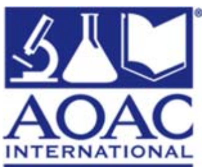

2017 AOAC OFFICIAL METHODS BOARD AWARDS

2014 - 2016 SPSFAM METHODS TO BE REVIEWED FOR

2017 METHOD OF THE YEAR

OFFICIAL METHODS OF ANALYSIS OF AOAC INTERNATIONAL

METHOD OF THE YEAR
OMB may select more than one method in this category each year.

Selection Criteria
The minimum criteria for selection are:
a. The method must have been approved for first or final action within the last three years.
b. Generally, some unique or particularly noteworthy aspect of the method is highlighted as making it worthy of the award, such as innovative technology or application, breadth of applicability, critical need, difficult analysis, and/or range of collaborators.
c. The method demonstrates significant merit in scope or is an innovative approach to an analytical problem.

Selection Process:
a. AOAC staff lists all eligible methods for consideration and forwards that list with supporting documentation (e.g. ERP chair recommendation(s)) to the Chair of the Official Methods Board (OMB).
b. The Chair forwards the list along with any supporting information to the members of the OMB.
c. The OMB selects the Method of the Year. The winner is selected by 2/3 vote. If necessary, the OMB chair may cast tie-breaking vote.

Award
An appropriate letter of appreciation and thanks will be sent to the author(s) of the winning method. The corresponding author will be announced at the appropriate session of the AOAC INTERNATIONAL annual meeting, with presentation of an award. All authors will be acknowledged at the annual meeting, will receive an award and a letter of appreciation. The name of the winner(s), with supporting story, will be carried in the announcement in the *ILM*.

TABLE OF CONTENTS FOR METHODS

SPSFAM METHODS REVIEWED IN 2014 - 2016

AOAC 2016.12	Ethanol in Kombucha	**3**
AOAC 2016.04	Four Arsenic Species in Fruit Juice	**8**
AOAC 2015.01	Heavy Metals in Food	**17**

FOOD COMPOSITION AND ADDITIVES

Determination of Ethanol in Kombucha Products: Single-Laboratory Validation, First Action 2016.12

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Kombucha is a fermented nonalcoholic beverage that has drawn government attention due to the possible presence of excess ethanol (≥0.5% alcohol by volume; ABV). A validated method that provides better precision and accuracy for measuring ethanol levels in kombucha is urgently needed by the kombucha industry. The current study validated a method for determining ethanol content in commercial kombucha products. The ethanol content in kombucha was measured using headspace GC with flame ionization detection. An ethanol standard curve ranging from 0.05 to 5.09% ABV was used, with correlation coefficients greater than 99.9%. The method detection limit was 0.003% ABV and the LOQ was 0.01% ABV. The RSD_r ranged from 1.62 to 2.21% and the Horwitz ratio ranged from 0.4 to 0.6. The average accuracy of the method was 98.2%. This method was validated following the guidelines for single-laboratory validation by AOAC INTERNATIONAL and meets the requirements set by AOAC SMPR 2016.001, "*Standard Method Performance Requirements* for Determination of Ethanol in Kombucha."

Kombucha is a traditional fermented drink that is prepared by fermenting sweetened green or black tea with the addition of "tea fungus," which is a symbiotic colony of bacteria and yeast (1, 2). This traditional Asian fermented beverage has gained significant popularity in the United States in recent years (1, 3). The U.S. market for kombucha products is expected to reach $1.8 billion in 2020 (1). Kombucha is usually marketed as a nonalcoholic beverage in the United States (1). To qualify as a nonalcoholic beverage in the United States, the products are required to contain an ethyl alcohol content of less than 0.50% alcohol by volume (ABV; 3). However, some kombucha products have been reported to have alcohol levels at or above 0.5% ABV (4–11). Another consideration for this type of beverage is the continuous fermentation of the product during transportation and storage, causing an increased ethanol level in the product at the time of purchase. Regulations regarding the alcohol content in kombucha are addressed by the U.S. Tax and Trade Bureau (3).

Even though some studies have been conducted on the beverage, there is no fully validated method for determining ethyl alcohol content in kombucha in the literature. Methods for determining the ethyl alcohol (ethanol) content in other beverages, such as beer, wine, and vinegar, have been published extensively in the literature (12–16). Existing methods have many drawbacks, including large RSD_r values, low accuracy, and not being suitable for kombucha products. The kombucha industry is in need of a fully validated method that can provide better precision and accuracy. GC with flame-ionization detection (FID) is one of the most common methods used, such as in beer ethanol determination (AOAC *Official Method*[SM] **984.14**; 13) and wine ethanol determination (AOAC *Official Method* **983.13**; 14), and may be a great candidate for kombucha ethanol determination (17, 18).

To address the problem, AOAC INTERNATIONAL issued a call for methods that determine ethanol content in kombucha products. The candidate method needs to meet the *Standard Method Performance Requirements* (SMPRs®) established by the AOAC Stakeholder Panel on Strategic Food Analytical Methods (SMPR 2016.001; 19). The single-laboratory validation (SLV) requirements in the SMPRs are provided in Table 1.

This study provides a fully validated method for determining ethanol in kombucha products using headspace GC–FID. The validation of the method followed the SLV guidelines set out by AOAC (20) and by the SMPRs for the determination of ethanol in kombucha (19). This method was developed from a forensic method for measuring ethanol in human plasma (21). The method is suitable for ethanol determination in mixtures such as foods, beverages, and botanical materials.

Received November 29, 2016. Accepted by SG January 26, 2017.
This method was approved by the AOAC Expert Review Panel for Kombucha as First Action.

The Expert Review Panel for Kombucha Methods invites method users to provide feedback on the First Action methods. Feedback from method users will help verify that the methods are fit-for-purpose and are critical for gaining global recognition and acceptance of the methods. Comments can be sent directly to the corresponding author or methodfeedback@aoac.org.

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Table 1. SMPRs for the determination of ethanol in kombucha products

Parameter	Value, %
Analytical range	0.1–2.8 ABV
LOQ	≤0.05 ABV
Accuracy[a]	97–102
Repeatability, RSD_r	≤4
Reproducibility, RSD_R	≤6

[a] Mean spiked recovery over the range of the assay.

AOAC Official Method 2016.12
Ethanol in Kombucha Products
Headspace Gas Chromatography with
Flame-Ionization Detection
First Action 2016

A. Principle

This is a GC method utilizing a headspace autosampler and FID for the determination of ethanol in kombucha samples.

B. Apparatus

(a) *Chromatography system*.—Agilent 7890 GC system equipped with an FID and a Combi-PAL headspace autosampler (Agilent Technologies, Santa Clara, CA).
(b) *Headspace vials*.—Screw-top vials and crimp-top vials (Resteck, Bellefonte, PA).
(c) *Magnetic Teflon-lined caps*.—Restek.
(d) *Volumetric flasks*.
(e) *Micropipets*.

C. Headspace Conditions

(a) *Incubation temperature*.—80°C.
(b) *Syringe temperature*.—85°C.
(c) *Heating time*.—15–20 min.

D. GC Conditions

(a) *Column*.—J&W DB-WAXetr (0.53 mm × 30 m, 2 µm film).
(b) *Initial GC oven temperature*.—40°C.
(c) *Oven temperature gradient*.—Hold at 40°C for 10 min, increase 25°C/min until 240°C is reached, and hold at 240°C for 1 min.
(d) *Run time*.—20 min.
(e) *FID temperature*.—250°C.
(f) *Injector temperature*.—150°C.
(g) *Carrier gas*.—He at 7 mL/min.
(h) *Injection volume*.—200 µL.

E. Reagents

(a) *Ethanol*.—ACS reagent grade, >99.8% (Sigma-Aldrich, St. Louis, MO).
(b) *1-Propanol*.—ACS reagent grade, >99.5% (Sigma-Aldrich).
(c) *Water*.—ACS reagent (Sigma-Aldrich).

F. Standard Reference Materials

(a) *Propyl alcohol (1-propanol)*.—Purity 99.98% (Sigma-Aldrich).
(b) *Ethanol reference standard*.—Absolute 200 proof, purity 99.97% (Sigma-Aldrich).
(c) *Ethanol reference standard*.—Absolute 200 proof, purity 99.5% (Sigma-Aldrich).
(d) *Ethanol–water*.—Certified Reference Material, 100 mg/dL (0.1267% ethanol ABV at 20°C; Cerilliant Corp., Round Rock, TX).

Standard Reference Material, **F(a)**, was used as the internal standard. Standard Reference Material, **F(b)**, was used for preparing the standard stock solutions and standard curves. Standard Reference Materials, **F(c)** and **F(d)**, were used in the accuracy evaluation.

