

AOAC Official Method 2015.01

Heavy Metals in Food

Inductively Coupled Plasma–Mass Spectrometry

First Action 2015

Note: The following is not intended to be used as a comprehensive training manual. Analytical procedures are written based on the assumption that they will be performed by technicians who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

{Applicable for the determination of heavy metals [arsenic (As), CAS No. 7440-38-2; cadmium (Cd), CAS No. 7440-43-9; lead (Pb), CAS No. 7439-92-1; and mercury (Hg), CAS No. 7439-97-6] at trace levels in food and beverage samples, including solid chocolate, fruit juice, fish, infant formula, and rice, using microwave digestion and inductively coupled plasma–mass spectrometry (ICP-MS).}

Caution: Nitric acid and hydrochloric acid are corrosive. When working with these acids, wear adequate protective gear, including eye protection, gloves with the appropriate resistance, and a laboratory coat. Use an adequate fume hood for all acids.

Hydrogen peroxide is a strong oxidizer and can react violently with organic material to give off oxygen gas and heat. Adequate protective gear should be worn.

Many of the chemicals have toxicities that are not well established and must be handled with care. For all known chemicals used, consult the Material Safety Data Sheet (MSDS) in advance.

The inductively coupled plasma–mass spectrometer emits UV light when the plasma is on. UV resistant goggles should be worn if working near the plasma.

The instrument generates high levels of radio frequency (RF) energy and is very hot when the plasma is on. In the case of an instrument failure, be aware of these potential dangers.

Safely store interference reduction technology (IRT) gases, such as oxygen, in a closed, ventilated cabinet. Use adequate caution with pressurized gases. Prior training or experience is necessary to change any gas cylinders. Oxygen gas can cause many materials to ignite easily.

Following microwave digestion, samples are hot to the touch. Allow the samples to cool to room temperature before opening the digestion vessels to avoid unexpected depressurization and potential release of toxic fumes.

A. Principle

Food samples are thoroughly homogenized and then prepared by microwave digestion and the addition of dilute solutions of gold (Au) and lutetium (Lu). The Au is used to stabilize the Hg in the preparation, and the Lu is used to assess the potential loss of analyte during the microwave digestion process.

A prepared, diluted, aqueous sample digestate is pumped through a nebulizer, where the liquid forms an aerosol as it enters a spray chamber. The aerosol separates into a fine aerosol mist and larger aerosol droplets. The larger droplets exit the spray chamber while the fine mist is transported into the ICP torch.

Inside the ICP torch, the aerosol mist is transported into a high-temperature plasma, where it becomes atomized and ionized as it passes through an RF load coil. The ion stream is then focused by a single ion

lens through a cylinder with a carefully controlled electrical field. For instruments equipped with dynamic reaction cell (DRC) or collision cell IRT, the focused ion stream is directed into the reaction/collision cell where, when operating with a pressurized cell, the ion beam will undergo chemical modifications and/or collisions to reduce elemental interferences. When not operating with a pressurized cell, the ion stream will remain focused as it passes through the cell with no chemical modification taking place.

The ion stream is then transported to the quadrupole mass filter, where only ions having a desired mass-to-charge ratio (m/z) are passed through at any moment in time. The ions exiting the mass filter are detected by a solid-state detector and the signal is processed by the data handling system.

B. Equipment

Perform routine preventative maintenance for the equipment used in this procedure.

An ultra-clean laboratory environment is critical for the successful production of quality data at ultra-low levels. All sample preparation must take place in a clean hood (Class 100). Metallic materials should be kept to a minimum in the laboratory and coated with an acrylic polymer gel where possible. Adhesive floor mats should be used at entrances to the laboratory and changed regularly to prevent the introduction of dust and dirt from the outside environment. Wear clean-room gloves and change whenever contact is made with anything non-ultra-clean. The laboratory floor should be wiped regularly to remove any particles without stirring up dust. *Note:* "Ultra-clean" (tested to be low in the analytes of interest) reagents, laboratory supplies, facilities, and sample handling techniques are required to minimize contamination in order to achieve the trace-level detection limits described herein.

(a) Instrumentation.--ICP-MS instrument, equipped with IRT with a free-running 40 MHz RF generator; and controllers for nebulizer, plasma, auxiliary, and reaction/collision flow control. The quadrupole mass spectrometer has a mass range of 5 to 270 atomic mass units (amu). The turbo molecular vacuum system achieves 10^{-6} torr or better. Recommended ICP-MS components include an RF coil, platinum skimmer and sampler cones, Peltier-cooled quartz cyclonic spray chamber, quartz or sapphire injector, micronebulizer, variable speed peristaltic pump, and various types of tubing (for gases, waste, and peristaltic pump). *Note:* The procedure is written specifically for use with a PerkinElmer ELAN DRC II ICP-MS (www.perkinelmer.com). Equivalent procedures may be performed on any type of ICP-MS instrument with equivalent IRT if the analyst is fully trained in the interpretation of spectral and matrix interferences and procedures for their correction, including the optimization of IRT. For example, collision cell IRT can be used for arsenic determination using helium gas.

