Determination of Inorganic Arsenic in Rice using IC-ICP-MS

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Key Words

Arsenic, Codex Alimentarius, Rice, ICP-MS, IC

Goal

Demonstrate how the coupling of IC with ICP-MS helps meet regulatory requirements in the field of arsenic determination.

Introduction

The ability of rice to accumulate relatively large quantities of arsenic in its grain, in contrast to other cereals, like wheat, is well established[1]. Generally rice contains dimethylarsenic acid (DMA), small amounts of monomethylarsonic acid (MMA) and inorganic arsenic (iAs). In rare cases other organo-arsenic compounds, like tetramethylarsenic, have been recorded[2]. The arsenic content and the species present depend on the rice variety, soil and growth conditions[3]. Consumers in Europe or North America have no control over the latter two and often no origin information is provided on pre-packaged rice, making it impossible for the average consumer to make an informed choice about which rice to eat. Introducing legislation on maximum levels of total arsenic in rice (and other foods) is difficult, due to the highly variable health effects of individual arsenic species. Whereas inorganic arsenic is highly toxic and carcinogenic, DMA is far less toxic and the main arsenic containing compound in fish, arsenobetaine, is considered to be non-toxic[4]. Legislation for arsenic content in foods therefore requires the dependable identification and quantification of inorganic arsenic in the presence of potentially dozens of arsenic-containing compounds. This in turn requires the use of highly selective separation and detection systems. One of the most efficient of these is the combination of strong-anion-exchange chromatography for separation with ICP-MS for specific arsenic detection.

Rice is a relative easy matrix for this method, since it contains generally not more than 4 different arsenic species, two of which (arsenite and arsenate) are interchangeable into one using hydrogen peroxide during sample extraction. Rice also does not contain much chloride, reducing therefore the risk of molecular-ion interferences on m/z 75. This can further be reduced by using collision cell technology. One potential problem with rice, which should not be forgotten, is the amount of other soluble organic molecules present, especially the presence of starch. These can, during very long run-sequences lead to deterioration of the separation efficiency of the column, especially when special high-throughput run conditions with very short run times are used.

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Methods and materials

Samples

In addition to the certified reference materials BCR-211 (As species certified) and NIST 1568a, 48 different rice samples bought between 2012 and 2015 in shops or market places, mainly in the UK but also in other countries (e.g. Egypt) were used . Ready packed rice from shops within Europe rarely indicates the geographic origin on the package, so some samples are of unknown origin. All samples, not ground already, were finely ground using a coffee grinder and mortar and pestle. During method development, 3 independent extracts on 2 different days of NIST1568 and BCR 211 were prepared and measured. Additionally rice noodles (finely ground, iAs 35 µg·kg^{-1}, DMA 13 µg·kg^{-1}, MMA < 0.16 µg·kg^{-1}) were spiked with the four As-species (iAs and DMA 90 µg·kg^{-1}, MMA 8 µg·kg^{-1}) under investigation and the recovery determined.

Chemicals

Concentrated nitric acid (69% p.a., from Fisher Chemical) and hydrogen peroxide (30% p.a., from Sigma) were used for the extraction of arsenic species. Ammonium carbonate (from BDH) was used for the preparation of the eluent. Sodium arsenite (Chemservice), sodium arsenate dibasic heptahydrate (Sigma), disodium methyl arsenate (MMA, ChemService) and Sigma cacodylate trihydrate (DMA, Sigma) were used for the preparation of standard stock solutions. Yttrium standard solution (1 g/L, Specpure, Alfa Aesar) was used as continuous internal standard added via a Y-piece before the nebulizer. 18 MΩ·cm water (Millipore) was used for the preparation of all solutions.

Extraction of Arsenic Species

0.1 g sample (weighed to 0.1 mg) was extracted with 10 g solvent containing 2% (v/v) nitric acid and 3% (v/v) hydrogen peroxide using a temperature program up to 95 °C in a MARS 5 CEM microwave system[5]. Samples were centrifuged after cooling and the supernatant used for speciation.

