Elemental Analysis Manual

for Food and Related Products

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U.S. Department of Health & Human Services

FDA U.S. Food and Drug Administration

Elemental Analysis Manual

for Food and Related Products

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

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GLOSSARY

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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, rice breakfast cereals, rice crackers, rice cakes, and rice beverages. 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 (As(III)) and arsenate (As(V)). Additionally, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are determined. Concentrations of individual arsenic species determined in samples are reported based on elemental arsenic concentrations (i.e., µg of As per kg food). Other matrices may be analyzed by this procedure if performance is verified in the matrix of interest and at the analyte(s) concentration of interest.

Analysts using the method should be experienced in the use of HPLC and ICP-MS, including the identification of chromatographic and matrix interferences and procedures for their correction. Additionally, personnel should be thoroughly trained in the handling and analysis of samples for the determination of trace elements in food products including procedures for minimizing or eliminating contamination.

The analytical limits listed in 4.11 Table 1 are presented as an example of results achievable for rice and rice products when using the method and equipment specified herein. Analytical limits will vary depending on instrumentation and actual operating conditions used.

4.11 Table 1. Typical Analytical Limits

Analyte	Abbreviation	ASDL[a,b] (ng/g)	ASQL[a,b] (ng/g)	LOD[b,c] (µg/kg)	LOQ[b,c] (µg/kg)
Arsenite	As(III)	0.05	0.4	2.5	20
Arsenate	As(V)	0.05	0.4	2.5	20
Monomethylarsonic acid	MMA	0.05	0.4	2.5	20
Dimethylarsinic acid	DMA	0.05	0.4	2.5	20

[a] Based on 0.2 ng/g standard, n=10.

[b] Calculated as in EAM §3.2[1]

[c] Based on approximate sample dilution factor of 50 used for rice

4.11.2 SUMMARY OF METHOD

HPLC-ICP-MS provides for sensitive, selective determination of arsenic species in rice.[2-11] In this method, analytical samples of rice and rice-containing food products are composited and portions of the composite are mixed with a 0.28 M HNO_3 solution and heated at 95°C for 90 minutes.[5,6] The extracts are initially diluted with deionized water, 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 phosphate dibasic at pH 8.25 (±0.05).[12] The ICP-MS is used as an arsenic-specific detector monitoring *m/z* 75 for arsenic-containing chromatographic peaks and is operated in helium collision cell mode to eliminate interference from possible co-eluting chloride species. 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 personal protective equipment 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.

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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.01.02, 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, version B.01.01. (Agilent Technologies)

(2) High performance liquid chromatograph (HPLC)—Agilent 1100 series 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) 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 4.11 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 4.11 Table 2, using the "Timetable" tab that allows for the ISTD injection. A 20-50 µL injection loop is used. For the peristaltic pump, an approximate flow rate of 0.1-0.3 mL/min should be used as it must refill the injection loop between injections.

(4) Analytical and guard HPLC columns—model PRP X100 column, 4.1 × 250 mm, stainless steel, 10µm, Hamilton Co. (cat. no. 79433) and respective guard column cartridge (cat. no. 79446, 5-pack of cartridges).

(5) Block digestion system—48-place, 50 mL, temperature range from ambient to at least 100°C (SCP Science model DigiPREP MS).

(6) Laboratory mill—laboratory grade mill. (Retsch, Inc. model ZM100 with 0.5 mm ring sieve)

(7) Auto-sampler vials and caps—use plastic, SUN-Sri 8-425, 600 µL, (Fisher cat. no. 14-823-313) 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, soak vials using 2% nitric acid for approximately one hour and rinse 4X with deionized water. Check again for contamination.

(8) High density polyethylene (HDPE) amber bottles— for preparation and storage of stock standards.

(9) Centrifuge tubes—polypropylene conical tubes with caps, 50 and 15 mL. Check representative centrifuge tubes placing 1% HNO_3 in the tubes for a period of time and then analyzing this solution for total arsenic to ensure no arsenic is detected above the ASDL.

(10) Pipettes—automatic pipettes capable of accurate delivery from 10 µL up to 10.00 mL with assorted tips.

(11) Syringes—disposable, general use and non-sterile, 10 mL, Luer-Lok tip.

(12) Syringe filters—disposable, 0.45 µm Nylon or PTFE membrane with polypropylene housing and Luer-Lok inlet.

