

Elemental Analysis Manual

for Food and Related Products

Archive Notes

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4.7 INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRIC DETERMINATION OF ARSENIC, CADMIUM, CHROMIUM, LEAD, MERCURY, AND OTHER ELEMENTS IN FOOD USING MICROWAVE ASSISTED DIGESTION

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GLOSSARY

(*link removed*)

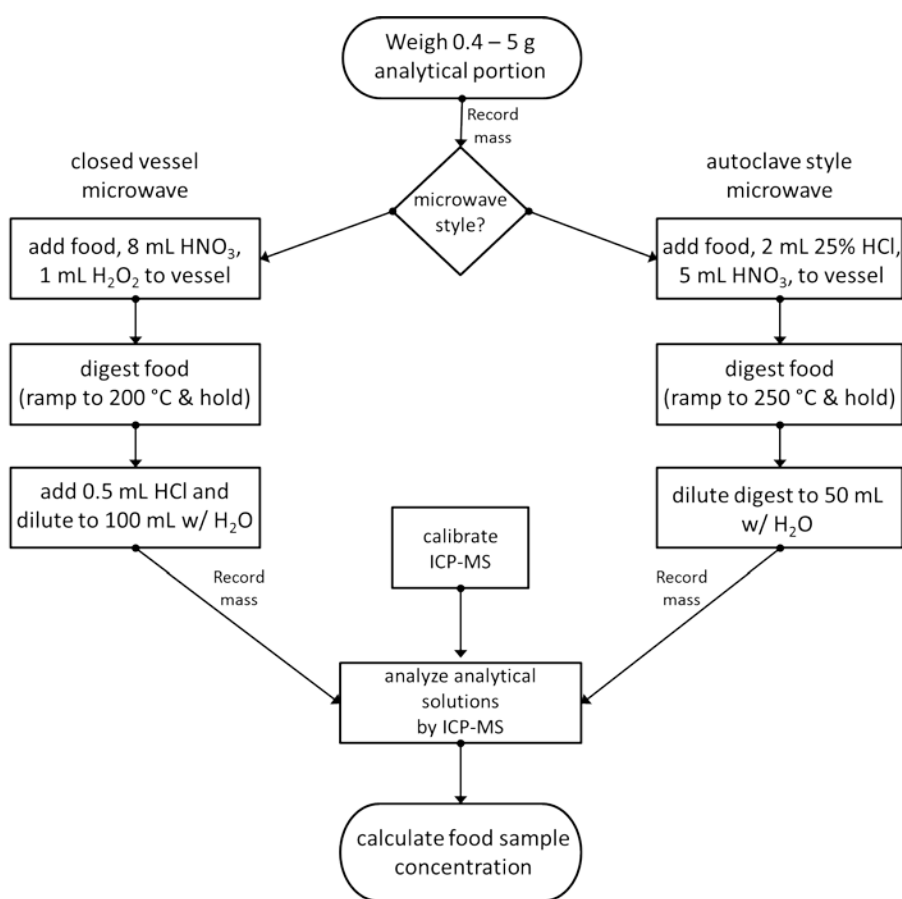
4.7.1 SCOPE AND APPLICATION

This method describes procedures for determining total acid-extractable concentrations of arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, nickel, and zinc in food by microwave assisted acid decomposition and inductively coupled plasma-mass spectrometry (ICP-MS).

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 (*link removed*) and are listed in 4.7 Table 1 but will vary depending on the specific instrumentation, analytical portion mass, blank quality, sensitivity and operating conditions.

4.7 Table 1. Typical Analytical Limits

Element	Symbol	ASDL ^a (µg/kg)	LOD ^b (µg/kg)	LOQ ^b (µg/kg)
Arsenic	As	0.0044	0.4	3.7
Cadmium	Cd	0.0031	0.3	2.6
Lead	Pb	0.0032	0.3	2.6
Chromium	Cr	0.0032	1.2	9.7
Manganese	Mn	0.0155	1.5	12.8
Molybdenum	Mo	0.0151	1.5	12.5
Nickel	Ni	0.0139	1.4	11.5
Copper	Cu	0.0315	3.2	26.1
Zinc	Zn	0.0935	9.3	77.3
Mercury	Hg	0.0018	0.2	1.5
^a . Based on fortified method blanks; n = 15 (see §3.2)				
^b . Based on 0.5 g analytical portion and 50 g analytical solution				

