



U.S. Department of Health & Human Services



U.S. Food and Drug Administration

# **Elemental Analysis Manual**

## **for Food and Related Products**

The following is a section of the Elemental Analysis Manual for Food and Related Products.

For additional information and to view other sections of the manual, visit the Elemental Analysis Manual for Food and Related Products web page at

<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006954.htm>.



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# Elemental Analysis Manual

## for Food and Related Products

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

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GLOSSARY

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#### 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 (iAs, the sum of arsenite, As(III) and arsenate, As(V)) in clear (free of solids) fruit juice and fruit juice concentrates. Dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are also determined with this method. A solution containing arsenobetaine (AsB) and As(III) is analyzed to demonstrate adequate separation between unretained arsenic-containing species and 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.

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.

The analytical limits listed in 4.10 Table 1 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.

**4.10 Table 1. Typical Analytical Limits**

Analytical parameter	Abbreviation	ASDL <sup>a,b</sup> (ng/g)	ASQL <sup>a,b</sup> (ng/g)	LOD <sup>c,d</sup> (RTD, µg/kg)	LOQ <sup>c,d</sup> (RTD, µg/kg)	LOD <sup>d,e</sup> (Conc, µg/kg)	LOQ <sup>d,e</sup> (Conc, µg/kg)
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

<sup>a</sup>Based on replicate injections of fortified method blanks, results taken from EAM Method 4.11's multilaboratory validation reports where average ASDLs were 0.047 ng/g As(III), 0.056 ng/g As(V), 0.041 ng/g DMA, and .041 ng/g MMA).  
<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.  
<sup>e</sup>Based on 30-fold dilution of juice concentrate.

#### 4.10.2 SUMMARY OF METHOD

Ready-to-drink (RTD) clear (i.e., no pulp) juice is prepared by diluting an analytical portion approximately 5-fold with water. Commercial juice concentrates and consumer juice concentrates (i.e. canned frozen juice concentrate) require dilution to approximate RTD strength prior to this 5-fold dilution. Arsenic species are analyzed by HPLC-ICP-MS using a PRP-X100 anion exchange column for separation. Arsenic species are identified by peak retention time match with arsenic species standards. Concentrations are calculated based on peak area for analytical solutions compared to response of standard solutions. The ICP-MS is used as an

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arsenic-specific detector monitoring  $m/z$  75 for arsenic-containing chromatographic peaks and is operated in helium collision cell mode to eliminate interference from possible co-eluting chloride species.

#### 4.10.3 SAFETY CONSIDERATIONS

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

#### 4.10.4 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)—Agilent model 7500ce or 7700x with ICP-MS ChemStation, version B.04.00, or MassHunter, version A.02.01 (with Agilent 7700) or B.01.01 (with Agilent 7500), for instrumental control software. The ICP-MS should be equipped with an octopole reaction cell using He as collision gas and should interface with or be configured to remote start by the HPLC instrument for integrated operation. Chromatographic ICP-MS data is processed using MassHunter. (Agilent Technologies)
- (2) High performance liquid chromatograph (HPLC)—Agilent 1200 series can be controlled with the Instant Pilot control module and should be equipped with a binary pump, an autosampler, degasser and a column compartment. (Agilent Technologies)
- (3) HPLC analytical column—Hamilton PRP-X100 anion exchange column, 250 × 4.1 mm, stainless steel, 10 $\mu$ m particle size (Hamilton cat. no. 79433) with PRP-X100 guard column (Hamilton cat. no. 79446 for 5-pack of cartridges).
- (4) A 6-port switching valve either integrated in the HPLC column compartment or externally provided is used to inject a post column internal standard (ISTD) (See 4.10 Figure 1). The ISTD (2 ng As(V)/g in mobile phase) is delivered to the switching valve using a peristaltic pump (model MP4 from Gilson, Inc.) and a combination of PEEK tubing and standard pump tubing. The HPLC method is modified as indicated in 4.10 Table 2, using the "Timetable" tab that allows for the ISTD injection. A 20-50  $\mu$ L 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.
- (5) Glass or plastic HPLC autosampler vials—use plastic, SUN-Sri 8-425, 600  $\mu$ L, (Fisher cat. no. 14-823-313) of acid-cleaned glass vials to minimize or eliminate possible inorganic arsenic contamination. Check representative vials with blank deionized water injections to determine if inorganic arsenic is detected. If necessary, soak vials using

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2% nitric acid for approximately one hour and rinse 4 times with deionized water. Check again for contamination.

- (6) High density polyethylene (HDPE) amber bottles—for preparation and storage of stock standards.
- (7) Centrifuge tubes—polypropylene conical tubes with caps, 15 mL. Check representative centrifuge tubes placing 1% HNO<sub>3</sub> in the tubes for a period of time and then analyzing this solution for total arsenic to ensure no arsenic is detected above the ASDL.
- (8) Vortex mixer—used to mix diluted fruit juices and fruit juice concentrates.
- (9) Plastic Syringes—used in filtering juice samples, disposable, general use and non-sterile, 5 or 10 mL, Luer-Loc tip.
- (10) Syringe filters—used in filtering juice samples, disposable, 0.45 μm Nylon or PTFE membrane with polypropylene housing and Luer-Loc inlet.
- (11) Analytical balance—precision of 0.0001 g.
- (12) Pipettes—automatic pipettes capable of accurate delivery from 10 μL up to 10.00 mL with assorted tips.
- (13) pH meter—with appropriate calibration buffers (pH 7 and 10).