(13) Analytical balance—precision of 0.0001 g.

(14) Centrifuge—bench top centrifuge capable of 3000 rpm with buckets and carriers for 50 mL tubes.

(15) Standard laboratory oven—gravity-flow convection oven. Temperature range: 50-225°C.

(16) Desiccators—for storage of dry samples with indicating Drierite (>98% $CaSO_4$) desiccant. Available from W.A. Hammond Drierite Company.

(17) Vortex mixer—for mixing solutions.

(18) pH meter—with appropriate calibration buffers (pH 7 and 10).

4.11.5 REAGENTS AND STANDARDS

(1) De-ionized water (DIW)—18 MΩ cm de-ionized water, Millipore Milli-Q system.

(2) Nitric acid (HNO_3), 68-70%—CAS 7697-37-2, F.W. 63.01, OPTIMA ultra-pure grade, Fisher Scientific (cat. no. A467-500).

(3) Ammonium phosphate dibasic ($(NH_4)_2HPO_4$)—CAS 7783-28-0, F.W. 132.06, purity ≥ 99%. Due to arsenic contamination in various lots from several manufacturers, the $(NH_4)_2HPO_4$ used in this procedure must be verified to have a low arsenic content. See section §4.11.9, 4a-4e.

(4) Ammonium hydroxide (NH_4OH), 20%—CAS 1336-21-6, F.W. 35.05, OPTIMA ultra-pure grade, Fisher Scientific (cat. no. A470-250).

(5) Hydrogen Peroxide (H_2O_2), 30%—CAS 7722-84-1, F.W. 34.01, OPTIMA ultra-pure grade, Fisher Scientific (cat. no. P170-500).

(6) Arsenite Stock Standard (As(III))—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.

(7) Arsenate Stock Standard (As(V))—1000 mg/L As(+5) in H_2O, Spex Certiprep (cat. no. SPEC-AS5) 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) Certified Reference Materials (CRMs)

- National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1568a and/or 1568 Rice Flour. Certified for total arsenic concentrations.
- National Metrology Institute of Japan (NMIJ) CRM 7503-a Arsenic Compounds and Trace Elements in White Rice Flour. Certified for As(III) As(V) and DMA, as well as total arsenic concentration. Available from Waco Chemicals USA.
- NIST SRM 1643e, Trace Elements in Water. Certified for total arsenic concentration.

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 $(NH_4)_2HPO_4$ 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 (0.28 M HNO_3)

This solution is used to extract the arsenic species from the samples. Prepare by adding 25.3 g of concentrated HNO_3 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 method blank and 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. For clarity, report mass fraction of analytical solutions on ng/g basis and mass fraction of test samples on µg/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) DMA stock standard—≈1000 µg/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—≈1000 µg/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—≈1000 µg/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. The concentration of the AsB stock standard is approximately 1000 µg/g and does not need to be further verified.

Working Standards

The arsenic concentration of the DMA and MMA standards must be verified, typically using ICP-MS analysis. It is recommended that the As(III), and As(V) concentrations also be verified, but this is not required. Determine the total arsenic concentrations in 1 µg/g standards of MMA and DMA using a certified total arsenic standard. It is also advisable to analyze a certified reference material such as NIST SRM 1643e Trace Elements in Water with certified total arsenic level for additional confidence. Calculate the As concentration of the MMA and DMA working standard solutions. Use these concentrations to correct the stock standard concentrations and apply these values in all future calculations. Once the stock standards of DMA and MMA have been verified and corrected, future preparations of the respective working standards do not need to be verified. Record all weights to calculate standard concentrations.

Additionally, the retention times and purity of the working standards for 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 re-verified for both total As and for species purity periodically (*e.g.*, monthly). Inter-conversion of As(III)/As(V) standards is most likely to be seen and comparison to the original analysis for purity is recommended.

(1) As(III) working standard—1 µg/g As of As(III). Prepare As(III) working standard by weight in DIW using the 1000 mg/L As(III) 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 supplied with a Certificate of Analysis and the certified value is traceable to a NIST SRM.

(2) As(V) working standard—1 µg/g As of As(V). Prepare As(V) working standard by weight in DIW using the 1000 mg/L As(V) 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 supplied with a Certificate of Analysis and the certified value 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. Once the stock standard concentration of MMA has been verified, future preparations of the respective working standard do not need to be verified.

