

Elemental Analysis Manual

for Food and Related Products

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4.11 Arsenic Speciation in Rice and Rice Products Using High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometric Determination

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4.11.1 SCOPE AND APPLICATION

The method provides a procedure for arsenic speciation analysis of rice and rice-containing food products including white and brown rice, Basmati rice, rice breakfast cereals, rice cakes, and rice milk. The method utilizes high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) to determine inorganic arsenic (iAs) as the sum of two inorganic forms of arsenic, arsenite (AsIII) and arsenate (AsV). Additionally, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are determined and arsenobetaine (AsB) is used as a representative unretained arsenic-containing species in order to

ensure adequate separation from AsIII. 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.

4.11.2 SUMMARY OF METHOD

Analytical samples of rice and rice-containing food products are composited. Portions of the composite are mixed with a 0.28M HNO₃ solution and heated at 95°C for 90 minutes. The extracts are initially diluted with DIW, centrifuged, filtered, and then diluted further while adjusting pH prior to analysis by HPLC-ICP-MS. The arsenic species are separated using an isocratic anion exchange HPLC separation with a mobile phase of 10 mM ammonium hydrogen phosphate, dibasic at pH 8.25 (±0.05). The ICP-MS is used as an arsenic-specific detector monitoring *m/z* 75 for arsenic-containing chromatographic peaks. Arsenic species are identified by peak retention time match with arsenic species standards. Quantification of arsenic species is achieved using peak areas and an external calibration curve. Signal drift is corrected by adding a post column internal standard.

4.11.3 SAFETY CONSIDERATIONS

Use appropriate PPE including safety glasses, gloves and lab coat when handling concentrated solutions containing nitric acid or 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.

4.11.4 EQUIPMENT AND SUPPLIES

Disclaimer: The use of trade names in this method constitutes neither endorsement nor recommendation by the U. S. Food and Drug Administration. Equivalent performance may be achievable using apparatus and materials other than those cited here.

- (1) ICP-MS, Agilent model 7500ce or 7700 with ICP-MS ChemStation, version B.04.00 instrumental control software. The ICP-MS should be equipped with octopole reaction cell using He as collision gas or equivalent. Instrument should interface with or be configured to remote start by HPLC instrument for integrated operation. Chromatographic ICP-MS data is processed using MassHunter, version B.01.01. (Agilent Technologies)
- (2) HPLC Agilent 1100 series or equivalent is controlled with the Instant Pilot control module and it is equipped with a binary pump, an autosampler, degasser and a column compartment. (Agilent Technologies)
- (3) An integrated 6-port switching valve found in the HPLC column compartment is used to inject a post column internal standard (ISTD) (See Figure 1). The ISTD (2 ng AsV/g in

mobile phase) is delivered to the switching valve using a peristaltic pump (model MP4 from Gilson, Inc. or equivalent) and a combination of PEEK tubing and standard pump tubing. The LC method is modified as indicated in Table 1, using the "Timetable" tab that allows for the ISTD injection. A 20-50 μ L injection loop is used. For the peristaltic pump, an approximate flow rate of 0.1mL/min should be used as it must refill the injection loop between injections.

- (4) Analytical and guard columns. Model PRP X100 column, 4.1 X 250 mm, stainless steel, 10 μ m. (Hamilton part #: 79433) and respective guard column (Hamilton part #: 79446, 5-pack of cartridges)
- (5) HDPE amber bottles for preparation of stock standards.
- (6) Centrifuge tubes. 50 and 15 mL polypropylene conical tubes with caps. Check representative centrifuge tubes with blank deionized water injections to determine if inorganic arsenic is detected.
- (7) Pipettes. Automatic pipettes capable of accurate delivery from 10 μ L up to 10.00 mL with assorted tips.
- (8) Syringes. Disposable, general use and non-sterile, 10 mL, Luer-Lok tip.
- (9) Syringe filters: Disposable, 0.45 μ m Nylon or PTFE membrane with polypropylene housing and Luer-Lok inlet.
- (10) Analytical balance with precision of 0.0001 g. (Mettler-Toledo model AG204 or equivalent)
- (11) Centrifuge. Bench top centrifuge capable of 3000 rpm with buckets and carriers for 50 mL tubes (Marathon 8K, Fisher or equivalent)
- (12) Auto-sampler vials and caps. Use plastic (Sun-Sri, 0.3 mL, 8-425 #14-823-313, Fisher) or acid-washed 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 wash vials using 2% nitric acid soak for approximately one hour and rinse 4X with deionized water.
- (13) Block digestion system. Model miniMOD, 24-place, ambient to 180°C (CPI International) or equivalent.
- (14) Standard laboratory oven: Gravity-flow convection oven. Temperature range: 50-225°C.
- (15) Desiccators to store dry samples.
- (16) Centrifugal Grinding Mill (Retsch model ZM100) or similar laboratory grade mill.
- (17) Vortex mixer.
- (18) pH meter (Accumet Basic, Fisher Scientific) or equivalent with appropriate calibration buffers (pH 7 and 10).