(b) Gases.--High-purity grade liquid argon (>99.996%). Additional gases are required for IRT (such as ultra-x grade, 99.9999% minimum purity oxygen, used for determination of As in DRC mode with some PerkinElmer ICP-MS instruments).

(c) Analytical balance.--Standard laboratory balance suitable for sample preparation and capable of measuring to 0.1 mg.

(d) Clean-room gloves.--Tested and certified to be low in the metals of interest.

(e) Microwave digestion system.--Laboratory microwave digestion system with temperature control and an adequate supply of chemically inert digestion vessels. The microwave should be appropriately vented and corrosion resistant.

(1) The microwave digestion system must sense the temperature to within $\pm 2.5^\circ\text{C}$ and automatically adjust the microwave field output power within 2 s of sensing. Temperature sensors should be accurate

to $\pm 2^{\circ}\text{C}$ (including the final reaction temperature of 190°C). Temperature feedback control provides the primary control performance mechanism for the method.

(2) The use of microwave equipment with temperature feedback control is required to control the unfamiliar reactions of unique or untested food or beverage samples. These tests may require additional vessel requirements, such as increased pressure capabilities.

(f) *Autosampler cups*.--15 and 50 mL; vials are precleaned by soaking in 2-5% (v/v) HNO_3 overnight, rinsed three times with reagent water/deionized water (DIW), and dried in a laminar flow clean hood. For the 50 mL vials, as these are used to prepare standards and bring sample preparations to final volume, the bias and precision of the vials must be assessed and documented prior to use. The recommended procedure for this is as follows:

(1) For every case of vials from the same lot, remove 10 vials.

(2) Tare each vial on an analytical balance, and then add reagent water up to the 20 mL mark. Repeat procedure by adding reagent water up to the 50 mL mark.

(3) Measure and record the mass of reagent water added, and then calculate the mean and RSD of the 10 replicates at each volume.

(4) To evaluate bias, the mean of the measurements must be within $\pm 3\%$ of the nominal volume. To evaluate precision, the RSD of the measurements must be $\leq 3\%$ using the stated value (20 or 50 mL) in place of the mean.

(g) *Spatulas*.--To weigh out samples; should be acid-cleaned plastic (ideally Teflon) and cleaned by soaking in 2% (v/v) HNO_3 prior to use.

C. Reagents and Standards

Reagents may contain elemental impurities that could negatively affect data quality. High-purity reagents should always be used. Each reagent lot should be tested and certified to be low in the elements of interest before use.

(a) *DIW*.--ASTM Type I; demonstrated to be free from the metals of interest and potentially interfering substances.

(b) *Nitric acid (HNO_3)*.--Concentrated; tested and certified to be low in the metals of interest.

(c) *Hydrogen peroxide (H_2O_2)*.--Optima grade or equivalent, 30-32% assay.

(d) *Stock standard solutions*.--Obtained from a reputable and professional commercial source.

(1) *Single-element standards*.--Obtained for each determined metal, as well as for any metals used as internal standards and interference checks.

(2) *Second source standard*.--Independent from the single-element standard; obtained for each determined metal.

(3) *Multi-element stock standard solution*.--Elements must be compatible and stable in solutions together. Stability is determined by the vendor; concentrations are then verified before use of the standard.

(e) *Internal standard solution*.--For analysis of As, Cd, Pb, and Hg in food matrices, an internal standard solution of 40 $\mu\text{g/L}$ rhodium (Rh), indium (In), and thulium (Tm) is recommended. Rh is analyzed in DRC

mode for correction of the As signal. In addition, the presence of high levels of elements, such as carbon and chlorine, in samples can increase the effective ionization of the plasma and cause a higher response factor for arsenic in specific samples. This potential interference is addressed by the on-line addition of acetic acid (or another carbon source, such as methanol), which greatly increases the effective ionization of incompletely ionized analytes, and decreases the potential increase caused by sample characteristics. The internal standard solution should be prepared in 20% acetic acid.

(f) *Calibration standards.*--Fresh calibration standards should be prepared every day, or as needed.