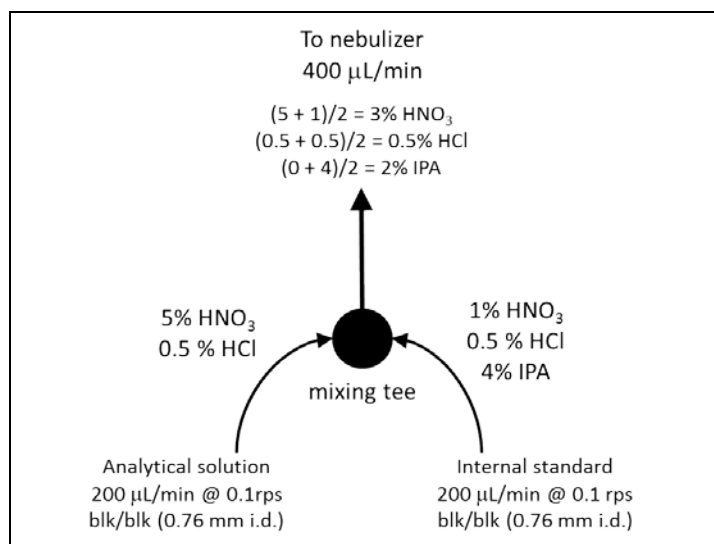
4.7.3 EQUIPMENT AND SUPPLIES

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 for polyatomic interference attenuation. Method was developed on Agilent™ models 7500ce and 7700x and directions are specific to Agilent brand equipment. Use of the method with other brands of instruments or models may require procedural modifications. Any such modifications must be validated according to FDA guidelines⁶ and method quality control (§4.7.6) must pass.
- (2) Nebulizer and spray chamber—Concentric quartz and Scott quartz double pass at 2 °C.
- (3) 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.

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- (4) 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. Teflon[®] FEP, PFA, PP, LDPE or HDPE are recommended materials. Non-metal spatulas should be used for sampling food portions.
 - (5) 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.
 - (6) Analytical balance—Capable of measuring to 0.1 mg.
 - (7) Top Loading balance—Capable of measuring to 0.01 g.
 - (8) 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.
 - (9) Clean air hood/canopy—Class 100 polypropylene metal free hoods/canopies are recommended for sample handling.
 - (10) 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.
 - (11) 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 but all QCs must pass 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 element and isopropanol concentrations
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- (12) Drain tubing—Recommended drain tubing is yellow:blue (1.52 mm i.d.) or larger which drains $> 650 \mu\text{L}/\text{min}$ from the spray chamber. Using smaller drain tubing will result in spray chamber flooding.



4.7 Figure 2. Recommended peristaltic pump tubing schematic

4.7.4 REAGENTS AND STANDARDS

Use high purity or trace metals grade reagents at all times. Blank levels will be $< \text{ASQL}$ 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 are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.

Reagents

- (1) Reagent water—Water meeting specifications for ASTM Type-I water³.
- (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) Nitric acid 20% (v/v)—Dilute 200 mL (284 g) trace metals grade nitric acid to 1000 mL with reagent water.

Recommendation: Prepare solution in an empty bottle originally used for concentrated nitric acid. Dilute gravimetrically on a top loading balance with a capacity of at least 1500 g. Tare bottle. Fill with approximately 600 mL reagent water. Note mass. Add approximately 250 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 nitric acid added should be 284 g ($200 \text{ mL} \times 1.42 \text{ g/mL} = 284 \text{ g}$). Add reagent water until a total solution mass of 1084 g is reached (800 g water + 284 g HNO_3). Cap bottle and mix.

- (2) Hydrochloric acid 25% (v/v)—Dilute 500 mL (590 g) high purity HCl to 2,000 mL with reagent water.

Recommendation: Prepare solution in an empty bottle originally used for concentrated hydrochloric acid. Dilute gravimetrically on a top loading balance with a capacity of at least 2500 g. Tare bottle. Fill with approximately 1000 mL reagent water. Note mass. Add approximately 550 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 590 g ($500 \text{ mL} \times 1.18 \text{ g/mL} = 590 \text{ g}$). Add reagent water until a total solution mass of 2090 g is reached (1500 g water + 590 g HCl). Cap bottle and mix.

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- (3) Diluent and rinse solution 5% HNO₃ & 0.5% HCl (v/v)—Dilute 100 mL HNO₃ (142 g) and 10 mL (11.8 g) HCl to 2,000 mL with reagent water.

