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Fast Analysis of Arsenic Species in Infant Rice Cereals using LC-ICP-QQQ

Routine determination of inorganic As in less than two minutes

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Introduction

Arsenic contamination of food can be harmful to human health. To assess the risk, several speciation methods have been developed to separate the toxic inorganic forms of As (iAs)—a class 1 carcinogen—from less toxic or non-toxic forms.

In a previous study (1), a speciation method specified in US FDA EAM: Section 4.11 (2) was used to separate four arsenic species in 31 baby rice cereals. The arsenic species included the inorganic forms; As(III) (arsenite) and As(V) (arsenate), and two organic forms; monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). The four species were separated using isocratic anion-exchange HPLC, and ICP-MS was used to detect arsenic-containing chromatographic peaks.

This study aimed to develop a rapid and reliable screening method for inorganic arsenic (iAs) analysis, to assist the food industry in meeting existing and future regulations. As shown in Table 1, the FDA has proposed an action limit of 100 ppb for iAs in infant rice cereals. This limit is in line with the European Union's limit for rice used for the production of food for infants and young children.

Table 1. Example regulatory maximum concentrations governing iAs in rice and rice-based products.

Regulating body	Action or Maximum Concentration for iAs (ppb)	Rice Type or Rice Product
US FDA (3)	100 (proposed)	Infant rice cereals
Codex Alimentarius Commission (4, 5)	200	Polished (white) rice
	350	Husked (brown) rice
European Union (6)	200	Polished rice
	250	Parboiled and husked rice
	300	Rice waffles, wafers, crackers, and cakes
	100	Rice used for the production of food for infants and young children
China (7)	150	Rice grains

The methodology described in this application note is based on a previous method developed by Jackson (8), where As species were determined using HPLC coupled to a triple quadrupole ICP-MS (ICP-QQQ). HPLC-ICP-QQQ was also used in this study, but instead of analyzing the iAs species separately, As(III) was intentionally oxidized to As(V) with hydrogen peroxide before analysis (9, 10). By converting As(III) and analyzing all inorganic species as As(V), this method was able to separate MMA and DMA from iAs (as As(V)) in less than 2 minutes. The analysis time is 10 times faster than the current FDA methods used for As speciation (2). The same fast HPLC-ICP-QQQ approach has also been applied to As speciation in wine (11).

Oxygen was used as a reaction gas in the collision/reaction cell (CRC) of the ICP-QQQ to resolve the Cl-based spectral interferences on As-75 for total As measurements. For the speciation measurements, the potential Cl-based interferences are resolved chromatographically, so ICP-QQQ with MS/MS is not essential. While this analysis could be done on a single quadrupole ICP-MS such as the Agilent 7800 or 7900 ICP-MS, ICP-QQQ offers higher sensitivity and lower detection limits where both As speciation and total As analysis is required. Results are presented that demonstrate the accuracy and reproducibility of the new method. The method was further validated by analyzing four rice standard and certified reference materials.

Experimental

Standards

The As(III) and As(V) standards were bought from Spex Certiprep (Metuchen, NJ, USA). The MMA and DMA standards were bought from Chem Service (West Chester, PA, USA). An arsenobetaine (AB) standard was also purchased from Chem Service to be used as a flow injection marker (internal standard) for post-column injection. Calibration standards were prepared at 0.1, 0.5, 1.0, 5.0, 10, and 20 µg/L (ppb) for each of DMA, MMA, and total iAs (sum of As(III) and As(V)).

Standard/certified reference materials

Four SRM/CRMs were used as quality control materials for the As speciation measurements and total As measurements (without HPLC separation). The SRM used was the National Institute of Standards and Technology (NIST) 1568a Rice Flour. The three CRMs were the National Metrology Institute of Japan (NMIJ) 7503a White Rice Flour, the NMIJ 7532a Brown Rice Flour, and the Joint Research Centre (JRC) ERM-BC211 - Arsenic in Rice.

Samples and sample preparation

Six baby rice cereals were purchased from a local store in Berkeley, CA, USA. Each cereal was produced by a different manufacturer.

