

# Elemental Analysis Manual

## for Food and Related Products

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## 4.10 High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometric Determination of Four Arsenic Species in Fruit Juice

Version Draft 0.82 (August 2010)  
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GLOSSARY

### 4.10.1 SCOPE AND APPLICATION

This method describes a procedure for using high performance liquid chromatography (HPLC) in combination with inductively coupled plasma-mass spectrometry (ICP-MS) to determine inorganic arsenic (both arsenite (AsIII) and arsenate (AsV)) in clear (free of solids) fruit juice. Dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are also determined with this method. Arsenobetaine (AsB) is included in the multi-analyte working standard solution as a representative unretained arsenic-containing species in order to ensure adequate separation from As(III). Other matrices may be analyzed by this procedure if performance is verified in the matrix of interest and at the concentration of interest. The limits listed in 4.10 Table 1 are presented as an example of results achievable using the method and equipment specified herein and will vary depending on instrumentation and actual operating conditions.

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 be used only by personnel thoroughly trained in the handling and analysis of samples for determination of trace elements in food products.

**4.10 Table 1. Analytical Limits**

Analytical parameter	Abbreviation	ASDL <sup>a,b</sup> (µg/kg)	LOD <sup>c,d</sup> (µg/kg)	LOQ <sup>c,d</sup> (µg/kg)
Arsenite	As(III)	0.05	0.23	1.8
Arsenate	As(V)	0.09	0.45	3.5
Monomethylarsonic acid	MMA	0.06	0.29	2.2
Dimethylarsenic acid	DMA	0.05	0.26	2.0
<sup>a</sup> Based on fortified method blanks (0.2 ng/g each species, n=10, analyzed twice per day over 5 days). <sup>b</sup> Calculated as in EAM §3.2.2 <sup>c</sup> Based on 5-fold dilution of ready-to-drink juice. <sup>d</sup> Calculated as in EAM §3.2.3.				

#### 4.10.2 SUMMARY OF METHOD

Analytical samples of ready-to-drink clear juice are prepared by diluting an analytical portion approximately 5-fold with mobile phase (dilute juice concentrates 6-fold with water prior to 5-fold dilution with mobile phase). Arsenic species are separated by injecting 100 µL of these analytical solutions onto a PRP-X100 anion exchange column. Arsenic species are identified by peak retention time match with arsenic species standards. Concentrations are calculated based on response to standard solutions compared to integrated peak area for analytical solutions.

#### 4.10.3 EQUIPMENT AND SUPPLIES

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*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.*

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- (1) Inductively coupled plasma-mass spectrometer (ICP-MS)—Equipped with octopole reaction cell using He as collision gas. Instrument should interface with or be configured to remote start by HPLC instrument for integrated operation.
- (2) High performance liquid chromatograph (HPLC)—Consisting of analytical pump and autosampler capable of delivering basic aqueous mobile phase through analytical column and programmed sample injections.
- (3) HPLC analytical column— Hamilton PRP-X100 anion exchange column, 250 x 4.1 mm, 10 µm particle size (Hamilton cat. no. 79433) with PRP-X100 guard column (Hamilton cat. no. 79446 for 5-pack).
- (4) Glass or plastic HPLC autosampler vials—acid-cleaned, unless demonstrated to be free of arsenic contamination (plastic tends to be cleaner than glass).
- (5) Syringes for filtering juice—Disposable, general use and non-sterile, 5 or 10 mL, Luer-Loc tip.
- (6) Syringe filters for filtering juice—Disposable, 0.45 µm PVDF (polyvinylidene fluoride) membrane with polypropylene housing and Luer-Loc inlet.

#### 4.10.4 REAGENTS AND STANDARDS

Verify total arsenic concentration in intermediate standards against NIST SRM 1643e (trace elements in water). Arsenic intermediate standards may be kept indefinitely in tightly sealed polypropylene containers stored at 4°C, but should be rechecked for both total As and for species purity periodically (e.g., every 6 months). Multi-analyte working standard solution and check solution should be prepared fresh on day of use.

