High throughput determination of inorganic arsenic in rice using hydride generation-ICP-MS

Application note

Food safety

Authors

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Abstract

A fast and sensitive method using hydride generation (HG) with ICP-MS has been developed for the separation and detection of inorganic arsenic (iAs) in foodstuffs. The iAs measured values in 31 commercial rice products all fell below the current legislative maximum level (ML) of 150 µg/kg set by China. Comparing the results obtained by HG-ICP-MS and HPLC-ICP-MS showed comparable limits of detection were achieved.

HG-ICP-MS is ideally suited to the large scale screening of food samples, such as rice, as analysis can be performed in a fraction of the time typically required by a conventional HPLC-ICP-MS approach, with no compromise in analytical performance.

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Introduction

The concentration of potentially toxic chemicals such as arsenic in foodstuffs is closely monitored to ensure food safety. However, the toxicity of arsenic depends on the chemical form or "species" of the element that is present rather than total concentration. Inorganic arsenic (iAs) species, arsenite (As(III)) and arsenate (As(V)), are known to be carcinogenic and highly toxic, whereas the common organoarsenic species monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are less toxic [1]. Because of these different toxicities, a large amount of research has focused on developing robust and reliable methodology to separate the different arsenic species to allow specific quantitative analysis of the toxic forms.

While chromatographic separation of different species followed by species-specific quantification using ICP-MS is widely accepted as the preferred analytical approach for the speciation of trace elements in many food sample types, there are various non-chromatographic strategies that are less time-consuming and more cost-effective than high-pressure liquid chromatography (HPLC) for the speciation of arsenic [2,3].

In this paper, we focus on using hydride generation (HG) with ICP-MS for the speciation of iAs in rice samples. Rice is an important food source for a large percentage of the world's population, but it does contain relatively high concentrations of iAs due to the plants' uptake of As from soils. Available As in soils can be both naturally occurring and due to anthropogenic sources such as As-based pesticides that were widely used until the 1970s. Clearly there is an urgent food-safety requirement for a simple and quick analytical method to screen large number of samples for iAs.

Current legislation

China has legislation in place defining maximum levels (MLs) of iAs in rice (0.15 mg/kg) [4], and MLs for total arsenic in rice of 0.3 mg/kg exist in South America's trading bloc [5]. Following the eighth meeting of the CODEX Committee on Contaminants in Food, the World Health Organization (WHO) proposed a guideline for inorganic As in polished rice of 0.2 mg/kg [6]. The United States and European Union have yet to legislate on iAs in rice but the US FDA and European Committee

for Standardization (CEN) have initiated projects aimed at establishing standard methods for the determination of iAs in food.

In this study, we evaluate an alternative method for the determination of iAs in rice samples to illustrate that speciation analysis for iAs can be performed without the need for time-consuming chromatography.

Experimental

Chemicals and standards

All standards and samples were prepared using ultrapure water (>18 MΩ cm).

Chemical/Standard	Purpose	Source
1002 mg/L certified As stock solution	Calibration of total As and measurements with HG	Merck, UK
Sodium dimethylarsinic acid (DMA, 98%)	HPLC-ICP-MS measurements	ChemService, USA
Rhodium: 1 µg/L	Internal standard for HG measurements	Specpure, Alfa Aesar, Germany
Rhodium: 25 µg/L	Internal standard for total arsenic/ speciation	Specpure, Alfa Aesar, Germany
Nitric acid (69 %)		Fluka, UK
Ammonium nitrate (98+%)		Sigma-Aldrich, UK
Ammonium solution (28%) and ammonium carbonate		BDH, UK
Hydrogen peroxide (>30 % w/v), ammonium phosphate, sodium hydroxide (*LR grade)		Fisher Scientific, UK
Hydrochloric acid (32%, *LR grade)	Hydride generation reaction	Fisher Scientific, UK
Sodium borohydride (99 %)		Acros Organics, UK
Antifoam B emulsion		Sigma-Aldrich (USA)

All chemicals used were at least of Analytical Reagent (AR) grade unless otherwise stated.