(1) Dilute the multi-element stock standard solutions into 50 mL precleaned autosampler vials with 5% HNO₃ in such a manner as to create a calibration curve. The lowest calibration standard (STD 1) should be equal to or less than the limit of quantitation (LOQ) when recalculated in units specific to the reported sample results.

(2) See Table **2015.01A** for recommended concentrations for the calibration curve.

Standard	As, µg/L	Cd, µg/L	Pb, µg/L	Hg, µg/L
0	0.00	0.00	0.000	0.00
1	0.01	0.01	0.005	0.01
2	0.02	0.02	0.010	0.05
3	0.10	0.10	0.050	0.10
4	0.50	0.50	0.250	0.50
5	5.00	5.00	2.500	2.00
6	20.00	20.00	10.000	5.00

(g) *Initial calibration verification (ICV) solution.*--Made up from second source standards in order to verify the validity of the calibration curve.

(h) *Calibration solutions.*--Daily optimization, tuning, and dual detector calibration solutions, as needed, should be prepared and analyzed per the instrument manufacturer's suggestions.

(i) *Certified Reference Materials (CRMs).*--CRMs should preferably match the food matrix type being analyzed and contain the elements of interest at certified concentrations above the LOQ. Recommended reference materials include NIST SRM 1568a (Rice Flour), NIST SRM 1548a (Typical Diet), NRCC CRM DORM-3 (Dogfish Muscle), and NIST SRM 2976 (Mussel Tissue).

(j) *Spiking solution.*--50 mg/L Au and Lu in 5% (v/v) HNO₃. Prepared from single-element standards.

D. Contamination and Interferences

(a) Well-homogenized samples and small reproducible aliquots help minimize interferences.

(b) *Contamination.*—(1) Contamination of the samples during sample handling is a great risk. Extreme care should be taken to avoid this. Potential sources of contamination during sample handling include using metallic or metal-containing homogenization equipment, laboratory ware, containers, and sampling equipment.

(2) Contamination of samples by airborne particulate matter is a concern. Sample containers must remain closed as much as possible. Container lids should only be removed briefly and in a clean environment during sample preservation and processing, so that exposure to an uncontrolled environment is minimized.

(c) *Laboratory.*--(1) All laboratory ware (including pipet tips, ICP-MS autosampler vials, sample containers, extraction apparatus, and reagent bottles) should be tested for the presence of the metals of interest. If necessary, the laboratory ware should be acid-cleaned, rinsed with DIW, and dried in a Class 100 laminar flow clean hood.

(2) All autosampler vials should be cleaned by storing them in 2% (v/v) HNO₃ overnight and then rinsed three times with DIW. Then dry vials in a clean hood before use. Glass volumetric flasks should be soaked in about 5% HNO₃ overnight prior to use.

(3) All reagents used for analysis and sample preparation should be tested for the presence of the metals of interest prior to use in the laboratory. Due to the ultra-low detection limits of the method, it is imperative that all the reagents and gases be as low as possible in the metals of interest. It is often required to test several different sources of reagents until an acceptable source has been found. Metals contamination can vary greatly from lot to lot, even when ordering from the same manufacturer.

(4) Keep the facility free from all sources of contamination for the metals of interest. Replace laminar flow clean hood HEPA filters with new filters on a regular basis, typically once a year, to reduce airborne contaminants. Metal corrosion of any part of the facility should be addressed and replaced. Every piece of apparatus that is directly or indirectly used in the processing of samples should be free from contamination for the metals of interest.

(d) *Elemental interferences.*--Interference sources that may inhibit the accurate collection of ICP-MS data for trace elements are addressed below.

(1) *Isobaric elemental interferences.*--Isotopes of different elements that form singly or doubly charged ions of the same m/z and cannot be resolved by the mass spectrometer. Data obtained with isobaric overlap must be corrected for that interference.

(2) *Abundance sensitivity.*--Occurs when part of an elemental peak overlaps an adjacent peak. This often occurs when measuring a small m/z peak next to a large m/z peak. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Proper optimization of the resolution during tuning will minimize the potential for abundance sensitivity interferences.

(3) *Isobaric polyatomic interferences.*--Caused by ions, composed of multiple atoms, which have the same m/z as the isotope of interest, and which cannot be resolved by the mass spectrometer. These ions are commonly formed in the plasma or the interface system from the support gases or sample components. The objective of IRT is to remove these interferences, making the use of correction factors unnecessary when analyzing an element in DRC mode. Elements not determined in DRC mode can be corrected by using correction equations in the ICP-MS software.

(e) *Physical interferences.*--(1) Physical interferences occur when there are differences in the response of the instrument from the calibration standards and the samples. Physical interferences are associated with the physical processes that govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass spectrometer interface.

