^{40}Ar$, $^{37}Cl^{37}Cl$, $^{58}Fe^{16}O$, $^{58}Ni^{16}O$ | $^{74}Se$, $^{148}Nd^{++}$, $^{148}Sm^{++}$ |
| 103 | Rh | $^{63}Cu^{40}Ar$, $^{87}Sr^{16}O$ | $^{206}Pb^{++}$ |
| 193 | Ir | $^{177}Hf^{16}O$ | |
| 209 | Bi | $^{193}Ir^{16}O$ | |

### Recommended pre-analysis or semi-quant scan

An optional pre-analysis scan checks for the presence of ISTD elements and high levels of analyte which will require additional dilutions.

(1) Analyze analytical solutions (one replicate from each sample) in semi-quant (KED mode). Use 1% $HNO_3$ for ISTD uptake.

(2) ISTD levels are considered significant if the counts in the analytical solution contribute ≥ 2% of the counts in an analytical solution for an ISTD isotope.

  a. Use a different ISTD isotope if ISTD is present in analytical solution at a significant level.

  b. If recommended ISTD isotopes cannot be used, optional ISTD isotopes are listed in 4.7 Table 7. $^{103}$Rh is a suitable ISTD for all analytes if necessary.

(3) Some foods may contain tungsten which can interfere with $^{202}$Hg via $^{186}W^{16}O$. The absence of W must be confirmed if $^{202}$Hg is used.

**4.7 Table 7. Optional internal standard isotopes**

| Recommended ISTD | Optional ISTD |
|---|---|
| $^{103}$Rh | $^{105}$Pd |
| $^{209}$Bi | $^{205}$Tl, $^{175}$Lu |
| $^{193}$Ir | $^{195}$Pt, $^{197}$Au |

### Determination of analyte concentration using external standard calibration curve

An example of an analytical sequence is shown in 4.7 Table 8.

(1) Calibrate using the calibration blank and at least 3 multi-element standards.

(2) Include the calibration blank as a point on the calibration curve (0 µg/kg calibrant).

(3) Ignore y-intercept.

(4) Both un-weighted and weighted linear regressions are acceptable as long as QC passes. $1/x^2$ and $1/(\text{st. dev})^2$ weighted regressions are suggested.

(5) Check standardization performance

  a. Linear regression correlation coefficient (r) must be ≥ 0.9975.

(6) Check instrument measurement performance and analyze analytical solutions

  a. Analyze the highest standard, standard blank and ICV in this order. This order will show whether the rinse time is adequate.

  b. Analyze initial calibration verification (ICV) solution to verify standardization. Recovery must be 100 ± 10% to proceed. If ICV fails, reanalyze one time. If ICV fails again, determine source of problem and remedy before proceeding.

  c. Continuing calibration verification solution (CCV) must be analyzed at a frequency of 10% and at the end of the analytical sequence. Recovery must be 100 ± 10% to proceed. If CCV fails, reanalyze one time. If CCV fails again, reanalyze samples analyzed after the last acceptable CCV. If CCV fails a third time, restart analytical sequence and/or prepare new digests, standards, and QC solutions.

- d. RSD of replicate integrations must be ≤ 10% for all solutions when instrument response > 5 times ASQL or greater than 5,000 cps, whichever is greater. If RSD exceeds 10%, determine source of noise and remedy before proceeding.
- e. Continuing calibration blank (CCB) analyzed at a frequency of 10% and at the end of the analytical sequence and must be < ASQL to proceed. If CCB fails, reanalyze one time. If CCB fails again, reanalyze samples analyzed after the last acceptable CCB. If CCB fails a third time, restart analytical sequence with a longer washout time and/or prepare new digests, standards and QC solutions.
- f. Analytical solution concentrations must be less than the highest standard concentration. Gravimetrically dilute analytical solution if necessary.

(7) Suppression or enhancement of ISTD response indicates a matrix effect. ISTD response must be 60-120%. Monitor internal standard signals and dilute any analytical solution where % recovery falls below 60% or exceeds 120%.

- a. It is helpful to monitor $^{13}C$ as an ISTD element. Carbon enhanced ionization can cause false positives of high ionization potential isotopes. Use $^{13}C$ as an easy proxy to determine if samples may need further dilution due to high carbon content (e.g., greater than 200% $^{13}C$ vs. standard blank).
- b. In the same way as $^{13}C$, monitor $^{37}Cl$ as an ISTD element. Foods high in salt (NaCl) may form Cl-containing polyatomic interferences (see table 6).

