Challenge

Separation and quantification of toxic As species in rice

Solution

Combination of LC and highly sensitive ICP-MS to determine As species down to sub microgram per kilogram concentrations

Speciation of Arsenic in Rice by LC-ICP-MS on PlasmaQuant MS Elite

Introduction

According to the World Health Organization guide lines, the permissible level for total arsenic in drinking water is 10 ng/mL. Although no such limit exists for food products, the Food and Agriculture Organization / World Health Organization (FAO/WHO) recommend an intake no greater than 15 µg per kg body weight per week.

Next to drinking water, rice consumption is a major source of arsenic that concerns approximately 3 billion people. World rice consumption has risen from 156 million in 1960 to 496.6 million metric tons in 2013. Moreover, studies show that arsenic exposure is more critical in rice than in any other food stuff. For example, the arsenic level in rice is 10 times higher than in wheat and barley. In addition to direct ingestion, using rice straw for cattle feed increases the risk of arsenic exposure.

The toxicity of arsenic depends not only on the total concentration, but also its chemical forms as these differ in terms of mobility, toxicity and bioavailability. The inorganic trivalent arsenic (AsIII) and pentavalent arsenic (AsV) are the most toxic forms, whereas other common forms including the organic monomethyl arsenic (MMA) and dimethyl arsenic (DMA) have significantly reduced toxicities. Rice typically contains a high proportion of the inorganic forms of arsenic, emphasizing the importance of arsenic speciation in the analysis of rice samples.

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Instrumentation

The LC system used was a BRUKER Advance HPLC system with a 100 µL sample loop and a Hamilton PRP-X100 (4.6 mm x 150 mm, 5 µm) anion exchange column. The ASpect MS software allows automatic optimization of the ion optics and plasma gas flows. Prior to connecting the LC to the ICP-MS, it was optimized for maximum sensitivity on the ^{75}As isotope. ICP-MS and LC conditions are summarized in Tables 1 and 2, respectively.

Table 1: PlasmaQuant® MS Elite ICP-MS operating conditions

Parameter	Specification
Plasma Gas Flow	9.0 L/min
Auxiliary Gas Flow	1.00 L/min
Nebulizer Gas Flow	1.00 L/min
Plasma RF Power	1.30 kW
Monitored Ion	^{75}As
Scan Mode	Time Resolved
Dwell Time	500 ms
Spraychamber Temp.	3 °C
Ion Optics	Optimized for ^{75}As sensitivity

Table 2: LC operating conditions

Parameter	Specification
Mobile Phase	A : 12.5 mM ammonium carbonate, 1 % MeOH B : 60.0 mM ammonium carbonate, 1 % MeOH
Flow Rate	1 mL/min
Run Time	10 min
Column	Anion exchange, Hamilton PRP-X100, 4.6 mm x 150.0 mm, 5 µm
Column Temperature	40 °C
Sample Injection	50 µL
Detection	PlasmaQuant® MS Elite ICP-MS

Samples and Reagents

Deionized water (18.2 MΩ/cm, Millipore MiliQ, Billerica, MA, USA) was used for all solution preparations (mobile phases, standard solutions and samples).
Where specified, solutions were acidified with 67 % w/w nitric acid (NORMATOM, VWR).

Mobile phase (LC)

Ammonium carbonate Puratronic® (Alfa Aesar) and methanol absolute ULC/MS (Biosolve BV, 5555 Valkenswaard) were used to produce both A (12.5 mM ammonium carbonate, 1 % MeOH) and B (60 mM ammonium carbonate, 1 % MeOH) mobile phases. Mobile phases were prepared daily. Gradient was used as shown on table 2.

Calibration standards

Calibration solutions of arsenic trioxide (AsIII, Acros Organics), arsenic pentoxide (AsV, Sigma-Aldrich), monosodium acid methane arsonate (MMA, Supelco) and cacodylic acid (DMA, Fluka) were prepared daily. Calibration ranged from 0.1 to 1 µg/l for all arsenic species.

Sample preparation

Long grain basmati rice, purchased from a French supermarket, was ground and dried. The sample then underwent two different extraction methods as detailed below. National Institute of Standards and Technology, Certified Reference Material 1568b (Rice Flour) was also prepared using the same extraction methods.

Extraction 1 - Arsenic speciation

Approximately 0.2 g of sample was weighed into each of 3 x 50 mL PFA microwave tubes and 10 mL of 0.5 % v/v HNO_3 added and left to stand overnight at room temperature. Samples were microwave digested with a 3 stage temperature ramping program (table 3). Samples were allowed to cool and each tube was then filled to 50.0 mL with ultrapure water. Arsenobetaine (BCR 626, IRMM) was added as an internal standard at 1 µg/L to correct for any potential drift and matrix effect.