Arsenic was extracted from the rice matrix according to FDA method EAM 4.11 (2). Infant rice cereal (1 g) was weighed into a centrifuge tube and 10 mL of 0.28 mol/L HNO_3 was added. The capped tube was placed in a preheated block digestion system at 95 °C for 90 minutes. The mixture was then diluted with 6.6 mL H_2O, centrifuged, and filtered. Equal 0.5 mL portions of rice extract, H_2O_2, and mobile phase were pipetted into a 2 mL plastic HPLC vial as the test solution. Each sample was prepared in duplicate.

Instrumentation

An Agilent 1260 HPLC fitted with a Hamilton PRP-X100 5 µm 50 x 2.1 mm column was coupled to an Agilent 8800* Triple Quadrupole ICP-MS (ICP-QQQ). The mobile phase was 40 mM ammonium carbonate ((NH_4)$_2$$CO_3$, trace metal grade 99.999%, Sigma Aldrich) with 3% v/v methanol (Optima LC/MS grade, Fisher Chemical) adjusted to a pH of 9.0 with ammonium hydroxide (Optima Grade, Fisher Scientific). The ICP-QQQ was equipped with a standard sample introduction system comprising a glass concentric nebulizer, quartz spray chamber, quartz torch with 2.5 mm i.d. injector, and nickel-tipped interface cones. Peak integration was carried out according to FDA EAM §4.10 (12) and 4.11.15 (2). The instrument operating conditions are summarized in Table 2.

Table 2. HPLC-ICP-QQQ operating conditions.

ICP-QQQ	
Forward power	1550 W
Sampling depth	8.0 mm
Spray chamber temp.	2 °C
Carrier gas	0.95 L/min
Makeup gas	0.20 L/min
Extract 1	0 V
Octopole bias	−5.0 V
Energy discrimination	−7 V
O_2 cell gas flow rate	0.31 mL/min
Scan mode	MS/MS
Q1/Q2 mass	75/91 u
HPLC	
Mobile phase flow	0.5 mL/min
Injection volume	5 µL
Sample temperature	4 °C
ISTD injection volume	5 µL

Results and Discussion

Development of a fast method

The focus of the method was to reduce the analysis time per sample compared to the current FDA method for As speciation. In common with Jackson's method (8), a small injection volume, short ion-exchange column, high mobile phase linear velocity, and oxygen cell gas mode were used.

Figure 1 shows overlaid chromatograms for a representative calibration set of 0.5, 1.0, 5.0, and 20 µg/kg standards. All As species are baseline separated in less than two minutes. Simply by oxidizing As(III) to As(V) and analyzing all iAs in the form of As(V), the analysis time was reduced significantly compared to approximately 20 minutes for the current FDA regulatory method (2).
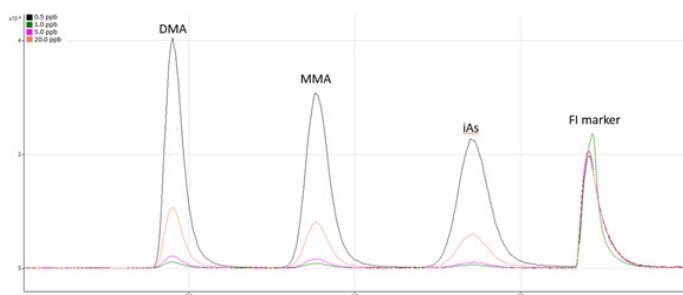

Figure 1. Overlay of the 0.5, 1.0, 5.0, and 20.0 µg/kg As species calibration standards. An AB internal standard (flow injection marker; fourth peak) was added post column via an external switching valve.