(2) Physical interferences can be associated with the transfer of solution to the nebulizer at the point of nebulization, transport of aerosol to the plasma, or during excitation and ionization processes in the plasma. High levels of dissolved solids in a sample can result in physical interferences. Proper internal

standardization (choosing internal standards that have analytical behavior similar to the associating elements) can compensate for many physical interferences.

(f) Resolution of interferences.—(1) For elements that are subject to isobaric or polyatomic interferences (such as As), it is advantageous to use the DRC mode of the instrument. This section specifically describes a method of using IRT for interference removal for As using a PerkinElmer DRC II and oxygen as the reaction gas. Other forms of IRT may also be appropriate.

(a) Arsenic, which is monoisotopic, has an m/z of 75 and is prone to interferences from many sources, most notably from chloride (Cl), which is common in many foods (e.g., salt). Argon (Ar), used in the ICP-MS plasma, forms a polyatomic interference with Cl at m/z 75 [$^{35}\text{Cl} + ^{40}\text{Ar} = ^{75}(\text{ArCl})$].

(b) When arsenic reacts with the oxygen in the DRC cell, $^{75}\text{As}^{16}\text{O}$ is formed and measured at m/z 91, which is free of most interferences. The potential ^{91}Zr interference is monitored for in the following ways: ^{90}Zr and ^{94}Zr are monitored for in each analytical run, and if a significant Zr presence is detected, then $^{75}\text{As}^{16}\text{O}$ measured at m/z 91 is evaluated against the ^{75}As result. If a significant discrepancy is present, then samples may require analysis using alternative IRT, such as collision cell technology (helium mode).

(c) Instrument settings used (for PerkinElmer DRC II): DRC settings for $^{91}(\text{AsO})$ and ^{103}Rh include an RPq value of 0.7 and a cell gas flow rate of 0.6 L/min. Cell conditions, especially cell gas flow rates, may be optimized for specific analyte/matrix combinations, as needed. In such cases, the optimized methods will often have slightly different RPq and cell gas flow values.

(2) For multi-isotopic elements, more than one isotope should be measured to monitor for potential interferences. For reporting purposes, the most appropriate isotope should be selected based on review of data for matrix interferences and based on the sensitivity (or relative abundance) of each isotope. The table below lists the recommended isotopes to measure. Low abundance isotopes are not recommended for this method as it is specifically applicable for ultra-low level concentrations (8-10 ppb LOQs). See Table **2015.01B**.

Element	Isotope, amu	Isotopic abundance, %	Potential interferences
Cd	111	13	MoO ⁺
	114	29	MoO ⁺ , Sn ⁺
Hg	200	23	WO ⁺
	202	30	WO ⁺
Pb ^a	Sum of 206, 207, and 208	99	OsO ⁺

^a Allowance for isotopic variability of lead isotopes.

(g) Memory effects.—Minimize carryover of elements in a previous sample in the sample tubing, cones, torch, spray chamber, connections, and autosampler probe by rinsing the instrument with a reagent blank after samples high in metals concentrations are analyzed. Memory effects for Hg can be minimized through the addition of Au to all standard, samples, and quality control (QC) samples.

E. Sample Handling and Storage

(a) Food and beverage samples should be stored in their typical commercial storage conditions (either frozen, refrigerated, or at room temperature) until analysis. Samples should be analyzed within 6 months of preparation.

(b) If food or beverage samples are subsampled from their original storage containers, ensure that containers are free from contamination for the elements of concern.

F. Sample Preparation

(a) Weigh out sample aliquots (typically 0.25 g of as-received or wet sample) into microwave digestion vessels.

(b) Add 4 mL of concentrated HNO₃ and 1 mL of 30% hydrogen peroxide (H₂O₂) to each digestion vessel.

(c) Add 0.1 mL of the 50 mg/L Au + Lu solution to each digestion vessel.

(d) Cap the vessels securely (and insert into pressure jackets, if applicable). Place the vessels into the microwave system according to the manufacturer's instructions, and connect the appropriate temperature and/or pressure sensors.

(e) Samples are digested at a minimum temperature of 190°C for a minimum time of 10 min. Appropriate ramp times and cool down times should be included in the microwave program, depending on the sample type and model of microwave digestion system. Microwave digestion is achieved using temperature feedback control. Microwave digestion programs will vary depending on the type of microwave digestion system used. When using this mechanism for achieving performance-based digestion targets, the number of samples that may be simultaneously digested may vary. The number will depend on the power of the unit, the number of vessels, and the heat loss characteristics of the vessels. It is essential to ensure that all vessels reach at least 190°C and be held at this temperature for at least 10 min. The monitoring of one vessel as a control for the batch/carousel may not accurately reflect the temperature in the other vessels, especially if the samples vary in composition and/or sample mass. Temperature measurement and control will depend on the particular microwave digestion system.