Standards

Single species standards were prepared at arsenic concentrations of 1000 mg·kg^{-1} in water. These were further diluted to working standards of 10 mg·kg^{-1} (also as single species) in water. From these mixtures of species, different concentrations suitable for the determination of arsenic species in rice were prepared fresh each day in the extraction solvent in a concentration range from 0 to 5 (20) µg·kg^{-1}.

Instrument Configuration

Chromatographic separations were carried out using a Thermo Scientific™ Dionex™ AS-7 Anion Exchange Column and the Thermo Scientific™ Dionex™ ICS-5000 IC. Due to the completely metal-free solvent pathway, this system is perfectly suited for elemental speciation studies. The Thermo Scientific™ iCAP™ Qc ICP-MS was used as highly sensitive and selective detector. High selectivity is achieved through the use of a He pressurized QCell in Kinetic Energy Discrimination (KED) mode that efficiently reduces polyatomic interferences in all sample types. Uniquely, the QCell He KED mode also includes a low mass filter that ensures the lowest background noise – particularly important for applications at low concentration levels. For the sensitive analysis of (monoisotopic) arsenic at m/z 75, there is no possibility to use a different isotope for analysis and therefore the flexible, non-matrix specific interference reduction QCell He KED mode was employed in order to minimize polyatomic interferences such as $^{40}Ar^{35}Cl$ that would otherwise return a potentially serious false positive. The instrument was optimized daily for optimum performance in He KED mode using the supplied autotune protocol. The ICS-5000 IC was connected with a 25 cm Peek-tubing directly to the iCAP Q ICP-MS nebulizer. Details of the instrumental set-up are presented in Table 1 and Figure 1.

Table 1. ICS-5000 IC and iCAP Qc ICP-MS operating parameters.

Ion Chromatography	Thermo Scientific Dionex ICS-5000 IC
Column	IonPac AG7 (guard column 2x50 mm) + IonPac AS7 (2 x 250 mm)
Flow Rate	0.5 mL·min^{-1}
Sample Loop	25 µL
Autosampler Tray Temp	4 °C
Column Compartment	40 °C
Eluent A	Water
Eluent B	200 mM(NH$_4$)$_2$CO$_3$
Gradient	0-2 min: 15% B 2-5 min: 15–100% B 5-10 min: 15% B
ICP-MS	Thermo Scientific iCAP Q ICP-MS
Interface Cones	Ni sampler and skimmer (with High Matrix insert)
Reaction Cell Gas	He
Reaction Cell Gas Flow Rate	4.5 mL·min^{-1}
Nebulizer and Spray Chamber	Micromist and Quartz cyclonic spray chamber
Forward Power	1550 W
Nebulizer Flow Rate	1.3 L·min^{-1}

Figure 1. Instrument configuration with the Thermo Scientific Dionex ICS-5000 IC and Thermo Scientific iCAP Q ICP-MS.