### 4.7 Table 8. Analytical Sequence Example

| Grouping | Solution | QC Criteria |
|---|---|---|
| | tune report | sensitivity, RSD, $MO^+$, $M^{++}$ |
| | stability check | ≤10% RSD |
| | calibration standards | r ≥ 0.9975 |
| | REE++ correction stds | |
| | standard blank | IDL check |
| | high standard solution | memory check |
| | standard blank | ≤ASQL |
| | ICV | 90% - 110% recovery |
| | MBK 1 | ≤MBKc<br>2/3 MBKs ≤MBKc |
| | MBK 2 | ≤MBKc |
| | MBK 3 | ≤MBKc |
| | RM | 80% - 120% recovery |
| | FMB (optional) | 90% - 110% recovery |
| | sample 1 | < high cal. std |
| | sample 1 duplicate | ≤20% RPD |
| | sample 1 FAS (optional) | 90% - 110% recovery |
| | sample 1 FAP | 80% - 120% recovery |
| Unknowns - Set 1 | sample 2 | |
| | sample 2 duplicate | ≤20% RPD |
| | sample 3 | |
| | sample 3 duplicate | ≤20% RPD |
| | sample 4 | |
| | sample 4 duplicate | ≤20% RPD |
| | CCV | 90% - 110% |
| | CCB | ≤ ASQL |
| | sample 5 | < high cal. Std |
| | sample 5 duplicate | ≤20% RPD |
| | sample 5 FAS (optional) | |
| | sample 5 FAP (optional) | |
| Unknowns - Set 2 | sample 6 | |
| | sample 6 duplicate | ≤20% RPD |
| | sample 7 | |
| | sample 7 duplicate | ≤20% RPD |
| | sample 8 | |
| | sample 8 duplicate | ≤20% RPD |
| | CCV | 90% - 110% |
| | CCB | ≤ ASQL |

Precision required: All solutions must be ≤10% RSD when analyte ≥5xASQL or greater than 5,000 cps..

### 4.7.8 CALCULATIONS

Calculate the concentration (mass fraction) of the analyte in the analytical portion according to:

$$Concentration\left(\frac{\mu g}{kg}\right) = [S - MBK_{\mathrm{L}}] * DF * \frac{M}{m * MCF}$$

where:

$S$ = concentration of analyte in analytical solution (or diluted analytical solution) (ng/g)

$MBK_L$ = laboratory MBK (ng/g) (subtract if average of the three MBK is greater than ASDL)†

$M$ = Mass (g) of analytical solution (usually 50 – 100 g)

$m$ = mass of analytical portion (g)

$DF$ = dilution factor (1 if analytical solution not diluted)

$MCF$ = mass correction factor (1 if no water or other solvent was added to aid homogenization)

Report concentration to no more than 3 significant figures. Concentration may be converted to other convenient units (*e.g.*, mg/kg, ng/kg for solids or ng/L for liquids providing that the specific gravity is known).

† If $MBK_L$ is not well established or when multiple analysts work on a single analytical portion, subtract the average MBK from the analysis batch (omit the subtraction if this average is <ASDL)

Marginal spike recoveries are calculated as follows:

$$\%\ Recovery = \left[\frac{C_{x+s} - C_x}{\frac{C_s M_s}{M_x}}\right] * 100$$

where:

$C_{x+s}$ = concentration determined in spiked sample (µg/kg)

$C_x$ = concentration determined in unspiked sample (µg/kg)

$C_s$ = concentration of spiking solution (µg/kg)

$M_S$ = mass of spiking solution added to analytical portion (g)

$M_X$ = mass of analytical portion (g)

### 4.7.9 REPORT

Report results after all quality control criteria for an analytical sequence have been met. Report average concentration when replicate analytical portions are analyzed.

- Report results that are ≥LOQ as concentration followed by the units of measurement.
- Report results that are ≥LOD and <LOQ as concentration followed by the units of measurement and the “Trace” data qualifier that indicates analyte is present at a trace level that is below the limit of reliable quantification. Trace values are documented by a “TR” after the result.
- Report results that are <LOD as 0 followed by the units of measurement and the qualifier that indicates analyte is below the level of reliable detection or is not detected (ND).

*Example: LOQ = 10 µg/kg; LOD =3 µg/kg. Levels found for three different samples were 10 µg/kg, 5 µg/kg and 2 µg/kg.*

*10 µg/kg is ≥LOQ; report 10 µg/kg*

*5 µg/kg is ≥LOD but also <LOQ; report 5 µg/kg (TR)*

*2 µg/kg is <LOD; report 0 µg/kg (ND)*

### 4.7.10 METHOD VALIDATION

*In-house validation (single lab).*

EAM 4.7 has undergone a level 2 single lab validation (SLV) as described in FDA’s *Guidelines for the Validation of Chemical Methods for the FDA Foods Program* (Office of Foods and Veterinary Medicine).[6] The cumulative LODs and LOQs for method blanks measured over the course of 1 year during the SLV (n = 143) are listed in Table 1a and are adequate for the intended purpose of this method. Additional elements were added and LODs and LOQs for those are listed in Table 1b. All quality control criteria were followed and met during the SLV. Analyses were performed on 25 different foods that were similar to those collected in FDA’s Total Diet Study but purchased from local grocers. Foods were analyzed several (N ≥ 5) times. Fortified analytical portions (3 spike levels each) were also prepared and analyzed. Repeatability and reproducibility of the method was < 20% relative standard deviation for elements with concentrations > LOQ. Fortification and reference material recoveries were in the range of 80-120%.

