APPLICATION BRIEF

HPLC-ICP-MS

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Arsenic Speciation Analysis in Rice Following Method GB 5009.11-2014 to Ensure Safety

Introduction

Rice represents a dietary staple for over half of the world's population. Due to its gluten-free properties and high mineral content, the pulverized form is often one of the first solid foods to be introduced to babies. It is also the most popular grain for people with Celiac disease and for those on gluten-free diets. In recent years, however, concern has been raised over the presence of high levels of arsenic (As) in rice, where this carcinogenic metalloid can be accumulated at levels far exceeding ambient concentrations.[1] There are a number of potential sources of As in rice, such as the soil in which it is grown, the water which is used for irrigation, as well as atmospheric fallout. High concentrations of arsenic in soils and water can be either due to the natural geology of the area and leaching of this metalloid or can be introduced through a variety of different anthropogenic activities.[2]

Inorganic As species (As III and As V) are the prevalent forms of As in the terrestrial environment and are also the most toxic. The reducing and anoxic conditions often found in rice paddy fields can promote the interconversion and reduction of some As species to the most common toxic and carcinogenic form of As (As III). After As from the ambient environment is taken up by the rice plant, it may be somewhat metabolized and methylated into less toxic As species, where the extent of such methylation depends largely upon the part of the plant (roots, shoots, leaves, seeds). Consequently, if the total concentration of As in rice crops were to be evaluated, this would simply provide a sum of all of the different species of arsenic in the rice and could cause us to draw inaccurate conclusions about its potential toxicity. It is for this reason that speciation studies are necessary.

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Since China is the world's largest producer of rice, the Chinese government released a national standard method for food safety, GB 5009.11 in 2014 to aid the evaluation of As species in rice and rice products. In this document, HPLC-ICP-MS is identified as the preferred analytical technique. In 2017, GB 2762 was released, imposing maximum allowable limits (0.2 mg/kg) on the inorganic arsenic content. One of the challenges encountered in many As speciation methods is the need for a solvent gradient to be able to control the elution of the various arsenic species. This requires a robust plasma with accurate impedance matching, which is able to cope with the changing solvent gradient and the effect which this may have upon ionization. Moreover, since this method may increase the wear and tear on the pump seal, a pump possessing a mechanism which is able to effectively wash behind the pump seal would dramatically reduce seal damage, limiting downtime and the need for maintenance visits.

In this study, five arsenic species were characterized in a commercial rice sample and certified reference material using a gradient anion-exchange method in accordance with GB 5009.11. The analysis was performed using a PerkinElmer NexSAR™ HPLC-ICP-MS speciation solution, consisting of a NexSAR inert HPLC coupled to a NexION® ICP-MS.

Experimental

Sample Preparation

Calibration standards with concentrations of 0.2, 1, 5, 10, 50 and 100 µg/L were prepared in 0.15 M HNO_3 (Ultrapure, 68%,Suzhou JINGRUI Chemical Co. Ltd., Suzhou, Jiangsu, China) from the following reagents: As III from arsenite (75.5±1.2 µg/g), As V from arsenate (17.5±0.4 µg/g), monomethylarsonic acid (MMA; 25.1±0.8 µg/g), dimethylarsinic acid (DMA; 52.9±1.8 µg/g) and arsenobetaine (AsB; 38.8±1.1 µg/g) were from the National Institute of Metrology, China (NIM, Beijing, China). These species were selected for evaluation due to the requirements of the national standard and their high abundance in rice. Concentrations of the calibration standards were chosen according to the recommended concentrations as specified in GB 5009.11. Each species was identified according to its elution time by analyzing each arsenic species separately.