(1) Note: a predigestion scheme for samples that react vigorously to the addition of the acid may be required.

(2) The method performance data presented in this method was produced using a Berghof Speedwave 4 microwave digestion system, with the program listed in Table 2015.01C (steps 1 and 2 are a predigestion step).

Step	Temp., °C	Ramp, min	Hold, min
1	145	1	1
2	50	1	1
3	145	1	1
4	170	1	10
5	190	1	10

(3) Equivalent results were achieved using the program listed in Table **2015.01D** on a CEM MARS 6 microwave digestion system using the 40-position carousel and 55 mL Xpress digestion vessels.

Table 2015.01D. Digestion program for CEM MARS 6 microwave			
Step	Temp., °C	Ramp, min	Hold, min
1	190	20	10
2	Cool down	NA	10

(4) For infant formula samples, the program described in Table **2015.01E** has been shown to work effectively.

Table 2015.01E. Digestion program for infant formula			
Step	Temp., °C	Ramp, min	Hold, min
1	180	20	20
2	Cool down	NA	20
3	200	20	20
4	Cool down	NA	20

(f) Allow vessels to cool to room temperature and slowly open. Open the vessels carefully, as residual pressure may remain and digestate spray is possible. Pour the contents of each vessel into an acid-cleaned 50 mL HDPE centrifuge tube and dilute with DIW to a final volume of 20 mL.

(g) Digestates are diluted at least 4x prior to analysis with the 1% (v/v) HNO₃ diluent. When the metals concentration of a sample is unknown, the samples may be further diluted or analyzed using a total quantification method prior to being analyzed with a comprehensive quantitative method. This protects the instrument and the sample introduction system from potential contamination and damage.

(h) Food samples high in calcium carbonate (CaCO₃) will not fully digest. In such cases, the CRM can be used as a gauge for an appropriate digestion time.

(i) QC samples to be prepared with the batch (a group of samples and QC samples that are prepared together) include a minimum of three method blanks, duplicate for every 10 samples, matrix spike/matrix spike duplicate (MS/MSD) for every 10 samples, blank spike, and any matrix-relevant CRMs that are available.

G. Procedure

(a) *Instrument startup.*--(1) Instrument startup routine and initial checks should be performed per manufacturer recommendations.

(2) Ignite the plasma and start the peristaltic pump. Allow plasma and system to stabilize for at least 30 min.

(b) *Optimizations.*--(1) Perform an optimization of the sample introduction system (e.g., X-Y and Z optimizations) to ensure maximum sensitivity.

(2) Perform an instrument tuning or mass calibration routine whenever there is a need to modify the resolution for elements, or monthly (at a minimum), to ensure the instrument's quadrupole mass filtering performance is adequate. Measured masses should be ± 0.1 amu of the actual mass value, and the resolution (measured peak width) should conform to manufacturer specifications.

(3) Optimize the nebulizer gas flow for best sensitivity while maintaining acceptable oxide and double-charged element formation ratios.

(4) Perform a daily check for instrument sensitivity, oxide formation ratios, double-charged element formation ratios, and background. If the performance check is not satisfactory, additional optimizations (a "full optimization") may be necessary.

(c) Internal standardization and calibration.--(1) Following precalibration optimizations, prepare and analyze the calibration standards prepared as described in **C(e)**.

(2) Use internal standardization in all analyses to correct for instrument drift and physical interferences. Refer to **D(e)(2)**. Internal standards must be present in all samples, standards, and blanks at identical concentrations. Internal standards can be added using a second channel of the peristaltic pump to produce a responses that is clear of the pulse-to-analog detector interface.

(3) Multiple isotopes for some analytes may be measured, with only the most appropriate isotope (as determined by the analyst) being reported.

(4) Use IRT for the quantification of As using the Rh internal standard.

(d) Sample analysis.--(1) Create a method file for the ICP-MS.

(2) Enter sample and calibration curve information into the ICP-MS software.

(3) Calibrate the instrument and ensure the resulting standard recoveries and correlation coefficients meet specifications (**H**).

(4) Start the analysis of the samples.

(5) Immediately following the calibration, an initial calibration blank (ICB) should be analyzed. This demonstrates that there is no carryover of the analytes of interest and that the analytical system is free from contamination.

(6) Immediately following the ICB, an ICV should be analyzed. This standard must be prepared from a different source than the calibration standards.