G. Sample Collection

A total of seven commercial kombucha products were obtained from a local market in Carmel, IN. The products were selected based on their high popularity, which was determined by a preliminary market survey conducted on nine food retailers in Carmel. The labeled alcohol level and the ingredients of the products were also considered during the product selection process to ensure the best coverage of the products in the market. An additional unflavored tea product, **G(h)**, formulated by KeVita, Inc. (Ventura, CA) to ensure that no ethanol was in the product, was used as the blank samples. All samples were sealed properly and stored in a (5 ± 3°C) refrigerator before analysis. Six samples, **G(a–f)**, were used in the precision evaluation. A seventh sample, **G(g)**, was used for the determination of the method LOD and LOQ, and the ethanol-free sample **G(h)**, was used in the accuracy determination.
(a) Elderberry-flavored kombucha (manufacturer 1).
(b) Berry-flavored kombucha (manufacturer 2).
(c) Raspberry-flavored kombucha (manufacturer 3).
(d) Unflavored kombucha (manufacturer 3).
(e) Ginger-lemon-flavored kombucha (manufacturer 4).
(f) Apple-flavored kombucha (manufacturer 4).
(g) Pineapple-peach-flavored kombucha (manufacturer 5).
(h) Ethanol-free unflavored tea (KeVita, Inc).

H. Standard and Sample Preparation

(a) *Ethanol stock solution*.—Mix 5 mL ethanol reference standard, **F(b)**, with 95 mL water.
(b) *Internal standard stock solution*.—Mix 5 mL 1-propanol, **F(a)**, with 95 mL water.
(c) *Ethanol calibration solution*.—Dilute the ethanol stock solution, **H(a)**, with water to reach final concentrations of 0.05, 0.10, 0.25, 0.25, 1.002, 2.54, 4.07, and 5.09% ABV ethanol standard solution with 1% internal standard stock solution, **H(b)**. Transfer a 10 mL portion of the individual ethanol standard solution into a 20 mL headspace vial.
(d) *Sample preparation*.—Weigh 0.01–0.02 g sample, **G(a–h)**, into a volumetric flask. Add a sufficient amount of internal standard stock solution, **H(b)**, to the vial to reach a final concentration of 1% 1-propanol by volume before diluting to 10 mL with water. Transfer 10 mL of the sample solution into a 20 mL headspace vial.

I. Analysis

(a) *GC–FID system*.—Set up the GC–FID system according to the conditions listed in **C** and **D**.

(b) *Analysis*.—Make single injections of each sample and standard solution. Measure chromatographic peak response (area).

(c) *Identification*.—Identify ethanol and 1-propanol peak in the sample solution by comparison with the retention time of the ethanol standard solution.

J. SLV Parameters

(a) *Selectivity and specificity*.—Chromatographs of the samples and the ethanol standard were evaluated to determine the selectivity and specificity of the method. Blank sample, **G(h)**, demonstrated no interfering matrix effects in the analysis of ethanol.

(b) *Linearity*.—Seven-point calibration curves were prepared from the ethanol standard solutions (0.05–5.09% ABV) on separate days in triplicate. Calibration curves were built based on the ratio of the ethanol signal response to the internal standard (1-propanol) signal response, and linearity was visually confirmed. Linear regression was then used to determine the correlation coefficient (r) of the curves. Linearity was considered acceptable if all curves had r^2 values >0.999.

(c) *LOD and LOQ*.—The LOD of the method was determined using method detection limit (MDL) guidelines from the U.S. Environmental Protection Agency. A preliminary study was conducted to determine the ethanol level in the kombucha samples. One sample, **G(g)**, was found to contain the lowest amount of ethanol (approximately 0.05% ABV). Thus, four replicates of this sample were analyzed on 3 different days. The MDL was calculated based on the formula given in **K**. The LOQ of the method was calculated as 10× the SD determined for the MDL.

(d) *Precision*.—Four replicates of six samples, **G(a–f)**, were analyzed over 3 different days. Statistical analysis was performed to determine within-day, between-day, and overall precision of the method. The Horwitz Ratio (HorRat) was calculated using the calculation in **K**.

(e) *Recovery*.—Recovery of the method was evaluated first through a spike recovery study. The ethanol-free sample, **G(h)**, was spiked with the ethanol reference standard, **F(c)**, at three different levels: 0.13, 1.30, and 3.30% ABV on 3 different days in duplicate. Recovery was also determined by analyzing the certified ethanol reference standard, **F(d)**, in duplicate on 2 days.

K. Calculations

The concentration of ethanol in the injected sample solution was calculated as

$$AC = \frac{(y - \varepsilon)}{\beta}$$

where AC = the ethanol concentration in the injected sample solution (µg/mL); y = the ratio of the peak area of ethanol to the peak area of 1-propanol in the solution; ε = the intercept of the calibration curve; and β = the slope of the calibration curve.

The concentration of ethanol in the original sample, measured in micrograms per milliliter, was calculated as

$$AM = \frac{AC * VV}{SM}$$

where AM = the concentration of ethanol in the original sample (µg/mL); AC = the concentration of ethanol in the injected sample solution (µg/mL); VV = the volume of sample solution in the headspace vial (mL); and SM = the mass of the sample (g).

The concentration of ethanol in the original sample, measured in % ABV, was calculated as

$$AV = \frac{AM * GK}{GE * 10000}$$

where AV = the concentration of ethanol in the original sample (% ABV); AM = the concentration of ethanol in the sample (µg/mL); GE = the specific gravity of ethanol (0.789 g/mL at 20°C); and GK = the specific gravity of kombucha (1.02 g/mL at 20°C).

The HorRat was calculated as

$$HorRat = \frac{RSD_r}{PRSD_r}$$

where $PRSD_r$ = the predicted RSD_r. The $PRSD_r$ value was $C^{-0.15}$, where C = the concentration of the analyte expressed as a mass fraction.

The MDL of the method was calculated as

$$MDL = s * t_{(0.01,\ n-1)}$$

where s = the sample SD of the concentration determined for the replicates; and $t_{(0.01, n-1)}$ = the t statistic value at α = 0.01 and $n - 1$ degrees of freedom.

Results and Discussion

Selectivity and Specificity

Resolution was sufficient between the analyte peaks and other peaks in the samples, and all analyte peaks were consistent, with no splits, shoulders, or other indications of interference by coeluting compounds (Figure 1). There were no interfering peaks observed at the retention times of ethanol and the internal standard in any of the spiked or blank samples evaluated.

Linearity

An extended calibration range of 0.05–5.09% ABV was used for linearity demonstration. The correlation coefficient (r) for each day was 1.0000, 1.0000, and 0.9997, with an average of 0.9998. All the prepared standard curves appeared linear and had r^2 values >0.999. The coverage of the calibration curve

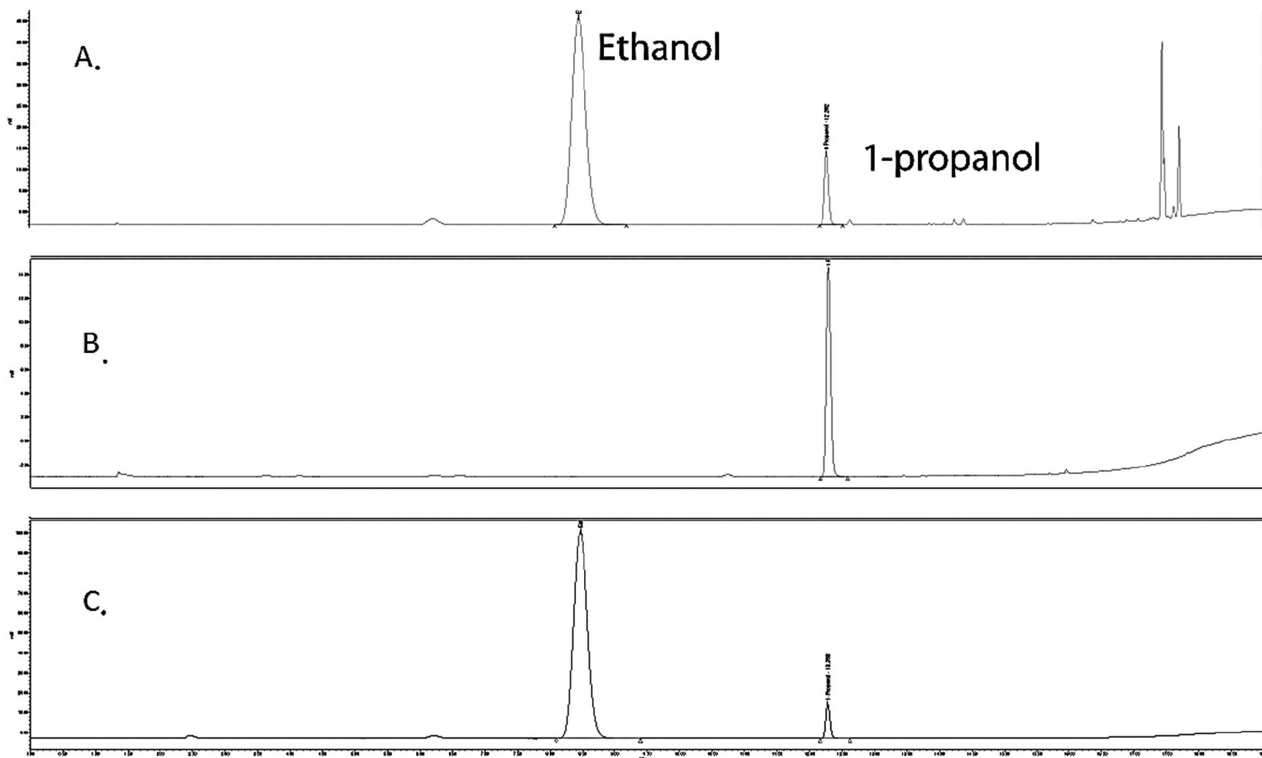

Figure 1. Gas chromatograms of commercial kombucha products and ethanol references. (A) Representative commercial kombucha sample; (B) blank sample, G(h); (C) blank sample, G(h), spiked with ethanol standard solution at 3.30% ABV.

included the analytical range of 0.1–2.8% ABV required by SMPR 2016.001 for kombucha products.