4.11.5 REAGENTS AND STANDARDS

- (1) De-ionized water (DIW) MilliQ 18 MΩ cm de-ionized water or equivalent.
- (2) Nitric acid (HNO₃), CAS 7697-37-2, F.W. 63.01, OPTIMA ultra-pure grade, Fisher Scientific or equivalent.
- (3) Ammonium phosphate dibasic ((NH₄)₂HPO₄), CAS 7783-28-0, F.W. 132.06, purity ≥ 99.99%, Sigma-Aldrich (cat. no. 379980) or equivalent.
- (4) Ammonium hydroxide (NH₄OH), 20% CAS 1336-21-6, F.W. 35.05, Ultrex II, Ultrapure Reagent, J.T. Baker or equivalent.
- (5) Hydrogen Peroxide (H₂O₂), 30% CAS 7722-84-1, F.W. 34.01, OPTIMA ultra-pure grade, Fisher Scientific or equivalent.
- (6) Arsenite Stock Standard (AsIII), 1000 mg/L As(+3) in 2% HCl, Spex Certiprep (cat. no. SPEC-AS3) or equivalent standard with the certified value of arsenic traceable to a NIST Standard Reference Material.
- (7) Arsenate Stock Standard (AsV), 1000 mg/L As(+5) in H₂O, Spex Certiprep (cat. no. SPEC-AS5) or equivalent standard with the certified value of arsenic traceable to a NIST Standard Reference Material.
- (8) Dimethylarsinic acid (DMA), CAS 75-60-5, F.W. 138.01, purity ≥98%, ChemService Inc. (cat. no. PS-51)
- (9) Disodium methyl arsonate hexahydrate (monomethylarsonic acid, MMA), CAS 144-21-8 or 5967-62-4, F.W. 291.9, purity ≥98%, ChemService Inc. (cat. no. PS-281)
- (10) Arsenobetaine (AsB), CAS 64436-13-1, F.W.178.06, purity ≥95%, Fluka (cat. no. 11093)
- (11) Standard Reference Material (SRM) 1568a and/or 1568 Rice Flour, certified for total arsenic concentrations, National Institute of Standards and Technology (NIST)
- (12) Certified Reference Material (CRM) 7503-a Arsenic Compounds and Trace Elements in White Rice Flour, National Metrology Institute of Japan (NMIJ), available from Waco Chemicals USA
- (13) Standard Reference Material (SRM) 1643e, Trace Elements in Water, National Institute of Standards and Technology (NIST)

4.11.6 REAGENT AND STANDARD PREPARATION

Mobile Phase Preparation:

The chromatographic mobile phase consists of aqueous 10 mM ammonium phosphate dibasic at a pH of 8.25 (±0.05). Prepare by adding 1.32 g (NH₄)₂HPO₄ to a 1-L HPLC reservoir bottle (or 1-L volumetric flask) containing 990 g DIW. Adjust the pH to 8.25 (±0.05) with 20% ammonium hydroxide, and fill to 1000 g (or 1-L) with DIW. Mobile phase should be prepared

fresh daily as necessary to minimize changes in pH from the atmosphere.

Extraction Solution (0.28M HNO₃):

This solution is used to extract the arsenic species from the samples. Prepare by adding 25.3 g of HNO₃ to approximately 500 mL of DIW and diluting to 1000 g (or 1-L).

pH Adjustment Solution Preparation:

This solution is used to dilute the sample extract 1:3 and adjust the pH of the sample extracts. Adjusting sample pH more closely to that of the mobile phase improves the chromatography and prolongs the HPLC column lifetime. The pH adjustment solution is prepared by adding approximately 0.9 g of 20% ammonium hydroxide to 100 g of mobile phase. The pH of this solution should be adjusted to 9.85 ± 0.05 . This solution should be prepared fresh daily as necessary to minimize changes in pH from the atmosphere.

Standard 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.

Stock Standards:

All stock standard solutions are prepared in water. Stock standards should be stored refrigerated. Stock standards should be brought to room temperature and mixed well before using. **Record all weights to calculate standard concentrations.**

- (1) DMA stock standard. $\approx 1000 \mu\text{g/g}$ As in the form of DMA, Accurately weigh ≈ 9.2 mg dimethylarsinic acid into a tared 30-mL HDPE bottle and add 5 g DIW (accurately weighed). Cap and shake by hand to mix.
- (2) MMA stock standard. $\approx 1000 \mu\text{g/g}$ As in the form of MMA, Accurately weigh ≈ 19 mg disodium methyl arsonate hexahydrate into a tared 30-mL HDPE bottle and add 5 g DIW (accurately weighed). Cap and shake by hand to mix.
- (3) AsB stock standard. $\approx 1000 \mu\text{g/g}$ As in the form of AsB, Accurately weigh ≈ 12 mg arsenobetaine into a tared 30-mL HDPE bottle and add 5 g DIW (accurately weighed). Cap and shake by hand to mix.

Working Standards:

The arsenic concentration of the DMA and MMA standards must be verified, typically using ICP-MS analysis. It is recommended that the AsIII, and AsV concentrations also be verified, but this is not required. Determine the total arsenic concentrations in $1 \mu\text{g/g}$ 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 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 purity of the working standards (AsIII, AsV, 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 AsIII, AsV, DMA and MMA may be kept indefinitely in tightly sealed polypropylene containers stored in the dark at 4°C, but should be rechecked for both total As and for species purity periodically (*e.g.*, monthly). Interconversion of AsIII/AsV standards is most likely to be seen and comparison to the original analysis for purity is recommended.