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*Safety Note: Reagents should be considered as potential health hazards, and exposure to these materials should be minimized as much as possible.*

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- (1) Reagent water—Water that meets specifications for ASTM Type I water<sup>1</sup>.
- (2) Arsenobetaine (AsB) solid (e.g. Fluka cat. no. 11093)—Purity  $\geq 95\%$ , formula wt. 178.06.
- (3) Arsenite (As(III)) stock solution (e.g. Spex CertiPrep cat. no. SPEC-AS3)—1000 mg As/L in 2% HCl.
- (4) Dimethylarsinic acid (DMA) solid (e.g. Chem Service cat. no. PS-51)—Purity  $\geq 98.9\%$ , formula wt. 137.99.
- (5) Disodium methyl arsonate hexahydrate (monomethylarsonic acid (MMA)) solid (e.g. Chem Service cat. no. PS-281)—Purity  $\geq 98.5\%$ , formula wt. 291.9.
- (6) Arsenate (As(V)) stock solution (e.g. Spex CertiPrep cat. no. SPEC-AS5)—1000 mg As/L in H<sub>2</sub>O.
- (7) Reference Material certified for arsenic—e.g. NIST SRM 1643e
- (8) Ammonium hydrogenphosphate (e.g. Sigma-Aldrich cat. no. 379980)—Purity  $\geq 99.99\%$ , formula wt. 132.06.
- (9) Germanium elemental standard solution (e.g. Inorganic Ventures cat. no. MSGE-10  $\mu\text{g/mL}$ ) —10  $\mu\text{g Ge/L}$  in 2% HNO<sub>3</sub>.
- (10) Mobile phase, aqueous 10 mM ammonium hydrogenphosphate, dibasic, pH 8.25—Add 1.32 g (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> to 1 L HPLC reservoir bottle, add 990 g reagent water, adjust pH to 8.25 with ammonium hydroxide, fill to 1000 g with reagent water.
- (11) AsB stock solution, As = 1000  $\mu\text{g/g}$  in H<sub>2</sub>O — Tare a 125 mL polypropylene bottle. Weigh 0.250 g AsB into bottle. Add reagent water to 100 g total.
- (12) DMA stock solution, As = 1000  $\mu\text{g/g}$  in H<sub>2</sub>O—Tare a 125 mL polypropylene bottle. Weigh 0.184 g DMA into bottle. Add reagent water to 100 g total.
- (13) MMA stock solution, As = 1000  $\mu\text{g/g}$  in H<sub>2</sub>O—Tare a 125 mL polypropylene bottle. Weigh 0.390 g MMA into bottle. Add reagent water to 100 g total.
- (14) AsB intermediate solution, As = 1000 ng/g in H<sub>2</sub>O—Tare 125 g polypropylene bottle. Pipet 100  $\mu\text{L}$  (0.1 g) of 1000  $\mu\text{g/g}$  As(III) stock solution into bottle. Dilute to 100 g total with reagent water.
- (15) AsIII intermediate solution, As = 1000 ng/g in H<sub>2</sub>O—Tare 125 mL polypropylene bottle. Pipet 100  $\mu\text{L}$  (0.1 g) of 1000 mg/L As(III) stock solution into bottle. Dilute to 100 g total with reagent water.
- (16) DMA intermediate solution, As = 1000 ng/g in H<sub>2</sub>O—Tare 125 mL polypropylene bottle. Pipet 100  $\mu\text{L}$  (0.1g) of 1000  $\mu\text{g/g}$  DMA stock solution into bottle. Dilute to 100 g total with reagent water.