Linear calibrations

The calibration curves for DMA, MMA, and iAs showed good linearity (Figure 2). All As concentrations in the rice samples were within the linear range except iAs, which was measured at a maximum concentration of 150% of the highest calibration standard.
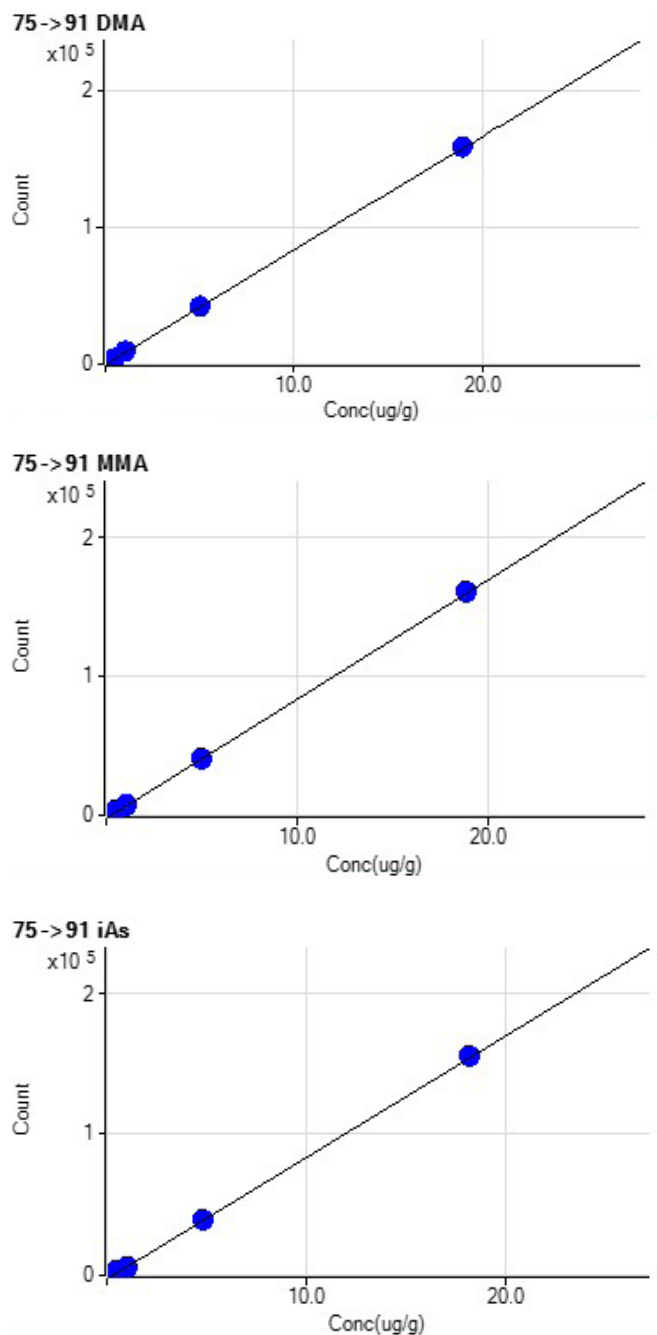

Figure 2. Calibration curves for DMA, MMA, and total iAs (sum of converted As(III) and As(V)).

Detection limits

The limits of detection (LOD) and limits of quantitation (LOQ) given in Table 3 are based on repeated measurements of the 0.05 µg/kg (ppb) mixed standard, n=15.

Table 3. LOD (3 sigma), LOQ (30 sigma), and estimated LOQ in rice for each As species.

	LOD, µg/kg	LOQ, µg/kg	Estimated rice LOQ, µg/kg (50x dilution)
DMA	0.018	0.175	8.8
MMA	0.026	0.258	12.9
iAs	0.022	0.221	11.0

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Analyis of rice RMs

Arsenic species were determined in four rice reference materials using the new HPLC-ICP-QQQ method. The total As concentration in each sample was also determined by direct ICP-QQQ analysis (no HPLC separation). Table 4 lists the reference and measured concentrations for DMA, MMA, iAs, and total As. Only one of the reference materials—NIST 1568b—had a reference value for MMA. The HPLC-ICP-QQQ measured concentrations were compared to the reference values, where available. Species recoveries ranged from 93 to 123% of their certified values when concentrations were above the LOQ. The recoveries for total As were also acceptable, ranging from 92 to 112%.

To check the quality of the data, z-scores were also calculated. Z-scores are the number of standard deviations from the mean, with values between −3 and +3 being sufficient for regulatory purposes. The percent recovery for DMA in ERM BC211 RM was biased high, but the z-score was 2.1. The recovery for MMA in NIST 1568b RM was 128% but the reference concentration was below the method's LOQ. The z-score was 0.94.