- (1) AsIII working standard. 1 µg/g As of AsIII. Prepare AsIII working standard by weight in DIW using the 1000 mg/L AsIII commercial standard. Pipet 100 µL (0.1 g) of 1000 mg/L As(III) stock solution into a 125-mL HDPE bottle. Dilute to 100 g total with DIW. This standard does not require concentration verification because the stock is traceable to a NIST SRM.
- (2) AsV working standard. 1 µg/g As of AsV. Prepare AsV working standard by weight in DIW using the 1000 mg/L AsV commercial standard. Pipet 100 µL (0.1 g) of 1000 mg/L As(V) stock solution into a 125-mL HDPE 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) MMA working standard. 1 µg/g As of MMA. Prepare MMA working standard by weight in DIW using the 1000 µg/g MMA stock standard. Pipet 100 µL (0.1 g) of 1000 µg/g MMA stock standard into a 125-mL HDPE 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) DMA working standard. 1 µg/g As of DMA. Prepare DMA working standard by weight in DIW using the 1000 µg/g DMA stock standard. Pipet 100 µL (0.1 g) of 1000 µg/g DMA stock standard into a 125-mL HDPE 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) AsB working standard. 1 µg/g As of AsB. Prepare AsB working standard by weight in DIW using the 1000 µg/g AsB stock standard. Pipet 100 µL (0.1 g) of 1000 µg/g AsB stock standard into a 125-mL HDPE bottle. Dilute to 100 g total with DIW.
- (6) Multi-analyte spiking standard. 1 µg/g As each of AsIII, AsV, MMA, and DMA. Prepare multi-analyte spiking standard by weight in DIW using the 1000 µg/g DMA and MMA stock standards and the 1000 mg/L AsIII and AsV stock standards. Pipet 100 µL (0.1 g) of each stock standard into a 125-mL HDPE bottle. Dilute to 100 g total with DIW. Use the calculated arsenic concentrations from the 1 µg/g DMA and MMA working standards above in the calculations of DMA and MMA concentrations of this standard. For AsIII and AsV, use the labeled concentration. This multi-analyte standard may be used for a period of one month as long as it is stored in a tightly sealed polypropylene amber container at 4°C.

Calibration Standards:

Prepare a minimum of four mixed analyte standards in DIW for instrument calibration. **Record all weights to calculate standard concentrations.** Multi-analyte calibration standards 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 at 4°C in the dark and standard chromatograms are inspected for possible interconversion of arsenic species.

- (1) 200 ng/g AsIII, DMA, MMA, and AsV. Take 1.0 g of each 1 µg/g working standard and dilute to 5g with DIW. Mix thoroughly. **THIS STANDARD SHOULD NOT BE USED FOR CALIBRATION.**
- (2) 20 ng/g AsIII, DMA, MMA, and AsV. Take 0.5 g of 200 ng/g AsIII, DMA, MMA, and AsV, and dilute to 5 g with mobile phase. Mix thoroughly.
- (3) 5 ng/g AsIII, DMA, MMA, and AsV. Take 0.125 g of 200 ng/g AsIII, DMA, MMA, and AsV, and dilute to 5 g with mobile phase. Mix thoroughly.
- (4) 1 ng/g AsIII, DMA, MMA, and AsV. Take 0.25 g of 20 ng/g AsIII, DMA, MMA, and AsV, and dilute to 5 g with mobile phase. Mix thoroughly.
- (5) 0.25-0.5 ng/g AsIII, DMA, MMA, and AsV. For example, take 0.25 g of 5 ng/g AsIII, DMA, MMA, and AsV, and dilute to 5 g with mobile phase for 0.25 ng/g. Mix thoroughly. Note: this standard should be at or slightly above the laboratory's ASQL.
- (6) Calibration check standard. 10 ng/g AsIII, DMA, MMA, and AsV. Take 0.5 g of 200 ng/g AsIII, DMA, MMA, and AsV, and dilute to 10 g with mobile phase.

Additional Standards:

- (1) Internal standard at 2 ng/g AsV. Take 1 g of 1 µg/g AsV and dilute to 500 g with mobile phase. This solution is injected post-column and used as an internal standard (ISTD) to monitor and correct for signal drift. Fresh ISTD solution should be re-prepared if the signal obtained decreases significantly.
- (2) Resolution check standard. 5 ng/g AsIII and AsB. Take 0.5 g of the AsIII working standard (1 µg/g AsIII) and 0.5 g of AsB working standard (1 µg/g AsB) and dilute to 100 g with mobile phase. A new resolution check standard should be prepared when ~50% of AsIII has been converted to As(V).
- (3) Standard for LOD and LOQ Determination. Prepare a standard containing AsIII, DMA, MMA, and AsV at a concentration between the approximate ASDL and ASQL (*e.g.*, 0.2 ng/g each in mobile phase).

4.11.7 ANALYTICAL SAMPLE PREPARATION PROCEDURE

Record all weights (to 0.0001g) to calculate concentration of arsenic species in the sample. If not received as ground, homogenized composites, solid samples must be ground and homogenized prior to analysis taking care not to contaminate the samples.

Rice, Basmati Rice, and Rice Cereals

- (1) Dry at least 10g of the homogenized and ground samples of rice and Basmati rice in a laboratory oven at 85°C until a constant weight is obtained. Calculate the moisture content of the original sample. Store dried samples in a dessicator. Samples of rice cereal do not

require drying.