*Interlaboratory trial.*

EAM 4.7 has undergone a level 4 multi-lab validation (MLV) as described in FDA’s *Guidelines for the Validation of Chemical Methods for the FDA Foods Program* (Office of Foods and Veterinary Medicine).[6,7] Fourteen laboratories participated in the collaborative study, including nine Food Emergency Response Network state laboratories and five federal FDA laboratories. Laboratories tested 8 labeled standard reference materials and 12 blinded foods: mayonnaise, dark chocolate, sunflower seeds, hamburger with cheese, brown rice flour (blinded reference material included as a test food), infant formula, canned smoked oysters, sardines in tomato paste, swordfish, mineral water, cinnamon, and a multivitamin. The blinded test foods represented every sector of the AOAC food triangle. Participants measured the mass fraction of each element in each sample in triplicate. Horwitz Ratio (HorRat) values were better than 1.5 for all As, Cd, Cu, Hg, Mo, Ni, Pb, and Se measurements when at least eight laboratories reported results greater than LOQ. The HorRat values were better than 1.5 for all Mn and Zn measurements except for the multivitamin and for all Cr measurements except for sunflower seeds, in which nonhomogeneity was identified. The average HorRat value of the blinded test foods was 0.66 for results greater than LOQ (n = 4206). The study showed that the method performed satisfactorily as a standard method for extractible elemental analysis of food.

The MLV results were reviewed by both FDA Chemical Methods Validation Subcommittee and peer review by the Journal of AOAC International. The MLV results were published in Journal of AOAC International.[7]

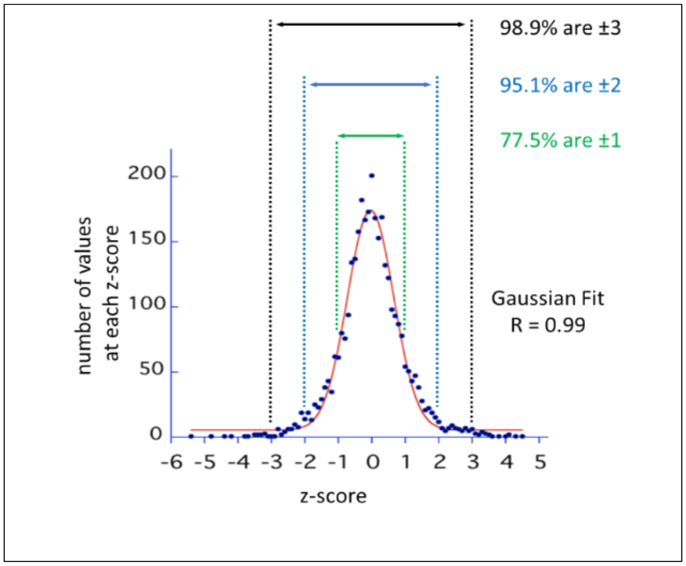
In addition, an interlaboratory study with the Food and Drug Administration's (FDA) New York Human and Animal Food Laboratory (NYHAFL) was conducted in 2021-23 to include six additional elements: Co, Sr, Tl, Sn, U & V. The analyte extension validation followed all guidelines for a Level 2 or single laboratory validation and met all acceptance criteria for analyte extensions. The analyte extension results were published in Journal of AOAC International.[8]

*Uncertainty*.

A nominal 10% combined standard uncertainty is assigned for results at or greater than LOQ (figure 3). The multi-laboratory validation study results confirmed this uncertainty. Measurement accuracy should be better for typical measurements, where levels can be far greater than LOQ. A result above LOD but below LOQ is considered qualitative and is not reported with an uncertainty.

A more detailed discussion of method uncertainty is presented in §3.3. This method conforms to the information contained in that discussion. Derivation of an estimated uncertainty specific to an analysis is discussed §3.3.

**4.7 Figure 3. z-score distribution of reference material results**



(n = 3,378) from the multi-laboratory validation study.[7]

### 4.7.11 METHOD REVISION HISTORY

- Version 1.2 (February 2020)
- Version 1.3 (April 2025): Editorial changes and the addition of cobalt, strontium, tin, uranium, and vanadium.

### 4.7.12 REFERNCES

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