APPLICATION NOTE

HPLC/ICP - MS

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Arsenic Speciation Analysis in White Rice by HPLC/ICP-MS Using the NexION 300D/350D

Introduction

There has been a rising concern about the presence of arsenic in rice, especially in societies which consume large quantities of rice. Arsenic can enter rice naturally through the environment or through the application of pesticides. Because not all arsenic species are toxic, the ability to measure the different forms is important.

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 demonstrates the ability to measure various arsenic forms in white rice, building upon previous work.[1,2]

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Experimental

Sample Preparation

Rice samples included: NIST 1568a Rice Flour; three samples of rice purchased in a local grocery store, each from a different region of Korea; and two instant rice products, also purchased from a local grocery store.

Uncooked rice was ground into a fine powder, and 0.5 g was transferred to a 15 mL sample tube. Next, 4.5 g of 0.2% HNO_3 (v/v) was added to the tube, which was then mixed with a vortex mixer for 10 seconds. The tube was place in a hot block at 120 °C for four hours and then allowed to cool. The cooled solution was centrifuged at 4000 rpm for 30 minutes and then filtered through a 0.45 µm PTFE membrane. For final analysis, 0.1 mL of the filtered solution was combined with 0.9 mL of deionized water in a 1.5 mL HPLC autosampler vial and mixed for 10 seconds with a vortex mixer.

For comparison, an analysis for total As was also performed in the same samples. Sample preparation involved adding 0.5 g of ground rice powder to a 50 mL tube, to which 4 mL of concentrated nitric acid and 1 mL concentrated hydrogen peroxide were added; the contents were mixed with a vortex mixer for 10 seconds. The tube was then placed in a hot block at 120 °C for 30 minutes, cooled, and brought to a final volume of 50 mL with deionized water.

Instrumental Conditions

All analyses were done using a PerkinElmer Flexar™ HPLC system coupled to a PerkinElmer NexION® 300D ICP-MS. Tables 1 and 2 show the HPLC and ICP-MS conditions used for this work. Separation is accomplished with a reversed-phase column in four minutes using an isocratic chromatographic method. The same ICP-MS conditions were used for both speciation and total analyses, with iridium (Ir) being used as an internal standard for total analysis. All measurements were made against external calibration standards.

Results

Figure 1 shows the separation of a 5 ppb standard containing five common arsenic species: As3+, As5+, monomethyl arsenic (MMA), dimethyl arsenic (DMA), and arsenobetaine (AsB). All peaks are baseline resolved and elute in less than four minutes. The chromatogram in Figure 1 is actually an overlay of five consecutive injections of the same standard, with Table 3 showing the statistics of this analysis. With retention times having RSDs < 0.5% and recoveries within 100 +/- 2%, the reproducibility of the methodology is demonstrated.

Table 1. HPLC Conditions

Parameter	Condition
Instrument	Flexar HPLC System
Separation scheme	Isocratic
Flow rate (mL/min)	1.5
Injection volume (µL)	50
Column	C18
Column temperature	Room temperature

Table 2. ICP-MS Conditions

Parameter	Condition
Instrument	NexION 300D ICP-MS
Spray chamber	Glass cyclonic
Nebulizer	Glass concentric
Analyte monitored	AsO+ (m/z 91)
Cell gas	O_2 = 0.5 mL/min
RPq	0.45
Dwell time (ms)	500

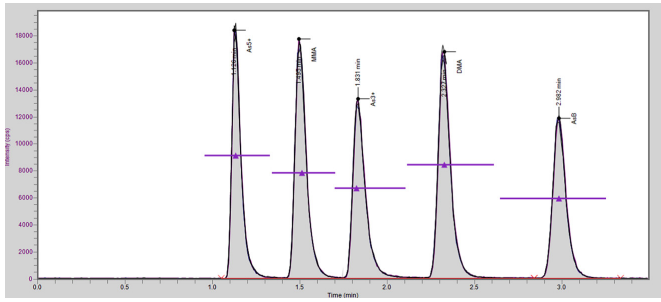

Figure 1. Five consecutive injections of a 5 ppb mixed standard.