#### 4.10.5 REAGENTS AND STANDARDS

- (1) Reagent water—Water that meets specifications for ASTM Type I water<sup>1</sup>, such as 18 MΩ•cm de-ionized water (DIW) from a Millipore Milli-Q system.
- (2) Arsenobetaine (AsB)—CAS 64436-13-1, F.W.178.06, purity ≥95%, Fluka (cat. no. 11093).
- (3) Arsenite (As(III)) stock solution—1000 mg/L As(+3) in 2% HCl. Spex CertiPrep cat. no. SPEC-AS3 with the certified value of arsenic traceable to a NIST Standard Reference Material.
- (4) Dimethylarsinic acid (DMA)—CAS 75-60-5, F.W. 138.01, purity ≥98%, Chem Service Inc. (cat. no. PS-51).
- (5) Disodium methyl arsonate hexahydrate(monomethylarsonic acid (MMA)) solid (e.g., Chem Service cat. no. PS-281)—Purity ≥98.5%, formula wt. 291.9.
- (6) Arsenate (As(V)) stock solution—1000 mg/L As(+5) in H<sub>2</sub>O. Spex CertiPrep (cat. no. SPEC-AS5) with the certified value of arsenic traceable to a NIST Standard Reference Material.
- (7) Certified Reference Material (CRM)—National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1643e Trace Elements in Water. Certified for a total arsenic concentration of 58.98 μg/kg.
- (8) Ammonium phosphate dibasic ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>)—CAS 7783-28-0, F.W. 132.06, purity ≥ 99%. Due to arsenic contamination in various lots from several manufacturers, the (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> used in this procedure must be verified to have a low arsenic content (see

Section 4.10.8, 4a-4e).

- (9) Ammonium hydroxide ( $\text{NH}_4\text{OH}$ ), 20%—CAS 1336-21-6, F.W. 35.05, Ultrex II, Ultrapure Reagent, J.T. Baker or equivalent.

#### 4.10.6 REAGENT AND STANDARD PREPARATION

##### Mobile Phase Preparation

Mobile phase, aqueous 10 mM ammonium phosphate dibasic, pH 8.25 ( $\pm 0.05$ )—Add 1.32g  $(\text{NH}_4)_2\text{HPO}_4$  to 1L HPLC reservoir bottle, add 990 g DIW, adjust pH to 8.25 ( $\pm 0.05$ ) 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.

##### Standards Preparation

Calculations for the preparation of standards of arsenic species are based on elemental arsenic concentration (as opposed to the molecular weight of the compound). All standard preparation must be made based on a mass/mass basis. For clarity, report mass fraction of analytical solutions on ng/g basis and mass fraction of test samples on  $\mu\text{g}/\text{kg}$  basis.

##### *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 one 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 one year.

- (1) AsB stock solution, As = 1000  $\mu\text{g}/\text{g}$  in DIW—Tare a 15 mL polypropylene centrifuge tube. Weigh 0.025 g AsB into tube. Add DIW to 10 g total.
- (2) DMA stock solution, As = 1000  $\mu\text{g}/\text{g}$  in DIW—Tare a 15 mL polypropylene centrifuge tube. Weigh 0.0184 g DMA into tube. Add DIW to 10 g total.
- (3) MMA stock solution, As = 1000  $\mu\text{g}/\text{g}$  in DIW—Tare a 15 mL polypropylene centrifuge tube. Weigh 0.039 g MMA into tube. Add DIW to 10 g total.

##### *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  $\mu\text{g}/\text{g}$  standards of MMA and DMA using a calibration curve prepared using a verified total arsenic standard. It is also advisable to analyze a certified reference material such as NIST SRM 1643e Trace Elements in Water, along with the standards for additional confidence. Calculate the As concentration of the

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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 retention times 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 less than 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 reverified for both total As and for species purity periodically (*e.g.*, monthly).

Interconversion of As(III)/As(V) standards is most likely to be seen and comparison to the original analysis for purity is recommended.

- (1) AsB working standard, As = 1 µg/g in H<sub>2</sub>O—Tare 125 g HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 µg/g AsB stock solution into bottle. Dilute to 100 g total with DIW.
- (2) As(III) working standard, As = 1 µg/g in H<sub>2</sub>O—Tare 125 mL HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 mg/L As(III) stock solution into 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<sub>2</sub>O—Tare 125 mL HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 µg/g DMA stock solution into bottle. Dilute to 100 g total with DIW. Analyze for total arsenic as described above and use calculated arsenic concentration in all future calculations.
- (4) MMA working standard, As = 1 µg/g in H<sub>2</sub>O—Tare 125 mL HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 µg/g MMA stock solution into bottle. Dilute to 100 g total with DIW. Analyze for total arsenic as described above and use calculated arsenic concentration in all future calculations.
- (5) As(V) working standard, As = 1 µg/g in H<sub>2</sub>O—Tare 125 mL HDPE or polypropylene bottle. Pipet 100 µL (~0.1 g, accurately weighed) of 1000 mg/L As(V) stock solution into 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) Multi-analyte spiking solution, As(III) DMA MMA and As(V) 1000 ng/g As each—Prepare multi-analyte 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 multi-analyte 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 checked for As(III), As(V), DMA, and MMA concentrations periodically (*e.g.*, monthly).