Recommendation: Use an empty bottle originally used for concentrated hydrochloric or nitric acid. Dilute gravimetrically on a top loading balance with a capacity of at least 2500 g if making 2L 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 HNO₃ (double distilled, 67-70%). Dilute with reagent water to 2L or 2044 g. It is recommended to add concentrated acids either with a high purity bottle top acid dispenser or Teflon PFA squeeze bottle.

- (4) Internal standard solution (ISTD)—Multi-element solution prepared by diluting an appropriate volume of stock standard. ISTD matrix is 1% HNO₃, 0.5% HCl and 4% isopropanol. The presence of isopropanol will help equalize arsenic sensitivity due to residual carbon post digestion⁴. The dilution factor of the internal standard solution 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.
- ISTD solution may be prepared volumetrically. The exact concentration is not as important as maintaining the same concentration over an analytical sequence.
 - ISTD elements and suggested concentrations: 100 ng Ge/g, 50 ng Rh/g, 50 ng Ir/g, and 20 ng Bi/g.
- (5) Recommended Tuning Solution—2 µg/L Li, Co, Y, Ce, and Tl solution in 5% HNO₃ – 1% HCl used to tune ICP-MS.

The method specifies sample tubing and internal standard 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 may be used. 10 mg/kg stock solutions are recommended to minimize the number of dilutions and intermediate solutions. Custom made multi-element solutions may be economically viable when one considers the time savings they provide. Alternatively, prepare in the laboratory from high purity (99.99%) metals or salts.
- Standards can be purchased on a mass/mass basis to eliminate density correction factors. If standards are mass/volume, a density correction is necessary (refer to EAM §3.4.4 ([link removed](#)) for gravimetric standard solution preparation).

- b. Standard solutions must be used prior to expiration. Solutions may slowly become more concentrated due to 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, Pb: 10 µg/g
 - Mn: 50 µg/g
 - Cu, Zn: 100 µg/g
- (2) Intermediate standard solutions—Dilute stock standards with 5% HNO₃ – 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.
- All calibration standards should be prepared on a mass/mass basis (refer to §3.4.4 *(link removed)* for gravimetric preparation). Standard certificates of analyses often provide density information.
- (3) Standard solutions—Dilute intermediate standard with 5% HNO₃ – 0.5% HCl to prepare multi-element working standards. Store in Teflon[®] FEP, PP or HDPE bottles.
- a. Hg concentration should be less than 2.5 ng/g to minimize memory effects and wash out times.
 - b. High concentrations of Mn, Cu and Zn are often present in foods compared to As, Cr, Cd, Pb, Ni, Mo and Hg.

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)	Level 6 (ng/g)
Hg	0	0.004	0.01	0.1	1.0	2.5
As	0	0.04	0.1	1.0	10.0	25.0
Cr	0	0.04	0.1	1.0	10.0	25.0
Cd	0	0.04	0.1	1.0	10.0	25.0
Ni	0	0.04	0.1	1.0	10.0	25.0
Mo	0	0.04	0.1	1.0	10.0	25.0
Pb	0	0.04	0.1	1.0	10.0	25.0
Mn	0	0.20	0.5	5.0	50.0	125.0
Cu	0	0.40	1.0	10.0	100.0	250.0
Zn	0	0.40	1.0	10.0	100.0	250.0

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- (4) Standard blank—5% HNO₃–0.5% HCl.
 - (5) Initial calibration verification (ICV)—Dilute an appropriate volume of stock ICV solution gravimetrically with 5% HNO₃–0.5% 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).
 - (6) Continuing calibration verification (CCV)—Use a mid-level standard.

4.7.5 DIGESTION PROCEDURE

Terms and definitions:

- (1) A “digestion batch” is defined as digests from a single rotor undergoing the same digestion program at the same time. For example, a CEM MARS Xpress digestion batch will have up to 40 vessels, a Milestone UltraWAVE batch will have 15 (15 mL) vessels, a Milestone UltraCLAVE batch will have 40 (18 mL) vessels, *etc.*
- (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.

The following operations should be performed in a clean environment to reduce contamination. An exhausting hood must be used when working with nitric acid. See §2.3.1 [\(link removed\)](#) for additional information on performing microwave digestions.