A commercial rice sample was purchased from a local store for analysis and a certified reference material (CRM, GBW(E)100348, Beijing China) used for method validation. The sample (0.5 g) was accurately weighed in triplicate on an analytical balance (Mettler Toledo, Columbus, Ohio, USA) to account for heterogeneity in the sample and placed in a 15 mL polypropylene tube (Crystalgen, Commack, New York, USA). Nitric acid (10 mL of 0.15 M HNO_3) was added to each sample and the tube swirled to ensure that the sample was fully immersed in the acid. Thereafter, the samples were placed in a drying oven (DHG-9000, Shanghai Bluepard Instruments Co. LTD. Shanghai, China) for 3 hours at 90 °C and shaken by hand for 1 min every ½ hour. After extraction, the samples were allowed to cool and were centrifuged (TDL-60B, ShangHai Anting Scientific Instrument Factory, Shanghai, China) at 8000 rpm for 15 min. The supernatant was decanted and filtered through a 0.45 µm syringe filter (PTFE, hydrophilic, Millex, Sigma Aldrich™, St. Louis, Missouri, USA) to remove any remaining particulate matter and the remaining pellet disposed of. A methodology blank and the CRM were prepared in the same way as the samples to ensure that the method did not contribute toward the observed As concentrations or skew the analytical results, ensuring the accuracy of the analysis. A spiked rice sample (5 µg/L of all As species) was also analyzed as part of the sample sequence to further validate the analytical method for the individual As species in the sample.

The mobile phase was prepared from HNO_3 (Suzhou JINGRUI Chemical Co. Ltd.), H_3PO_4 (85%, Fisher Scientific, Hampton, New Hampshire, USA) and NH_4OH (≥25%, Sigma Aldrich, Burlington, Massachusetts, USA). During this work, the calibration standards, rice extract, CRM and blanks were decanted into metal-free polypropylene HPLC vials and analyzed without dilution. To account for the changing gradient of the chromatographic baseline, a bypass sample was analyzed as part of the sequence and subtracted from the blank, sample and CRM chromatograms.

Instrumentation

All analyses were performed using a NexSAR Speciation Analysis Ready HPLC system (PerkinElmer Inc., Shelton, Connecticut, USA) comprised of the NexSAR 200 Inert HPLC Pump, Cooled Inert Autosampler, and Solvent Tray with Degasser. The system was coupled to a NexION ICP-MS (PerkinElmer Inc.). Details pertaining to the HPLC and ICP-MS conditions are shown in Tables 1 and 2, respectively. Samples and standards were run in standard mode where a correction equation was applied to compensate for the $ArCl^+$ polyatomic interference. All analyses and the collection of data were performed using Clarity™ chromatography software (DataApex, Prague, The Czech Republic).

Table 1. NexSAR Inert HPLC Conditions.

Component/Parameter	Type/Value
Chromatography	Anion exchange chromatography
Mobile Phase	A: 10 mM ammonium phosphate B: 10 mM ammonium nitrate
pH	8.6
Flow	1 mL/min
Separation Scheme	Gradient
Run Time	16 min
Injection Volume	100 µL
Column Temperature	30 °C
Injection Type	Full loop
LC Vials	HPLC tested PP vials, 1.5 mL

Table 2. NexION ICP-MS Conditions.

Component/Parameter	Type/Value
Nebulizer	MEINHARD® Plus Glass Type C
Spray Chamber	Glass cyclonic
RF Power	1600 W
Injector	2.0 mm I.D. quartz
Mode	Standard
Dwell Time	500 ms

Results and Discussion

The correlation coefficients for the standards (0.2-100 µg/L, n=6) of AsB, As III, DMA, MMA and As V were 0.999965, 0.999979, 0.999999, 0.999999 and 0.999989 respectively (Figure 1a-e), demonstrating excellent linearity across the expected concentration range for As in rice as specified by GB 5009.11. The overlay of the calibration standards from 0.2-100 µg/L (Figure 2) demonstrates the consistent, reliable and reproducible flows which can be delivered by the NexSAR 200 Inert HPLC Pump, thereby ensuring that peaks are correctly identified and are consistently sharp, allowing good accuracy and signal-to-noise (S/N) ratios to be achieved.