Results

Method evaluation

A hyphenated IC-ICP-MS method suitable for arsenic speciation should fulfil some criteria. First, the sample matrix should not lead to drifts in retention time during a sequence running over eight or more hours. Other important criteria are that the time required for separation should not be exceedingly long to enable relatively high throughput measurements and a reasonable peak shape for improved automatic peak detection and quantification should be achieved. With these aspects in mind, several eluent mixtures and gradient settings were tested for the separation of arsenic species in rice using a Thermo Scientific™ Dionex™ IonPac™ AS7 Column. Ammonium carbonate buffer at pH 9.2 proved to be the most suitable buffer additive. For the separation of DMA and MMA a relatively low buffer concentration of 30 mM ammonium carbonate gave the best results during long-term (>6 hours) sequence runs. The disadvantage of this low buffer concentration is the strongly retained arsenate (eluting between 25 and 30 minutes) resulting in a broad arsenate peak (which in turn gives reduced sensitivity for this species). Therefore, different gradient conditions were tested to reach a total time for the chromatogram of 10 minutes or less per separation (including re-equilibration time of the column). For a matrix like rice containing generally only DMA, iAs and small amounts of MMA, a short steep gradient pushing iAs off the column relatively quickly is suitable. This approach would not however be suitable for more complex matrices like fish where the risk of co-elution of other As species (and therefore overestimation of iAs) would be high. The gradient used started with 30 mM ammonium carbonate for 2 minutes followed by a steep increase over 3 minutes to 200 mM ammonium carbonate followed by a 5 minute re-equilibration time of the column. The iCAP Q ICP-MS measured over the whole 10 minutes of the gradient. DMA eluted around 90 s, MMA at 120 s and iAs at 360 s (Figure 2). Due to the use of

hydrogen peroxide as part of the extraction solution arsenite is oxidised to arsenate and measured together with arsenate.

Evaluation of the developed method (and in particular the stability of the gradient), was performed by measuring independent samples (including rice certified reference material and rice noodles, spiked and non-spiked) and standards on two different days (total run time in both cases was 10 hours). Both CRMs, NIST 1568a and BCR 211 (where BCR-211 is certified for DMA and iAs, as shown in Table 2) have been used for method development of arsenic speciation over a number of years, resulting in a large number of published values. On another day, the evaluated method was used to measure the arsenic species in the rice samples.

The calibration curves for all three species were near identical on all three days of measurement (2 days method evaluation one day samples) with R^2= 0.997 ± 0.0022, slope 0.19 ± 0.026 and intercept 0.0068 ± 0.011 (Figures 3-5). This, in principle would allow species unspecific calibration using only one As-species during calibration. The retention times of the species varied less than 10 seconds over all three days showing good reproducibility of the chromatographic separation. There was no statistical significant difference between the extracts of both days for NIST 1568a and BCR 211 (T-test p >0.2 for all species and both samples). The spike recovery from the rice noodles was also not different between days with an average recovery for DMA, MMA and iAs of 101 ± 4.7% with no difference between species. Detection and quantification limits for all three species are sufficiently low, with iAs (eluting last) having the highest LOQ at 6.5 µg/kg rice when applying an average dilution factor of 90 (Table 2). The sum of extraction efficiency and column recovery for all samples was 102 ± 5.4%. The reproducibility of multiple injections of the same sample was better than 2%. The developed method was therefore deemed fit for purpose.

Table 2. Results for LOD, LOQ, BEC, CRM results and spike recovery from method verification, where BCR-211 data include 2 samples measurements from day 3 (day samples were measured) and LOD was determined from 10 blank injections (Note: LOD for MMA directly was not possible to determine, since no signal occurred at this RT).