#### *Calibration Standards:*

Prepare a minimum of four mixed analyte standards in DIW for instrument calibration. Record all weights to calculate standard concentrations in ng/g units. Multi-analyte calibration standards

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and calibration check standards should be prepared fresh on day of use. However, multi-analyte calibration standards may be used for up to 1 week if kept in the dark at 4°C and standard chromatograms do not show evidence of inter-conversion of arsenic species.

- (1) 200 ng/g each As(III), DMA, MMA, and As(V)—Tare a 15 mL HDPE or polypropylene tube. Pipet 1mL (~1.0 g) each of As(III), DMA, MMA and As(V) 1 µg/g working standards into tube. Dilute to 5 g total with DIW and mix thoroughly. This standard is used in preparation of calibration standards, but not analyzed.
- (2) For quantification using calibration plot:
  - a. 10 ng/g each As(III), DMA, MMA, and As(V)—Pipet 500µL (~0.500 g) of the 200 ng/g multi-analyte solution into a tared HDPE or polypropylene tube. Dilute to 10 g total with DIW and mix thoroughly.
  - b. 4ng/g each As(III), DMA, MMA, and As(V)—Pipet 200µL (~0.200 g) of the 200 ng/g multi-analyte standard solution into a tared HDPE or polypropylene tube. Dilute to 10 g total with DIW and mix thoroughly.
  - c. 1 ng/g each As(III), DMA, MMA, and As(V)—Pipet 1.0 mL (~1.0g) of the 10ng/g multi-analyte calibration standard solution into a tared HDPE or polypropylene tube. Dilute to 10 g total with DIW and mix thoroughly.
  - d. 0.4 – 0.5 ng/g each As(III), DMA, MMA, and As(V)—Pipet 500 µL (~0.500 g) of the 10 ng/g multi-analyte 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 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 multi-analyte standard solution into a tared HDPE or polypropylene tube. Dilute to 10 g with DIW and mix thoroughly.

*Additional Standards:*

- (1) Arsenobetaine/As(III) resolution check solution, 5 ng/g each—Pipet 50 µL (~0.05 g) each of AsB and As(III) 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 internal standard 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) Reference material NIST 1643e—Prepare a 15-fold dilution. Pipet 0.5 mL (~0.5 g) of NIST 1643e into a tared HDPE or polypropylene tube. Dilute to 7.5 g total with DIW.

#### 4.10.7 ANALYTICAL SAMPLE PREPARATION PROCEDURE

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



arsenic species in the sample.

### **Commercial juice concentrates**

Measure and record the degree Brix ( $^{\circ}\text{Bx}$ ) in commercial juice concentrates. For commercial concentrates, equivalent inorganic arsenic calculated for ready-to-drink (100% juice) is based on  $^{\circ}\text{Bx}$  in the juice concentrate, inorganic arsenic concentration determined in the juice concentrate, and minimum  $^{\circ}\text{Bx}$  value for 100% juice listed in 4.10 Table 5.

Transfer approximately 1g of concentrate to a tared 15 mL polypropylene centrifuge tube and record mass. Dilute to 6 g with DIW, record the final mass and mix thoroughly. Take this solution through the sample preparation procedure for ready-to-drink juice.

### **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 ready-to-drink juice.

In the absence of manufacturer's directions, measure and record  $^{\circ}\text{Bx}$  in juice concentrates. Transfer approx. 1g of concentrate to a tared 15mL polypropylene centrifuge tube and record mass. Dilute to 4 g total with DIW, record final mass and mix thoroughly. This should approximately reflect typical label instructions for dilution. Take this solution through the sample preparation procedure for ready-to-drink juice.

### **Ready-to-drink (RTD) juices**

Pipet 2mL (~ 2g) juice into a tared 15-mL polypropylene centrifuge tube and record mass of analytical portion. Dilute to 10g with DIW in the tube and record total mass of analytical solution. Cap and mix thoroughly. Draw ~4 mL of the analytical solution into syringe and dispense through a 0.45  $\mu\text{m}$  Nylon or PTFE syringe filter (discard first ~1mL to waste) into a 15mL polypropylene centrifuge tube. Transfer ~1 mL of diluted juice to an autosampler vial prior to analysis. Store unused portion up to 48 hours at 4°C in the event the sample needs to be re-analyzed.

### **Fortified analytical portions (FAP) for RTD samples**

Prepare an analytical portion fortified with As(III), DMA, MMA and As(V) at a level of 25  $\mu\text{g}/\text{kg}$  each by combining 2 mL (~2 g) RTD juice and 0.05 mL (~ 0.05g) of the 1000ng/g multi-analyte 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  $\mu\text{m}$  Nylon or PTFE syringe filter (discard first ~1mL to waste) into a 15mL polypropylene centrifuge tube. Transfer ~1 mL of FAP diluted juice to an autosampler vial for analysis. Store unused portion up to 48 hours at 4°C in the event the sample needs to be re-analyzed.

### **FAPs for commercial juice concentrates**

Prepare an analytical portion fortified with As(III), DMA, MMA and As(V) at a level of

150µg/kg each by combining approximately 1 g concentrate and 0.15mL (~0.15 g) of the 1000 ng/g multi-analyte spiking solution in a 15mL polypropylene centrifuge tube. Dilute to 6 g total with DIW. Pipet 2 mL (~2 g) of this solution to a 15mL polypropylene centrifuge tube, and 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 ~1mL to waste) into a 15mL polypropylene centrifuge tube. Transfer ~1 mL of FAP diluted juice to an autosampler vial for analysis. Store unused portion up to 48 hours at 4°C in the event the sample needs to be re-analyzed.