Food preparation and homogenization procedures are found in §2.1 through §2.2.2 [\(links removed\)](#). Elements of interest (e.g. Cr, Ni, Mo, Co and Fe) may leach out of stainless steel and contaminate foods, especially when foods are acidic or tough to grind. Care should be taken to evaluate potential for leaching of these elements during contact with metallic (stainless steel) equipment. Substitute stainless steel grinding components for titanium or tungsten carbide when possible.

Considerations of acid concentration in analytical solutions: HCl is added to stabilize Hg.² Nitric acid is lost or consumed during digestion by reaction with the organic sample, high temperature decomposition and venting as a vapor. Assuming 50% acid consumption during digestion, the final matrix concentration is 4-5% HNO₃ and 0.5% HCl. The ISTD matrix is 1% HNO₃ and 0.5% HCl. Analytical solution and ISTD are combined in a 1:1 ratio inside a mixing tee immediately prior to nebulization. The final matrix aspirated into the spray chamber is approximately 3% HNO₃ 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 dissolve solids introduced into the plasma.

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.
- (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. Less than the maximum mass should be used for samples high in salt or fat.
 - c. For beverages and liquids, use an analytical portion mass of 5 g. A maximum 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.
 - d. Use 1 g reagent water for method blanks (MBK) and optional fortified method blanks (FMB).
 - e. For dry foods and dry CRMs adding 1 g of reagent water can help control exothermic reactions.
- (4) Add 8.0 mL of high purity nitric acid (11.3 g) to vessel liner, washing down any material on walls. Using 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 class 100 clean hood for 20 minutes or 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 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) 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

<i>Digestion Programs for CEM MARS XPress™ with 12-Position Carousel with Ramp to Temperature Feature</i>	
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 (Watts)	1200
Control Pressure (psi) ^a	800
Ramp Time (min)	25
Hold Time (min)	15
Control Temperature (°C)	200
^a Only use with non-venting vessels.	

- (7) 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 (1.2 g) high purity HCl. Add more reagent water for a final volume of 100 mL and record final analytical solution mass. The mass of a 100 mL 5% HNO₃ – 0.5% HCl analytical solution is approximately 102 g.

Note: Gravimetric dilution is recommended into a trace element free 100 mL polyethylene or polypropylene bottle. Total dilution factor will be approximately 200 (0.5 g analytical portion to 102 g analytical solution).

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.
- (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. Less than the maximum mass should be used for samples high in salt or fat.
 - c. For beverage and liquid samples, use an analytical portion mass of 5 g. A maximum 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.
 - d. Use 1 g reagent water for method blanks (MBK) and optional fortified method

blanks (FMB).

- e. For dry foods and dry CRM materials adding 1 g of reagent water can help control exothermic reactions during the digestion.
- (4) Add 2 mL 25% HCl to each vessel. A bottle top acid dispenser is recommended.
- Gently agitate microwave vessel to promote sample wetting.
- (5) Add 5.0 mL (7.1 g) of high purity nitric acid into each vessel, washing down any material on 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 class 100 clean hood for 20 minutes or until reaction subsides.
- 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.
- (6) After the HNO₃ and HCl additions, the analytical portion should be completely wetted and well-mixed. Soft agitation in an ultrasonic bath may be useful to assist mixing.
- A small amount of deionized 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.
- a. Milestone UltraCLAVE: 300 mL H₂O, 30 mL H₂O₂, and 3 mL H₂SO₄.
 - b. Milestone UltraWAVE: 130 mL H₂O, 5 mL H₂O₂, and 2 mL H₂SO₄.
 - c. Replace the base load after each digestion cycle.
- (8) Cap on 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 N₂ 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

^aProgram adapted from Milestone application note UW-17 (fresh food or feed sample)

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- (10) After the digestion finishes, allow chamber temperature to cool to 60 °C and release pressure no faster than 8 bar/min. Effervescence 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 50 g with reagent water. Record weight of final analytical solution to nearest 0.01 g.
 - (12) Assuming 50% oxidative acid consumption, the final matrix composition is 5% HNO₃ and 0.5% HCl.