Table 3: Microwave temperature ramping program

Temperature (°C)	Ramp Time (min)	Hold Time (min)
0 – 55	5	**10**
55 – 75	5	**10**
75 – 95	5	**30**

Extraction 2 - Total arsenic

Approximately 0.2 g of sample was weighed into each of 3 x 50 mL PFA microwave tubes and 2 mL of HNO_3 added and left to stand overnight at room temperature. The same microwave digestion program as shown in table 3 was applied. Samples were allowed to cool and each tube was filled to 50.0 mL with ultrapure water.

Blank solutions were prepared following both extraction methods.

Results and Discussion

Speciation method calibration

Successful speciation of four arsenic species (AsIII, DMA, MMA, AsV) and arsenobetaine (internal standard) was achieved in less than 10 minutes using a gradient LC method. Excellent calibrations for each of the arsenic species were obtained with regression coefficients (r^2) ≥ 0.9993 when calibrating from 0.1 to 1 µg/L as illustrated in Figure 1.

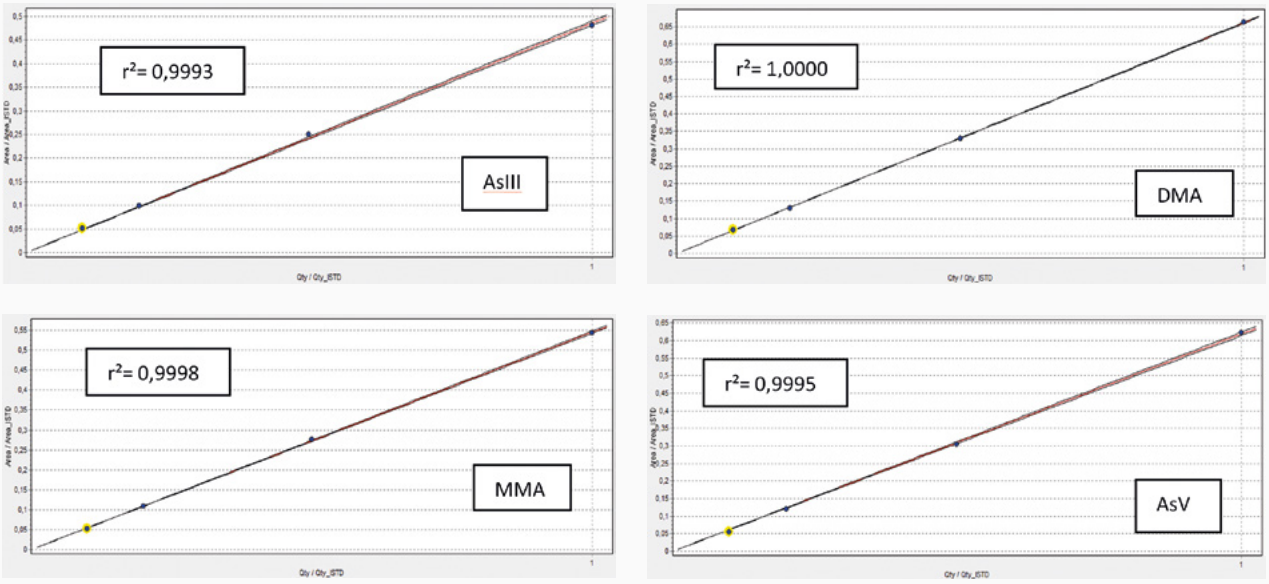

Figure 1: Calibration curves for AsIII, DMA, MMA and AsV

Speciation method

Table 4 shows the determination of As species in a basmati rice sample prepared in triplicate using extraction method 1.

Table 4: Triplicate analyses of basmati rice sample for As species

	AsIII	DMA	MMA	AsV	Sum of the 4 species
Mean [As] (µg/kg)	162	60	ND	95	317
% RSD	4.3	6.7	--	11.2	5.7