#### **Method blank (MBK)**

Take 2 g DIW through the sample preparation procedures described above for RTD juice as well as juice concentrates.

#### **4.10.8 DETERMINATION PROCEDURE**

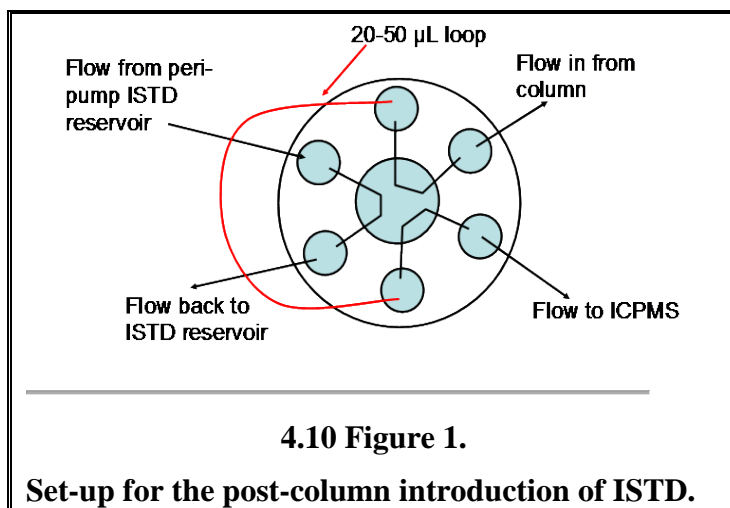
4.10 Table 2 is an example of operating conditions used for this analysis. Operating conditions and settings are suggestions only, will vary with instrument, and should be optimized for the equipment used.

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 composition	10 mM (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>
Plasma gas flow rate (L/min)	15	Mobile phase pH	8.25 (±0.05)
Auxiliary (makeup) gas flow rate (L/min)	0.1	Mobile phase flow rate (mL/min)	1.0
Nebulizer (carrier) gas flow rate (L/min)	1.0	Injection volume (µL)	100
Sampling depth (mm)	8.5	Degasser	ON
Peristaltic pump speed (rps)	0.3 (~1mL/min)	Column temperature	Ambient
Spray chamber temperature (°C)	2	Column compartment time table for introduction of ISTD	0.1 min, Column Position 1 1.0 min, switch to Column Position 2 2.0 min, switch back to Column Position 1
Ions (mass-to-charge ratio)	75 77	Acquisition time	1200 s (20 min)
Dwell time (sec/point)	0.8 s ( <i>m/z</i> 75) 0.2 s ( <i>m/z</i> 77)		
Reaction/collision cell mode	ON, ~2.0 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. 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 analysis using He mode.
- (2) Use peristaltic pump to introduce a 1 ng/g to 10ng/g As solution (in mobile phase) directly into the nebulizer. Ensure signal for *m/z* 75 response is within normal range. Be sure to rinse the ICP-MS system well when finished tuning.
- (3) For post-column As internal standard, connect a small (20 to 50 µL) loop across 2 ports of the 6-way 2 position column switching valve, with LC flow and peristaltic pump internal standard (ISTD) reservoir flow tubes connected similar to 4.10 Figure 1. In the HPLC method timetable, column switching valve should be triggered at 1 min and triggered to switch back at 2 min. Start the peri-pump and verify that no bubbles are present.



- (4) Connect ICP-MS and HPLC. Start HPLC flow (1 mL/min).
- If this is the first time using a source of  $(\text{NH}_4)_2\text{HPO}_4$  for the mobile phase it needs to be tested for arsenic contamination. Follow steps 4a-4e and if acceptable proceed to step 5. If the  $(\text{NH}_4)_2\text{HPO}_4$  source has already been found to be acceptable, follow step 4a and then proceed to step 5.
    - Ensure proper flow and adequate drainage of ICP spray chamber (>1mL/min).
    - Check for leaks.
    - Allow time for column and plasma to equilibrate (>15 min).
    - Ensure that backpressure is acceptable. Increasing backpressure can be indicative of column problems.
  - Set the ICP-MS conditions as in 4.10 Table 2, but rather than setting up an acquisition method, test the following in the tune window.
  - After eluting DIW through the HPLC to the ICP-MS (through the HPLC column) for at least 30 minutes, monitor  $m/z$  75 (integration time of 0.8 seconds) in the tune window for at least 30seconds and record the average response (in CPS).
  - Switch the eluent to the mobile phase (using the new source of  $(\text{NH}_4)_2\text{HPO}_4$ ). After eluting the mobile phase for at least 30 minutes, monitor  $m/z$  75 (integration time of 0.8 seconds) in the tune window for at least 30seconds and record the average response (in CPS).
  - 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 to 1. If it is not, try another source of  $(\text{NH}_4)_2\text{HPO}_4$  or contact the method authors. If it is <6, proceed to step 5.