(7) A minimum of three reagent/instrument blanks should be analyzed following the ICV. These instrument blanks can be used to assess the background and variability of the system.

(8) A continuing calibration verification (CCV) standard should be analyzed after every 10 injections and at the end of the run. The CCV standard should be a mid-range calibration standard.

(9) An instrument blank should be analyzed after each CCV (called a continuing calibration blank, or CCB) to demonstrate that there is no carryover and that the analytical system is free from contamination.

(10) Method of Standard Additions (MSA) calibration curves may be used any time matrix interferences are suspected.

(11) Post-preparation spikes (PS) should be prepared and analyzed whenever there is an issue with the MS recoveries.

(e) Export and process instrument data.

H. Quality Control

(a) The correlation coefficients of the weighted-linear calibration curves for each element must be ≥ 0.995 to proceed with sample analysis.

(b) The percent recovery of the ICV standard should be 90-110% for each element being determined.

(c) Perform instrument rinses after any samples suspected to be high in metals, and before any method blanks, to ensure baseline sensitivity has been achieved. Run these rinses between all samples in the batch to ensure a consistent sampling method.

(d) Each analytical or digestion batch must have at least three preparation (or method) blanks associated with it if method blank correction is to be performed. The blanks are treated the same as the samples and must go through all of the preparative steps. If method blank correction is being used, all of the samples in the batch should be corrected using the mean concentration of these blanks. The estimated method detection limit (EMDL) for the batch is equal to 3 times the standard deviation (SD) of these blanks.

(e) For every 10 samples (not including quality control samples), a matrix duplicate (MD) sample should be analyzed. This is a duplicate of a sample that is subject to all of the same preparation and analysis steps as the original sample. Generally, the relative percent difference (RPD) for the replicate should be $\leq 30\%$ for all food samples if the sample concentrations are greater than 5 times the LOQ. RPD is calculated as shown below. An MSD may be substituted for the MD, with the same control limits.

$$RPD = 200 \times \frac{|S1 - S2|}{S1 + S2}$$

where S1 = concentration in the first sample and S2 = concentration in the duplicate.

(f) For every 10 samples (not including quality control samples), an MS and MSD should be performed. The percent recovery of the spikes should be 70-130% with an RPD $\leq 30\%$ for all food samples.

(1) If the spike recovery is outside of the control limits, an MSA curve that has been prepared and analyzed may be used to correct for the matrix effect. Samples may be corrected by the slope of the MSA curve if the correlation coefficient of the MSA curve is ≥ 0.995 .

(a) The MSA technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique attempts to compensate for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift.

(b) The best MSA results can be obtained by using a series of standard additions. To equal volumes of the sample are added a series of standard solutions containing different known quantities of the analyte(s), and all solutions are diluted to the same final volume. For example, addition 1 should be prepared so that the resulting concentration is approximately 50% of the expected concentration of the native sample. Additions 2 and 3 should be prepared so that the concentrations are approximately 100% and 150%, respectively, of the expected native sample concentration. Determine the concentration of each solution and then plot on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated to zero absorbance, the point of interception of the abscissa is calculated MSA-corrected concentration of the analyte in the sample. A linear regression program may be used to obtain the intercept concentration.

(c) For results of the MSA technique to be valid, take into consideration the following limitations:

(i) The apparent concentrations from the calibration curve must be linear (0.995 or greater) over the concentration range of concern.

(ii) The effect of the interference should not vary as the ratio of analyte concentration to sample matrix changes, and the MSA curve should respond in a similar manner as the analyte.

(2) If the sample concentration levels are sufficiently high, the sample may be diluted to reduce the matrix effect. Samples should be diluted with the 1% (v/v) HNO₃ diluent. For example, to dilute a sample by a 10x dilution factor, pipette 1 mL of the digested sample into an autosampler vial, and add 9 mL of the 1% (v/v) HNO₃ diluent. MS/MSD sets should be performed at the same dilution factor as the native sample.

(3) Spike at 1-10 times the level of a historical sample of the same matrix type, or, if unknown, spike at 1-5 times a typical value for the matrix. Spiking levels should be no lower than 10 times the LOQ.

(g) Percent recoveries of the CRMs should be 75-125% of their certified value.

(h) Percent recoveries of the CCV standards should be within 85-115%. Sample results may be CCV-corrected using the mean recovery of the bracketing CCVs. This should only be done after careful evaluation of the data. The instrument should show a trending drift of CCV recoveries and not just a few anomalous outliers.

(i) CCBs should be monitored for the effects of carryover and for possible system contamination. If carryover of the analyte at levels greater than 10 times the MDL is observed, the sample results may not be reportable.