(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. Once the stock standard concentration of DMA has been verified, future preparations of the respective working standard do not need to be verified.

(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 As(III), As(V), 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 As(III) and As(V) 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. 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 mobile phase 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 do not show evidence of inter-conversion of arsenic species.

(1) 200 ng/g As(III), As(V), DMA and MMA—Take 1.0 g of each 1 µg/g working standard and dilute to 5 g with DIW. Mix thoroughly. This standard is used in preparation of calibration standards, but not analyzed.

(2) 20 ng/g As(III), As(V), DMA and MMA—Take 0.5 g of 200 ng/g As(III), As(V), DMA and MMA, and dilute to 5 g with mobile phase. Mix thoroughly.

(3) 5 ng/g As(III), As(V), DMA and MMA—Take 0.125 g of 200 ng/g As(III), As(V), DMA and MMA, and dilute to 5 g with mobile phase. Mix thoroughly.

(4) 1 ng/g As(III), As(V), DMA and MMA—Take 0.25 g of 20 ng/g As(III), As(V), DMA and MMA, and dilute to 5 g with mobile phase. Mix thoroughly.

(5) 0.25-0.5 ng/g As(III), As(V), DMA and MMA—For example, take 0.25 g of 5 ng/g As(III), As(V), DMA, MMA, 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 As(III), As(V), DMA and MMA. Take 0.5 g of 200 ng/g As(III), As(V), DMA, MMA, and dilute to 10 g with mobile phase.

Additional Standards

(1) Internal standard, 2 ng/g As(V)—Take 1 g of 1 µg/g As(V) 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 solution, 5 ng/g each As(III) and AsB—Take 0.5 g of the As(III) working standard (1 µg/g As(III) and 0.5 g of AsB working standard (1 µg/g AsB) and dilute to 100 g with mobile phase. A new resolution check solution should be prepared when significant oxidation of As(III) to As(V) is noted.

(3) Standard for LOD and LOQ Determination—Prepare a standard containing As(III), As(V), DMA and MMA 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 0.0001 g) to calculate the concentration of arsenic species in the sample. If not received as ground, homogenized composites, solid samples must be first ground and homogenized prior to analysis. The method of grinding the sample is not as critical as taking care not to contaminate the samples. Ground samples are not required to be sieved. However, if the precision of results deviate from the recommendation, re-grinding the sample may be necessary.

Rice, Rice Cereals, and Rice Crackers

(1) Typically, raw or natural rice samples are dried to constant weight (typically within approximately 1%) prior to analysis. However, drying of all samples may not be required. In general, processed rice products should be analyzed without drying. Refer to sampling assignments for further direction. If required, dry a minimum of 10 g of the homogenized, ground samples in a laboratory oven at 85°C until a constant weight is obtained. Calculate the moisture content of the original sample (this may be accomplished by the laboratory performing total arsenic analysis). Store dried samples in a desiccator.

(2) Weigh a 50 mL centrifuge tube with lid and record weight.

(3) Weigh ≈1.0 g of the ground composite into the pre-weighed 50 mL centrifuge tube (with lid), record weight.

(4) 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 quick vortex mixing and then rinsing the walls of the container with the other 5 mL of 0.28 M HNO_3)

(5) Cap all tubes tightly and place in preheated block digestion system at 95°C for 90 min.

(6) Let samples cool. Add approximately 6.7 g DIW and record weight.

(7) Centrifuge samples at 3000 rpm for 10 min.

(8) 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.

(9) Dilute 1 g of filtrate from one method blank with 2 g of pH Adjustment Solution. Perform the same dilution with 1 g of one sample extract. Confirm that the pH of the resulting 1:3 dilutions of the blank and sample are 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 method blanks and samples for analysis (step 10). Due to possible contamination, the portion of pH adjusted extract that has come in contact with the pH electrode must not be analyzed.

(10) For all samples, transfer 1 g of filtrate to a 15-mL tared centrifuge tube. Add 2 g of pH Adjustment Solution. Record the initial and final weight. Transfer a portion of solution to a plastic or an acid-washed glass auto-sampler vial for analysis by HPLC-ICP-MS.

Rice Cakes

Compared to rice flour, puffed rice cakes appear to be less dense and absorb significant amounts of water during the extraction process making it necessary to use a smaller sample size for the extraction.