*LR = Laboratory Reagent

Samples and reference materials

Samples included 31 different rice products purchased from local stores, and 12 samples of rice grown under controlled arsenic-exposed conditions. Sub-samples (30 g) of the commercially sourced rice samples were ground to a fine homogeneous powder using a coffee grinder. Two rice reference materials IMEP-107 rice (Institute for Reference Materials and Measurements

Geel, Belgium) and National Institute of Standards and Technology (NIST) 1568a rice flour (Gaithersburg, MD, USA) were used as quality control for iAs concentration measurements.

Sample preparation
For the measurement of total As, 0.15 g of each rice sample was digested in 1 mL concentrated HNO_3 and 2 mL H_2O_2 (30 % w/w) using open vessel digestion in a CEM Mars microwave system. All samples were diluted to a final volume of 30 mL using deionized water.

For the speciation of iAs by HPLC-ICP-MS, rice samples (0.1 g) were extracted in 10 mL of 1% HNO_3 and 1% H_2O_2 (5 min 50 °C, 5 min 75 °C, 10 min 95 °C). The same extracts were also analyzed by HG-ICP-MS. The calibration standards were also prepared in 1% HNO_3 and 1% H_2O_2, the same as the samples. Each sample was centrifuged at 13,000 rpm for 10 mins prior to analysis.

Instrumentation - Hydride generation
A Hydride Generation (HG) accessory for Agilent's ICP-MS Integrated Sample Introduction System (ISIS) is used for the high sensitivity analysis of gaseous hydride forming elements such as As. Figure 1 shows the setup of the HG-ICP-MS used in this study. The samples were introduced via an ASX-500 autosampler and transported to the hydride generator by the ISIS peristaltic pump (PP1). The sample was mixed with HCl (5M) and $NaBH_4$ (2% (w/v)) + antifoam B emulsion in a mixing coil before entering the gas liquid separator (GLS). The gaseous sample containing iAs as volatile hydrides was then transported to the ICP-MS spray chamber in an argon gas flow controlled via the make-up gas line of the ICP-MS. The Rh internal standard (IS) was introduced to the spray chamber of the ICP-MS via a conventional pneumatic nebulizer, creating wet plasma conditions. Details of the method development can be found in a previous paper [7]. Optimized operating conditions are given in Table 1.
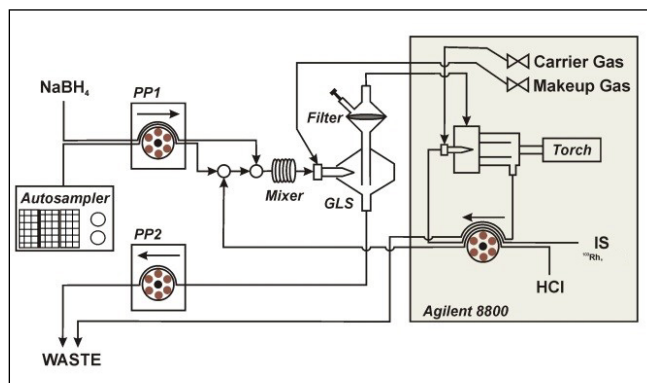

Figure 1. Schematic of the HG-ICP-MS configuration used in this study

Table 1. Operating parameters for hydride generation

Parameter	Value
Sample flow rate (mL/min)	0.5
HCl flow rate (mL/min)	2.5
$NaBH_4$ flow rate (mL/min)	0.5
Reaction coil volume (mL)	0.23
Ar flow rate for HG (L/min)	0.3
Ar flow rate for nebulization of IS (L/min)	0.85–0.95

When treated with $NaBH_4$ under acidic conditions, iAs is very efficiently converted into volatile arsine (AsH_3), whereas organically bound As compounds are not converted, or form only less volatile arsine species such as dimethylarsine $(CH_3)_2AsH$, which has a boiling point of 35 °C. Adding high concentrations of HCl further reduces the production of the less volatile arsines, and iAs is almost exclusively converted to arsine, enabling the measurement of iAs alone, without requiring species separation using chromatography.

Instrumentation—ICP-MS
The HG was coupled to an Agilent 8800 Triple Quadrupole ICP-MS (ICP-QQQ) for the detection of arsenic. ICP-QQQ was used for initial method development because it facilitates monitoring and control of possible interferences compared to conventional quadrupole ICP-QMS. This was important since a high concentration of HCl is necessary for selective formation of arsines using HG, increasing the chance of chlorine contributing to the $^{40}Ar^{35}Cl$ interference on As at m/z 75.