LOD and LOQ

The results from the 12 independent analyses showed that the MDL was 0.003% ABV and that the LOQ of the method was 0.01% ABV, which is lower than the LOQ value of ≤0.05% ABV specified in SMPR 2016.001 (Table 1).

Precision

Results of the precision evaluation for the six samples are summarized in Table 2.

The overall RSD_r values ranged from 1.62 to 2.21%, which are within the AOAC range for the sample concentration (20) and the SMPR limit of ≤4% (Table 1). The HorRat values, which ranged from 0.4 to 0.6 for all the samples, are within the AOAC guideline of 0.5–2.0 (20).

Table 2. Precision determinations for ethanol in kombucha beverages

Kombucha sample	Mean, % ABV	RSD_r, %	HorRat
Elderberry-flavored	2.18	2.14	0.6
Berry-flavored	0.11	2.21	0.4
Raspberry-flavored	2.22	1.62	0.5
Unflavored	1.56	1.67	0.5
Ginger-lemon-flavored	1.21	1.80	0.5
Apple-flavored	1.30	2.18	0.6

Accuracy

Results of the spike recovery study are summarized in Table 3. The mean recovery for each of the three levels tested was found to be 99.6, 100.4, and 100.4%. The lowest recovery (96.2%) was found in the low-level ethanol-spiked kombucha sample on day 3. Table 4 shows the accuracy of the method for analyzing the certified ethanol reference standard, **F(d)**. The average recovery over 2 days was 98.2% ABV. Overall, the results from the recovery assessments are within AOAC guidelines and meet the requirements of AOAC SMPR 2016.001 for the determination of ethanol in kombucha, which states that recovery should be 97–102% over the range of the assay (Table 1).

Conclusions

The method, validated following AOAC *Guidelines for Single Laboratory Validation of Chemical Methods for Dietary*

Table 3. Spike recovery of ethanol using matrix at three different levels[a]

Day	Low, %	Medium, %	High, %
1	98.3	99.7	99.9
	99.9	99.5	99.1
2	99.7	99.5	98.4
	100.4	99.6	99.2
3	103.2	100	102.5
	96.2	104.2	103.4
Mean	99.6	100.4	100.4

[a] Low = 0.13% ABV; medium = 1.30% ABV; and high = 3.3% ABV.

Table 4. GC-FID analysis of the certified ethanol reference standard results

Day	Accuracy, %
1	98.0
	99.2
2	98.5
	97.1
Mean	98.2

Supplements and Botanicals (20), demonstrated acceptable performance for the determination of ethanol content in kombucha products using GC–FID. The SMPRs approved by the AOAC Stakeholder Panel on Strategic Food Analytical Methods have been met, thereby supporting the First Action status of the method. This method will serve as an improved tool for industry, government, and academia in their respective efforts in investigating and ensuring the safety and quality of kombucha.

Acknowledgments

We acknowledge KeVita, Inc. for their support and the donation of standardized kombucha materials used for control reference samples. We also thank Michael Chan (British Columbia Institute of Technology, Centre for Applied Research and Innovation, Burnaby, BC, Canada) for his valuable input on method validation protocols.

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OFFICIAL METHODS

Determination of Four Arsenic Species in Fruit Juice by High-Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry: Single-Laboratory Validation, First Action 2016.04

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Submitted for publication April 2016.
Adopted as First Action *Official Method*SM by the Expert Review Panel on Heavy Metals.
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.
Approved on March 14, 2016
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DOI: 10.5740/jaoacint.16-0154

AOAC Official Method 2016.04
Four Arsenic Species in Fruit Juice
High-Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry
First Action 2016

A. Principle

For the analysis of various arsenic species present in fruit juices high pressure liquid chromatography (HPLC) is used to separate the arsenic compounds and inductively coupled plasma-mass spectrometry (ICP-MS) quantitatively detects them at the ng/g concentration level. Samples should be analyzed for total arsenic concentration and compared the sum of the individual arsenic species.

B. Scope and Application

This method describes a procedure for using HPLC in combination with ICP-MS to determine inorganic arsenic {iAs, the sum of arsenite [As(III)] and arsenate [As(V)]} in clear (free of solids) fruit juice and fruit juice concentrates (1). Due to difficulties controlling As(III) and As(V) interconversion, these compounds are not reported individually, only as iAs. Dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are also determined with this method.

This method should be used by analysts experienced in the use of HPLC and ICP–MS, including the identification of chromatographic and matrix interferences and procedures for their correction, and should only be used by personnel thoroughly trained in the handling and analysis of samples for the determination of trace elements in food products.

The analytical limits listed in Table **2016.04A** are presented as an example of results achievable for juice and juice concentrates when using the method and equipment specified herein. Analytical limits will vary depending on instrumentation and actual operating conditions used.

C. Summary of the Method

Ready-to-drink (RTD), clear (i.e., no solids) juice is prepared by diluting, approximately 5-fold, an analytical portion with water. Commercial and consumer juice concentrates (e.g., canned frozen juice concentrate) require dilution to

Table 2016.04A. Typical analytical limits

Analytical parameter	Abbreviation	ASDL, ng/g[a,b]	ASQL, ng/g[a,b]	LOD; RTD, µg/kg[c,d]	LOQ; RTD, µg/kg[c,d]	LOD; concn, µg/kg[d,e]	LOQ; concn, µg/kg[d,e]
Arsenite	As(III)	0.05	0.4	0.25	2.0	1.5	12
Arsenate	As(V)	0.05	0.4	0.25	2.0	1.5	12
Monomethylarsonic acid	MMA	0.05	0.4	0.25	2.0	1.5	12
Dimethylarsinic acid	DMA	0.05	0.4	0.25	2.0	1.5	12

[a] Based on replicate injections of fortified MBKs. The results are taken from the multilaboratory validation reports of EAM Method 4.10, where average ASDLs were 0.047 ng/g for As(III), 0.056 ng/g for As(V), 0.041 ng/g for DMA, and 0.041 ng/g for MMA.

[b] Calculated as in EAM Section 3.2.2.

[c] Based on a 5-fold dilution of RTD juice.

[d] Calculated as in EAM Section 3.2.3.

[e] Based on a 30-fold dilution of juice concentrate.

approximate RTD strength prior to this 5-fold dilution. Arsenic species are analyzed by HPLC–ICP–MS, using a PRP-X100 (Hamilton, Reno, NV) anion exchange column for separation. Arsenic species are identified by peak retention time (RT) matched with arsenic species standards. Concentrations are calculated based on peak area for analytical solutions compared with the response of standard solutions. The ICP–MS is used as an arsenic-specific detector, monitoring *m/z* 75 for arsenic-containing chromatographic peaks, and is operated in helium collision cell mode to eliminate any interference from possible coeluting chloride species.

Caution: Use appropriate personal protective equipment (including safety glasses, gloves, and a laboratory coat) when handling concentrated solutions containing toxic arsenic compounds. Analysts should consult and must be familiar with their laboratory's chemical hygiene and safety plan and Safety Data Sheets for all reagents and standards listed. Refer to instrument manuals for safety precautions regarding use. All waste generated must be handled appropriately.

D. Equipment and Supplies

(a) *ICP–MS*.—Agilent Model 7500ce or 7700x with respective instrumental control software (Agilent Technologies, Palo Alto, CA). The ICP–MS should be equipped with an octopole reaction cell using He as the collision gas and should interface with or be configured to start remotely by the HPLC instrument for integrated operation. Chromatographic ICP–MS data are processed using MassHunter data analysis software that accompanies the instrument control software.

(b) *HPLC*.—Agilent 1200 series that can be controlled with Instant Pilot control module and equipped with a binary pump, autosampler, degasser, and a column compartment (Agilent Technologies).

(c) *HPLC analytical column*.—Hamilton PRP-X100 anion exchange column, 250 × 4.1 mm, stainless steel, 10 µm particle size (Hamilton Cat. No. 79433), with PRP-X100 guard column (Hamilton Cat. No. 79446 for five-pack of cartridges).

(d) *Six-port switching valve*.—Either integrated in the HPLC column compartment or externally provided. To be used to inject a postcolumn internal standard (IS; *see* Figure **2016.04A**). The IS [2 ng As(V) per gram in the mobile phase] is delivered to the switching valve using a peristaltic pump (Model MP4; Gilson, Inc., Middleton, WI) and a combination of polyetheretherketone and standard pump tubing. The HPLC method is modified as indicated in Table **2016.04B**, using the "Timetable" tab that allows for IS injection. A 20–50 µL injection loop is used. For the peristaltic pump, an approximate flow rate of 0.1–0.3 mL/min should be used, as it must refill the injection loop between injections.