(1) Weigh ≈0.67 g of the ground composite into a pre-weighed 50 mL centrifuge tube (with lid), record weights. Samples of rice cakes do not typically require drying.

(2) Follow steps 4 – 10 as listed above under **Rice, Rice Cereals, and Rice Crackers**.

(3) The LOD and LOQ for rice cakes samples are approximately 3.8 and 30 µg/kg, respectively based on an approximate dilution factor of 75.

Rice Beverage

Rice beverages (sometimes labeled as “rice drink” or “rice milk”) generally contain low levels of total arsenic compared to rice flour making it necessary to use a larger sample size for the extraction.

(1) Shake the sample vigorously prior to sampling.

(2) Weigh ≈2.0 g of rice beverage into a pre-weighed 50 mL centrifuge tube (with lid), record weights. Samples of rice milk are not evaporated or lyophilized prior to analysis.

(3) Follow steps 4 – 10 as listed above under **Rice, Rice Cereals, and Rice Crackers**.

(4) The LOD and LOQ for rice beverage samples are approximately 1.3 and 10 µg/kg, respectively, based on an approximate dilution factor of 25.

4.11.8 QUALITY CONTROL SAMPLE PREPARATION PROCEDURES

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

(1) Method blanks (MBK). Take 1 g DIW through the entire sample preparation procedure described in §4.11.7.

(2) Fortified analytical portions (FAP). Prepare an analytical portion fortified with As(III), As(V), DMA, and MMA at a level of approximately 50% of the total arsenic concentration found in the sample. For example, a sample containing 250 µg/kg total arsenic would be fortified at a level of 125 µg/kg each (As(III), As(V), DMA, and MMA) by taking a 1 g analytical portion and spiking with 125 µL (≈ 0.125 g) of 1 µg/g multi-analyte spiking standard. This has generally been found to be an effective spike level.

Alternatively, the spiking level can be prepared between 50% and 150% of the iAs and DMA levels detected. If total As results are not available, a spike level of 125 µg/kg each (As(III), As(V), DMA, and MMA) has generally been found to be an effective spike level.

(3) Oxidized sample extracts. To investigate possible interferences on the As(III) determination, a second portion of at least one sample extract from each batch will require an additional analysis. As(III) in the sample extract is oxidized to As(V) using 30% H_2O_2 prior to analysis. Prepare oxidized sample extracts by taking 1 g of filtered extract + 2 g of pH adjustment solution + 0.4 g of H_2O_2. Shake well and then allow the mixture to stand for at least 5-10 minutes prior to analysis to complete the oxidation. Record all weights to calculate a new dilution factor and estimate the unknown peak concentration.

4.11.9 DETERMINATION PROCEDURE

4.11 Table 2 provides operating conditions used for this analysis. Operating conditions and settings may be optimized for the equipment used.

(1) Follow instrument standard operating procedure for startup and initialization. After ~30 minutes 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.

4.11 Table 2. Typical HPLC-ICP-MS Operating Parameters

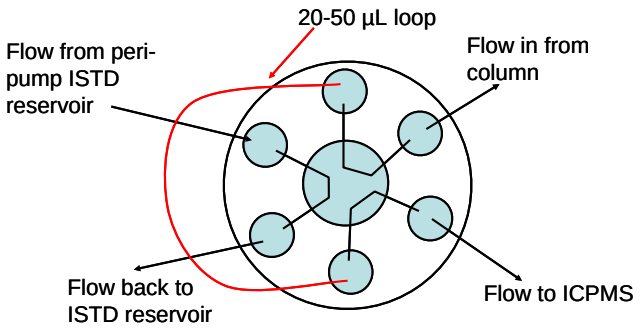
ICP-MS Conditions		HPLC Conditions	
RF power	1500 W	Mobile phase composition	10 mM $(NH_4)_2HPO_4$
Plasma gas flow	15 L/min	Mobile phase pH	8.25 (±0.05)
Auxilliary (makeup) gas flow	0.1 L/min	Mobile phase flow rate	1 mL/min
Nebulizer (carrier) gas flow	1.1 L/min	Injection volume	100 µL
Nebulizer type	Glass concentric	Degasser	On
Sampling depth	8.5 mm	Column temperature	Ambient
Peristaltic pump speed	0.3 rps (~1 mL/min)	Column compartment time table for introduction of ISTD	0.1 min, Column Position 1
Spray chamber temp.	2°C		1.0 min, switch to Column Position 2
Collision cell	He @ 2.0 mL/min		2.0 min, switch back to Column Position 1
Data acquisition mode	Time-resolved, *m/z* 75 for $^{75}As^+$, and *m/z* 77 for $^{40}Ar^{37}Cl^+$	Acquisition time	1200 s (20 min)
Dwell time	0.8 s (*m/z* 75), 0.2 s (*m/z* 77)		
Replicates per ion	1		