The preferred approach to measuring As using ICP-QQQ is to use a mass shift method whereby As^+ is converted

to AsO$^+$ by reaction with oxygen (O$_2$) cell gas, and then measured as its product ion at *m/z* 91. The ICP-QQQ is able to operate in MS/MS mode, where both quadrupoles operate as unit mass filters. If Q1 is set to *m/z* 75, only As and any on-mass interferences are introduced into the reaction cell, so potential interferences on the target product ion ^{75}As^{16}O$^+$ at *m/z* of 91 are excluded. However, in an initial study [7], no difference was found between the HG results obtained in O$_2$ reaction mode (where As is measured indirectly as AsO$^+$ at *m/z* 91) and no gas mode (where As is measured directly at *m/z* 75), indicating that no significant chloride interferences were present, despite the high concentration of HCl. It is therefore possible that a quadrupole ICP-MS instrument, such as the Agilent 7900 ICP-MS, can be used to measure arsenic using the same hydride generation method.

In order to compare the HG-ICP-QQQ results with data obtained using an established HPLC-ICP-MS method, an Agilent 1100 HPLC was connected to the ICP-QQQ. A Hamilton PRP X-100 anion exchange column (10 μm, 4.6 × 250 mm) was used, with a mobile phase of 20 mM ammonium carbonate (pH 8.5) at a flow rate of 1 mL/min.

Table 2: ICP-QQQ operating conditions

	ICP-QQQ for total As	HG-ICP-QQQ	HPLC-ICP-QQQ
Parameters	**Value**		
RF power (W)	1550		
Carrier gas flow (L/min)	1.19	0.93	1.13
Make up gas flow (L/min)*		0.3	
Spray chamber temperature (°C)	2		
Sample depth (mm)	8		
Exact 1 lens (V)	-4	0.5	0
Exact 2 lens (V)	-185	-170	-200
Quad mode	Single quad	MS/MS	
Cell gas mode and flow rate (%)	No gas	O$_2$ (30)	

*Used as hydride generation sweep gas

Results and Discussion

Quality control

The total As concentrations determined by ICP-QQQ in NIST 1568a and IMEP-107 were in good agreement with the respective certified and proficiency test values, as shown in Table 3a. The speciation results for iAs in NIST 1568a and IMEP-107 obtained using HG-ICP-QQQ were in good agreement with the values obtained using HPLC-ICP-QQQ and with the reported values (Table 3b).

Table 3a. Total As concentrations determined by ICP-QQQ in NIST 1568a and IMEP-107

Total As		
	ICP-QQQ (μg/kg)	Certified value (μg/kg)
NIST 1568a	295 ± 6, (n=3)	290 ± 30
IMEP-107	173 ± 1, (n=3)	*172 ± 18

* Proficiency test

Table 3b. iAs results obtained using HG-ICP-QQQ and HPLC-ICP-QQQ

Inorganic As			
	HG-ICP-QQQ (μg/kg)	HPLC-ICP-QQQ (μg/kg)	Reported value (μg/kg)
NIST 1568a	94 ± 8 (n=3)	105 ± 4 (n=3)	94 ± 12 [7]
IMEP-107	100 ± 11 (n=15)	110 ± 12 (n=15)	107 ± 14 [8]

The column recovery was good for both QC materials; 101 ± 4% (n=3) for NIST 1568a and 98 ± 9% (n=12) for IMEP-107. IMEP-107 was analyzed with every batch of samples and from these multiple measurements the "within-day" RSD and "between measurement days" RSD was calculated. The daily RSDs were an average of 3% for both HG and HPLC and the RSD of all replicates for all measurement days was 11% for both HG and HPLC. The results show that the reproducibility and repeatability of the two methods was similar. A blank sample was analyzed with every batch of samples.

Comparison of HG-ICP-QQQ and HPLC-ICP-QQQ results

The extraction efficiency of total arsenic for the various types of rice grains was generally good, with an average of 91 ± 10 % (from a range of 73-111%). With regard to MLs, all the samples studied contained less than 150 μg/kg iAs, and were therefore below both the existing Chinese ML and the proposed joint FAO/WHO Codex ML for iAs. The column recovery of the HPLC was quantitative (94 ± 10%) for all samples.