- (2) Weigh ≈ 1.0 g of the ground composite sample into a pre-weighed 50mL centrifuge tube (with lid), record weights.
- (3) Add 10 mL 0.28 M HNO_3 and record weight. Vortex for 10-30 sec. (It is helpful to do this in two steps; adding 5 mL of 0.28 M HNO_3 followed by a quick vortexing and then rinsing the walls of the container with the other 5 mL of 0.28 M HNO_3).
- (4) Cap all tubes tightly and place in preheated block digestion system at 95°C for 90 min.
- (5) Let samples cool. Add approximately 6.7 g DIW and record weights.
- (6) Centrifuge samples at 3000 rpm for 10 min.
- (7) Filter the supernatant with a 0.45 μm Nylon syringe filter attached to a 10 mL disposable syringe. Discard the first ~ 1 mL through the filter to waste.
- (8) Dilute 1g of filtrate from one method blank and one sample extract with 2g of pH Adjustment Solution. Confirm that the pH of the resulting 1:3 dilution has a pH between 6 and 8.5. If not within this range, re-prepare or adjust the pH of the pH Adjustment Solution until the proper pH range is achieved in method blanks and sample extracts. Only after this is confirmed can the pH Adjustment Solution be used to dilute the remaining samples.
- (9) For remaining samples, transfer 1g of filtrate to a 15 mL tared centrifuge tube. Add 2g of pH Adjustment Solution. Record the initial and final weight. Transfer a portion solution to a plastic or an acid-washed glass autosampler vial for analysis by HPLC-ICP-MS.

Rice Cakes

- (1) Weigh ≈ 0.67 g of the ground composite sample into a pre-weighed 50mL centrifuge tube (with lid), record weights.
- (2) Add 10 mL 0.28 M HNO_3 and record weight. Vortex for 10-30 sec. (It is helpful to do this in two steps; adding ~ 6.7 mL of 0.28 M HNO_3 followed by a quick vortexing and then rinsing the walls of the container with the other ~ 3.3 mL of 0.28 M HNO_3).
- (3) Cap all tubes and place in the block digestion system at 95°C for 90 min.
- (4) Let samples cool. Add approximately 6.7 g DIW and record weights.
- (5) Centrifuge samples at 3000 rpm for 10 min.
- (6) Filter the supernatant with a 0.45 μm Nylon syringe filter attached to a 10 mL disposable syringe. Discard the first ~ 1 mL through the filter to waste.
- (7) Transfer 1g of filtrate to a 15 mL centrifuge tube. Add 2g of pH Adjustment Solution. Record the initial and final weight. Transfer a portion solution to a plastic or an acid-washed glass autosampler vials for analysis by HPLC-ICP-MS.

Rice Milk, Brown Rice Syrup, etc.

Procedures for other rice-containing foods will be added as they become available. .

4.11.8 QUALITY CONTROL SAMPLE PREPARATION PROCEDURES

Record all weights (to 0.0001g) to calculate concentration of arsenic species in the dry sample.

- (1) Method blanks (MB). Take 1 g DIW through the entire sample preparation procedure described in section 4.11.7. Note: the pH of the method blank after addition of the pH Adjustment Solution should be between 6 and 8.5. If not, adjust the pH Adjustment Solution until this pH range is met; only after this is confirmed can the pH Adjustment Solution be used to dilute the remaining samples.
- (2) Fortified analytical portions (FAP). Prepare an analytical portion fortified with AsIII, AsV, DMA, and MMA at a level of approximately 50% of the total arsenic concentration found in the sample. For example, a sample containing 100 ng/g total arsenic would be fortified at a level of 50 ng/g each (AsIII, AsV, DMA, and MMA) by taking 1 g of sample and spiking with 50 µL of 1 µg/g multi-analyte spiking standard. This has generally been found to be an effective spike level. Alternatively, the spiking level should be kept between 50% and 150% of the specific analyte level detected.
- (3) Fortified method blanks (FMB). Prepare a fortified method blank (1 g DIW) containing AsIII, DMA, MMA, and AsV at a concentration of approximately 50 ng/g each (AsIII, AsV, DMA, and MMA) by taking 1 g of DIW and spiking with 50 µL of 1 µg/g multi-analyte spiking standard. The FMB is an optional quality control sample.
- (4) Oxidized sample extracts. To investigate possible interferences on the AsIII determination, a second portion of at least one sample extract from each batch will require an additional analysis. AsIII in the sample extract is oxidized to AsV using 30% hydrogen peroxide prior to analysis. Prepare oxidized sample extracts by taking 1g of filtered extract + 2g of pH adjustment solution and adding ~ 0.4g of H₂O₂. Record all weights. Shake well and allow to stand for at least 5-10 minutes prior to analysis of this mixture.

4.11.9 DETERMINATION PROCEDURE

Table 1 provides operating conditions used for this analysis. Operating conditions and settings may be optimized for the equipment used.

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 a 1 ng/g to 10 ng/g As solution in mobile phase directly into the nebulizer. Ensure signal for m/z 75 response is within normal range for the instrument being used. Be sure to rinse the ICP-MS system well when finished tuning.
- (3) For post-column As internal standard, connect a small (20-50 µL) loop across 2 ports of the 6-way 2 position column switching valve, with LC flow and peri-pump IS reservoir flow tubes connected similar to figure below. In the HPLC method timetable, column switching valve should be triggered at 1.0 min and triggered to switch back at 2.0 min. Start the peri-pump and verify that no bubbles are present.

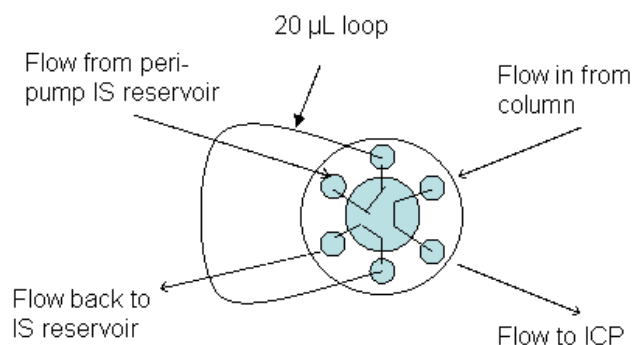


Figure 1. Set-up for the post-column introduction of ISTD.