(2) Use a 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 HPLC flow and peristaltic pump ISTD reservoir flow tubes connected similar to 4.11 Figure 1. In the HPLC method timetable, column switching valve should be triggered at 1.0 min and triggered to switch back at 2.0 min (see 4.11 Table 2). Start the peristaltic pump and verify that no bubbles are present.

(4) Connect ICP-MS and HPLC. Start HPLC flow (1 mL/min) and increase the peristaltic pump speed to ensure adequate drainage of ICP spray chamber (>1 mL/min).

(4a) If this is the first time using a source of $(NH_4)_2HPO_4$ 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 $(NH_4)_2HPO_4$ source has already been found to be acceptable, follow step 4a and then proceed to step 5.



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

- Ensure proper flow and adequate drainage of ICP spray chamber (>1 mL/min).
- Ensure there are no leaks.
- Allow time for the HPLC column and plasma to equilibrate (>15 min).
- Ensure that backpressure is acceptable. Increasing backpressure can be indicative of column problems.

(4b) Set the ICP-MS conditions as in 4.11 Table 2, but rather than setting up an acquisition method, test the following in the tune window.

(4c) 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 30 seconds and record the average response (in CPS).

(4d) Switch the eluent to the mobile phase (using the new source of $(NH_4)_2HPO_4$). After eluting the mobile phase for at least 30 minutes, monitor m/z 75 (integration time of 0.8 seconds) in the tune window for at least 30 seconds and record the average response (in CPS).

(4e) 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 $(NH_4)_2HPO_4$ or contact the method authors. If it is <6, proceed to step 5.

(5) Set ICP-MS acquisition method (see 4.11 Table 2) for time-resolved collection of m/z 75 and 77 with integration (dwell) times of 0.8 and 0.2 seconds, 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 solution containing 5 ng/g As(III) and AsB to ensure adequate resolution. AsB serves as a marker for unretained arsenic species including tetramethylarsonium which has been recently identified at low levels (<10% of total arsenic) in rice grain.[13]

(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 4.11 Table 3. Check retention times, peak shape and response of both ISTD and arsenic species in the *m/z* 75 chromatograms. See 4.11 Table 4 for typical retention times and sensitivities (peak area per ng/g). 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.

- Check the *m/z* 77 chromatograms of samples for indications of possible argon chloride ($^{40}Ar^{35}Cl^+$ at *m/z* 75 and $^{40}Ar^{37}Cl^+$ at *m/z* 77) interferences in the *m/z* 75 chromatograms. Peaks detected in the *m/z* 77 chromatograms arising from $^{40}Ar^{37}Cl^+$ will also have peaks with matching 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 ($^{77}Se^+$).
- 4.11 Figure 2 provides example chromatograms obtained for the resolution check solution, a 5 ng/g calibration standard, and SRM 1568a Rice Flour diluted extract.

4.11 Table 3. Typical Analytical Batch Sequence

Solution	Purpose	QC Criteria
Vial DIW Blank	Verify clean auto-sampler vials	≤ ASDL
Resolution Check Solution	Check separation between unretained species (represented by AsB) and As(III)	
0.25-0.5, 1, 5, 20 ng/g Calibration Standards*	Standardize instrument	r^2>0.99
MBK 1	Verify absence of contamination	≤ ASDL
Rice CRM/SRM (SRM 1568a/1568 or CRM 7503-a)	Demonstrate accuracy	
Ten (10) analytical solutions (includes replicate preparations and FAPs)	Determine arsenic species/concentrations	RSD ≤ 15% FAP 100 ± 20%
10 ng/g Calibration Check Standard*	Verify standardization	100 ± 15% for DMA, MMA, iAs
MBK 2	Verify absence of contamination	≤ ASDL
Ten (10) analytical solutions (includes replicate preparations and FAPs)	Determine arsenic species concentrations	RSD ≤ 15% FAP 100 ± 20%
10 ng/g Calibration Check Standard	Verify Standardization	100 ± 15%
Oxidized Sample Extract*	Investigate potential As(III) interference	