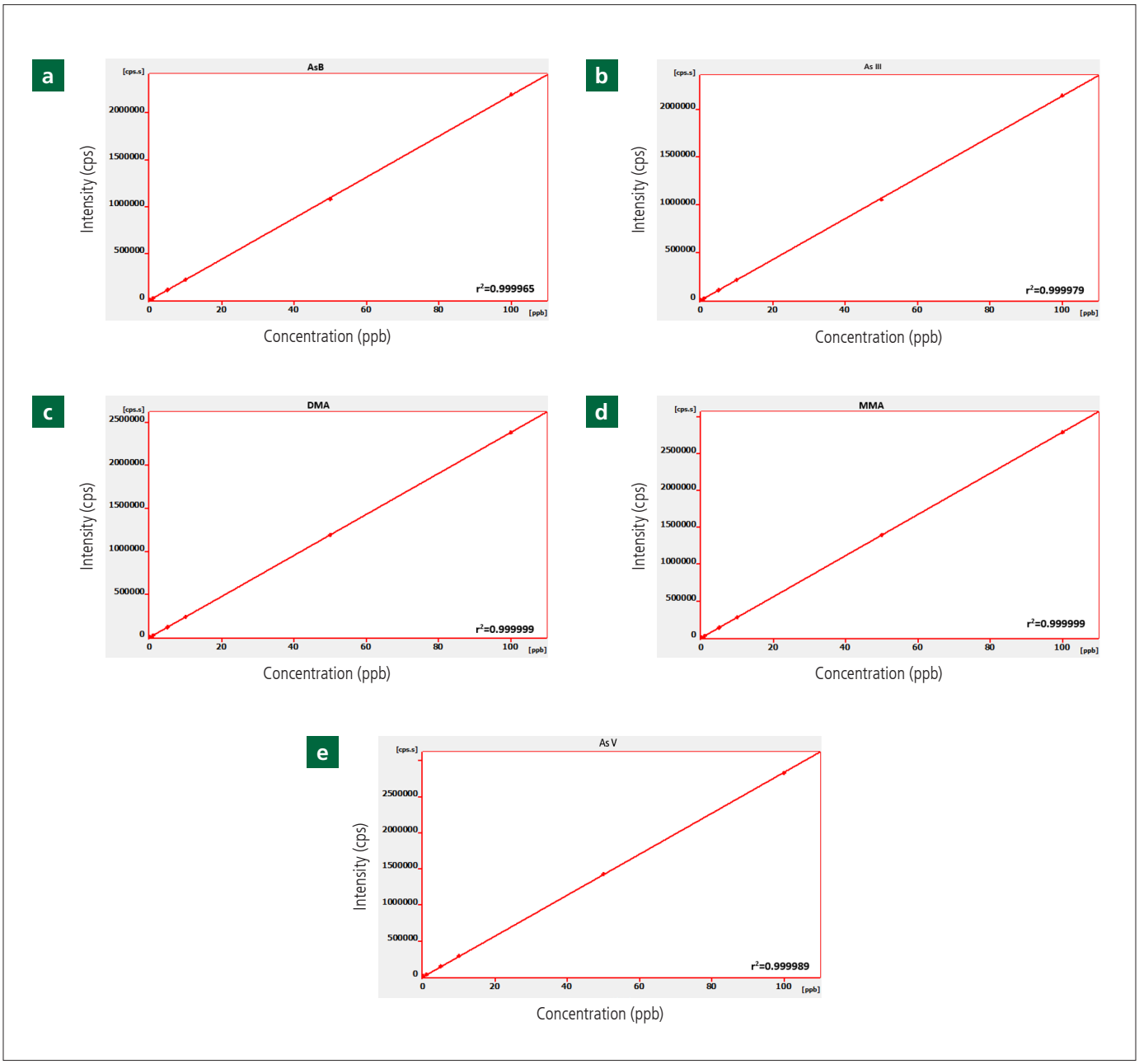

Figure 1. Linear regression of calibration standards ranging in concentration from 0.2-100 µg/L for (a) AsB, (b) As III, (c) DMA, (d) MMA, (e) As V in the mobile phase (pH 8.6) and the respective correlation coefficients.

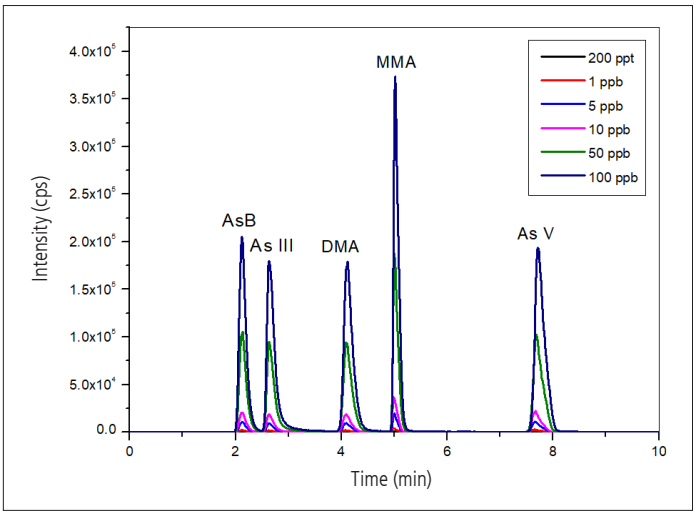

Figure 2. Bypass subtracted overlay of calibration standards (0.2-100 µg/L, n=6) in the mobile phase at pH 8.6.