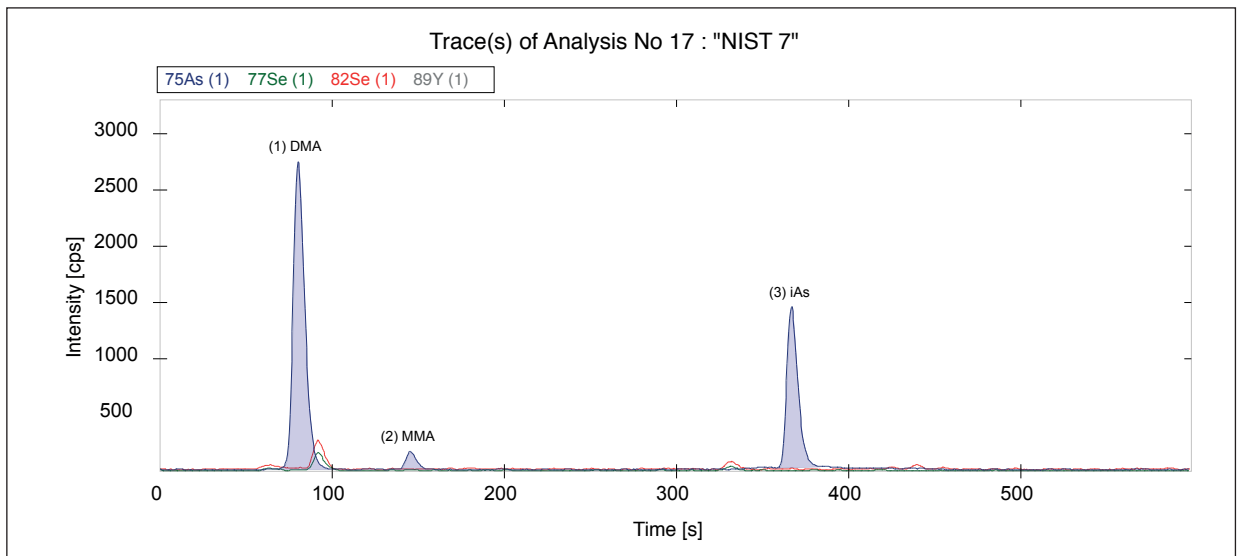|	DMA	MMA	iAs
LOD (µg·kg⁻¹ solution)	0.030 ± 0.017		0.11 ± 0.039
LOD (µg·kg⁻¹ rice) incl. DL of 90	2.7 ± 1.5		10 ± 3.6
BEC (µg·kg⁻¹ solution, from program)	0.019 ± 0.0040	0.0047 ± 0.0035	0.085 ± 0.0025
NIST 1568a (n=6)	181 ± 7.9	11 ± 4.9	118 ± 11.2
Literature	143–185		80–115
BCR 211 (n=8)	118 ± 3.6	14 ± 0.91	122 ± 2.1
BCR-211 (certified)	119 ± 13		124 ± 11
Spike Recovery % (n=6)	96 ± 3.2	103 ± 2.0	104 ± 3.4

Figure 2. Example chromatogram (screen shot from Qtegra ISDS Software, showing automatic identification and peak area determination for As-species).
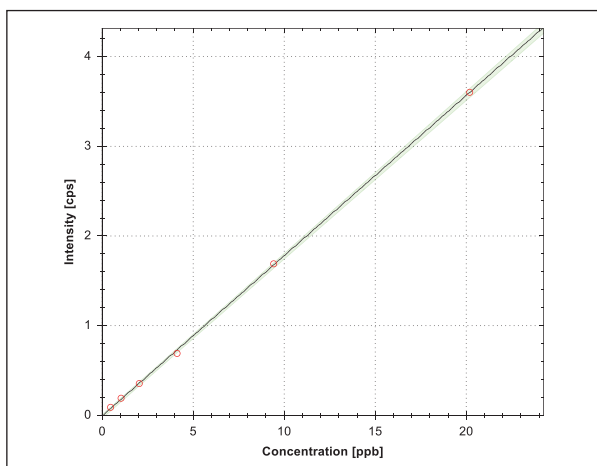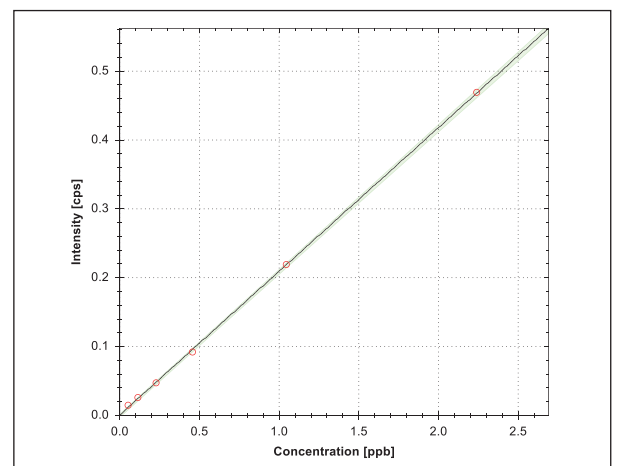
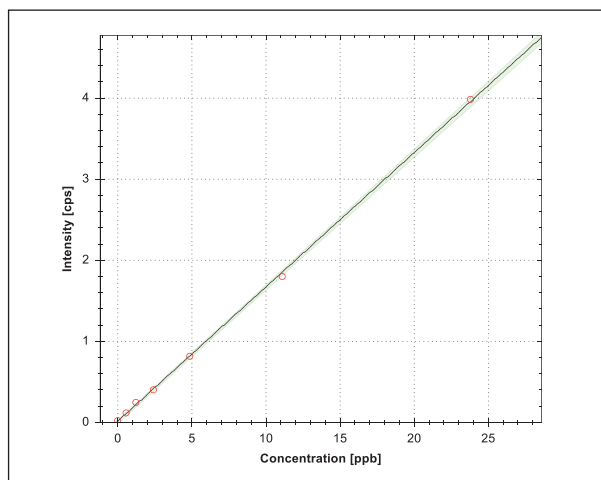