(j) Absolute response of any one internal standard should not vary from the original response in the calibration blank by more than 60-125%. Some analytical samples, such as those containing concentrations of the internal standard and tissue digestates, can have a serious effect on the internal standard intensities, but this does not necessarily mean that the analytical system is out of control. In some situations, it is appropriate to reprocess the samples using a different internal standard monitored in the analysis. The data should be carefully evaluated before doing this.

(k) The recovery of the Lu that was spiked into the sample preparation prior to digestion should be evaluated to assess any potential loss of analyte during the process. The concentration of Lu in the sample preparation is 0.25 mg/L, and for samples diluted 4x at the instrument, this is equivalent to 62.5 µg/L at the instrument (if samples are diluted more than 4x, this must be taken into account). The Lu recovery should be no less than 75% of the original spiked concentration.

(l) Refer to Table **2015.01F** for a summary of all recommended quality control samples, minimum frequency at which they are to be analyzed, acceptance criteria for each, and appropriate corrective action if the acceptance criteria are not met.

Table 2015.01F. Summary of quality control samples

QC sample	Measure	Minimum frequency	Acceptance criteria	Corrective action
Calibration standards	Linearity of the calibration curve	Analyzed once per analytical day	Correlation coefficient ≥ 0.995 , 1st standard \leq MRL, low standard recovery = 75-125%, all other standard recoveries = 80-120%	Reanalyze suspect calibration standard. If criteria still not met, then re-prepare standards and recalibrate the instrument.
Internal standards	Variation in sample properties between samples and standards	Each standard, blank, and sample is spiked with internal standard	60-125% recovery compared to calibration blank	If the responses of the internal standards in the following CCB are within the limit, rerun the sample at an additional 2x dilution. If not, then samples must be reanalyzed with a new calibration.
Lu digestion check spike	Assessment of potential loss during digestion	Added to every digested samples	Recovery $\geq 75\%$	Re-prepare the sample
Initial calibration verification (ICV)	Independent check of system performance	One following instrument calibration	Recovery = 90-110%	Correct problem prior to continuing analysis. Recalibrate if necessary.
Continuing calibration verification (CCV)	Accuracy	At beginning and end of analysis and one per 10 injections	Recovery = 85-115%	Halt analysis, correct problem, recalibrate, and reanalyze affected samples
Method blanks (MB)	Contamination from reagents, lab ware, etc.	Minimum of three per batch	Mean \leq MRL; SD \leq MDL or MBs $< 1/10$ th sample result	Determine and eliminate cause of contamination. Affected samples must be re-prepared and reanalyzed.
Method duplicates (MD)	Method precision within a given matrix	Minimum of one per 10 samples	RPD $\leq 30\%$ or $\pm 2x$ LOQ if results $\leq 5x$ LOQ	If RPD criteria not met, then sample may be re-prepared and reanalyzed, but this is not required. Sample matrix may be inhomogeneous. A post-digestion duplicate (PDD) can be analyzed to evaluate instrument precision.

Matrix spikes/matrix spike duplicates (MS/MSD)	Method accuracy and precision within a given matrix	Minimum of one per 10 samples	Recovery = 70-130% and RPD \leq 30%	If RPD > 30%, results must be qualified
Post-preparation spike (PS)	Check for matrix interference	When required (samples spiked too low/high, dilution test fails, etc.)	Recovery = 75-125%	Analyze samples using MSA or results flagged accordingly
Laboratory fortified blank (LFB) or blank spike (BS)	Method accuracy	Minimum of one per batch	Recovery = 75-125%	If LFB recovery is outside of the control limit, then batch must be re-prepared and reanalyzed
Certified Reference Material (CRM)	Method accuracy	Must be matrix-matched to samples; minimum of one per batch	Recovery = 75-125% unless limits set by CRM manufacturer are greater or element/CRM specific limits have been established	If CRM true value is \geq 5x the LOQ and recovery is outside of the control limit, then batch must be re-prepared and reanalyzed

I. Method Performance

(a) Limit of detection (LOD) and LOQ were determined through the analysis of 23 method blanks (see Table **2015.01G**). LOD was calculated as 3 times the SD of the results of the blanks, and LOQ was calculated as 2 times the value of the LOD, except where the resulting LOQ would be less than the lowest calibration point, in which case LOQ was elevated and set at the lowest calibration point and LOD was calculated as 1/3 of the LOQ. All LOQs achieved are \leq 10 $\mu\text{g}/\text{kg}$ for all food matrices and \leq 8 $\mu\text{g}/\text{kg}$ for liquid matrices, such as infant formula.