(e) *Glass or plastic HPLC autosampler vials*.—Use plastic SUN-Sri 8-425, 600 µL (Cat. No. 14-823-313; Fisher, Pittsburgh, PA) or acid-cleaned glass vials to minimize or eliminate possible inorganic arsenic contamination. Check representative vials with blank deionized water (DIW) injections to determine if inorganic arsenic is detected. If necessary, soak vials using 2% nitric acid for ~1 h and rinse four times with DIW. Check again for contamination.

(f) *High-density polyethylene (HDPE) amber bottles*.—For preparation and storage of stock standards.

(g) *Centrifuge tubes*.—Polypropylene conical tubes with caps, 15 mL. Check representative centrifuge tubes, placing 1% HNO_3 in the tubes for a period of time, and then analyzing this solution for total arsenic to ensure no arsenic is detected above the analytical solution detection limit (ASDL).

(h) *Vortex mixer*.—To mix diluted fruit juices and fruit juice concentrates.

(i) *Plastic syringes*.—To filter juice samples: disposable, general-use, and nonsterile with 5 or 10 mL Luer-Lock tip (Fisher).

(j) *Syringe filters*.—To filter juice samples, disposable, 0.45 µm nylon or PTFE membrane with polypropylene housing and Luer-Lock also from Fisher.

(k) *Analytical balance*.—Precision of 0.0001 g.

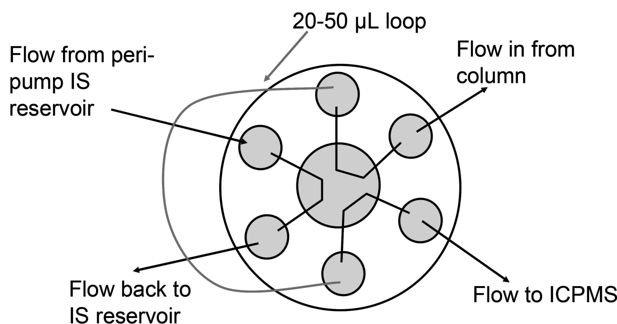


Figure 2016.04A. Setup for the postcolumn introduction of IS.

Table 2016.04B. Typical HPLC–ICP–MS operating conditions for Agilent 7500c ICP–MS and 1200 HPLC

Conditions	Setting
ICP–MS	
Radio Frequency power, W	1550
Plasma gas flow rate, L/min	15
Auxiliary (makeup) gas flow rate, L/min	0.1
Nebulizer (carrier) gas flow rate, L/min	1.0
Sampling depth, mm	8.5
Peristaltic pump speed, rps	0.3 (~1 mL/min)
Spray chamber temperature, °C	2
Ions (mass-to-charge ratio)	75
	77
Dwell time, seconds per point	0.8 s (*m/z* 75)
	0.2 s (*m/z* 77)
Reaction/collision cell mode	On, ~2.0 mL/min He
HPLC	
Mobile-phase composition	10 mM $(NH_4)_2HPO_4$
Mobile-phase pH	8.25 (±0.05)
Mobile-phase flow rate, mL/min	1.0
Injection volume, µL	100
Degasser	On
Column temperature	Ambient
Column compartment timetable for the introduction of IS	0.1 min, column position 1
	1.0 min, switch to column position 2
	2.0 min, switch back to column position 1
Acquisition time	1200 s (20 min)

(l) *Pipets*.—Automatic pipets capable of accurate delivery from 10 µL up to 10.00 mL with assorted tips.

(m) *pH meter*.—With appropriate calibration buffers (pH 7 and 10).

E. Reagents and Standards

(a) *Reagent water*.—Water that meets specifications for ASTM International type I water (2), such as 18 MΩ·cm DIW from a Millipore Milli-Q system (EMD Millipore, Billerica, MA).

(b) *AsB*.—CAS No. 64436-13-1, F.W.178.06, purity ≥95% (Cat. No. 11093; Sigma-Aldrich, St. Louis, MO).

(c) *As(III) stock solution*.—1000 mg/L As(+3) in 2% HCl [Cat. No. SPEC-AS3, with the certified value of arsenic traceable to a National Institute of Standards and Technology (NIST) Standard Reference Material (SRM); Spex CertiPrep, Spex CertiPrep].

(d) *DMA*.—CAS No. 75-60-5, F.W. 138.01, purity ≥98% (Cat. No. PS-51; Chem Service Inc., West Chester, PA).

(e) *MMA solid*.—Purity ≥98.5%, formula wt. 291.9 (e.g., Chem Service Inc. Cat. No. PS-281).

(f) *As(V) stock solution*.—1000 mg/L As(+5) in H_2O (Spex CertiPrep Cat. No. SPEC-AS5, with the certified value of arsenic traceable to a NIST SRM).

(g) *Certified Reference Material (CRM)*.—NIST SRM 1643e *Trace Elements in Water*. Certified for a total arsenic concentration of 58.98 µg/kg.

(h) *Ammonium phosphate dibasic [$(NH_4)_2HPO_4$]*.—CAS No. 7783-28-0, F.W. 132.06, purity ≥99%. Due to arsenic contamination in various lots from several manufacturers, the $(NH_4)_2HPO_4$ used in this procedure must be verified as having a low arsenic content [**I(d)***(1)-(5)*].

(i) *Ammonium hydroxide (NH_4OH), 20%*.—CAS No. 1336-21-6, F.W. 35.05, Ultrex II Ultrapure Reagent (Avantor, Center Valley, PA).

F. Reagent and Standard Preparation

(a) *Mobile-phase preparation*.—Mobile-phase, aqueous 10 mM ammonium phosphate dibasic, pH 8.25 (±0.05). Add 1.32 g $(NH_4)_2HPO_4$ to 1 L HPLC reservoir bottle, add 990 g DIW, adjust pH to 8.25 (±0.05) with 20% ammonium hydroxide, and fill to 1000 g with DIW. Mobile phase should be prepared fresh daily as necessary to minimize changes in pH from the atmosphere.

(b) *Standards preparation*.—Calculations for the preparation of standards of arsenic species are based on the elemental arsenic concentration (as opposed to the molecular weight of the compound). All standard preparations must be made based on a mass-to-mass basis. For clarity, report mass fraction of analytical solutions on nanograms-per-gram basis and mass fraction of test samples on micrograms-per-kilogram basis.

(c) *Stock standards*.—Commercially available stock standards of As(III) and As(V) are used "as is" and may be stored at room temperature or refrigerated. Stock standard solutions of DMA, MMA, and AsB are prepared in DIW. All stock standards should be brought to room temperature and mixed well prior to use. Record all weights to calculate standard concentrations. Stock standards of DMA, MMA, and AsB may be kept and used for up to 1 year in tightly sealed HDPE or polypropylene containers stored in the dark at 4°C. Expiration dates for commercial stock standards of As(III) and As(V) are typically 1 year.—(*1*) *AsB stock solution, As = 1000 µg/g in DIW*.—Tare a 15 mL polypropylene centrifuge tube. Weigh 0.025 g AsB in a tube. Add DIW to 10 g total.

(*2*) *DMA stock solution, As = 1000 µg/g in DIW*.—Tare a 15 mL polypropylene centrifuge tube. Weigh 0.0184 g DMA in a tube. Add DIW to 10 g total.

(*3*) *MMA stock solution, As = 1000 µg/g in DIW*.—Tare a 15 mL polypropylene centrifuge tube. Weigh 0.039 g MMA in a tube. Add DIW to 10 g total.

(d) *Working standards*.—The arsenic concentration of the DMA and MMA standards must be verified, typically using ICP–MS analysis. It is recommended that the As(III) and As(V) concentrations also be verified, but this is not required. Determine the total arsenic concentrations in 1 µg/g standards of MMA and DMA using a calibration curve prepared using a verified total arsenic standard. It is also advisable to analyze a CRM such as NIST SRM 1643e *Trace Elements in Water*, along with the standards for additional confidence. Calculate the As concentration of the MMA and DMA working standard solutions. Use these concentrations to recalculate the stock standard concentrations and apply these values in all future calculations. Record all weights to calculate standard concentrations. Additionally, the RTs and purity of the working standards [As(III), As(V), DMA and MMA] must be verified via HPLC–ICP–MS analysis of a 100 ng/g single-compound standard. Impurity peaks should account for <2% of the total

peak area. Single-analyte 1 µg/g working standards of As(III), As(V), DMA, and MMA may be kept for up to 3 months in tightly sealed HDPE or polypropylene containers stored in the dark at 4°C, but should be periodically reverified (e.g., monthly) for both total As and for species purity. Interconversion of As(III)/As(V) standards is most likely to be seen and comparison with the original analysis for purity is recommended.—(*1*) *AsB working standard, As = 1 µg/g in H_2O*.—Tare a 125 g HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 µg/g AsB stock solution into the bottle. Dilute to 100 g total with DIW.

(*2*) *As(III) working standard, As = 1 µg/g in H_2O*.—Tare a 125 mL HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 mg/L As(III) stock solution into the bottle. Dilute to 100 g total with DIW. This standard does not require concentration verification because the stock is traceable to a NIST SRM.