Figure 3. DMA calibration.

Figure 4. MA calibration.

Figure 5. iAs calibration.

Application of developed method to rice samples available on the market

To date, the majority of legislation on arsenic content in food stuff is concerned with inorganic arsenic. Maximum permitted levels for inorganic arsenic in rice were introduced several years ago in China (< 0.15 mg·kg^{-1})[6] and Australian maximum permitted levels for cereals are 1 mg·kg^{-1}total As[7]. In Europe legislation for maximum permitted levels of iAs in white polished rice is in preparation, whereas the subject is still under discussion in the US. The FAO/WHO - Codex Alimentarius commission proposed a maximum As level of 0.2 mg·kg^{-1} in polished rice in 2014[8]. In this study a total of 48 rice samples of different origin were measured, of which 7 were rice products intended for babies and toddlers. Of these, nine were clearly above the threshold of 150 µg inorganic As·kg^{-1} rice and an additional five were closed to the threshold currently being discussed within the EU (Figure 6). Around 20% of the measured samples would have failed the limit currently in force in China.

In contrast only 12% of the tested samples (3 of them from the baby rice category) would have failed the FAO/WHO limit. The majority of the samples above the thresholds were in the baby rice and whole rice (brown rice) categories. In particular, the very high failure rate of samples intended for babies and toddlers (5 out of 7 analyzed) is a cause of concern. This consumer group has a much higher food intake in relation to their body weight than adults and is especially vulnerable. These high levels of iAs are unchanged or even higher compared to values determined in baby rice products in 2008[9]. As for other samples, basmati rice products were all below the threshold as were white rice samples from a variety of local markets in North Africa, Sri Lanka, India and Japan, but not necessarily supermarket bought white rice from the UK. The relative amount of iAs was on average 70 ± 13% of the total As present and 5 samples also contained large quantities of DMA (>100 µg/kg). The data are presented in Table 3.
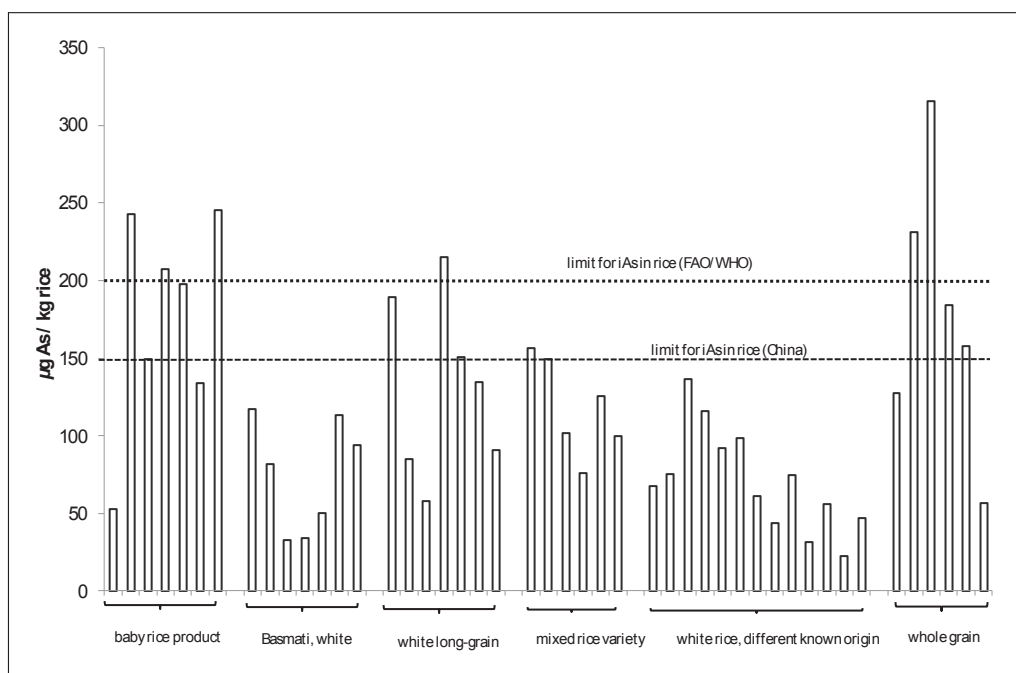

