



HPLC/ICP-MS

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Arsenic Speciation Analysis in Brown Rice by HPLC/ICP-MS

Introduction

Arsenic (As) is a well-known toxic element which has been highly regulated, especially for drinking water. Although regulatory limits

have been set for total arsenic, its toxicity varies widely and is dependent on its chemical form. For example, inorganic forms of arsenic are highly toxic and carcinogenic. However, organic forms (such as monomethylarsonic acid, dimethylarsinic acid, and arsenobetaine) are recognized as non-toxic or as having low toxicity.

The Joint Expert Committee on Food and Additives (JECFA) recognizes the importance of monitoring inorganic arsenic intake. In 1988, they established a provisional tolerable weekly intake (PTWI) of 0.015 mg/kg body weight inorganic arsenic. However, this recommendation was withdrawn in 2010.

In Japan, the average total arsenic intake/person/day is divided between seafood (53.6%), vegetables and seaweed (35.4%), rice (7.1%), and other sources.¹ It is known that the majority of arsenic in marine organisms is in the form of arsenobetaine, which is non-toxic. However, because of the large quantities of rice consumed in Japan, it is important to know what forms of arsenic are present in rice.



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In recent years, it has become common to measure different forms of arsenic using HPLC/ICP-MS: HPLC separates the forms and ICP-MS detects them as they elute from the column. The advantage of ICP-MS as an HPLC detector is that it is very sensitive and can measure trace levels, as demonstrated by its use to measure impurities in a wide range of electronic materials and environmental samples.

This work focuses on the use of HPLC/ICP-MS to measure various arsenic compounds in brown rice.

Experimental

Calibration and Sample Preparation

As part of an inter-laboratory study, brown rice samples with known arsenic concentrations were distributed to eight labs. The sample preparation, HPLC conditions, and analysis scheme were defined by the institution coordinating the study.

Sample preparation consisted of adding 10 mL ultrapure water to 0.1 g rice powder and heating for 4 hours at 90 °C. After cooling to room temperature, the samples were centrifuged and filtered with a 0.45 µm syringe filter.

Standards were prepared by mixing DMA, MMA, As(III), and As(V); AsB was also added as an internal standard. Calibration curves, established with 0.5, 1, 2, 5, 10, 20, and 30 ppb standards, had correlation coefficients > 0.999 for all compounds.

Instrumental Conditions

All analyses were done using a PerkinElmer Flexar™ HPLC system coupled to a PerkinElmer NexION® 300D ICP-MS. When performing elemental analyses with ICP-MS,

polyatomic interferences are a concern. For example, if a sample contains chloride, ArCl^+ and CaCl^+ may interfere with arsenic measurements since they all exist at m/z 75. The NexION 300 series ICP-MS is equipped with a Universal Cell, which allows the effects of polyatomic interferences to be removed either through chemical reactions or collisions. Although collisions with helium as a cell gas remove the interferences, there is also a significant decrease in analyte sensitivity. In this work, methane was used in the Universal Cell to remove any ArCl^+ or CaCl^+ polyatomic interferences on As^+ through chemical reactions without significantly reducing As^+ sensitivity. Both the HPLC and ICP-MS conditions used in this work are shown in Tables 1 and 2, respectively.

Table 1. HPLC Conditions

Parameter	Condition
Instrument	Flexar HPLC
Column	4.6 mm × 250 mm, 5 µm particles
Mobile phase	10 mmol/L 1-butanesulfonic acid (sodium salt) 4 mmol/L tetramethyl ammonium hydroxide, 4 mmol/L malonic acid, 0.05 % methanol, 0.03% nitric acid
Flow	0.8 mL/min
Column temperature	Room temperature
Injection volume	10 µL

Table 2. ICP-MS Conditions

Parameter	Condition
Instrument	NexION 300D ICP-MS
RF power	1600W
Analyte	m/z ^{75}As
Cell conditions	Methane, 0.3 mL/min

Results

The concentrations of each compound were determined by measuring peak area; Chromera HPLC software was used for instrument control, sample analyses, and data processing. Figure 1 shows the chromatograms of two brown rice samples; each contained As(V), As(III), and DMA, with AsB added as the internal standard. Each sample measurement required 7 minutes.