4.7.6 METHOD QUALITY CONTROL

Failure of any of the QC elements described below to meet performance criteria may require reanalysis of samples analyzed prior to the loss of method control measures. The following is the minimum number of quality control samples analyzed with each analytical sequence:

- 2 method blanks (MBKs)
 - Minimum of 2 MBKs analyzed in an analytical sequence and concentration of both MBKs are \leq MBK_C. If 3 or more MBKs are analyzed then at least two-thirds of MBKs are \leq MBK_C (§4.0.2.6) (*link removed*). MBKs exceeding MBK_C should be uncommon.
 - If MBK_C has not been established, subtract average MBK if $>$ ASDL as in §4.7.8.
 - If a failure occurs due to contamination, the source of contamination should be investigated and remedied. If a failure occurs due to interference, increase helium flowrate and/or KED 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.
- Precision Check
 - Demonstrate instrument stability by analyzing a midrange multi-element standard containing the analytes (*e.g.* CCV). Relative standard deviation (RSD) of ion signals must be \leq 10%. If RSD $>$ 10%, determine and correct problem before standardization. Stability problems are usually related to sample introduction.
- 1 certified reference material (CRM)

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% 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, the entire analytical sequence may need to be reanalyzed and/or new analytical portions be digested.

Analyze duplicate analytical portions at a frequency of 10%. Analyze at least one duplicate analytical portion of each food sample type and for any foods where non-homogeneity is a concern. It is highly recommended that duplicate analytical portions are analyzed 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. It is recommended that the food sample analyzed in duplicate also be spiked for FAP.
 - FAP preparation: Spike 50-200% of the native elemental 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)
 - 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, reanalysis of samples analyzed after the last acceptable FAP is required. A new FAP at an appropriate spike level may need to be prepared.
- 1 fortified analytical solution (FAS) per sample type. It is recommended that the food sample analyzed in duplicate also be spiked for FAS.
 - FAS preparation: Spike 50-200% 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).
 - 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, reanalysis of samples analyzed after the last acceptable FAS is required. A new FAS at an appropriate spike level may need to be prepared.
- Optional fortified method blank
 - FMB preparation: Spike approximately 2x ASQL – 8x ASQL
 - FMB % marginal recovery: 90 – 110%

4.7.7 DETERMINATION PROCEDURE

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

4.7 Table 5. ICP-MS Instrument Parameters

Element	Monitored isotopes	Recommended ISTD	Recommended reporting isotope	Minimum integration time (sec)	Analysis Mode
Chromium	⁵² , ⁵³ Cr	¹⁰³ Rh	⁵² Cr	0.3	Helium
Manganese	⁵⁵ Mn	¹⁰³ Rh	⁵⁵ Mn	0.1	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
Molybdenum	⁹⁵ , ⁹⁸ Mo	¹⁰³ Rh	⁹⁵ Mo	0.1	Helium
Cadmium	¹¹¹ , ¹¹⁴ Cd	¹⁰³ Rh	¹¹¹ Cd	0.3	Helium
Lead	²⁰⁶ , ²⁰⁷ , ²⁰⁸ Pb	²⁰⁹ Bi	Sum isotopes	0.1	Helium
Mercury	²⁰¹ , ²⁰² Hg	¹⁹³ Ir	²⁰¹ Hg	0.5	Helium
Neodymium	¹⁴⁶ Nd	—		0.1	Helium
Samarium	¹⁴⁷ Sm	—		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 ([link removed](#)) for additional details on ICP-MS.
- (2) Perform manufacturer recommended or laboratory start-up procedures.
- (3) Program data acquisition method as shown in 4.7 Table 5.
 - a. Elements that are not requested may be removed to save time. Ensure that proper internal standard isotopes are still measured.
 - b. 3 Pb isotopes are summed to account for isotopic variations between standards and samples. Use the method edit function to sum Pb isotopes.

$$208: (206)*1 + (207)*1 + (208)*1$$
 - c. Use spectrum helium mode and kinetic energy discrimination (Agilent specific nomenclature – other manufacturers will have different names).
 - 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. The rinse time must be great enough so that a standard blank solution analyzed after the highest standard results in all analytes <ASQL.

-
- e. 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.
 - f. Use 3 points per peak and 3 replicates for integration. Use the mean of the integrations for reporting. Minimum integration times are listed in 4.7 Table 5.
- (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. Tune for maximum sensitivity while minimizing interferences. 4.7 Table 6 shows possible interferences in digested foods.
- c. 1% HCl is added to the tuning solution to create chloride based interferences that would be found in food samples high in salt.
- d. Use 3 – 4 volts energy discrimination (difference between octapole and quadrupole biases) and 3 – 5 mL/min He flow rate.
- e. Keep a record of instrument parameters such as sample gas flow rate, sensitivity, oxide formation, doubly charged ratio, and stability (count rate %RSD).