The S/N ratio for a 50 ng/L standard (Figure 3) was 15, 13, 13, 17 and 23 for AsB, As III, DMA, MMA and As V respectively using the defined mobile phase, providing a theoretical detection limit of 11.5 ng/L for As III and DMA, 6.5 ng/L for As V, 8.8 ng/L for MMA, and 10 ng/L for AsB.

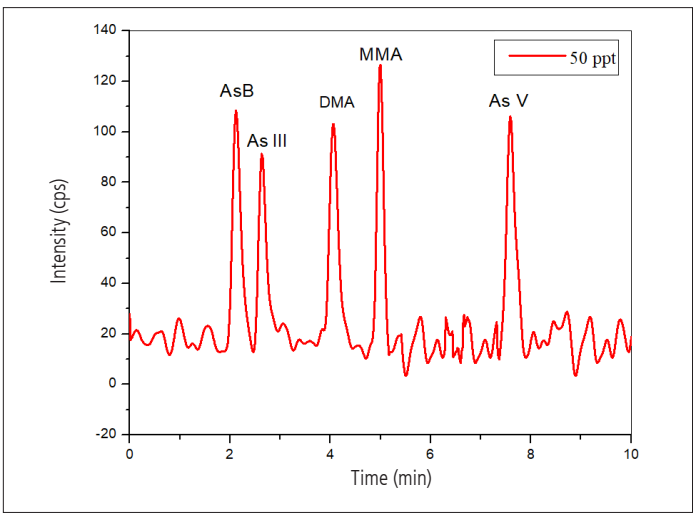

Figure 3. 50 ng/L standard (bypass subtracted) of five species of arsenic to determine the theoretical detection limits.

The overlay of the unspiked rice sample, the spiked (5 µg/L) rice sample and the 10 µg/L standard can be seen in Figure 4. This overlay demonstrates that the sample matrix does not have an effect on the retention time of different As species, proving the robustness of the method which is enhanced through the accuracy of the pump flows. Excellent spike recoveries of 105, 92, 95, 97 and 96% were achieved for AsB, As III, DMA, MMA and As V respectively (Figure 5), further validating the method and proving its accuracy for each individual As species in the sample matrix. The method was additionally validated through the use of a CRM (GBW(E) 100348), where Figure 6 shows an overlay of the CRM and the 10 µg/L calibration standard. The CRM, which had the same retention time as the standards, had a recovery of 100% for inorganic As and 99% for the total As content, further confirming the analytical accuracy of the method.

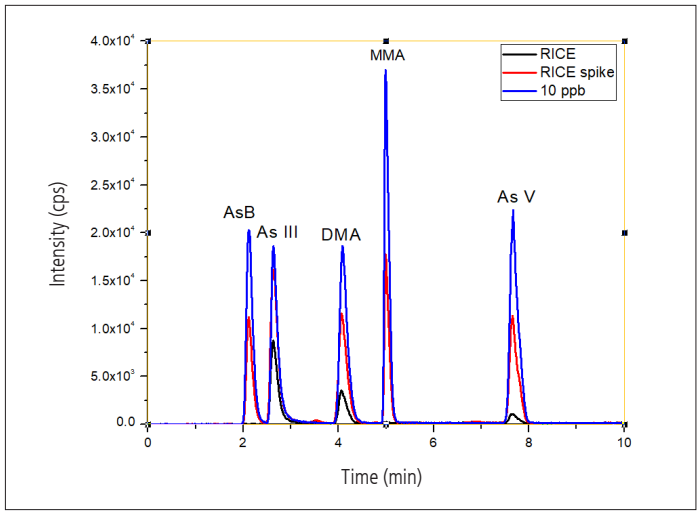

Figure 4. Overlay of chromatograms (bypass subtracted) for the rice sample, spiked rice sample and 10 µg/L standard.