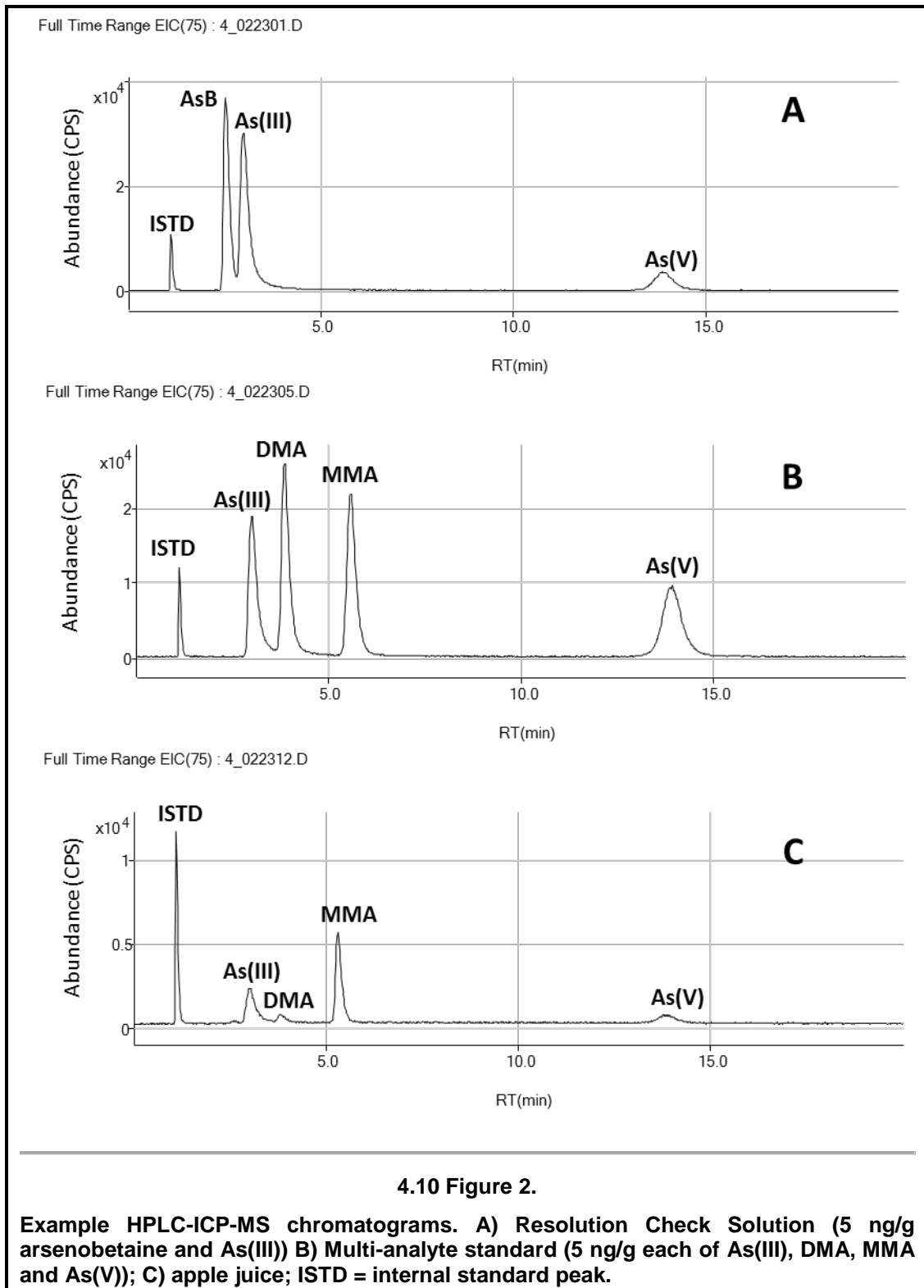
- 
- (5) Set ICP-MS acquisition method for time-resolved collection of  $m/z$  77 and 75 with integration (dwell) times of 0.2 and 0.8 s, respectively, and 1 replicate (read) per point. (see 4.10 Table 2)
  - (6) Analyze a blank (DIW only) to verify that the water and autosampler vials are arsenic-free.
    - a. Monitor instrument conditions to ensure operation is stable and within normal functioning range.
  - (7) Analyze arsenobetaine/As(III) resolution check solution to ensure adequate resolution.
  - (8) Create/edit the sequence file on the ICP-MS data system. Make sure that the injection list and HPLC method on the HPLC controller matches the ICP-MS sequence.
  - (9) Analyze calibration standards, method blanks, check solutions, sample extracts, fortified analytical portions, CRMs and any other quality control (QC) samples. A typical analytical batch is shown in 4.10 Table 3. Check retention times, peak shape and response of both ISTD and arsenic species in the  $m/z$  75 chromatograms. Typical retention times are as follows: As(III)  $2.9 \pm 0.2$  min, DMA  $3.9 \pm 0.2$  min, MMA  $5.5 \pm 0.3$  min, and As(V)  $12.7 \pm 0.5$  min. To some extent, the retention times and peak shapes are dependent on the age and performance of the LC column (especially the As(V) peak). However, significant differences between retention time of standards and samples (including spiked samples) within the same batch are not anticipated and should be investigated and corrected if noted.
    - a. 4.10 Figure 2 shows example chromatograms obtained for the resolution check solution, a 5 ng/g standard, and an apple juice sample.
    - b. Check the  $m/z$  77 chromatograms of samples for indications of possible argon chloride ( $^{40}\text{Ar}^{35}\text{Cl}^+$  at  $m/z$  75 and  $^{40}\text{Ar}^{37}\text{Cl}^+$  at  $m/z$  77) interferences in the  $m/z$  75 chromatograms. Peaks detected in the  $m/z$  77 chromatograms arising from  $^{40}\text{Ar}^{37}\text{Cl}^+$  will also have peaks with matching retention time 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, for example due to selenium species ( $^{77}\text{Se}^+$ ).
  - (10) Integrate  $m/z$  75 chromatograms.
    - a. The settings in 4.10 Table 4 are suggested integration parameters for  $m/z$  75 and provide a recommended starting point for integration. 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 imperative that manual integrations be as consistent as possible, especially within the same analytical batch.
    - b. After settings are correct, choose "Apply to All." This will apply these integration parameters to the ISTD, As(III), As(V), DMA and MMA peaks.
    - c. To eliminate peaks in the  $m/z$  77 trace from being integrated (this causes extended processing time), in the *DA Method Editor / EIC Integration Setup / IntParms (77)*, change the Peak Area [counts] > 10,000.
-