Table 3. Results from Five Consecutive Injections of a 5 ppb Mixed Standard

Species	Retention Time #1	Retention Time #2	Retention Time #3	Retention Time #4	Retention Time #5	AVG	SD	%RSD
As5+	1.126	1.126	1.134	1.134	1.134	1.131	0.004	0.387
MMA	1.495	1.495	1.504	1.495	1.504	1.499	0.005	0.329
As3+	1.831	1.831	1.823	1.831	1.831	1.829	0.004	0.196
DMA	2.318	2.327	2.318	2.327	2.318	2.322	0.005	0.212

Species	Results #1	Results #2	Results #3	Results #4	Results #5	AVG	SD	%RSD	% Recovery
As5+	4.928	4.931	4.899	5.008	4.913	4.936	0.042	0.858	98.716
MMA	4.91	4.924	4.913	4.912	4.916	4.915	0.005	0.111	98.3
As3+	4.974	4.96	4.927	5.01	5.021	4.978	0.038	0.766	99.568
DMA	5.007	4.998	4.96	4.931	4.973	4.974	0.03	0.612	99.476
AsB	5.032	5.075	5.081	5.108	5.1	5.079	0.03	0.583	101.584

To examine the effect of the rice matrix on the separation, chromatograms of the three rice samples from different regions of Korea were overlaid with the calibration standards, as shown in Figure 2. Since the retention times of the samples match those of the standards, the rice matrix does not affect the chromatography.

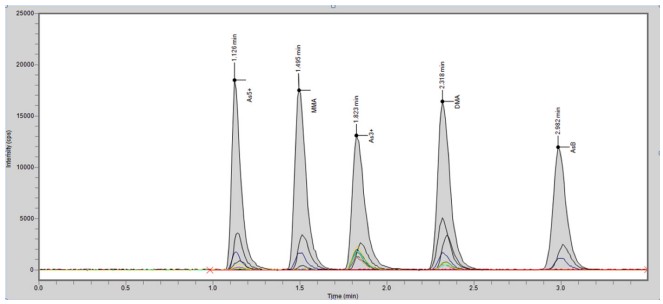

Figure 2. Chromatograms of three rice samples and three calibration standards.

Table 4 shows the quantitative results from both speciation and total analysis. For all samples, the sum of the species is within 10% of the total As content, demonstrating the accuracy of the method. Further validation is seen with the NIST SRM, where both total and the sum of the species are within 10% of the certified value.

Table 4. Quantitative Results

#	Name	Acid Digestion AsO 91 (ppb)	Speciation AsO 91 (ppb)	Speciation/Digestion Matching Rate (%)	NIST SRM Recovery (%) Acid Digestion	NIST SRM Recovery (%) Speciation
1	NIST SRM	287.36	265.60	92.43	99.09	91.59
2	Cooked Rice #1	68.03	73.40	107.89	N/A	N/A
3	Cooked Rice #2	84.66	81.80	96.62		
6	Market Rice #1	106.11	116.80	110.07		
5	Market Rice #2	95.46	91.00	95.33		
4	Market Rice #3	95.91	90.10	93.94		

Conclusion

This work has demonstrated the ability to separate and measure arsenic compounds present in rice. Sample preparation involves a non-aggressive extraction procedure to preserve the species as much as possible. The chromatography separates all species in less than four minutes using an isocratic method and is unaffected by the rice matrix. The results of the separation were validated by comparing the speciated and total results. These results prove that the combination of Flexar HPLC and NexION ICP-MS is suitable for the analysis of different forms of arsenic in white rice.

References

1. "Determination of Arsenic Species in Apple Juice by HPLC/ICP-MS," K. Neubauer, P. Perrone, W. Reuter, PerkinElmer Application Note

2. "As Speciation Analysis in Brown Rice Using HPLC/ICP-MS," K. Kobayashi, O. Shikino, PerkinElmer Application Note

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