- (4) Connect ICP-MS and HPLC. Start HPLC flow (1 mL/min).
 - a. Ensure proper flow and adequate drainage of ICP spray chamber (>1 mL/min).
 - b. Check for leaks.
 - c. Allow time for the HPLC column and plasma to equilibrate (>15 min).
 - d. Ensure that backpressure is acceptable. Increasing backpressure can be indicative of column problems.
- (5) Set ICP-MS acquisition method for time-resolved collection of m/z 75 and 77 with integration (dwell) times of 0.8 and 0.2 s, respectively, and 1 replicate (read) per point.
- (6) Analyze a blank (DIW only) solution to verify that water and chromatography vials are arsenic-free.
- (7) Analyze resolution check standard containing 5 ng/g As(III) and AsB to ensure adequate resolution.
- (8) Create/edit the sequence file on the ICP-MS data system. Make sure that the injection list and LC method on the LC controller matches the ICP-MS sequence.
- (9) Analyze calibration standards, method blanks, check standards, sample extracts, fortified analytical portions, CRMs and any other QC samples. An example analytical batch is shown in Table 2.
 - a. Check retention times, peak shape and response of both ISTD and arsenic species in the m/z 75 chromatograms. To some extent, the retention times and peak shapes are dependent on the age and performance of the LC column (especially the AsV peak). However, significant differences between retention time (RT) of standards and samples (including spiked samples) are not anticipated. See Table 3 for typical variations in RT.
 - 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 RT in the m/z 75 chromatograms.
 - c. Table 3 provides example retention times. These may vary somewhat from column to column, especially for AsV depending on the age/condition of the column used. Example sensitivities (slope of the calibration curve), ASDLs

(analytical solution detection limit) and ASQLs (analytical solution quantitation limit) obtained using an Agilent model 7700 ICP-MS for AsIII, AsV, DMA, and MMA are also presented in Table 3.

- d. Figure 2 provides example chromatograms obtained for the resolution check standard, a 5 ng/g calibration standard, and a SRM 1568a Rice Flour diluted extract.

(10) Integrate m/z 75 chromatograms.

- a. The following settings for m/z 75 in the DA Method Editor → Analyte List (not EIC Integration Setup) → Int/Parms. column are a recommended starting point for integration. All chromatograms should be visually inspected and manually integrated when necessary to ensure consistency and accuracy of integration.

General (tab)	
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	
Peak Filter (tab)	
Peak Area [counts] >2000 (fill in this bullet only)	
Leave all other input fields unchanged	

After settings are correct, choose “Apply to All.” This will apply these integration parameter to the ISTD, AsIII, DMA, MMA, and AsV peaks.

- b. 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 → Int/Parms. (77), change the Peak Area [counts] > 10,000.
- c. Unknown peaks
 - i. If unknown peaks are detected, they should be added to the analyte list (in DA Method Editor) and named Unk X (where X is the approximate retention time).
 - ii. 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.
 - iii. Once integrated, use the unknown’s peak area to estimate approximate concentration of the unknown (based on elemental arsenic concentration). See below.

Table 1. Typical HPLC-ICP-MS Operating Parameters

ICP-MS Conditions	
RF power	1500 W
Plasma gas flow	15 L/min
Auxilliary (makeup) gas flow	0.1 L/min
Nebulizer (carrier) gas flow	1.1 L/min
Nebulizer type	Glass concentric
Sampling depth	8.5 mm
Peristaltic pump speed	0.3 rps
Spray chamber temp.	2°C
Collision cell	He @ 2.0 mL/min
Data acquisition mode	Time-resolved, m/z 75 for $^{75}\text{As}^+$, and m/z 77 for $^{40}\text{Ar}^{35}\text{Cl}^+$
Dwell time	0.8 s (m/z 75), 0.2 s (m/z 77)
Replicates per ion	1
HPLC Conditions	
Mobile phase composition	10 mM $(\text{NH}_4)_2\text{HPO}_4$
Mobile phase pH	8.25 (± 0.05)
Mobile phase flow rate	1 mL/min
Injection volume	100 μL
Degasser	On
Column temperature	Ambient
Column compartment time table	Beginning of run, 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)

Table 2. Typical Analytical Batch Sequence

Run	Purpose
Vial DIW Blank	Verify clean autosampler vials
Resolution Check Solution	Check separation between unretained species (represented by AsB) and AsIII
0.25-0.5, 1, 5, 20 ng/g Calibration Stds	Standardize instrument
Method Blank 1	Verify absence of contamination
Rice CRM/SRM (SRM 1568a/1568 or CRM 7503-a)	Demonstrate accuracy
Ten (10) sample solutions (includes replicate preparations and FAPs)	Determine arsenic species/concentrations
10 ng/g Calibration Check Standard	Verify standardization
Method Blank 2	Verify absence of contamination
Ten (10) sample solutions (includes replicate preparations and FAPs)	Determine arsenic species/concentrations
10 ng/g Calibration Check Standard	Verify Standardization
Oxidized Sample Extract	Investigate potential AsIII interference

Table 3. Typical Retention Times, Sensitivities, ASDLs and ASQLs, and Method LODs and LOQs for Rice