**4.10 Table 3. Typical Analytical Batch Sequence**

Solution	Purpose	QC Criteria
DIW blank	Verify clean auto-sampler vials	≤ ASDL
Resolution check solution	Check separation between unretained species (represented by AsB) and As(III)	near-baseline separation
Multi-analyte calibrations standards	Standardize instrument	$r^2 > 0.99$
MBK 1	Verify absence of contamination	≤ ASDL
SRM NIST 1643e	Demonstrate accuracy	80-120% recovery
Ten (10) analytical solutions (includes replicates and FAPs)	Determine As species conc.	within calibration range, RSD ≤ 15%
Calibration check standard	Verify standardization	85-115% of expected
MBK 2	Verify absence of contamination	≤ ASDL
Ten (10) analytical solutions (includes replicates and FAPs)	Determine As species conc.	within calibration range, RSD ≤ 15%
Calibration check standard	Verify standardization	85-115% of expected
<sup>a</sup> This and any subsequent standard solution injections should agree with previous standard solution injection within ±10%.		

**4.10 Table 4. Recommended Data Analysis Method Editor Settings for *m/z* 75**

<b>General (tab)</b>	
Detector	
Data Point Sampling: 1	Start threshold: 0.3
Smoothing: (Checked)	Stop threshold: 0.5
Detection filtering: 5 point	Peak location: Top
Baseline Allocation	
Baseline reset (#points): > 10	
If leading or trailing edge: < 50	
Baseline preference: Drop else tangent skim	
<b>Peak Filter (tab)</b>	
Peak Area [counts]: >2000 (fill in this bullet only)	
Leave all other input fields unchanged	



- d. The signal to noise ratio (S/N) for questionable chromatographic peaks can be calculated using the MassHunter software. Auto-integrate the questionable peak and verify the proper integration. Manually adjust the integration if necessary. Select the icon, “Set Noise Region” and select 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 re-process the data. Questionable peaks must have a  $S/N > 3$  to be considered detected. Questionable peaks with  $S/N < 3$  shall be treated as non-detected.
- e. Unknown peaks
- If unknown peaks are detected with a signal-to-noise  $> 3:1$ , they should be added to the analyte list (in DA Method Editor) and named Unk X (where X is the approximate retention time). Unknown peaks are defined as peaks that do not match the expected retention times of As(III), As(V), DMA, or MMA.
  - 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.
  - Once integrated, use the unknown’s peak area to estimate approximate concentration of the unknown in the sample (based on elemental arsenic concentration). See §4.10.8.

#### 4.10.9 CALCULATIONS

When using the post-column injection internal standard, the Agilent MassHunter software, when configured properly, will automatically perform internal standard correction calculations. This process can be applied to estimate the concentrations of unknown peaks as well using the MassHunter software. To have the MassHunter software calculate the concentration of a given unknown peak, add the unknown peak to the Data Analysis method, then under FullQuant task go to the Basic Calibration Parameters table and check the box “CIC” which adds a column “Substitute” to the Analyte table below. From the drop-down list choose the nearest eluting arsenic standard and process the data as normal. Optionally, the calculation of the concentration of an unknown peak can be calculated manually using the following equation.

$$\text{Unk}_{\text{conc}} = \frac{\left( \frac{A_{\text{Unk}}}{A_{\text{ISTD}}} \right) - b}{m}$$

where

- $A_{\text{Unk}}$  = integrated peak area of unknown
- $A_{\text{ISTD}}$  = integrated peak area of post-column injection peak (ISTD)
- $m$  = slope of calibration curve of nearest eluting arsenic species
- $b$  = Y-intercept of calibration curve of nearest eluting arsenic species



*Calibration and Analytical Solution Concentrations*

Use a weighted calibration curve ( $1/x^2$ ) to calculate 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).

*Sample Concentrations*

Calculate the concentration of individual arsenic species in the samples as follows:

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

where

$[C_{\text{spl}}]$  = concentration of As(III), As(V), DMA, or MMA in the sample ( $\mu\text{g}/\text{kg}$ )

$[C_{\text{soln}}]$  = concentration of As(III), As(V), DMA or MMA in the analytical solution ( $\text{ng}/\text{g}$ )

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

where

$M_{\text{RTD}}$  = mass of 2-g aliquot of RTD equivalent, either RTD juice or the diluted concentrate (g)

$M_{\text{DIW}}$  = mass of 4-g portion of DIW (g)

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

where

$M_{\text{Conc}}$  = mass of the 1-g aliquot of the juice concentrate (g)

$M_{\text{DIW}}$  = mass of DIW used to dilute juice concentrate (g)

$M_{\text{RTD}}$  = mass of 1-g aliquot of diluted concentrate (g)