* It is recommended that DIW blanks be injected after injections with high As concentrations or H_2O_2 treatment to minimize carryover to the next injection

4.11 Table 4. Typical Retention Times and Sensitivities

Species	Retention Time (min)	Sensitivity (peak area /(ng/g))
As(III)	2.9 ± 0.2	55000
DMA	3.9 ± 0.2	71000
MMA	5.5 ± 0.3	65000
As(V)	12.7 ± 0.5	64000

(10) Integrate *m/z* 75 chromatograms.

- The settings in 4.11 Table 5 for m/z 75 in the *Data Analysis (DA) Method Editor* / *Analyte List* (not *EIC Integration Setup*) / *Int Parms* 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.

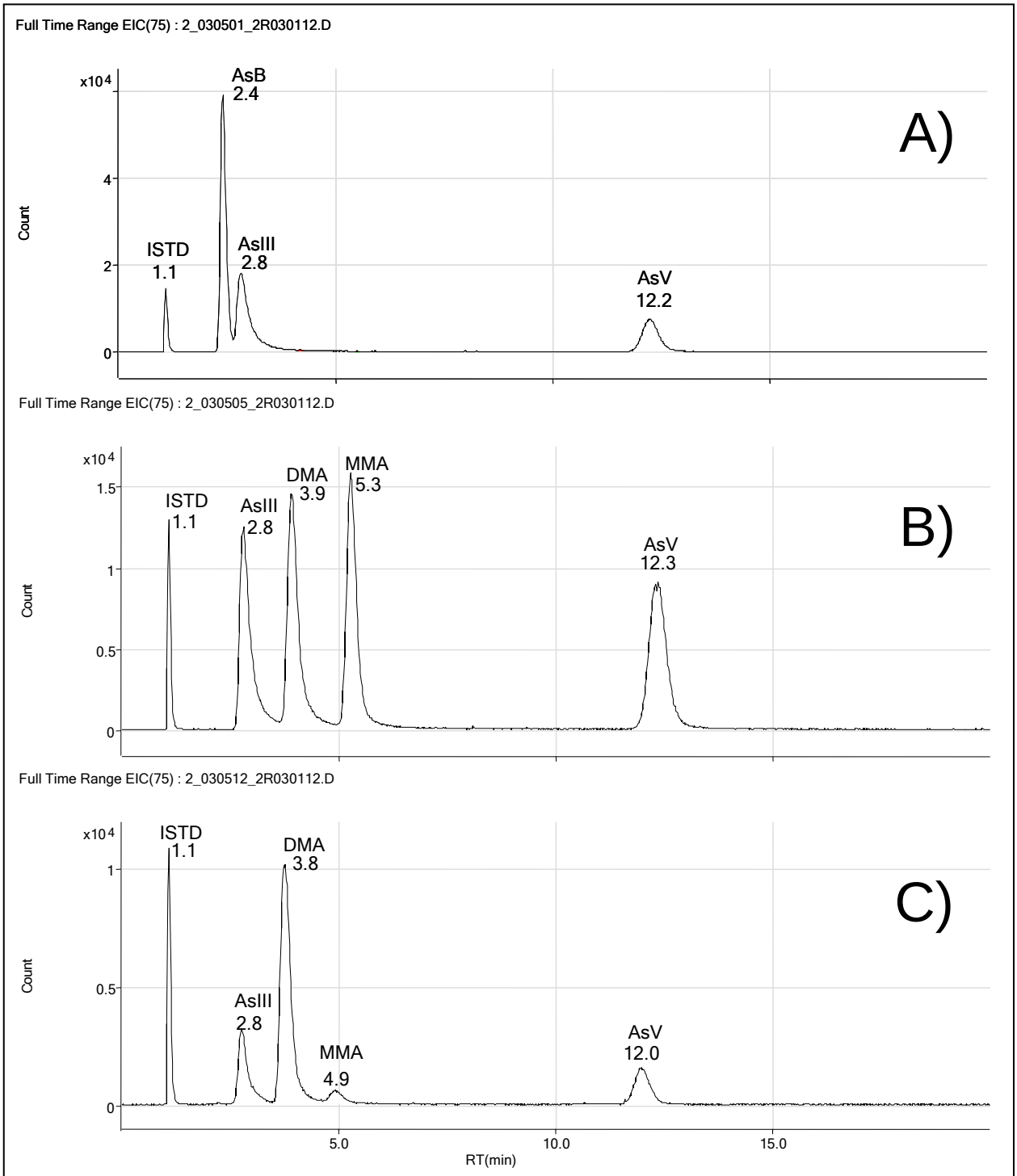
  After settings are correct, choose "Apply to All." This will apply these integration parameter to the ISTD, As(III), As(V), DMA and MMA peaks.