- (17) MMA intermediate solution, As = 1000 ng/g in H<sub>2</sub>O—Tare 125 mL polypropylene bottle. Pipet 100 µL (0.1 g) of 1000 µg/g MMA stock solution into bottle. Dilute to 100 g total with reagent water.
- (18) AsV intermediate solution, As = 1000 ng/g in H<sub>2</sub>O—Tare 125 mL polypropylene bottle. Pipet 100 µL (0.1 g) of 1000 mg/L As(V) stock solution into bottle. Dilute to 100 g total with reagent water.
- (19) Multi-analyte intermediate standard solution, each species = 200 ng/g As (due to possibility of interconversion of As(III) and As(V), this solution should be prepared on the day of use)—Tare a 15 mL polypropylene tube. Record weights to calculate concentration in ng/g. Pipet 500 µL (~0.5g) each of AsB, As(III), DMA, MMA and As(V) intermediate solutions into tube.
- (20) Multi-analyte working standard solution, each species = 5 ng/g As (due to possibility of interconversion of As(III) and As(V) , this solution should be prepared on the day of use)—Tare a 15 mL polypropylene tube. Record weights to calculate concentration in ng/g. Pipet 250 µL (~0.25g) of the 200 ng/g multi-analyte intermediate standard solution into tube. Dilute to 10g total with mobile phase (10 mM aqueous ammonium phosphate, pH 8.25). Transfer ~1 mL to autosampler vial.
- (21) Internal standard solution, Ge = 100 ng/g in mobile phase—Dilute 100 µL of a 10 µg/mL germanium standard solution to 100 g total with mobile phase in a 125 mL polypropylene bottle.
- (22) Check solution—Prepare a 2 ng/g mixed species standard for the check standard. Record weights to calculate concentration in ng/g. Pipet 100 µL (~0.1g) of the 200 ng/g multi-analyte intermediate standard solutions into a 15 mL polypropylene tube. Dilute to 10 g total with mobile phase. Transfer ~1 mL to autosampler vial.

#### 4.10.5 ANALYTICAL SAMPLE PREPARATION PROCEDURE

Fruit juice analytical solution (if sample is a juice concentrate, first dilute 1 g juice concentrate up to 6 g total with water, and record actual mass of juice and total mass of solution) — Invert juice container several times to ensure homogeneity. Draw ~4 mL juice into syringe and dispense through 0.45 µm syringe filter into a disposable polystyrene cup. Tare a 15 mL polypropylene tube. Pipet 500 µL (~ 0.5 g) filtered juice into tube and record mass of analytical portion. Pipet 2 mL (~2 g) mobile phase into the tube and record total mass of analytical solution. Cap and shake to mix. Prepare analytical solutions in triplicate. Transfer ~1mL of diluted juice to an autosampler vial for analysis. Store unused portion at 4°C in the event the sample needs to be re-analyzed.

Fortified analytical portion (FAP) — Prepare an analytical portion fortified with each species at a level of 2 ng/g by combining 0.5 mL filtered juice, 1 mL of the 5 ng/g mixed standard, and 1 mL of MP in a 15 mL polypropylene tube. Cap and shake to mix. Transfer ~1 mL to autosampler vial. For juice concentrates, prepare FAP by combining 1 g juice concentrate, 200 µL of the 200 ng/g multi-analyte intermediate standard, and 4.8 g of DIW. Dilute this further by combining 1mL fortified diluted juice concentrate and 4 mL MP in a 15 mL polypropylene tube. Cap and shake to mix. Transfer ~1 mL to an autosampler vial.

#### 4.10.6 DETERMINATION PROCEDURE

The determination procedure was developed using an Agilent 1200 HPLC and Agilent 7500c ICP-MS. 4.10 Table 2 is an example of operating conditions used for this analysis. Operating