Species	Retention Time (min)	Sensitivity (peak area /ppb)	ASDL (ng/g)	ASQL (ng/g)	Method LOD (µg/kg)	Method LOQ (µg/kg)
AsIII	2.9 ± 0.2	55000	0.05	0.4	2.5	20
DMA	3.9 ± 0.2	71000	0.05	0.4	2.5	20
MMA	5.5 ± 0.3	65000	0.05	0.4	2.5	20
AsV	12.7 ± 0.4	64000	0.05	0.4	2.5	20

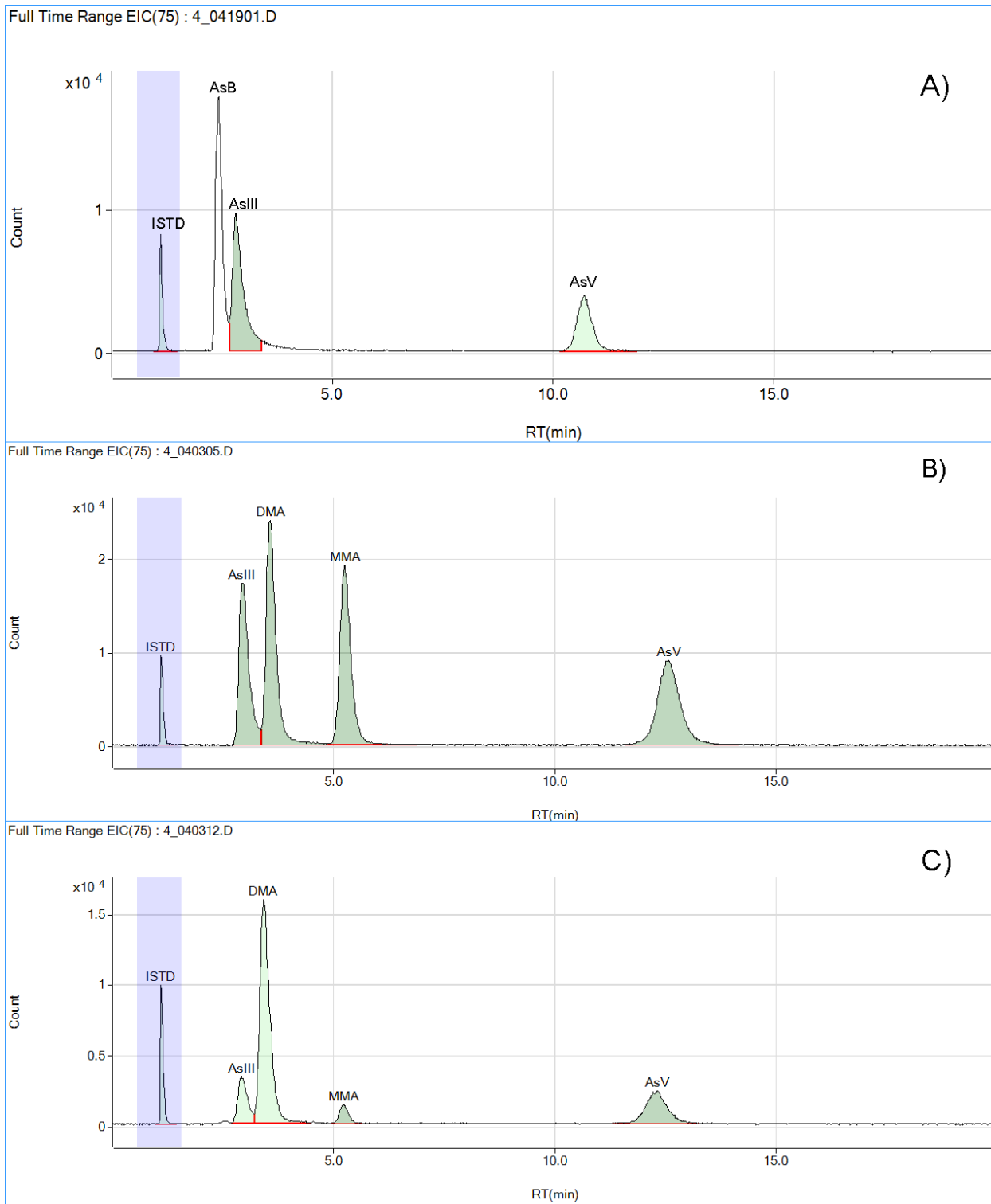


Figure 2. Example HPLC-ICP-MS chromatograms. A) Resolution Check Solution (5 ng/g arsenobetaine and AsIII) Note: some of the AsIII has converted to AsV in this example, B) multi-analyte calibration standard (5 ng/g each of AsIII, DMA, MMA, and AsV); C) SRM 1568a Rice Flour diluted extract; ISTD = internal standard flow injection peak

4.11.10 CALCULATIONS

Peak Area Integration:

When using post-column injection internal standard, the Agilent MassHunter software, when configured properly, will automatically perform internal standard correction calculations. However, this is only applicable to peaks for which an external calibration curve is generated; therefore, concentrations of unknown peaks (Unk_{conc}) will have to be calculated manually using the equation.

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

Where

A_{Unk} = integrated peak area of unknown

A_{ISTD} = integrated peak area of post-column injection peak (ISTD)

m = slope of trendline of calibration curve of nearest eluting arsenic species.

b = Y-intercept of plot 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 force the y-intercept through zero (use the IGNORE option for Intercept). This calculation is typically accomplished within the MassHunter software.