- 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.

- 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.
- 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 §4.11.10 under *Peak Area Integrations*.

4.11 Table 5. Recommended Data Analysis Method Editor Settings for m/z 75

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	



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

4.11.10 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.

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

where

A_{Unk} = integrated peak area of unknown
A_{ISTD} = integrated peak area of post-column injection peak (ISTD)
m = slope of 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:

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

where

$[C_{spl}]$ = The concentration of As(III), As(V). DMA, or MMA in the sample (µg/kg)
$[C_{soln}]$ = The concentration of As(III), As(V), DMA or MMA in the analytical solution (ng/g)

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

where

$M_{Extract}$ = mass of the 1-g aliquot of extract (g)
$M_{pH\ Adjustment\ Solution}$ = mass of the 2-g pH Adjustment Solution (g)
$M_{analytical\ portion\ +\ nitric\ +\ water}$ = mass of the extract (analytical portion + nitric acid + water) (g)
$M_{analytical\ portion}$ = mass of the analytical portion (g)

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

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

where

[As(III)] = concentration (µg/kg) of arsenite in rice product
[As(V)] = concentration (µg/kg) of arsenate in rice product

Note: [As(III)] and [As(V)] results ≥ 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 §4.11.6 REAGENT AND STANDARD PREPARATION, Working Standards

(2) Verify concentrations of DMA and MMA stock standards. See §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) according to FDA's Elemental Analysis Manual (EAM), §3.2 Analytical Figures of Merit.[1] 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 (*e.g.*, In 4.11 Table 1, 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

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>0.99. 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 10^{th} 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 ± 15% of the calculated concentration for DMA, MMA, and iAs (As(III)+As(V)). The control limits for individual As(III) and As(V) concentrations can be outside of the 100 ± 15% individually, as long as their sum as iAs is within 100 ± 15%. Control limits for the calibration check standard retention times (RT) are as follows: As(III) RT ± 0.2 minutes, DMA RT ± 0.2 minutes, MMA RT ± 0.3 minutes, and As(V) RT ± 0.5 minutes when compared to the 20 ng/g calibration standard.

(3) Method Blanks

A minimum of one method blank must be prepared and analyzed with every 10 or fewer sample 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, 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 RSD is 15% for iAs, DMA and MMA when detected > LOQ.

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

where

s = standard deviation of replicates (µg/kg)
C_{avg} = average concentration of replicates (µg/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 food matrix prior to adding the extract solution (0.28M HNO_3). 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 (see recovery). 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 ± 20% for iAs, DMA and MMA. The following equation demonstrates how to calculate spike recoveries for individual species.

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

where

C_{x+s} = concentration determined in spiked sample (µg/kg)
C_x = concentration determined in unspiked sample (µg/kg)
C_s = concentration of spiking solution (µg/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)).

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

where

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

(6) Reference Material

For each batch, one rice flour CRM or in-house reference material must be prepared and analyzed. It should be noted that CRMs generally require drying prior to analysis. If available, it is suggested that NIST SRM 1568a or 1568 Rice Flour be used. These materials are not currently certified for individual arsenic species. However, the values reported in 4.11 Table 6 have been extracted from the Certificates of Analysis (for total As), and best available literature (for individual As species) and shall be considered as the true values for comparison purposes. NMIJ CRM 7503-a may also be used as this CRM has certified values for As(III), As(V) and DMA. However, the present methodology does not prevent the oxidation of As(III) to As(V) and the total inorganic arsenic found should be compared to the sum of the certified concentrations of As(III) and As(V). Additionally, the certified value for DMA in CRM 7503a is typically going to be less than the method LOQ.