$M_{\text{DIW2}}$  = mass of DIW used to prepare analytical solution (g)

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

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

where

$[\text{As(III)}]$  = concentration ( $\mu\text{g}/\text{kg}$ ) of arsenite in RTD juice or juice concentrate

$[\text{As(V)}]$  = concentration ( $\mu\text{g}/\text{kg}$ ) of arsenate in RTD juice or juice concentrate

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

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

$$[C]_{\text{RTD}} = [C]_{\text{conc}} \times \left( \frac{\text{Brix}_{\text{RTD}(\text{min})}}{\text{Brix}_{\text{conc}}} \right)$$

where

- [C]<sub>conc</sub> = concentration of As(III), As(V), DMA, or MMA in the sample (µg/kg)  
 Brix<sub>RTD(min)</sub> = reference minimum °Bx value for single strength (RTD) juice given in 4.10 Table 5  
 Brix<sub>conc</sub> = measured °Bx value of juice concentrate

**4.10 Table 5. Minimum °Bx Values for RTD (single strength) Juices**

In enforcing these regulations, the 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 below where single-strength (100 percent) juice has at least the specified minimum Brix listed below<sup>2</sup>:

Juice	°Bx value for "100% Juice" <sup>a</sup>	Juice	°Bx value for "100% Juice" <sup>a</sup>
Apple	11.5	Mango	13.0
Apricot	11.7	Nectarine	11.8
Blackberry	10.0	Orange <sup>2</sup>	11.8
Blueberry	10.0	Papaya	11.5
Carrot	8.0	Passion Fruit	14.0
Cherry, dark, sweet	20.0	Peach	10.5
Cherry, red, sour	14.0	Pear	12.0
Cranberry	7.5	Pineapple	12.8
Currant (Black)	11.0	Pomegranate	16.0
Currant (Red)	10.5	Prune	18.5
Grape	16.0	Raspberry (Black)	11.1
Grapefruit <sup>b</sup>	10.0	Raspberry (Red)	9.2
Guava	7.7	Strawberry	8.0
Kiwi	15.4	Tomato	5.0

<sup>a</sup> Indicates Brix value unless other value specified.  
<sup>b</sup> Brix values determined by refractometer for citrus juices may be corrected for citric acid.

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#### 4.10.10 QUALITY CONTROL ELEMENTS

##### Prior to the Analysis of Samples

- (1) Verify retention times and purity of single component standards. See §4.10.6 REAGENT AND STANDARD PREPARATION, Working Standards.
- (2) Verify concentrations of DMA and MMA stock standards. See §4.10.6 REAGENT AND STANDARD PREPARATION, Working Standards.
- (3) For each HPLC-ICP-MS instrument used, establish an Analytical Solution Detection Limit (ASDL) and Analytical Solution Quantitation Limit (ASQL) according to EAM§3.2. The limits for arsenic speciation analysis shall be based on the standard deviation 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 ≈ 0.1-0.3 ng/g, for example). ASDL and ASQL are calculated as follows for As(III), As(V), DMA and MMA:

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

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

where

$s$  = standard deviation of replicates (ng/g)

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 it to all species for reporting purposes.

- (4) Calculate the method Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ are calculated using the ASDL or ASQL × 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).

##### Analysis of Samples

Failure of any of the QC elements described below to meet performance criteria shall require an explanation of what was done to correct the problem and may require reanalysis of samples analyzed prior to the loss of method control measures.

The following is the minimum number of quality control 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 shall be used. The calibration curves must be linear over the entire concentration range with  $r^2 > 0.995$ . If there is a failure to meet these criteria, the calibration must be repeated and new working standard preparations may be necessary.

(2) Calibration Check Standard

A calibration check standard shall be analyzed after every 10<sup>th</sup> analytical solution and after the last analytical solution analyzed to monitor retention time and quantitative accuracy. The calibration check standard should be run at a level that is near the mid-point of the analytical calibration curve (*e.g.*, 2 ng/g). If there is a failure to meet the criteria below, the standard may be re-analyzed one time. Additional failures require re-analysis of samples analyzed after the last acceptable calibration check standard.

Control limits for the calibration check standard are  $100 \pm 15\%$  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  $100 \pm 15\%$  individually, as long as their sum as iAs is within  $100 \pm 15\%$ . Control limits for the calibration check standard retention times (RT) are as follows: As(III) RT  $\pm 0.2$  minutes, DMA RT  $\pm 0.2$  minutes, MMA RT  $\pm 0.3$  minutes, and As(V) RT  $\pm 0.5$  minutes when compared to the 10 ng/g calibration standard.

(3) Method Blanks

A minimum of one method blank must be prepared and analyzed with every 10 or fewer analytical solutions analyzed. No arsenic species should be detected in the method blank. 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 method blanks has been identified as a potential source of contamination.

Control limits for the method blank: no arsenic species detected (S/N >3) above the ASDL.

(4) Precision of Replicate Analytical Portions

For each batch and at least once for each separate matrix type (*i.e.* different types of juice), three (3) replicate preparations and analyses of a sample must be performed. If there is a failure to meet the criterion below, the source of the imprecision should be investigated and minimized. Re-analysis of samples analyzed after the last sample analyzed with acceptable precision may be required.

Control limit for relative standard deviation (RSD) is 15% for iAs, DMA and MMA when detected  $\geq$ LOQ.