Sample Concentrations:

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

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

Where

$[C_{spl}]$ = The concentration of AsIII, AsV, DMA, or MMA in the sample ($\mu g/kg$)

$[C_{soln}]$ = The concentration of AsIII, AsV, DMA or MMA in the analytical solution.

$$Dilution\ Factor = \left(\frac{(M_{Extract} + M_{pH\ Adjustment\ Solution})}{M_{Extract}} \right) \times \left(\frac{M_{sample + nitric + water}}{M_{sample}} \right)$$

Where

$M_{Extract}$ = mass of the extract

$M_{pH\ Adjustment\ Solution}$ = mass of the pH Adjustment Solution

$M_{sample + nitric + water}$ = mass of the sample + nitric acid + water

M_{sample} = mass of the sample

Calculate the concentration of inorganic arsenic (iAs) in the rice product sample as follows:

$$[iAs] = [AsIII] + [AsV]$$

Where

[AsIII] = concentration (µg/kg) of arsenite in rice product

[AsV] = concentration (µg/kg) of arsenate in rice product

Note: [AsIII] and As[V] results \geq LOD are used in the calculation of [iAs]

4.11.11 QUALITY CONTROL ELEMENTS

Prior to the Analysis of Samples

- (1) Verify retention times and purity of single component standards. See section 4.11.6 REAGENT AND STANDARD PREPARATION, Working Standards
- (2) Verify concentrations of DMA and MMA standards. See section 4.11.6 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) using FDA's Elemental Analysis Manual (EAM), section 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 \approx 0.1-0.3 ng/g, for example). Briefly, ASQL will be approximately equal to 30 x standard deviation, while ASDL will be approximately $2 \times t_{0.95} \times \text{standard deviation} \times \sqrt{1+1/n}$. 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 (*e.g.*, In Table 3, the largest ASDL and ASQL obtained for the four species were 0.05 and 0.4 ng/g, respectively.)
- (4) Calculate the method Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ are calculated using the ASDL or ASQL x nominal dilution factor. This will be dependent on the dilution factor used for each sample type (*e.g.*, for rice the LOD = ASDL x 50).

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

A minimum of four calibration levels shall be used. The calibration curves must be linear over the entire concentration range with $r^2 > 0.99$. 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 10th sample solution and after the last sample 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.*, 10 ng/g). If there is a failure to meet the criterion 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 each species.

(3) Method Blanks

A minimum of one method blank must be prepared and analyzed with every 10 or fewer sample solutions analyzed. 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.

Control limits for the method blank: no arsenic species detected above the ASDL

(4) Precision of Replicate Analytical Portions

For each batch and at least once for each separate matrix type, three (3) replicate preparations and analyses of a sample must be performed. Replicate analytical portions should also be prepared and analyzed whenever homogeneity of analyte is suspect. 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 RSD is 10% for all analytes detected $> \text{LOQ}$

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

Where

s = standard deviation of replicates

C_{avg} = average concentration of replicates

(5) FAP Recovery

For each batch and at least once for each separate matrix type, one FAP shall be prepared and analyzed. (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 food matrix.

Fortification levels of AsIII, AsV, DMA, and MMA at a level of approximately 50% of the total arsenic concentration found in the sample may be used. Alternatively, the spiking level should be kept between 50% and 150% of the specific analyte level detected. 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.

Control limit for FAP (spike) recovery is $100 \pm 20\%$ for iAs, DMA and MMA. Note that spikes of AsIII and/or AsV must be evaluated based on the total iAs determined (AsIII + AsV).

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

Where

C_{x+s} = concentration determined in spiked sample

C_x = concentration determined in unspiked sample

C_s = concentration of spiking solution

M_s = mass of spiking solution added to sample portion

M_x = mass of sample portion

(6) Certified Reference Material

For each batch, one rice flour CRM must be prepared and analyzed. It should be noted that each of these CRMs require drying according to directions prior to analysis. NIST SRMs 1568a or 1568 may be used; however, these materials are not currently certified for individual arsenic species. In addition, CRM 7503-a Arsenic Compounds and Trace Elements in White Rice Flour from the National Metrology Institute of Japan (NMIJ) may be used. This CRM has certified values for AsIII, AsV and DMA. However, the present methodology does not adequately control AsIII/AsV redox chemistry and the total inorganic arsenic found should be compared to the sum of the certified concentrations of AsIII and AsV. The following values have been extracted from the Certificates of Analysis, and best available literature and shall be considered as the true values for comparison purposes:

Table 4. Rice Flour Certified Reference Materials

SRM/CRM	Total As (µg/kg)	AsIII (µg/kg)	AsV (µg/kg)	iAs (µg/kg)	DMA (µg/kg)	MMA (µg/kg)
1568a	290 ± 30*	60 ± 12**	39 ± 8**	100 ± 20**	171 ± 34**	11 ± 2**
1568	410 ± 50*	85 ± 17**	31 ± 6**	116 ± 23**	285 ± 57**	22 ± 4**
7503-a	98 ± 7*	71.1 ± 2.9*	13.0 ± 0.9*	84 ± 17**	13.3 ± 0.9*	nr

* Certified Value with Uncertainty expressed as a 95% Confidence Interval or 95% Confidence Interval plus an allowance for systematic error.

** Uncertainty expressed as ± 20% of the average value from the best available data

nr = not reported

Control limits for CRMs are listed in Table 4. The limits for iAs and DMA must be met. If the values obtained are not in the acceptable range, prepare an additional portion and re-analyze the CRM. Re-analysis of the entire sample batch may be required. Note that the certified value for DMA in CRM 7503-a is typically going to be less than the method LOQ.