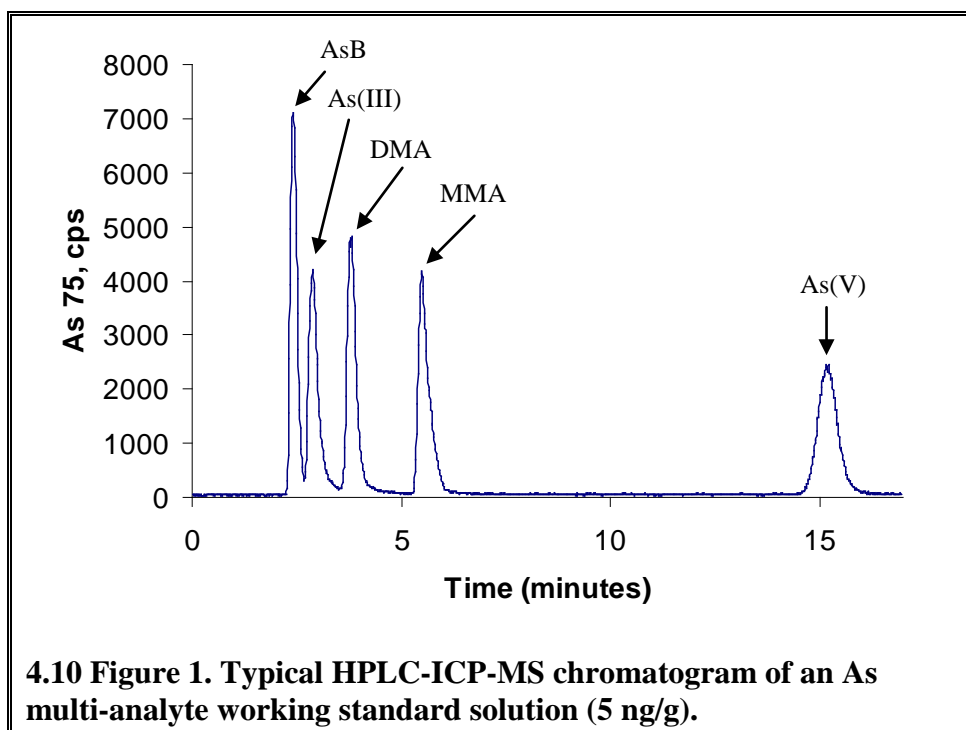
conditions and settings should be optimized for the equipment used.

**4.10 Table 2. Typical HPLC-ICP-MS Operating Conditions**

<i>Conditions for Agilent 7500c ICP-MS and Agilent 1200 HPLC</i>			
ICP-MS Conditions		HPLC Conditions	
RF Power (W)	1550	Mobile phase flow rate (mL/min)	1.0
Plasma gas flow rate (L/min)	15	Injection volume (μL)	100
Auxiliary (makeup) gas flow rate (L/min)	0.1	Degasser	ON
Nebulizer (carrier) gas flow rate (L/min)	1.0	Column temperature	not controlled
Sampling depth (mm)	7.5		
Peristaltic pump speed (rps)	0.3		
Spray chamber temperature (°C)	2		
Ions (mass-to-charge ratio)	75		
	72		
Integration time (sec/point)	0.8 (m/z 75)		
	0.2 (m/z 72)		
Total acquisition time (sec)	1020		
Reaction/collision cell mode	ON, 5.0 mL/min He		

### Instrument Setup

- (1) Follow instrument standard operating procedure for startup and initialization. After ~30 min warm-up, tune ICP-MS normally; check that performance meets default specifications.
- (2) Use peristaltic pump to introduce 10 ng/g As in mobile phase directly into the nebulizer. Ensure signal for m/z 75 response is within normal range.
- (3) Connect nebulizer tube to a 3-way tee. Internal standard (IS) should be delivered via peristaltic pump with a flow rate of approximately 0.04 mL/min into one port of the tee. The flow from the HPLC column should be connected to the third port of the tee.
  - All connections should use PEEK fittings.
- (4) Connect ICP-MS and HPLC. Start HPLC flow (1 mL/min).
  - Ensure proper flow and adequate drainage of ICP spray chamber (>1 mL/min).
  - Check for leaks.
  - Allow time for column and plasma to equilibrate.
- (5) Set ICP-MS acquisition method for time-resolved collection of m/z 72 and 75 with integration (dwell) times of 0.2 and 0.8 s, respectively, and 1 replicate (read) per point.
- (6) Analyze a blank (mobile phase only) solution to verify that mobile phase and chromatography vials are arsenic-free.
  - Monitor instrument conditions to ensure operation is stable and within normal functioning range.