ND=Not Detected

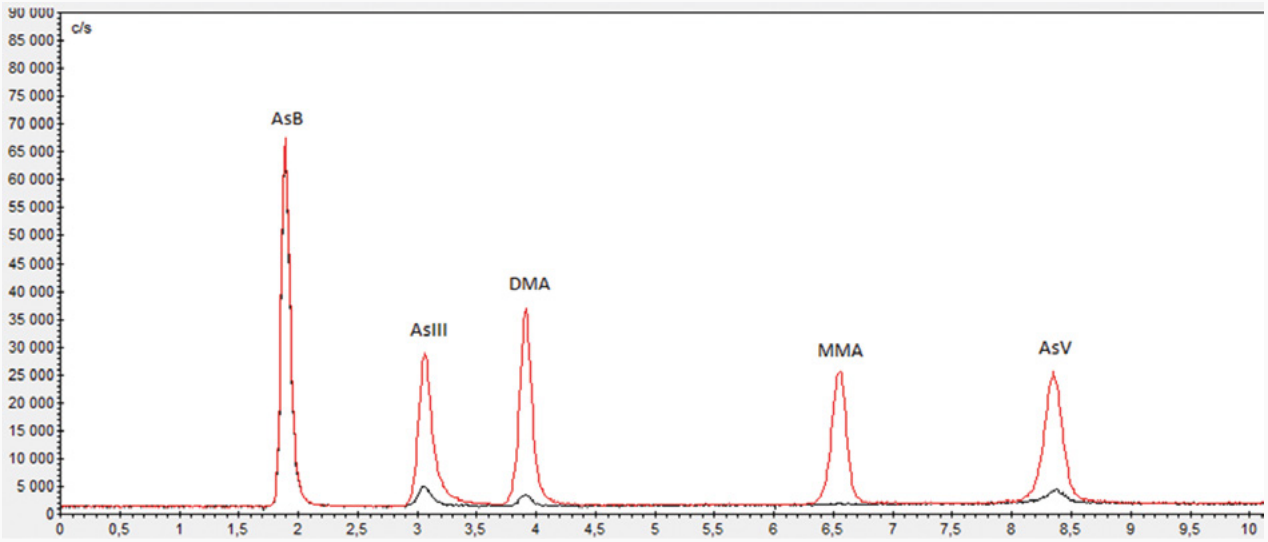

Figure 2: Overlay of 250 µg/kg spike (red) and basmati rice (black) chromatograms

Total As method

Samples prepared using extraction method 2 were analyzed directly by ICP-MS to verify to total As content in the basmati rice sample.

Table 5: Triplicate analyses of basmati rice sample for total As

	Total As
Mean [As] (µg/kg)	342
% RSD	0.9

Summing the four As species determined using extraction method 1 was found to have a recovery of 92.7 % of the total As concentration when determined directly using extraction method 2.

Extraction method accuracy

In order to test microwave extraction method 1 and its effects on the concentrations of the As species present in the sample, extractions were conducted on NIST's CRM 1568b (Rice Flour). Table 6 summarizes data and recoveries.

Table 6: Triplicate analyses of As species in NIST-1568b Reference Material

	AsIII	DMA	MMA	AsV	Inorganic As
Mean [As] (µg/kg)	37.7	189.6	10.7	35.5	73.2
% RSD	19.7	5.6	8.9	2.8	11.0
Certified value (µg/kg)	NA	180.0	11.6	NA	92.0
% Recovery	--	105	92	--	80

NA=Not Available

Long Term Stability

A sequence of 22 rice samples (6 hours) was run and a calibration check solution at 1 µg/L was measured every 5 samples. Table 7 summarizes the results.

Table 7: Long term stability test using a 1 µg/L calibration check solution over a sequence of 22 samples (6 hours)

	AsIII (µg/L)	DMA (µg/L)	MMA (µg/L)	AsV (µg/L)
Check 1	0.9881	0.9895	0.9966	1.000
Check 2	0.9499	0.9578	0.9975	0.9986
Check 3	0.9541	0.9566	1.006	1.0399
Check 4	0.9468	0.9605	0.9821	1.005
% RSD	1.92	1.57	0.99	1.95

Typical detection limits

Table 8 shows the method detection limits (MDL) for the four common organic and inorganic forms of arsenic in rice. All the measurements were made under routine laboratory conditions.
Detection limits were calculated using 3 times the standard deviation of blank samples (n=10).

Table 8: Typical detection limits of As species in rice using the PlasmaQuant® MS Elite

Arsenic species	Detection limit (µg/kg)
Arsenite (AsIII)	0.34
Dimethyl arsenic acid (DMA)	0.33
Monosodium acid methane arsonate (MMA)	0.34
Arsenate (AsV)	0.41

Conclusion

This work illustrates the superb performance of the PlasmaQuant MS Elite when coupled to a Bruker Advance HPLC system, measuring various arsenic species in a basmati rice sample in less than 10 minutes. Sample preparation and method accuracy were validated using NIST CRM 1568b and shown to be simple, yet reliable, following extraction and microwave digestion. Excellent precision and accuracy was observed and peak shapes were well defined and easily resolved using a gradient flow mobile phase. The combination of excellent chromatography and the high sensitivity of the PlasmaQuant MS Elite allows for sub parts per billion (<1 µg/kg) limits of detection for all measured arsenic species.

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