(*3*) *DMA working standard, As = 1 µg/g in H_2O*.—Tare a 125 mL HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 µg/g DMA stock solution into the bottle. Dilute to 100 g total with DIW. Analyze for total arsenic as described above and use the calculated arsenic concentration in all future calculations.

(*4*) *MMA working standard, As = 1 µg/g in H_2O*.—Tare a 125 mL HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 µg/g MMA stock solution into the bottle. Dilute to 100 g total with DIW. Analyze for total arsenic as described above and use the calculated arsenic concentration in all future calculations.

(*5*) *As(V) working standard, As = 1 µg/g in H_2O*.—Tare a 125 mL HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 mg/L As(V) stock solution into the bottle. Dilute to 100 g total with DIW. This standard does not require concentration verification because the stock is traceable to a NIST SRM.

(*6*) *Multianalyte spiking solution, As(III), DMA, MMA, and As(V); 1000 ng/g As each*.—Prepare the multianalyte spiking standard by weight in DIW using the 1000 µg/g DMA and MMA stock standards and the 1000 mg/L As(III) and As(V) stock standards. Pipet 100 µL (0.1 g) of each stock standard into a 125 mL HDPE or polypropylene bottle. Dilute to 100 g total with DIW. This multianalyte spiking standard may be used for up to 3 months if stored in tightly sealed polypropylene container in the dark at 4°C, but should be periodically checked (e.g., monthly) for As(III), As(V), DMA, and MMA concentrations.

(e) *Calibration standards*.—Prepare a minimum of four mixed analyte standards in DIW for instrument calibration. Record all weights to calculate standard concentrations in nanograms-per-gram units. Multianalyte calibration standards and calibration check standards should be prepared fresh on the day of use. However, multianalyte calibration standards may be used for up to 1 week if kept in the dark at 4°C and if standard chromatograms do not show evidence of interconversion of arsenic species.—(*1*) *200 ng/g each As(III), DMA, MMA, and As(V)*.—Tare a 15 mL HDPE or polypropylene tube. Pipet 1 mL (~1.0 g) each of As(III), DMA, MMA, and As(V) of the 1 µg/g working standards into the tube. Dilute to 5 g total with DIW and mix thoroughly. This standard is used in the preparation of calibration standards, but not analyzed.

(*2*) *For quantification using a calibration plot*.—(*a*) *As(III), DMA, MMA, and As(V); 10 ng/g each*.—Pipet 500 µL (~0.500 g) of the 200 ng/g multianalyte solution into a tared HDPE or polypropylene tube. Dilute to 10 g total with DIW and mix thoroughly.

(*b*) *As(III), DMA, MMA, and As(V); 4 ng/g each*.—Pipet 200 µL (~0.200 g) of the 200 ng/g multianalyte standard solution into a tared HDPE or polypropylene tube. Dilute to 10 g total with DIW and mix thoroughly.

(*c*) *As(III), DMA, MMA, and As(V); 1 ng/g each*.—Pipet 1.0 mL (~1.0 g) of the 10 ng/g multianalyte calibration standard solution into a tared HDPE or polypropylene tube. Dilute to 10 g total with DIW and mix thoroughly.

(*d*) *As(III), DMA, MMA, and As(V); 0.4–0.5 ng/g each*.—Pipet 500 µL (~0.500 g) of the 10 ng/g multianalyte calibration standard solution into a tared HDPE or polypropylene tube. Dilute to 10 g total with DIW and mix thoroughly. *Note*: This standard should be at or slightly above the laboratory's analytical solution quantitation limit (ASQL).

(*e*) *Calibration check standard*.—Prepare a 2 ng/g mixed-species standard for the check standard. Pipet 100 µL (~0.1 g) of the 200 ng/g multianalyte standard solution into a tared HDPE or polypropylene tube. Dilute to 10 g with DIW and mix thoroughly.

(f) *Additional standards*.—(*1*) *AsB/As(III) resolution check solution, 5 ng/g each*.—Pipet 50 µL (~0.05 g) each of AsB and As(III) of the 1 µg/g working standard solutions into a tared HDPE or polypropylene tube. Dilute to 10 g with DIW and mix thoroughly. A new resolution check solution should be prepared when significant oxidation of As(III) to As(V) is noted.

(*2*) *Arsenic IS solution, 2 ng/g*.—Pipet 1000 µL (~1 g) of the 1 µg/g As(V) working standard solution into a tared HDPE or polypropylene bottle and dilute to 500 g total with DIW.

(*3*) *CRM*.—Prepare a 15-fold dilution. Pipet 0.5 mL (~0.5 g) of NIST SRM 1643e into a tared HDPE or polypropylene tube. Dilute to 7.5 g total with DIW.

G. Analytical Sample Preparation Procedure

Allow refrigerated or frozen samples to come to room temperature. Invert the juice container several times to ensure homogeneity. Record all weights (to 0.0001 g) to calculate the concentration of arsenic species in the sample.

(a) *Commercial juice concentrates*.—Measure and record the degree Brix (°Bx) in the commercial juice concentrates. For commercial concentrates, the equivalent inorganic arsenic calculated for RTD (100% juice) is based on the °Bx in the juice concentrate, the inorganic arsenic concentration determined in the juice concentrate, and the minimum °Bx value for 100% juice listed in Table **2016.04C**. Transfer ~1 g concentrate into a tared 15 mL polypropylene centrifuge tube and record the mass. Dilute to 6 g with DIW, record the final mass, and mix thoroughly. Take this solution through the sample preparation procedure for RTD juice.

(b) *Consumer juice concentrates (usually canned, frozen)*.—For consumer juice concentrates, follow the manufacturer's directions for dilution and take this solution through the sample preparation procedure for RTD juice. In the absence of the manufacturer's directions, measure and record the °Bx in the juice concentrates. Transfer ~1 g concentrate into a tared 15 mL polypropylene centrifuge tube and record the mass. Dilute to 4 g total with DIW, record the final mass, and mix thoroughly. This should approximately reflect the typical label instructions for dilution. Take this solution through the sample preparation procedure for RTD juice.

Table 2016.04C. Minimum °Bx values for select RTD (single strength) juices[a]

Juice	°Bx value for "100% Juice"
Apple	11.5
Cranberry	7.5
Grape	16.0
Pear	12.0

[a] In enforcing these regulations, the U.S. Food and Drug Administration will calculate the labeled percentage of juice from concentrate found in a juice or juice beverage using the minimum Brix levels listed above, where single-strength (100%) juice has at least the specified minimum Brix listed above (3).

(c) *RTD juices*.—Pipet 2 mL (~2 g) juice into a tared 15 mL polypropylene centrifuge tube and record mass of analytical portion. Dilute to 10 g with DIW in the tube and record total mass of analytical solution. Cap and mix thoroughly. Draw ~4 mL analytical solution into syringe and dispense through a 0.45 µm nylon or PTFE syringe filter (discard first ~1 mL to waste) into a 15 mL polypropylene centrifuge tube. Transfer ~1 mL diluted juice to an autosampler vial prior to analysis. Store unused portion up to 48 h at 4°C in the event the sample needs to be reanalyzed.

(d) *Fortified analytical portions (FAPs) for RTD samples*.—Prepare an analytical portion fortified with As(III), DMA, MMA, and As(V) at a level of 25 µg/kg each by combining 2 mL (~2 g) RTD juice and 0.05 mL (~0.05g) of the 1000 ng/g multianalyte spiking solution in a 15 mL polypropylene centrifuge tube. Dilute to 10 g total with DIW and mix thoroughly (the spiking level is 5 ng/g each in this solution). Draw ~4 mL of the analytical solution into syringe and dispense through a 0.45 µm nylon or PTFE syringe filter (discard first ~1 mL to waste) into a 15 mL polypropylene centrifuge tube. Transfer ~1 mL of FAP diluted juice to an autosampler vial for analysis. Store unused portion up to 48 h at 4°C in the event the sample needs to be reanalyzed.

(e) *FAPs for commercial juice concentrates*.—Prepare an analytical portion fortified with As(III), DMA, MMA and As(V) at a level of 150 µg/kg each by combining ~1 g concentrate and 0.15 mL (~0.15 g) of the 1000 ng/g multianalyte spiking solution in a 15 mL polypropylene centrifuge tube. Dilute to 6 g total with DIW. Pipet 2 mL (~2 g) of this solution into a 15 mL polypropylene centrifuge tube, dilute to 10 g total with DIW, and mix thoroughly (the spiking level is 5 ng/g each in this solution). Draw ~4 mL analytical solution into the syringe and dispense through a 0.45 µm nylon or PTFE syringe filter (discard first ~1 mL to waste) into a 15 mL polypropylene centrifuge tube. Transfer ~1 mL FAP-diluted juice into an autosampler vial for analysis. Store the unused portion up to 48 h at 4°C in the event the sample needs to be reanalyzed.

(f) *Method blank (MBK)*.—Take 2 g DIW through the sample preparation procedures described above for RTD juice, as well as juice concentrates.

H. Determination Procedure

Table **2016.04B** is an example of the operating conditions used for this analysis. Operating conditions and settings are suggestions only, will vary with the instrument, and should be optimized for the equipment used.