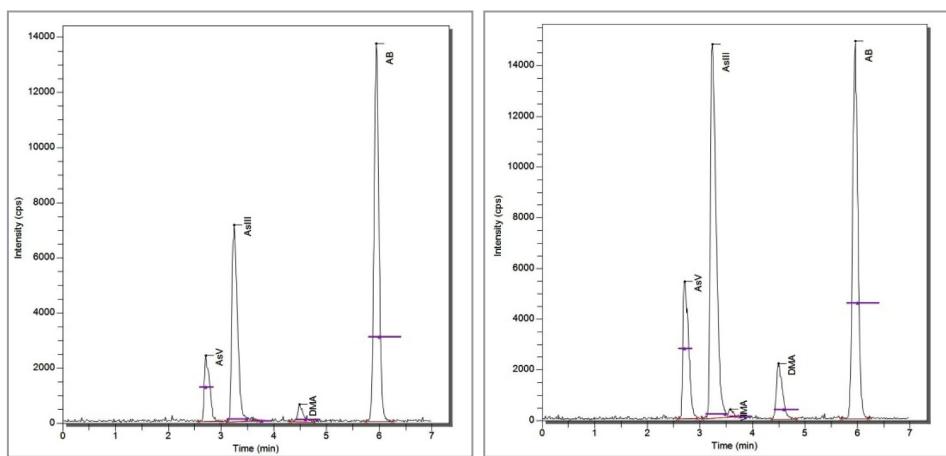


Figure 1. Chromatograms of rice samples A and C.

Table 3 shows the LODs and LOQs for each arsenic compound, along with the results for the five brown rice samples measured. The LODs and LOQs are based on the signal-to-noise ratios (S/N), with the LOD defined as S/N = 3 and the LOQ defined as S/N = 10.

Table 3. Results for Brown Rice Samples, LODs, and LOQs

Species	LOD	LOQ	Sample A	Sample B	Sample C	Sample D	Sample E
As(V)	0.00013	0.00044	0.021	0.043	0.044	0.040	0.053
As(III)	0.00012	0.00039	0.070	0.13	0.13	0.15	0.18
MMA	0.00014	0.00046	ND	ND	0.0013	ND	ND
DMA	0.00022	0.00072	0.0063	0.017	0.020	0.030	0.026

Units: mg-As/kg ND: Not detected

Figure 2 shows the results from the inter-laboratory study for all species in all five samples, as well as the total arsenic results. The error bars represent the range of results from the participating labs, while the red triangles show the average. The green circles represent the results from this work and fall at or near the center of the range for each sample, thus indicating the accuracy of the results.

The last plot in Figure 2 shows the results for total As in each sample. The blue diamonds (\blacklozenge) represent the results supplied by the institution running the study. These total As values were determined after sample decomposition in a microwave. The green circles (\bullet) show the sum of the individual species in each sample from this work. The red triangles (\blacktriangle) represent the average of the sum of all species from each of the participating labs. These results demonstrate the accuracy of the analysis and confirm that all of the As in the rice was extracted during the sample preparation for the chromatographic analysis.