A summary of the determined values for iAs, DMA and total As is given in Table 4a and b. The results for iAs determined using HG-ICP-MS and HPLC-ICP-MS show good agreement. The dominant arsenic species found in rice are iAs and DMA with only trace amounts of MMA. The method uses HCl (5 M) and $NaBH_4$ for the selective generation of arsines where AsH_3 is formed almost exclusively with only minor contribution of DMA as 2-4% as dimethylarsine. This is also confirmed by the results of rice grown under arsenic-exposed conditions (Table 4b). Though the samples contain DMA at high

concentration, iAs determined by HG-ICP-MS agrees well with the results obtained using HPLC-ICP-MS. MMA forms methylarsine at approximately 40% efficiency with the method; however, since MMA is generally absent from rice – or only present in trace amounts – this should not affect the quantification of iAs.

Table 4a. Speciation results of iAs in 31 rice products determined by HG-ICP-QQQ and HPLC-ICP-QQQ. Results are also given for DMA and MMA, and the total As concentration determined by ICP-QQQ. All data ± SD, with n=3 for speciation and n=2 or 3 for total As.

Rice product	HG iAs	HPLC iAs	HPLC DMA	HPLC MMA*	Total As
	(µg /kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)
Arborio Risotto	113 ± 13	120 ± 18	63 ± 7	<LOQ	236 ± 15
Organic ArbRis	109 ± 12	119 ± 13	60 ± 8	<LOD	150 ± 7
Basmati, 1	41 ± 4	53 ± 7	8 ± 1	<LOD	100 ± 12
Basmati, 2	76 ± 6	88 ± 6	28 ± 4	<LOD	91 ± 8
Basmati (white)	72 ± 11	69 ± 9	24 ± 1	<LOD	240 ± 5
Organic Basmati (white)	95 ± 3	104 ± 3	21 ± 2	<LOD	117 ± 13
Brown Rice	127 ± 6	137 ± 5	35 ± 2	<LOD	205 ± 2
Japanese Rice	101 ± 5	99 ± 5	123 ± 1	<LOQ	252 ± 10
Long Grain (white)	89 ± 2	85 ± 1	16 ± 1	<LOD	121 ± 11
Long Grain Rice, 1	103 ± 2	94 ± 1	218 ± 9	<LOQ	392 ± 23
Long Grain Rice, 2	40 ± 2	52 ± 10	39 ± 3	<LOQ	111 ± 8
Long Grain white	47 ± 2	61 ± 4	19 ± 4	<LOD	102 ± 9
Organic Long Grain (brown)	111 ± 7	131 ± 14	54 ± 7	<LOQ	207 ± 15
Organic (white)	65 ± 4	65 ± 2	11 ± 1	<LOD	92 ± 4
Paella, 1	60 ± 5	65 ± 2	38 ± 1	1.2 ± 0.1	136 ± 1
Paella, 2	66 ± 4	70 ± 3	17 ± 1	<LOD	121 ± 6
Spanish Paella	67 ± 2	67 ± 3	13 ± 1	<LOD	109 ± 7
Pudding Rice	124 ± 9	125 ± 11	44 ± 5	<LOD	202 ± 4
Rice Flour	40 ± 1	46 ± 5	19 ± 2	<LOD	102 ± 6
Carnaroli Risotto Rice	81 ± 2	82 ± 4	84 ± 2	<LOD	210 ± 15
Risotto Rice	97 ± 11	114 ± 10	72 ± 9	<LOQ	221 ± 17
FLG Thai (white)	88 ± 3	102 ± 3	52 ± 5	<LOD	197 ± 9
Thai Jasmine	61 ± 4	64 ± 3	49 ± 5	<LOD	143 ± 3
Thai Jasmine (white)	62 ± 4	62 ± 3	49 ± 2	<LOD	171 ± 5
Vietnamese Rice Paper	21 ± 2	28 ± 1	<LOQ	<LOD	58 ± 10
White Rice	71 ± 5	76 ± 5	14 ± 4	<LOQ	124 ± 1
Whole Grain	133 ± 2	127 ± 2	151 ± 12	7.2 ± 0.3	370 ± 19

*LOQs: HG-ICP-QQQ: 5 µg/kg, HPLC-ICP-QQQ: 1.1 µg/kg LODs: HG-ICP-QQQ: 1.5 µg/kg, HPLC-ICP-QQQ: 0.3 µg/kg

Table 4b. Speciation results of iAs determined by HG-ICP-QQQ and HPLC-ICP-QQQ for 12 rice grain samples from rice grown under arsenic-exposed conditions. Results are also given for DMA and MMA by HPLC-ICP-QQQ, and for the total As concentration determined by ICP-QQQ. All data ± SD, with n=3 for speciation and n=2 or 3 for total As.