I. Instrument Setup

(a) Follow instrument standard operating procedure for startup and initialization. After a ~30 min warm-up, tune the ICP–MS normally, checking that performance meets the default specifications. For a given ICP–MS instrument, it is recommended that the He gas flow rate for chromatographic analysis be 2–3 mL/min less than what is used for typical total arsenic analyses using He mode.

(b) Use the peristaltic pump to directly introduce a 1–10 ng/g As solution (in the mobile phase) into the nebulizer. Ensure the signal for a *m/z* 75 response is within the normal range. *Note*: Rinse the ICP–MS system well when finished tuning.

(c) For the postcolumn As IS, connect a small (20–50 µL) loop across two of the ports of the six-way two-position column switching valve, with the LC flow and peristaltic pump IS reservoir flow tubes connected in a manner similar to Figure **2016.04A**. In the HPLC method timetable column-switching valve should be triggered at 1 min and triggered to switch back at 2 min. Start the peripump and verify that no bubbles are present.

(d) Connect the ICP–MS and HPLC. Start HPLC flow (1 mL/min).—(*1*) If this is the first time a source of $(NH_4)_2HPO_4$ is being used for the mobile phase, you will need to test for arsenic contamination. Follow steps **I(d)***(1)-(5)* and if acceptable proceed to step **I(e)**. If the $(NH_4)_2HPO_4$ source has already been found to be acceptable, follow step **I(d)***(1)* and then proceed to step **I(e)**.—(*a*) Ensure proper flow and adequate drainage of the ICP spray chamber (>1 mL/min).

(*b*) Check for leaks.

(*c*) Allow time for the column and plasma to equilibrate (>15 min).

(*d*) Ensure that the backpressure is acceptable. Increasing backpressure can be indicative of column problems.

(*2*) Set the ICP–MS conditions as in Table **2016.04B**, but rather than setting up an acquisition method, test the following in the tune window.

(*3*) After eluting DIW through the HPLC to the ICP–MS (through the HPLC column) for at least 30 min, monitor *m/z* 75 (integration time of 0.8 s) in the tune window for at least 30 s and then record the average response (in counts per second (cps)).

(*4*) Switch the eluent to the mobile phase [using the new source of $(NH_4)_2HPO_4$]. After eluting the mobile phase for at least 30 min, monitor *m/z* 75 (integration time of 0.8 s) in the tune window for at least 30 s and then record the average response (in cps).

(*5*) Compare the average response of DIW and mobile phase for *m/z* 75. The ratio of mobile-phase response (cps) to DIW response (cps) should be less than 6:1. If it is not, try another source of $(NH_4)_2HPO_4$. If it is <6, proceed to step **I(e)**.

(e) Set the ICP–MS acquisition method for the time-resolved collection of *m/z* 77 and 75 with integration (dwell) times of 0.2 and 0.8 s, respectively, and one replicate (read) per point (*see* Table **2016.04B**).

(f) Analyze a blank (DIW only) to verify that the water and autosampler vials are arsenic-free. Monitor the instrument conditions to ensure that operation is stable and within the normal functioning range.

(g) Analyze the AsB/As(III) resolution check solution to ensure adequate resolution.

(h) Create/edit the sequence file on the ICP–MS data system. Ensure that the injection list and HPLC method on the HPLC controller match the ICP–MS sequence.

(i) Analyze calibration standards, MBKs, check solutions, sample extracts, FAPs, CRMs, and any other QC samples. A typical analytical batch is shown in Table **2016.04D**. Check RTs, peak shape and response of both IS and arsenic species in the *m/z* 75 chromatograms. Typical RTs are as follows: As(III) = 2.9 ± 0.2 min, DMA = 3.9 ± 0.2 min, MMA = 5.5 ± 0.3 min, and As(V) = 12.7 ± 0.5 min. To some extent, the RTs and peak shapes are dependent on the age and performance of the LC column [especially the As(V) peak]. However, significant (>7%) between the RT of the standards and samples (including spiked samples) within the same batch are not anticipated and should be investigated and corrected if noted.—(*1*) Figure **2016.04B** shows example chromatograms obtained for the resolution check solution, a 5 ng/g standard, and an apple juice sample.

(*2*) Check the *m/z* 77 chromatograms of samples for indications of possible argon chloride ($^{40}Ar^{35}Cl^+$ at *m/z* 75 and $^{40}Ar^{37}Cl^+$ at *m/z* 77) interferences in the *m/z* 75 chromatograms. Peaks detected in the *m/z* 77 chromatograms arising from $^{40}Ar^{37}Cl^+$ will also have peaks with matching RTs in the *m/z* 75 chromatograms. However, analysts should be aware that peaks may also be present in the *m/z* 77 chromatograms without corresponding peaks at *m/z* 75, as a result of, for example, selenium species ($^{77}Se^+$).

(j) Integrate *m/z* 75 chromatograms.—(*1*) The settings in Table **2016.04E** are suggested integration parameters for *m/z* 75 and provide a recommended starting point for integration; these parameters are specific to Agilent MassHunter data analysis software. All chromatograms should be visually inspected and manually integrated when necessary to ensure consistency and accuracy of integration. It is important to verify that peaks are properly identified by the integrator, and it is imperative that manual integrations be as consistent as possible, especially within the same analytical batch.

(*2*) After the settings are verified as correct, choose "Apply to All." This will apply these integration parameters to the IS, As(III), As(V), DMA, and MMA peaks.

(*3*) To eliminate peaks in the *m/z* 77 trace from being integrated (resulting in extended processing time), increase the minimum peak area counts for m/z 77 to ≥10,000.

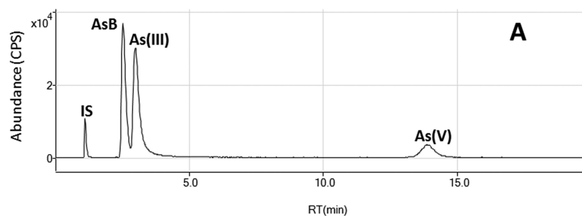
(*4*) The S/N for questionable chromatographic peaks can be calculated using MassHunter software. Autointegrate the questionable peak and verify proper integration. Manually adjust the integration if necessary. Select the "Set Noise Region" icon and the appropriate noise region near the peak of interest in the lower chromatogram. Ensure that the "S/N Ratio" option in the bottom window is checked under the "Show Peak Labels" dialog box, then reprocess the data. Questionable peaks must have an S/N > 3:1 to be considered detected. Questionable peaks with an S/N < 3:1 will be treated as nondetected.

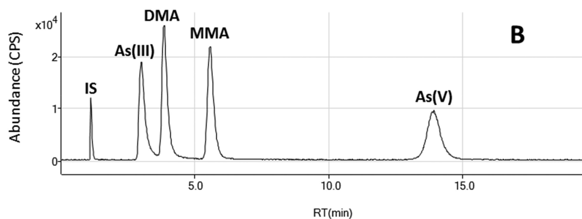
(*5*) *Unknown peaks*.—(*a*) If unknown peaks are detected with a S/N > 3:1, they should be added to the analyte list (in the Data Analysis Method Editor) and named "Unk X" (where "X" is the approximate RT). Unknown peaks are defined as peaks that do not match the expected RTs (as described previously) of As(III), As(V), DMA, or MMA.

(*b*) These peaks can be integrated using the above parameters, but care should be taken to ensure that unknown peaks are not integrated as known peaks and vice-versa.

Table 2016.04D. Typical analytical batch sequence and QC criteria

Solution	Purpose	QC criteria
DIW blank	Verify clean autosampler vials	≤ASDL
Resolution check solution	Check separation between unretained species (represented by AsB) and As(III)	Near-baseline separation
Multianalyte calibrations standards	Standardize instrument	$r^2 > 0.99$
MBK 1	Verify absence of contamination	≤ASDL
NIST SRM 1643e	Demonstrate accuracy	80–120% recovery
Ten analytical solutions (includes replicates and FAPs)	Determine As species concn	Within calibration range, RSD ≤ 15%
Calibration check standard	Verify standardization	85–115% of expected
MBK 2	Verify absence of contamination	≤ASDL
Ten analytical solutions (includes replicates and FAPs)	Determine As species concn	Within calibration range, RSD ≤ 15%
Calibration check standard	Verify standardization	85–115% of expected





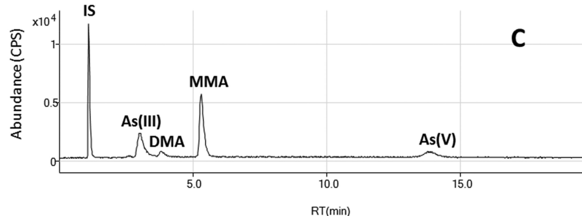


Figure 2016.04B. Example HPLC–ICP–MS chromatograms. (A) Resolution check solution [5 ng/g AsB and As(III)]. (B) Multianalyte standard [5 ng/g each of As(III), DMA, MMA, and As(V)]. (C) Apple juice. IS = internal standard peak.

Table 2016.04E. Recommended Data Analysis Method[a] settings for *m/z* 75

Data point sampling: 1	Start threshold: 0.3
Smoothing: enabled	Stop threshold: 0.5
Detection filtering: 5 point	Peak location: Top
Baseline reset (No. of points): >10	
If leading or trailing edge: <50	
Baseline preference: Drop else tangent skim[b]	
Peak area, counts: >2000	
All other parameters should be left at default values.	