4.7 Table 6. Possible interferences

m/z	Element	Polyatomic Interferences	Elemental Interferences
Analyte Isotopes			
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 ⁺⁺
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 ₂ H, ⁴⁰ Ar ²⁵ Mg, ⁴⁰ Ca ¹⁶ OH, ³⁶ Ar ¹⁴ N ₂ H	¹³⁰ Te ⁺⁺ , ¹³⁰ Xe ⁺⁺ , ¹³⁰ Ba ⁺⁺
66	Zn	⁵⁰ Ti ¹⁶ O, ³⁴ S ¹⁶ O ₂ , ³³ 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 ⁺⁺
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 ₂ H	
114		⁹⁸ Mo ¹⁶ O, ⁹⁸ Ru ¹⁶ O	¹¹⁴ Sn
201	Hg		
202		¹⁸⁶ W ¹⁶ O	
206	Pb	¹⁹⁰ Pt ¹⁶ O	
207		¹⁹¹ Ir ¹⁶ O	
208		¹⁹² Pt ¹⁶ O	
ISTD Isotopes			
74	Ge	³⁴ S ⁴⁰ Ar	⁷⁴ Se
103	Rh	⁶³ Cu ⁴⁰ Ar, ⁸⁷ Sr ¹⁶ O	²⁰⁶ Pb ⁺⁺
193	Ir	¹⁷⁷ Hf ¹⁶ O	
209	Bi	¹⁹³ Ir ¹⁶ O	

Optional Pre-Analysis Scan

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

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

- (2) ISTD levels are considered significant if the counts in the analytical solution contribute $\geq 2\%$ 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.
- (3) 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^{++}
 - a. 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.
 - b. Calculate the Nd and Sm correction factors from the ratio of the Nd^{++} and Sm^{++} signals at m/z 75 to the $^{146}\text{Nd}^+$ and $^{147}\text{Sm}^+$ signals at their nominal mass.
 - c. Enter these correction factors on ^{75}As using the Method Edit function.
- (4) Some foods may contain tungsten which can interfere with ^{202}Hg via $^{186}\text{W}^{16}\text{O}$. 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 , ^{72}Ge , ^{74}Ge
^{74}Ge	^{72}Ge , ^{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 standard blank and at least 4 multi-element standards. Hg should be no higher than 2.5 ng/g to minimize memory effects. Include the calibration blank as a point on the calibration curve (0 $\mu\text{g/kg}$ calibrant)
- (2) Use un-weighted linear regression.
- (3) Check Standardization Performance
 - a. Linear regression correlation coefficient (r) {intensity – [analyte counts/sec:(internal standard counts/sec)] versus concentration} is ≥ 0.9975 .
 - b. Analyze initial calibration verification (ICV) solution to verify standardization. Recovery must be $100 \pm 10\%$ to proceed. If ICV fails, reanalyze one time. If ICV

fails again, this indicates a problem with either standard solution or ICV (second source does not match). Determine source of problem and remedy before proceeding.

- (4) 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. Continuing calibration verification solution (CCV) must be analyzed at a frequency of 10% and at the end of the analytical sequence. Recovery must be $100 \pm 10\%$ to proceed. If CCV fails, reanalyze one time. If CCV fails again, reanalysis of samples analyzed after the last acceptable CCV is required.
 - c. RSD of replicate integrations must be $\leq 10\%$ for all solutions when instrument response $>$ ASQL. If RSD exceeds 10%, determine source of noise and remedy before proceeding.
 - d. 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, reanalysis of samples analyzed after the last acceptable CCB is required.
 - e. Analytical solution concentrations must be less than the highest standard concentration. Dilute analytical solution gravimetrically if necessary.
- (5) Suppression or enhancement of ISTD response may indicate a matrix effect is present. Monitor internal standard signals and dilute any analytical solution where the internal standard signal differs by more than 40% from the standard blank.

Determination of Analyte Concentration Using Standard Additions

Quantification by the method of standard additions can also be used.

- (1) Analyze 3 additional portions of analytical solution with added varying amounts of analyte.
- (2) Additions should be no less than 0.5 and no greater than 3 times native amount.
- (3) Correlation coefficient (r) of linear regression must be ≥ 0.9975 . If correlation coefficient is < 0.9975 , repeat analysis. If repeat analysis still fails to meet control limits then dilute sample by a factor of 2 and re-analyze using additions based on the level in analytical solution and the dilution factor.