Rice sample	HG iAs	HPLC iAs	HPLC DMA	HPLC MMA	Total As
	(µg /kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)
Low As exposure 1	229 ± 2	230 ± 2	66 ± 1	<LOD	323 ± 19
Low As exposure 2	184 ± 4	209 ± 8	275 ± 10	<LOD	484 ± 5
Low As exposure 3	213 ± 25	213 ± 8	168 ± 7	<LOQ	384 ± 21
Low As exposure 4	287 ± 7	288 ± 13	42 ± 4	<LOQ	350 ± 15
Low As exposure 5	372 ± 34	380 ± 29	101 ± 2	<LOQ	489 ± 20
Low As exposure 6	184 ± 4	184 ± 5	79 ± 1	<LOD	292 ± 11
High As exposure 1	208 ± 8	223 ± 4	91 ± 4	<LOD	332 ± 7
High As exposure 2	240 ± 2	220 ± 11	790 ± 5	7 ± 1	1015 ± 59
High As exposure 3	258 ± 14	237 ± 11	660 ± 25	5 ± 1	923 ± 6
High As exposure 4	426 ± 16	438 ± 22	214 ± 6	1.2 ± 0.4	630 ± 100
High As exposure 5	399 ± 55	374 ± 45	798 ± 61	1.7 ± 0.4	1259 ± 48
High As exposure 6	249 ± 14	245 ± 14	494 ± 8	<LOD	746 ± 35

The results in Table 4a show that the concentration of iAs in all of the commercial rice products fell below the proposed FAO/WHO Codex ML of 200 µg/kg and the Chinese ML of 150 µg/kg (even correcting for the low recovery of the iAs extract). If assessment of the As content had been based only on the total As concentration, 32% (10 out of 31) of the samples would have exceeded the proposed Codex ML and 42% (13 out of 31) would have exceeded the current Chinese ML. The proportion of As present as iAs ranged from 26—84% of the total As concentration for the commercial rice samples studied.

In the samples where MMA was detected, the concentration was below the LOQ in most cases, and did not exceed 7 µg/kg in any sample. This is in accordance with an extensive survey undertaken by the US FDA where over 1300 samples of rice products were measured [9]. In the US FDA survey, the level of MMA in 97% or more of the rice products fell below the LOD or LOQ (under 13 µg/kg). Only 1% of the samples contained more than 20 µg/kg, with the highest reported concentration being 25 µg/kg. It can be assumed that MMA is not a determining factor in the total As concentration of rice and, at these low concentrations, MMA does not influence the determination of iAs by HG-ICP-QQQ.

Conclusions

Inorganic arsenic (iAs) was quantified at low ppb levels in extracts of 43 rice-product samples using an Agilent Hydride Generator/ISIS coupled to an Agilent 8800 ICP-QQQ. Results obtained using HG-ICP-QQQ were in good agreement with HPLC-ICP-QQQ values across a wide linear range, with comparable limits of detection.

Following a simple sample preparation using microwave extraction, quick separation of iAs and DMA by HG-ICP-QQQ was performed online. A previous study has shown that HG-ICP-QQQ requires only 4 minutes total run time per sample (5 replicate measurements) compared to speciation with HPLC which commonly takes between 5 and 10 minutes for each sample for one replicate [10]. Data handling for the HG method is also straightforward as no peak-integration is necessary.

The new HG-ICP-MS method offers fast analysis time, high throughput, and simple, reliable operation. This makes it ideally suited to screening large numbers of food samples to meet the increasing demand for the routine determination of iAs in food, especially rice-based products.

More Information

For a full account of this work see publication: Hydride generation ICP-MS as a simple method for determination of inorganic arsenic in rice for routine biomonitoring by Ásta H. Pétursdóttir, Nils Friedrich, Stanislav Musil, Andrea Raab, Helga Gunnlaugsdóttir, Eva M. Krupp and Jörg Feldmann, *Anal. Methods,* 2014, 6, 5392-5396. DOI: 10.1039/C4AY00423J.

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