#### Determination of Analyte Concentration Using Response Factor

- (1) Analyze the 5 ng/g multi-analyte standard solution as the first and last solution, and additionally at least once every 10 analytical solutions.
- (2) Analyze analytical solutions and quality control solutions. A typical sequence for an analytical run is listed in 4.10 Table 4.
- (3) Integrate chromatograms. Integrator parameters used with the Agilent ChemStation<sup>®</sup> integrator are shown as an example in 4.10 Table 3. Parameters should be set to acquire the smallest arsenic peaks possible while rejecting background noise peaks. Once the parameters have been chosen, arsenic peaks that are not picked up by the auto integrator are considered insignificant and should be ignored.
- (4) Tabulate integrated peak areas and the average m/z 72 internal standard signal.
- (5) Check instrument performance
  - Replicate analyses of multi-analyte working standard have <10 %RSD
  - Check solution analyses have a recovery of  $100 \pm 10\%$
  - Ensure that retention times of the last multi-analyte working standard solution are comparable to the first ( $\pm 0.5$  min).

**4.10 Table 3. Agilent ChemStation® Integration Parameters**

Event	Value	Time (min)
Initial Area Reject	10000	Initial
Initial Peak Width	0.25	Initial
Shoulder Detection	OFF	Initial
Initial Threshold	8.5	Initial
Baseline Back	—	1.000
Threshold	8	10.000

**4.10 Table 4. Typical Analytical Sequence**

Solution	Purpose	QC Criteria
standard solution	standardize instrument	
MBK	verify absence of contamination	< MBK <sub>C</sub>
sample #1 rep 1	determine As species conc.	≤LDR
sample #1 rep 2		
sample #1 rep 3		
sample #1 FAP	spike recovery	80-120% recovery
sample #2 rep 1	determine As species conc.	≤LDR
sample #2 rep 2		
sample #2 rep 3		
sample #2 FAP	spike recovery	80-120% recovery
RM	accuracy	80-120% recovery
check solution	verify standardization	90-110% of expected
standard solution <sup>a</sup>	standardize instrument	90-110% of previous injection
<sup>a</sup> This and any subsequent standard solution injections should agree with previous standard solution injection within ±10%.		

#### 4.10.7 CALCULATIONS

Calculate an instrumental drift correction factor for each injection

$$CF_{XIS} = IS_{init} / IS_x$$

where

IS<sub>init</sub> = average signal (cps) for m/z 72 over the entire 17 minutes of the first injection

IS<sub>x</sub> = average signal (cps) for m/z 72 over the entire 17 minutes of each subsequent injection

Normalize each integrated peak area

$$A_{Xnorm} = A_X / CF_{XIS}$$

where

A<sub>x</sub> = integrated peak area

CF<sub>xIS</sub> = internal standard correction factor

Calculate five (one for each analyte in the multi-analyte working standard) response factors RF (area/ng/g)

$$RF = A_{X \text{ avg}} / C_{\text{std}}$$

where

$A_{X \text{ avg}}$  = average peak area of each analyte (X) from all injections of multi-analyte working standard solution (example: if three injections of multi-analyte standard were made, then  $A_{\text{avg}}$  for DMA is the average integrated area of three resulting DMA peaks).  
 $C_{\text{std}}$  = concentration of analyte (ng/g) in multi-analyte working standard solution

Calculate concentration of each species in analytical solution, S (ng/g)

$$S = A_{\text{as}} / RF$$

where

$A_{\text{as}}$  = integrated peak area of analyte in analytical solution  
 RF = response factor of analyte

Calculate concentration of each species in the ready-to-drink strength juice

$$\text{Concentration (ng/g)} = S \times (m_{\text{AS tot}} / m_{\text{RTD}})$$

where

$S$  = concentration of arsenic species in analytical solution  
 $m_{\text{AS tot}}$  = total mass of analytical solution, g  
 $m_{\text{RTD}}$  = mass of analytical portion (juice used to prepare analytical solution), g

Calculate concentration of total inorganic arsenic (TotInorgAs) in the ready-to-drink strength juice

$$\text{TotInorgAs (ng/g)} = [\text{As(III)}_{\text{RTD}}] + [\text{As(V)}_{\text{RTD}}]$$

where

$[\text{As(III)}_{\text{RTD}}]$  = concentration (ng/g) of arsenite in ready-to-drink juice  
 $[\text{As(V)}_{\text{RTD}}]$  = concentration (ng/g) of arsenate in read-to-drink juice

Use As(III) and As(V) results  $\geq$  LOD for calculating TotInorgAs.