The limits for MMA are presented; however, the MMA levels in these materials are generally going to be less than or near the method LOQ. Additionally Values for AsIII and AsV are provided for information only because the present methodology does not adequately control AsIII/AsV redox chemistry.

(7) FMB Recovery (optional)

A fortified method blank checks the accuracy of the fortification procedure without any matrix effects and is an optional quality control sample.

Control limit for FMB recovery is 100 ± 20%.

(8) Mass Balance

A mass balance shall be calculated between the sum of all arsenic species detected and the total As determined in each sample. 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. If the mass balance from a re-analysis is still not met, contact the method authors for further assistance.

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

Control limit for Mass Balance is 65% -135%.

(9) Oxidized Sample Extracts

For each batch and at least once for each separate matrix type, one oxidized sample extract should be prepared and analyzed. Additionally, any sample in which a noticeable chromatographic shoulder/peak is noted on or near the AsIII peak in the chromatogram, the oxidized sample extract must be prepared and analyzed.

The control limit for the oxidized sample extract is as follows: the unidentified peak remaining in the chromatogram with a RT very near that of AsIII after oxidation should be less than 10% of the sum total of arsenic species. Samples with the unidentified peak accounting for greater than 10% of the sum total of arsenic species must be reported immediately to the method authors.

4.11.12 REPORTING

Report results only when quality control criteria for a batch have been satisfactorily met. Report results for iAs (AsIII + AsV), DMA and MMA that are \geq LOQ as the mass fraction determined; “ $\mu\text{g/kg}$ ” are the preferred units. Report results that are \geq LOD and $<$ LOQ as the mass fraction determined and the qualifier that indicates analyte is present at a trace level that is below the limit of reliable quantification (e.g., TR). Report results that are $<$ LOD as “0”. For samples that have been dried prior to analysis, “dry wt” should be noted with the result. 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 method LOD and LOQ should be determined in each lab. The ASDL and ASQL values in Table 3 are presented only as examples.

Example: AsIII and AsV method LOQs = 20 $\mu\text{g/kg}$; AsIII and AsV method LODs = 2.5 $\mu\text{g/kg}$. Levels found for three different dried samples were 78 $\mu\text{g/kg}$ iAs, 17 $\mu\text{g/kg}$ iAs and 2.4 $\mu\text{g/kg}$ iAs .

78 $\mu\text{g/kg}$ is \geq LOQ; report 78 $\mu\text{g/kg}$, dry wt.

17 $\mu\text{g/kg}$ is \geq LOD but also $<$ LOQ; report 17 $\mu\text{g/kg}$ (TR), dry wt.

2.4 $\mu\text{g/kg}$ is $<$ LOD; report 0 $\mu\text{g/kg}$

4.11.13 METHOD VALIDATION

A Level 2 method validation was undertaken as described in the “Guidelines for the Validation of Chemical Methods for the FDA Foods Program.” The method LOD and LOQ obtained are adequate for the intended purpose of the method. The method was validated by analyses of three rice flour reference materials, recovery of analyte, and precision measurements. 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 samples tested. The iAs and DMA results for reference materials, SRM 1568, SRM 1568a, and CRM 7503-a agreed with certificate values or the best available literature values. Additionally, mass balances for all reference materials and samples tested were within the range of 70-130%.

4.11.14 REFERENCES

- (1) Conklin, S.D., FDA Elemental Analysis Manual, EAM 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).
- (2) Heitkemper, D.T., Vela, N.P., Stewart, K.R., and Westphal, C.S., *J. Anal. At. Spectrom.*, (2001) 16, 299-306.
- (3) Heitkemper, D.T., Kubachka, K.M., Halpin, P.R., Allen, M.N., and Shockey, N.V., *Food Additives and Contaminants, Part B*, (2009) 2, 112-120.
- (4) Creed, J., NERL, US EPA, personal communication.
- (5) Huang, J.-H., Ilgen, G., and Fecher, P., *J. Anal. At. Spectrom.* (2010) 25, 800-802.
- (6) Huang, J.-H., Fecher, P., Ilgen, G., Hu, K.-N., and Yang, J., *Food Chemistry* (2012) 130, 453-459.
- (7) Zhu, Y.-G., Sun, G.-X., Lei, M., Teng, M., Liu, Y.-X., Chen, N.-C., Wang, L.-H., Carey, A.M., Deacon, C., Raab, A., Meharg, A.A., and Williams, P.N., *Environ. Sci. Technol.*, (2008) 42, 5008-5013.
- (8) Guzman Mar, J.L., Hinojosa Reyes, L., Mizanur Rahman, G.M., and Skip Kingston, H.M., *J. Agric. Food Chem.*, (2009) 57, 3005-3013.
- (9) Narukawa, T., and Chiba, K., *J. Agric. Food Chem.*, (2010) 58, 8183-8188.
- (10) Jackson, B.P., Taylor, V.F., Karagas, M.R., Punshon, T., and Cottingham, K.L., *Environ. Health Perspectives*, <http://dx.doi.org/10.1289/ehp.1104619>, online 16 February 2012
- (11) FDA, Elemental Analysis Manual, for Food and Related Products. Section 3.2 Analytical Figures of Merit <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/ElementalAnalysisManualEAM/ucm205100.htm>. (*link removed*)
- (12) FDA, Office of Foods, “Guidelines for the Validation of Chemical Methods for the FDA Foods Program,” <http://www.fda.gov/ScienceResearch/FieldScience/ucm273423.htm> (*link removed*)