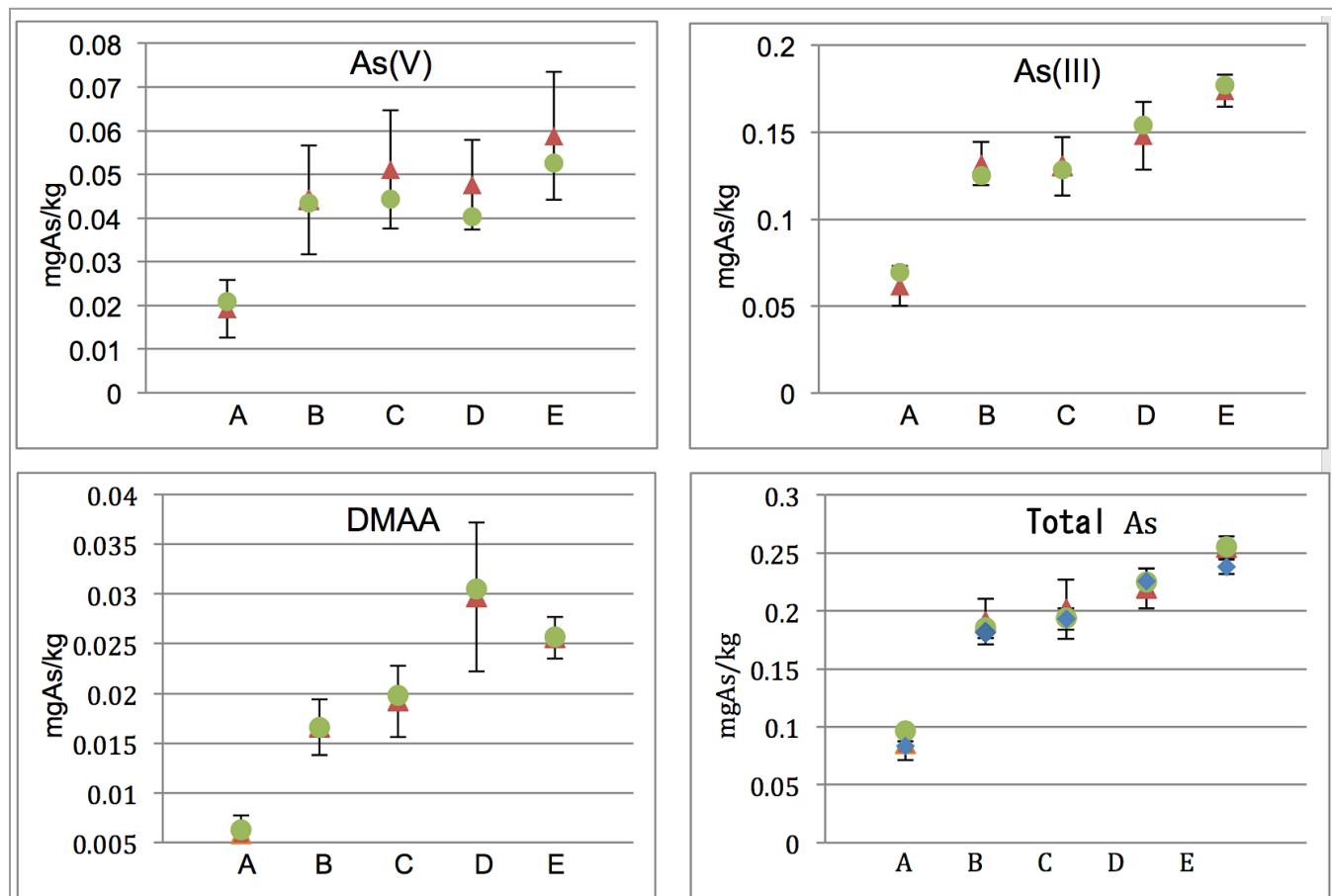


Figure 2. Results for As(V) (upper left), As(III) (upper right), DMAA (lower left), and Total As (lower right) \blacktriangle : Average results from the 8 participating labs; \bullet : Results from this work; \blacklozenge : Known concentrations

Summary

This work demonstrates the ability to separate and measure arsenic compounds present in brown rice samples by selection of proper HPLC and ICP-MS conditions. The results of this work correlate very well with those of the other organizations participating in the study. These results prove that the combination of Flexar HPLC and NexION ICP-MS is suitable for the analysis of different forms of arsenic in brown rice.

References

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