#### 4.10.8 METHOD VERIFICATION

The following is the minimum number of quality control samples to be analyzed with each batch of samples: 1 reference material (RM, for example NIST 1643e), 1 fortified analytical portion (FAP, 2 ng/g of each species), and 1 method blank (MBK). Replicate analytical portions should be analyzed for each sample whenever nonhomogeneity of analyte may be an issue.

A fortified method blank (FMB, 2 ng/g of each species) checks the accuracy of the fortification procedure without any matrix effects and is an optional quality control sample. Use same

fortification level as for the FAP.

### **Multi-analyte working standard and check solution**

Control limits for replicate injections of the multi-analyte working standard and check solution are  $100 \pm 10\%$  of the calculated concentration.

### **Reference Material**

Control limits for RM Recovery are  $100 \pm 20\%$  or within concentration uncertainty (converted to percent relative uncertainty) supplied on the certificate, whichever is greater. The z-score procedure, which allows for greater deviation and is discussed in EAM §3.5.3 (*link removed*), may also be used, although it requires additional calculations. If three or more RMs are analyzed, then only two-thirds of an element's RM recovery results must meet the control limit.

### **FAP Recovery**

Control limit for FAP recovery is  $100 \pm 20\%$ .

### **Relative Standard Deviation (RSD) of Replicate Analytical Portions**

Control limit for RSD is 10% (calculated as:  $\%RSD = (s/S_{avg}) \times 100\%$ )

where

$s$  = standard deviation of replicates

$S_{avg}$  = average concentration of replicates

### **FMB Recovery (optional)**

Control limit for FMB recovery is  $100 \pm 20\%$ .

## **4.10.9 REPORT**

Report results only when quality control criteria for a batch have been satisfactorily met. Report results for DMA, MMA, and Total Inorganic Arsenic (AsIII + AsV) that are  $\geq$ LOQ as the mass fraction determined followed by the units of measurement. Report results that are  $\geq$ LOD and  $<$ LOQ as the mass fraction determined followed by the units of measurement and the qualifier that indicates analyte is present at a trace level that is below the limit of reliable quantification (TR). Report results that are  $<$ LOD as 0 followed by the units of measurement and the qualifier that indicates analyte is below the level of reliable detection or is not detected (ND). Note that species present at concentrations  $<$ LOD will probably not be picked up by the auto-integrator. Due to variability between labs and instrumentation, values for LOD and LOQ should be determined in each lab. The values in Table 1 are presented only as examples.

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*Example: As(V) LOQ = 3.5 µg/kg; As(V) LOD = 0.45 µg/kg. Levels found for three different samples were 5 µg/kg, 1 µg/kg and 0.2 µg/kg.*

*5 µg/kg is  $\geq$ LOQ; report 5 µg/kg*

*1 µg/kg is  $\geq$ LOD but also  $<$ LOQ; report 1 µg/kg (TR)*

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

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#### 4.10.10 METHOD VALIDATION

Use of the PRP-X100 column with ammonium phosphate mobile phases for arsenic speciation has been previously reported with good results<sup>3-6</sup>.

*In-house validation.* The method was validated by analyses of reference materials, recovery of analyte, and precision measurements. Juices used for method validation included: red grape, purple grape, white grape, apple pear, cranberry, cherry (juice blend), and berry (juice blend). Precision of analyses for 3 analytical portions was  $\leq 10\%$  relative standard deviation for species present at concentrations  $>$ LOQ. Recovery of added analyte was in the range of 80-120% for all four species in all juices tested. As(V) results for NIST SRM 1640 agreed with certificate value for total arsenic, differing by less than 0.1%. Results for DMA, MMA, As(V) and total arsenic in NIST SRM 2669 were similar to certificate values (z-scores all  $< 2$ ). As(III) was not in agreement; however, total inorganic arsenic (sum of As(III) and As(V)) was in agreement with certificate value (z-score  $< 2$  using combined uncertainty for As(III) and As(V) calculated as root-sum-square).

*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 §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 §3.3.2 (*link removed*).

## REFERENCES

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