Table 2015.01G. Method blank results and LOD/LOQ, µg/kg

Method blanks	⁹¹ (AsO)	¹¹¹ Cd	¹¹⁴ Cd	Pb	²⁰⁰ Hg	²⁰² Hg
MB-01	2.83	0.229	0.270	1.90	1.61	0.95
MB-02	1.48	-0.088	0.270	0.14	1.48	1.13
MB-03	1.80	0.007	0.115	0.13	0.76	0.25
MB-04	1.03	0.154	0.288	0.12	1.46	0.33
MB-05	1.43	0.010	0.259	1.84	1.28	0.27
MB-06	1.07	0.105	0.096	3.02	0.87	0.76
MB-07	2.31	-0.002	0.297	2.67	0.89	0.44
MB-08	1.20	0.285	0.200	4.24	0.55	0.28
MB-09	1.05	0.002	0.182	0.09	0.96	0.25
MB-10	2.12	0.047	0.150	0.19	0.71	0.02
MB-11	2.09	-0.145	0.226	0.12	0.64	0.57
MB-12	1.44	0.037	0.165	0.18	0.45	0.50
MB-13	0.70	-0.122	0.160	0.17	0.81	0.19
MB-14	1.12	-0.001	0.074	0.14	0.85	0.21
MB-15	2.33	0.097	0.207	0.11	0.18	0.17
MB-16	1.53	-0.117	0.146	0.16	1.33	1.09
MB-17	1.79	-0.070	0.180	0.03	3.46	2.19
MB-18	1.90	0.049	0.115	0.06	3.30	2.36
MB-19	1.18	0.043	0.224	0.39	4.01	2.78
MB-20	1.24	-0.060	0.199	0.07	0.99	0.56
MB-21	0.92	0.165	0.120	0.03	0.73	0.33
MB-22	1.69	0.005	0.186	0.09	0.60	0.25
MB-23	2.13	0.171	0.152	0.08	0.41	-0.23
SD	0.54	0.113	0.063	1.18	1.01	0.77
LOD	1.6	0.50 ^a	0.50 ^a	3.5	3.0	2.3
LOQ	3.3	1.60 ^a	1.60 ^a	7.1	6.0	4.6

^a Adjusted to conform to lowest calibration point.

(b) Sample-specific LOQs for several matrices, based on LOQs determined by the default method, and adjusted for changes in sample mass for particular samples, are shown in Table 2015.01H. Values have been rounded up to the nearest part-per-billion.

Table 2015.01H. Sample-specific LOQs

Sample	LOQ, µg/kg (as received)			
	As	Cd	Pb	Hg
Infant formula	2	1	4	3
Chocolate	4	2	8	6
Rice flour	4	2	8	6
Fruit juice	1	1	2	2

(c) Numerous relevant CRMs were analyzed to establish method accuracy. Example percent recoveries are provided in Table **2015.01I** (recoveries have been omitted for CRMs that do not provide a certified value or if the certified value is less than the LOQ).

Table 2015.01I. Recoveries for numerous relevant CRMs

Certified Reference Material	As, %	Cd, %	Pb, %	Hg, %
DOLT-4 Dogfish Liver	104	97	87	114
DORM-3 Fish Protein	105	109	94	114
DORM-4 Fish Protein	105	91	91	81
NIST 1548a Typical Diet	103	95	113	NA
NIST 1568a Rice Flour	98	99	NA	NA
NIST 1946 Lake Superior Fish Tissue	119	NA	NA	101
TORT-2 Lobster Hepatopancreas	109	104	95	116
TORT-3 Lobster Hepatopancreas	113	89	86	86

(d) *Standard Method Performance Requirements*SM (AOAC SMPR 2012.007) for repeatability, reproducibility, and recovery for the method are shown in the Table **2015.01J**. See Appendix A (*J. AOAC Int.*, future issue) for detailed method performance information supporting acceptance of the method.

Table 2015.01J. AOAC SMPR 2012.007

Concentration range, µg/kg	Repeatability, %	Reproducibility, %	Recovery, %
LOQ–100	15	32	60-115
100–1000	11	16	80-115
>1000	7.3	8	80-115

(e) Detailed method performance information supporting acceptance of the method is on file with AOAC and the method author and is available upon request. Method validation samples were prepared and analyzed for all applicable matrices. In general, all SMPR criteria were met for As, Cd, Hg, and Pb in the matrices apple juice, infant formula, cocoa powder, and rice flour.

J. AOAC Int. (future issue)

AOAC SMPR 2012.007

J. AOAC Int. **96**, 704(2013)

DOI: 10.5740/jaoac.int.2012.007

Posted: May 28, 2015