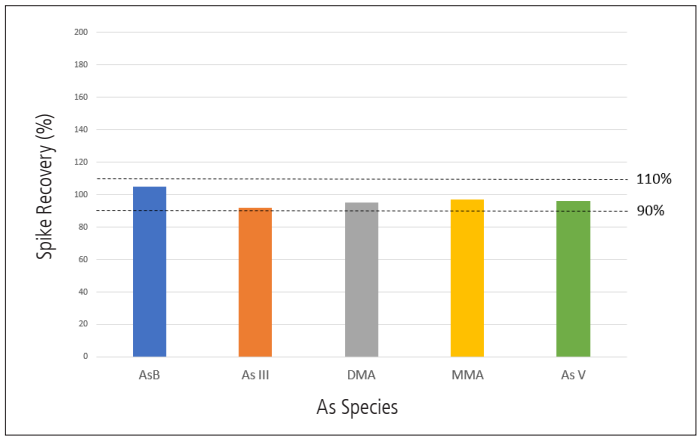

Figure 5. Spike recoveries for 5 µg/L spike of AsB, As III, DMA, MMA and As V in rice extract.

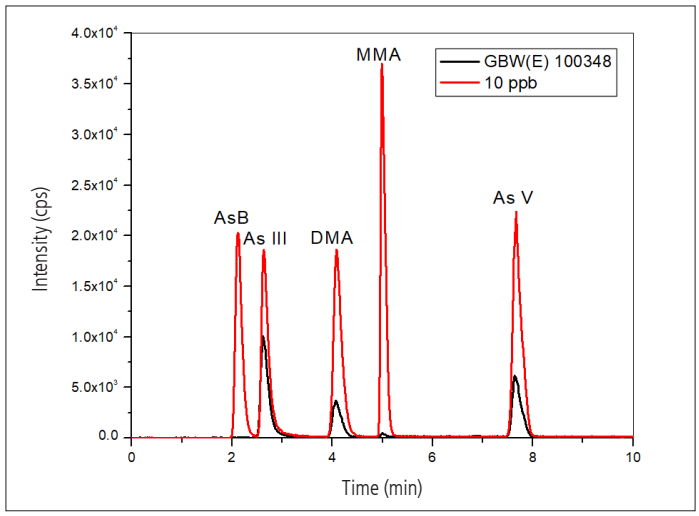

Figure 6. Chromatogram of certified reference material (bypass subtracted) GBW(E) 100348 in comparison to the 10 µg/L standard.

The concentration of As species in the rice sample purchased from a local store (Table 3) was evaluated using the proposed method. As III, As V and DMA were above the method detection limits where the sum of the inorganic As species (As III and As V) were below the regulated limit of 0.2 mg/kg (GB 2762). AsB and MMA were both below the method detection limits and are not reported here.

Table 3. Arsenic Species Concentrations in a Commercial Rice Sample.

Species	Average Concentration ± stdev (µg/g)
AsB	BD*
As III	0.099±0.004
DMA	0.039±0.001
MMA	BD*
As V	0.0105±0.001

*BD: below detection

Conclusion

This study evaluated the As species in a rice sample, spiked rice sample and rice CRM according to the method proposed by GB 5009.11 using a NexSAR Inert HPLC system coupled to a NexION ICP-MS. The results demonstrated that low concentrations of As species could be easily and accurately quantified using the proposed speciation solution and gradient method, where the robust plasma of the NexION and accurate flow of the mobile phase delivered by the HPLC pump were critical in being able to achieve this. In the commercial rice evaluated, the inorganic As content was found to be lower than the regulated amount.

References

1. Heikens, A., 2006. Arsenic Contamination of Irrigation Water, Soil and Crops in Bangladesh: Risk Implications for Sustainable Agriculture and Food Safety in Asia. Food and Agricultural Organization of the United Nations, Regional Office for Asia and the Pacific.
2. ATSDR, 2013. Arsenic Toxicity: Where is Arsenic Found? https://www.atsdr.cdc.gov/csem/csem.asp?csem=1&po=5

Consumables Used

Component	Description	Part Number
HPLC Vials	HPLC Tested Plastic Vials, 1.5 mL PP	N9301736
PEEK Tubing	Yellow, 0.007" ID, 1/16" OD (5 feet)	N9302678
PEEK Fittings	Fingertight for 1/16" OD PEEK Tubing	09920513
Nebulizer Connector	PEEK Connection Line for MEINHARD® Nebulizers	N8152484

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