[a] These integration parameter values are specific to the MassHunter Data Analysis software.

[b] Specific terminology used by MassHunter Data Analysis software for dropping a baseline from the start to the stop of the peak rather than a tangent line.

(*c*) Once integrated, use the unknown's peak area to estimate the approximate concentration of the unknown in the sample (based on elemental arsenic concentration). *See* **H**.

J. Calculations

When using the postcolumn injection IS, MassHunter, when configured properly, will automatically perform IS correction calculations. To calculate the concentration of a given unknown peak with MassHunter, add the unknown peak to the "Data Analysis" method, then under the "FullQuant" task, go to the "Basic Calibration Parameters" table and check the "CIC" box which then adds a "Substitute" column to the analyte table. From the drop-down menu, choose the nearest eluting arsenic standard and process the data as "normal." Alternatively, the calculation of the concentration of an unknown peak can be manually calculated using the following equation:

$$\mathrm{Unk_{concn}} = \frac{\left(\frac{A_{Unk}}{A_{IS}}\right) - (b)}{m}$$

where A_{Unk} = integrated peak area of the unknown, A_{IS} = integrated peak area of postcolumn injection peak (IS), m = slope of the calibration curve of the nearest eluting arsenic species, and b = y-intercept of the calibration curve of the nearest eluting arsenic species.

(a) *Calibration and analytical solution concentrations*.— Use a weighted calibration curve ($1/x^2$) to calculate the concentrations of individual arsenic species from the integrated peak areas in the analytical solutions. Do not choose an algorithm type where the y-intercept must pass through zero (use the "Ignore" option for "Intercept").

(b) *Sample concentrations*.— Calculate the concentration of the individual arsenic species in the samples as follows:

$$\left[C_{spl(\mu g/kg)}\right] = \left[C_{soln(ng/g)}\right] \times \text{Dilution factor} \times \left(\frac{1\ \mu g}{10^3 ng}\right) \times \left(\frac{10^3 g}{1\ kg}\right)$$

where $[C_{spl}]$ = concentration of As(III), As(V), DMA, or MMA in the sample (micrograms per kilogram), $[C_{soln}]$ = concentration of As(III), As(V), DMA, or MMA in the analytical solution (nanograms per gram).

$$\text{Dilution factor (RTD)} = \left(\frac{(M_{RTD} + M_{DIW})}{M_{RTD}}\right)$$

where M_{RTD} = mass of 2 g aliquot of RTD equivalent (either RTD juice or the diluted concentrate; grams) and M_{DIW} = mass of 4 g of the portion of DIW (grams).

$$\text{Dilution factor (concn)} = \left(\frac{(M_{concn} + M_{DIW})}{M_{concn}}\right) \times \left(\frac{(M_{RTD} + M_{DIW2})}{M_{RTD}}\right)$$

where M_{concn} = mass of the 1 g aliquot of the juice concentrate (grams), M_{DIW} = mass of the DIW used to dilute the juice concentrate (grams), M_{RTD} = mass of 1 g aliquot of the diluted concentrate (grams), and M_{DIW2} = mass of the DIW used to prepare analytical solution (grams).

Calculate the concentration of inorganic arsenic (iAs) in the RTD juice or juice concentrate sample as follows:

$$[\mathrm{iAs}] = [\mathrm{As(III)}] + [\mathrm{As(V)}]$$

where [As(III)] = concentration (micrograms per kilogram) of arsenite in RTD juice or juice concentrate and [As(V)] = concentration (micrograms per kilogram) of arsenate in RTD juice or juice concentrate

Note: [As(III)] and [As(V)] results ≥LOD are used in the calculation of [iAs].

For commercial concentrates, use the measured °Bx value to calculate the RTD-equivalent concentration of each species as follows:

$$[C]_{RTD} = [C]_{concn}\left(\frac{\mathrm{Brix}_{RTD(min)}}{\mathrm{Brix}_{concn}}\right)$$

where $[C]_{concn}$ = concentration of As(III), As(V), DMA, or MMA in the sample (micrograms per kilogram), $\mathrm{Brix}_{RTD(min)}$ = reference minimum °Bx value for single-strength RTD juice given in Table **2016.04C**, and Brix_{concn} = measured °Bx value of juice concentrate.

K. QC Elements

(a) *Prior to the analysis of samples*.—(*1*) Verify the RTs and purity of single-component standards. *See* **F(d)**.

(*2*) Verify concentrations of DMA and MMA stock standards. *See* **F(d)**.

(*3*) For each HPLC–ICP–MS instrument used, establish an ASDL and ASQL according to U.S. Food and Drug Administration's Elemental Analysis Manual. (EAM), Section 3.2. The limits for arsenic speciation analysis must be based on the SD of replicate (n = 10) analyses of a low-level mixed standard. The standard concentration used should be just above the estimated ASDL (e.g., each species is ~0.1 to 0.3 ng/g, for example). ASDL and ASQL are calculated as follows for As(III), As(V), DMA, and MMA:

$$\mathrm{ASDL} = 2 \times t_{0.95} \times \sqrt{1 + \frac{1}{n}} \times s$$

$$\mathrm{ASQL} = 30 \times s$$

where s = SD of replicates (nanograms per gram). Because these are estimates, it is suggested the laboratory use the largest ASQL and ASDL obtained from each of the four arsenic species and apply them to all species for reporting purposes.

(*4*) Calculate the method LOD and LOQ. The LOD and LOQ are calculated using the ASDL or ASQL multiplied by the nominal dilution factor. This will be dependent on the dilution factor used for each sample type (e.g., for RTD juice, the LOD = ASDL × 5; for juice concentrate, the LOD = ASDL × 30).

(b) *Analysis of samples*.—Failure of any of the below-described QC elements in meeting performance criteria will require an explanation of what was done to correct the problem and may require reanalysis of samples analyzed prior to the loss of the method control measures. The following is the minimum number of QC samples to be analyzed with each batch (maximum of 20 sample runs).—(*1*) *Calibration curve*.—For each analytical batch, a minimum of four calibration levels must be used. The calibration curves must be linear over the entire concentration range with $r^2 > 0.995$. If these criteria are not met, the calibration must be repeated and new working standard preparations may be necessary.

(*2*) *Calibration check standard*.—A calibration check standard must be analyzed after every 10th analytical solution and after the last analytical solution have been analyzed to monitor the RT and quantitative accuracy. The calibration check standard should be run at a level that is near the midpoint of the analytical calibration curve (e.g., 2 ng/g). If the below criteria are not met, the standard may be reanalyzed once. Additional failures require the reanalysis of samples analyzed after the last acceptable calibration check standard. Control limits for the calibration check standard are 100 ± 15% of the calculated concentration for DMA, MMA, and iAs [As(III) + As(V)]. The control limits for individual As(III) and As(V) concentrations can be outside of the 100 ± 15% individually, as long as their sum as iAs is within 100 ± 15%. Control limits for the calibration check standard RTs (RT) are as follows: As(III) RT ±0.2 min, DMA RT ±0.2 min, MMA RT ±0.3 min, and As(V) RT ±0.5 min when compared to the 10 ng/g calibration standard.

(*3*) *MBKs*.—A minimum of one MBK must be prepared and analyzed for every 10 or fewer analytical solutions analyzed. No arsenic species should be detected in the MBK. If there is a failure to meet this criterion, possible sources of contamination, including reagents, etc., should be identified and corrected prior to continuing with the analysis. As described previously, ammonium phosphate dibasic used in the preparation of mobile phase, sample extracts, and MBKs has been identified as a potential source of contamination. Control limits for the MBK: No arsenic species detected (S/N > 3:1) above the ASDL.

(*4*) *Precision of the replicate analytical portions*.—For each batch and at least once for each separate matrix type (i.e., different types of juice), three replicate preparations and analyses of a sample must be performed. If the below criterion is not met, the source of the imprecision should be investigated and minimized. Reanalysis of samples analyzed after the last sample analyzed with acceptable precision may be required. The control limit for the RSD is 15% for iAs, DMA, and MMA when detected ≥LOQ.

$$\text{RSD }(\%) = \left(\frac{s}{C_{avg}}\right) \times 100\%$$

where s = SD of replicates (micrograms per kilogram) and C_{avg} = average concentration of replicates (micrograms per kilogram).