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

where

s = standard deviation of replicates ( $\mu\text{g}/\text{kg}$ )

$C_{\text{avg}}$  = average concentration of replicates ( $\mu\text{g}/\text{kg}$ )

(5) Fortified Analytical Portion

For each batch and at least once for each separate matrix type, one FAP shall 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) shall be performed by

addition of standards to the juice matrix prior to dilution with DIW. If the recoveries are not acceptable, ensure that the spiking level is appropriate and re-prepare and re-analyze the FAP sample. Re-analysis 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 for the unfortified sample with no significant additional band broadening, shoulders or unexpected peaks. It is not unusual to observe a retention time shift of 0.3 to 0.5 minutes for MMA and As(V) when comparing standard to sample chromatograms.

Control limit for FAP (spike) recovery is  $100 \pm 20\%$  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 spiked sample ( $\mu\text{g/kg}$ )
- $C_x$  = concentration determined in unspiked sample ( $\mu\text{g/kg}$ )
- $C_s$  = concentration of spiking solution ( $\mu\text{g/kg}$ )
- $M_s$  = mass of spiking solution added to sample portion (g)
- $M_x$  = mass of sample portion (g)

Note that spikes of As(III) and/or As(V) must be evaluated based on the total iAs determined (As(III) + As(V)).

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

where

- $C_{\text{As(III)},x+s}$  = As(III) concentration determined in spiked sample ( $\mu\text{g/kg}$ )
- $C_{\text{As(V)},x+s}$  = As(V) concentration determined in spiked sample ( $\mu\text{g/kg}$ )
- $C_{\text{As(III)},x}$  = As(III) concentration determined in unspiked sample ( $\mu\text{g/kg}$ )
- $C_{\text{As(V)},x}$  = As(V) concentration determined in unspiked sample ( $\mu\text{g/kg}$ )
- $C_{\text{As(III)},s}$  = As(III) concentration of spiking solution ( $\mu\text{g/kg}$ )
- $C_{\text{As(V)},s}$  = As(V) concentration of spiking solution ( $\mu\text{g/kg}$ )
- $M_s$  = mass of spiking solution added to sample portion (g)
- $M_x$  = mass of sample portion (g)

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(6) Reference Material

For each batch, one CRM or in-house reference material must be prepared and analyzed. Unfortunately no juice reference material exists which is certified for arsenic. Since juice is comprised largely 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  $\sim 15\times$  to dilute down the acid content of this CRM. Control limit for the reference material is  $100 \pm 20\%$  for the mass balance with the certified total As value of  $58.98 \mu\text{g}/\text{kg}$ .

(7) Mass Balance

A mass balance shall 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 4.7). Often the 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, re-analysis 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.

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

Control limit for Mass Balance is 65% -115%.

#### 4.10.11 REPORT

Report results only when quality control criteria for a batch have been satisfactorily met. Report results for DMA, MMA, and Total Inorganic Arsenic (As(III) + As(V)) that are  $\geq \text{LOQ}$  as the mass fraction determined followed by the units of measurement. Report results that are  $\geq \text{LOD}$  and  $< \text{LOQ}$  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  $< \text{LOD}$  as 0 followed by the units of measurement. Note that species present at concentrations  $< \text{LOD}$  will probably not be picked up by the auto-integrator. Due to variability between laboratories and instrumentation, values for LOD and LOQ should be determined in each instrument system at each laboratory. The values in 4.10 Table 1 are presented only as examples.

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*Example: As(V) LOQ =  $3.5 \mu\text{g}/\text{kg}$ ; As(V) LOD =  $0.45 \mu\text{g}/\text{kg}$ . Levels found for three different RTD juice samples were  $5 \mu\text{g}/\text{kg}$ ,  $1 \mu\text{g}/\text{kg}$  and  $0.2 \mu\text{g}/\text{kg}$ .*

*$5 \mu\text{g}/\text{kg}$  is  $\geq \text{LOQ}$ ; report  $5 \mu\text{g}/\text{kg}$*

*$1 \mu\text{g}/\text{kg}$  is  $\geq \text{LOD}$  but also  $< \text{LOQ}$ ; report  $1 \mu\text{g}/\text{kg}$  (TR)*

*$0.2 \mu\text{g}/\text{kg}$  is  $< \text{LOD}$ ; report  $0 \mu\text{g}/\text{kg}$*

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#### 4.10.12 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>.

*Single lab validation.* The method was validated by analyses of reference materials, recovery of analyte, and precision measurements<sup>7</sup>. 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  $\geq$  LOQ. Recovery of added analyte was in the range of 80-120% for all four species in all juices tested. iAs results for NIST SRM 1640 Trace Elements in Natural Water agreed with the certificate value for total arsenic, differing by less than 0.1%. Results for DMA, MMA, 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 (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).

*QC data from surveys.* FDA conducted two surveys in 2011 using this method to gather information on arsenic species in fruit juices<sup>8</sup>. The analysis was performed in two labs, and the QC data from those surveys have been summarized. Values for iAs found in NIST 1643e were 54-63  $\mu\text{g}/\text{kg}$  (58.83  $\mu\text{g}/\text{kg}$  average, 99.8% of certified total,  $n=34$ ). The overall average mass balance was 85% (range 64-111%) in juice samples. %RSD ranged from 1.1 to 7.5% for iAs, DMA, MMA  $\geq$  LOQ in juices for which three or more replicate analytical portions were analyzed. Fortified analytical portions 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.

*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.

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