Control limits for CRMs are listed in 4.11 Table 6. The limits for iAs and DMA must be met as long as they are detected above a laboratory's LOQ. If the values obtained are not in the acceptable range, a second analytical solution may be prepared from the extract and re-analyzed one time. If the control limits are still not met, re-analysis of the entire sample batch may be required. The limits for MMA in SRM 1568a and 1568 are presented; however, the MMA levels in these materials are generally going to be less than or near the method LOQ. Control limits for in-house reference materials should be established as described in EAM §3.5 Reference Materials.[1]

4.11 Table 6. Control Limits for Rice Flour Certified Reference Materials

SRM/CRM	Total As (µg/kg)	iAs (µg/kg)	DMA (µg/kg)	MMA (µg/kg)
1568a	290 ± 30[a]	100 ± 20[b]	171 ± 34[b]	11 ± 2[b]
1568	410 ± 50[a]	116 ± 23[b]	285 ± 57[b]	22 ± 4[b]
7503-a	98 ± 7[a]	84 ± 17[b]	13.3 ± 0.9[a]	—

[a]Certified Value with Uncertainty expressed as a 95% Confidence Interval or 95% Confidence Interval plus an allowance for systematic error.
[b]Uncertainty expressed as ± 20% of the average value from the best available data.

(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. 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.

$$\%\text{MassBalance} = \frac{[\text{iAs}] + [\text{DMA}] + [\text{MMA}] + [\text{Unknownpeak(s)}]}{[\text{TotalAs}]} \times 100$$

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

(8) 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, for any sample in which a noticeable chromatographic shoulder/peak is noted on or near the As(III) peak in the chromatogram, the oxidized sample extract must be prepared and analyzed. If these shoulders/peaks are noted on several of the samples within a batch, limit the number of oxidized sample extracts to five samples with the highest apparent interference per batch as to minimize column damage due to H_2O_2.

The control limit for the oxidized sample extract is as follows: the unidentified peak remaining in the chromatogram 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 may require further testing.

4.11.12 REPORTING

Report results only when quality control criteria for a batch have been satisfactorily met. For clarity, report mass fraction of analytical solutions on ng/g basis and mass fraction of test samples on µg/kg basis. Report results for iAs (As(III) + As(V)), DMA and MMA that are ≥LOQ as the mass fraction determined; "µg/kg" are the preferred units. Report results that are ≥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 4.11 Table 1 are presented only as examples. Note for iAs, if either As(III) or As(V) is ≥ their respective LOQ, then iAs is considered ≥LOQ. If both As(III) and As(V) are <LOQ and at least one is >LOD, the iAs is considered to be at a trace level even if the sum of As(III) and As(V) is ≥LOQ.

Example: As(III) and As(V) method LOQs = 20 µg/kg; As(III) and As(V) method LODs = 2.5 µg/kg. Levels found for three different dried samples were 78 µg/kg iAs, 17 µg/kg iAs and 2.4 µg/kg iAs .

78 µg/kg is ≥LOQ; report 78 µg/kg, dry wt.

17 µg/kg is ≥LOD but also <LOQ; report 17 µg/kg (TR), dry wt.

2.4 µg/kg is <LOD; report 0 µg/kg

4.11.13 METHOD VALIDATION

The method has undergone both a Level 2 single laboratory validation and a Level 3 multi-laboratory validation as described in, "Guidelines for the Validation of Chemical Methods for the FDA Foods Program" with 6 participating laboratories.[14] The average method LODs and LOQs obtained among the 6 laboratories were just under those listed in 4.11 Table 1 and were adequate for the intended purpose of the method. The multi-laboratory validation included the determination of iAs, DMA and MMA in three rice flour reference materials and three rice matrices including, long grain white rice, long grain brown rice, and a brown rice cereal product. Triplicate portions of the validation samples and reference materials were analyzed by each laboratory. Method blanks and spiked method blanks at levels approximately equal to the method LOQ and 2.5X the method LOQ were analyzed by each laboratory. Fortified validation samples at three concentration levels were analyzed in duplicate by each of the laboratories. Repeatability and reproducibility of the method was ≤10% relative standard deviation for species present at concentrations >LOQ. The average spike recoveries were in the range of 80-120% in all samples tested. The iAs and DMA results for rice 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 90-115%.

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