(*5*) *FAP*.—For each batch and at least once for each separate matrix type, one FAP must be prepared and analyzed to verify peak identification and quantitative recovery. It is recommended that the same sample be used for FAP recovery and precision. Fortifications (spikes) must be performed by adding standards to the juice matrix prior to dilution with DIW. If the recoveries are not acceptable, ensure that the spiking level is appropriate and reprepare and reanalyze the FAP sample. Reanalysis of the entire sample batch may be required. For peak identification, the chromatograms for the unfortified and fortified samples must be compared. An appropriate increase in peak area must be observed. In addition, the peak shape in the fortified sample chromatograms should be similar to that of the unfortified sample with no significant additional band broadening, shoulders, or unexpected peaks. It is not unusual to observe an RT shift of 0.3–0.5 min for MMA and As(V) when comparing standard with sample chromatograms. The control limit for FAP (spike) recovery is 100 ± 20% for iAs, DMA, and MMA. The following equation demonstrates how to calculate spike recoveries for individual species:

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

where C_{x+s} = concentration determined in the spiked sample (micrograms per kilogram), C_x = concentration determined in the unspiked sample (micrograms per kilogram), C_s = concentration of spiking solution (micrograms per kilogram), M_s = mass of spiking solution added to the sample portion (grams), and M_x = mass of the sample portion (grams). *Note*: Spikes of As(III) and/or As(V) must be evaluated based on the total iAs determined [As(III) + As(V)].

$$\text{Recovery }(\%) = \left(\frac{\left(C_{As(III),x+s} + C_{As(V),x+s}\right) - \left(C_{As(III),x} + C_{As(V),x}\right)}{\left(\frac{C_{As(III),s} \times M_s}{M_x} + \frac{C_{As(V),s} \times M_s}{M_x}\right)}\right) \times 100\%$$

where $C_{As(III),x+s}$ = As(III) concentration determined in the spiked sample (micrograms per kilogram), $C_{As(V),x+s}$ = As(V) concentration determined in the spiked sample (micrograms per kilogram), $C_{As(III),x}$ = As(III) concentration determined in the unspiked sample (micrograms per kilogram), $C_{As(V),\,x}$ = As(V) concentration determined in the unspiked sample (micrograms per kilogram), $C_{As(III),s}$ = As(III) concentration of spiking solution (micrograms per kilogram), $C_{As(V),s}$ = As(V) concentration of spiking solution (micrograms per kilogram), M_s = mass of spiking solution added to the sample portion (g), and M_x = mass of the sample portion (grams)

(*6*) *Reference material*.—For each batch, one CRM or in-house reference material must be prepared and analyzed. Unfortunately no juice reference material exists that is certified for arsenic. Because juice is largely composed of water, reference materials such as NIST 1643e *Trace Elements in Water* represent a reasonable matrix match. Although 1643e is not certified for

arsenic species, As(V), and possibly As(III), should be the only peaks detected. NIST 1643e should be analyzed using a dilution factor of ~15× to dilute the acid content of this CRM down. The control limit for the reference material is 100 ± 20% for the mass balance with a certified total As value of 58.98 µg/kg.

(*7*) *Mass balance*.—A mass balance must be calculated between the sum of all arsenic species detected, and the total As determined in each sample (total As may be determined using EAM Section 4.7). Often, total arsenic analysis is performed by a different laboratory. This QC element ensures that the majority of the total arsenic in the sample is accounted for in the speciation analysis. If the mass balance does not meet the acceptable range, reanalysis of the sample *may* be required. For samples with all arsenic species concentrations near the LOQ, the mass balance requirements may be more difficult to meet.

$$\text{Mass balance}\ (\%) = \frac{[\text{iAs}] + [\text{DMA}] + [\text{MMA}] + [\text{Unknown peak(s)}]}{[\text{Total As}]} \times 100\%$$

The control limit for the mass balance is 65–115%.

L. Reporting

Report results only when QC criteria for a batch have been satisfactorily met. Report results for DMA, MMA, and total inorganic arsenic [As(III) + As(V)] that are ≥LOQ as the mass fraction determined, followed by the units of measurement. Report results that are ≥LOD and <LOQ as the mass fraction determined, followed by the units of measurement and the qualifier, "(TR)", that indicates analyte is present at a trace level that is below the limit of reliable quantification. Report results that are <LOD as "zero," followed by the units of measurement. Note that species present at concentrations <LOD will probably not be picked up by the autointegrator. Due to variability between laboratories and instrumentation, LOD and LOQ values should be determined for each instrument system at each laboratory. The values in Table **2016.04A** are presented only as examples.

Example: As(V) LOQ = 3.5 µg/kg and As(V) LOD = 0.45 µg/kg. Levels found for three different RTD juice samples were 5, 1, and 0.2 µg/kg, respectively; 5 µg/kg is ≥LOQ, thus report 5 µg/kg; 1 µg/kg is ≥LOD but also <LOQ, thus report 1 µg/kg (TR); and 0.2 µg/kg is <LOD, thus report 0 µg/kg.

M. Method Validation

Use of the PRP-X100 column with ammonium phosphate mobile phases for arsenic speciation has been previously reported with good results (4–7).

Single-Laboratory Validation.—The method was validated by reference material analyses, recovery of analyte, and precision measurements (8). Juices used in method validation included red grape, purple grape, white grape, apple, pear, cranberry, cherry (juice blend), and berry (juice blend). The precision of analyses for the three analytical portions was ≤10% RSD for species present at concentrations ≥LOQ. Recovery of the added analyte was in the range of 80–120% for all four species in all juices tested. As results for NIST SRM 1640 *Trace Elements in Natural Water* agreed with the certificate value for total arsenic, differing by <0.1%. Results for DMA, MMA, and As(V)—all present at levels above LOQ—and total arsenic in NIST SRM 2669 *Arsenic Species in Frozen Human Urine (Level II)* were similar to certificate values (z-scores all <2). As(III) was not in agreement, however, total inorganic arsenic [As(III) + As(V)] was in agreement with the certificate value [z-score < 2 using combined uncertainty for As(III) and As(V), calculated as root-sum-square].

QC Data from Surveys.—The U.S. Food and Drug Administration conducted two surveys in 2011 using this method to gather information on arsenic species in fruit juices (9). The analysis was performed in two laboratories, and the QC data from those surveys have been summarized. Values for iAs found in NIST 1643e were 54–63 µg/kg (58.83 µg/kg average, 99.8% of certified total, n = 34). The overall average mass balance was 85% (range of 64–111%) in juice samples. The RSD % for iAs, DMA, and MMA at concentrations ≥LOQ ranged from 1.1 to 7.5% in juices for which three or more replicate analytical portions were analyzed. FAPs gave average recovery ranges of 83–120% for iAs (101% average, n = 24), 86–106% for DMA (97% average, n = 17), and 83–111% for MMA (100% average, n = 17). Check standard recovery ranges were 93–115% for iAs, 90–112% for DMA, and 93–114% for MMA.

N. Uncertainty

A result above LOQ has an estimated combined uncertainty of 10%. Use a coverage factor of 2 to give an expanded uncertainty at about 95%. A result above LOD, but below LOQ, is considered qualitative and is not reported with an uncertainty.

A detailed discussion of method uncertainty is presented in EAM Section 3.3. This method conforms to the information contained in that discussion. Derivation of an estimated uncertainty specific to an analysis is also discussed EAM Section 3.3.2.

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OFFICIAL METHODS

Determination of Heavy Metals in Food by Inductively Coupled Plasma–Mass Spectrometry: First Action 2015.01

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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 ±2.5°C and automatically adjust the microwave field output power within 2 s of sensing. Temperature sensors should be accurate to ±2°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 with ±3% of the nominal volume. To evaluate precision, the RSD of the measurements must be ≤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.

Table 2015.01A. 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

(*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 µg/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% HNO_3 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.

(**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) HNO_3. 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) HNO_3 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% HNO_3 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

Table 2015.01B. Recommended isotopes for analysis

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.

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 [$^{35}Cl + ^{40}Ar = ^{75}(ArCl)$].

(*b*) When arsenic reacts with the oxygen in the DRC cell, $^{75}As^{16}O$ 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 $^{75}As^{16}O$ 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 $^{91}(AsO)$ 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**.

(**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 HNO_3 and 1 mL of 30% hydrogen peroxide (H_2O_2) 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).

(*3*) Equivalent results were achieved using the program listed

Table 2015.01C. Digestion program for Berghof Speedwave 4 microwave

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

in Table **2015.01D** on a CEM MARS 6 microwave digestion system using the 40-position carousel and 55 mL Xpress digestion vessels.

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

(**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) HNO_3 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 ($CaCO_3$) 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

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

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

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 ≤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 ≤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) HNO_3 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) HNO_3 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

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 ≤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 ≥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 ≤ MRL; SD ≤ MDL or MBs <1/10th 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 ≤ 30% or ±2x LOQ if results ≤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 ≤ 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 ≥5x the LOQ and recovery is outside of the control limit, then batch must be re-prepared and reanalyzed

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

Method blanks	91(AsO)	111Cd	114Cd	Pb	200Hg	202Hg
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.

appropriate corrective action if the acceptance criteria are not met.

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 ≤10 µg/kg for all food matrices and ≤8 µg/kg for liquid matrices, such as infant formula.

(**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

	LOQ, µg/kg (as received)			
Sample	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

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

Table 2015.01J. AOAC SMPR 2012.007 (ref. 1)

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

(**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).

(**d**) *Standard Method Performance Requirements*[SM] (AOAC SMPR **2012.007**; 1) for repeatability, reproducibility, and recovery for the method are shown in the Table **2015.01J**. *See* Appendix A (Appendix A is available on the *J. AOAC Int.* website as supplemental material, http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac) for detailed method performance information supporting acceptance of the method.

(**e**) *See* Appendix A for detailed method performance information supporting acceptance of the method. 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.

References

(1) *J. AOAC Int.* **96**, 704(2013) DOI: 10.5740/jaoac.int.2012.007