Quantitative results in infant rice products

Six baby rice cereal samples were measured in duplicate using the HPLC-ICP-QQQ speciation method. Table 5 lists the measured concentrations for DMA and iAs; MMA was only present above the LOQ (0.026 ppb) in two of the rice samples (E and F). There was no significant difference between the two duplicates run for each cereal sample, showing the reproducibility of the method.

The concentration of iAs in four of the six rice samples was below the US FDA's proposed action limit and the EU's maximum limit of 100 ppb for iAs in infant rice cereals. Samples C and D exceeded the regulatory limit.

Table 5. Quantitative results in µg/kg (ppb) for As species in six infant rice market basket samples measured in duplicate.

Sample Name	DMA	MMA	iAs	Proposed US FDA limit of 100 ppb for iAs
Baby rice cereal A_1	11.4	N/D	63.3	Pass
Baby rice cereal A_2	11.2	N/D	62.3	
Baby rice cereal B_1	12.5	N/D	53.6	Pass
Baby rice cereal B_2	14.9	N/D	56.4	
Baby rice cereal C_1	33.9	N/D	106.4	Fail
Baby rice cereal C_2	36.0	N/D	113.5	
Baby rice cereal D_1	15.4	N/D	102.6	Fail
Baby rice cereal D_2	15.1	N/D	103.6	
Baby rice cereal E_1	41.9	2.2	87.9	Pass
Baby rice cereal E_2	39.0	2.3	82.1	
Baby rice cereal F_1	46.4	8.7	89.4	Pass
Baby rice cereal F_2	46.7	9.0	90.4	

N/D = Not detected

Table 4. Quantitative results for As species and total As in rice reference materials.

Rice RM	DMA (mg/kg)		MMA (mg/kg)		iAs (mg/kg)		Total As (mg/kg)	
	Reference	Measured	Reference	Measured	Reference	Measured	Reference	Measured
NIST 1568b	180 ± 12	195 ± 4 (109%)	11.6 ± 3.5	14.9 ± 0.9 (128%)	92 ± 10	105 ± 1 (114%)	285 ± 14	315 ± 3 (110%)
NMIJ 7503a	13.3 ± 0.9	15.4 ± 0.1 (116%)	None reported	<LOD	84.1 ± 3[a]	79 ± 4 (94%)	98 ± 7	94 ± 4 (96%)
NMIJ 7532a	18.6 ± 0.8	18.7 ± 1.3 (101%)	None reported	2.2 ± 1.9	298 ± 8	277 ± 12 (93%)	320 ± 10	297 ± 12 (93%)
ERM BC-211	119 ± 13	146 ± 3 (123%)	None reported	19.9 ± 0.6	124 ± 11	124 ± 2 (100%)	260 ±13	290 ± 5 (112%)

a. NMIJ 7503a iAs uncertainty estimated as the square root of the sum of squares of the AsIII and AsV uncertainties. Reproduced with permission of The Royal Society of Chemistry. P. J. Gray et al, J. Anal. At. Spectrom., 2017, 32, 1031.

Conclusions

A fast and fit-for-purpose HPLC-ICP-QQQ method is described for the measurement of inorganic As and two organic As species in baby rice cereal. A full speciation analysis can be completed in under two minutes.

- By oxidizing As(III) to As(V) with H_2O_2 during sample preparation, total iAs was determined as As(V).

- The narrow bore column and 0.5 mL/min HPLC flow rate provided excellent sensitivity, which allowed low volume injections to be used.

- Sample run times were 10x faster than the current FDA 4.11 method for the determination of As in rice.

- The HPLC-ICP-QQQ method delivered improved sensitivity, limits of detection and limits of quantification compared to the FDA 4.11 method.

The reproducibility of the method was demonstrated by the good agreement between the quantitative results for duplicate measurements of six rice cereal samples. The results showed that two of the samples contained iAs above 100 ppb.

This method provides valuable information for the safety of rice and rice-based infant cereals, as well as allowing food producers to meet regulatory requirements.

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More Information

For a full account of this study, see Patrick J. Gray, Courtney K. Tanabe, Susan E. Ebeler, and Jenny Nelson, A fast and fit-for-purpose arsenic speciation method for wine and rice, *J. Anal. At. Spectrom.*, **2017**, 32, 1031–1034; DOI: 10.1039/C7JA00041C

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