4.7 Table 8. Analytical Sequence Example

Grouping	Solution	QC Criteria
	tune report	sensitivity, RSD, MO ⁺ , M ⁺⁺
	precision check	≤10% RSD
	calibration standards	r ≥ 0.9975
	standard blank	IDL check
	high standard solution	memory check
	standard blank	≤ASQL
	ICV	90% - 110% recovery
	MBK 1	<div> <div>2/3 of MBKs</div> <div>≤MBK_C</div> </div>
	MBK 2	
	MBK 3	
	RM	80% - 120% recovery
	FMB (optional)	90% - 110% recovery
Unknowns - Set 1	sample 1	≤10% RSD, < high cal. std
	sample 1 duplicate	≤20% RPD
	sample 1 FAS	90% - 110% recovery
	sample 1 FAP	80% - 120% recovery
	sample 2	
	sample 3	
	sample 4	
	sample 5	
	sample 6	
	sample 7	
	CCV	90% - 110%
	CCB	≤ ASQL
	sample 8	≤10% RSD, < high cal. std
	sample 8 duplicate	≤20% RPD
Unknowns - Set 2	sample 8 FAS (optional)	
	sample 8 FAP (optional)	
	sample 9	
	sample 10	
	sample 11	
	sample 12	
	sample 13	
	sample 14	
	CCV	90% - 110%
	CCB	≤ ASQL
Precision required: All solutions must be ≤10% RSD when analyte ≥ASQL.		

4.7.8 CALCULATIONS

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

$$\text{Concentration } (\mu\text{g}/\text{kg}) = [(S \times DF) - \text{MBK}_L] \times \frac{M}{m \times \text{MCF}}$$

where

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

MBK_L = laboratory MBK (ng/g) (subtract if 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).

[†] MBK_L subtraction may not be appropriate for all analyses or labs (*i.e.* when MBK_L is not well established for a new food matrix or when multiple analysts work on a single analytical portion). In this case replace MBK_L in the above equation with the average MBK concentrations from the digestion batch when $\text{MBK} > \text{ASDL}$ to check for spot contamination.

Marginal spike recoveries are calculated as follows:

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

where

C_{x+s} = concentration determined in spiked sample ($\mu\text{g}/\text{kg}$)

C_x = concentration determined in unspiked sample ($\mu\text{g}/\text{kg}$)

C_s = concentration of spiking solution ($\mu\text{g}/\text{kg}$)

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 $\geq \text{LOQ}$ as concentration followed by the units of measurement.
- Report results that are $\geq \text{LOD}$ and $< \text{LOQ}$ 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.

EAM 4.7 was validated following the guidelines set forth in reference 6 ([link removed](#)) and exceeds the standard method performance requirements approved by the AOAC Stakeholder Panel on Strategic Food Analytical Methods⁷.

The method was validated by analyses of reference materials and fortified analytical portions for accuracy and replicate portions for precision. Reference materials used for validation are listed in Appendix A.

Analyses were performed on 25 different foods that were similar to those collected in FDA’s Total Diet Study ([link removed](#)) but purchased from local grocers. Foods were analyzed several ($N \geq 5$) times. Fortified analytical portions (3 spike levels each) were also prepared and analyzed. Method validation results are found in Appendix A.

Uncertainty.

A result above LOQ has an estimated combined uncertainty of 10%. Use of a coverage factor of 2 to give an expanded uncertainty at about 95% confidence corresponds with the RM recovery control limit of $\pm 20\%$. 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 ([link removed](#)). This method conforms to the information contained in that discussion. Derivation of an estimated uncertainty specific to an analysis is discussed §3.3.2 ([link removed](#)).

Interlaboratory trial.

[Under development]

4.7.11 REFERENCES

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- (4) Larsen, E., and Sturup, S. (1994) Carbon-enhanced Inductively Coupled Plasma Mass Spectrometric Detection of Arsenic and Its Application to Arsenic Speciation, *J. Anal. At. Spectrom.* **9**, 1101-1105.
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- (7) AOAC International (2013) AOAC SMPR 2012.007 – Standard Method Performance Requirements for Heavy Metals in a Variety of Foods and Beverages, *J. AOAC Int.* **96**, 704.