Figure 6. Amount of inorganic As in 48 rice samples.

Table 3. Tabulated results of DMA, MMA and iAs in rice products (SM: supermarket UK, local market: sold non-packed, all in µg As · kg⁻¹ rice).

Product	Country of origin	DMA (µg·kg⁻¹)	MMA (µg·kg⁻¹)	iAs (µg·kg⁻¹)
Rice Flour + Vit. B1	unknown	30	1.4	53
Rice Flour + Vit. B1 + other vitamins	unknown	59	2.5	243
Rice Flour + Vit. B1	unknown	54	2.0	150
Organic Rice Flour + Vit. B1	unknown	131	3.5	207
Rice Flour + Vit. B1	unknown	158	3.4	198
Rice Flour + Vit. B1	unknown	48	1.7	134
Whole Grain Rice Flour + Vit. B1	unknown	52	2.8	245
White Basmati				
SM	unknown	38	1.6	117
SM	unknown	34	1.4	82
SM	Italy	23	N/A	33
Local Market	Pakistan	27	1.1	34
SM	unknown	43	1.1	50
SM	unknown	36	0.9	113
SM	unknown	25	0.4	94
White long grain rice				
SM	unknown	59	2.5	189
SM	unknown	38	2.5	85
Local Market	Pakistan	27	0.4	58
SM	unknown	74	1.9	215
Local Market	Europe	27	1.6	151
SM	unknown	22	0.9	134
SM	unknown	37	1.4	91
Mixed white rice (paella, risotto…)				
SM	unknown	96	3.2	157
SM	unknown	25	1.2	149
SM	unknown	24	1.1	102
SM	unknown	26	1.8	76
SM	unknown	57	1.1	126
SM	unknown	53	1.2	100
Local Market	India	30	1.3	67
Local Market	Japan	41	0.9	75
SM	unknown	79	2.1	137
Local Market	Thai	54	1.7	116
Local Market	Turkey	175	2.7	92
Local Market	Libya	17	0.8	98
Local Market	Egypt	9	N/A	61
Local Market	Egypt	8	N/A	44
Local Market	Egypt	16	0.7	75
Local Market	Bangladesh	30	0.9	32
Local Market	Sri Lanka	7	N/A	56
Local Market	Bangladesh	19	0.3	23
Local Market	India	13	N/A	47
SM	unknown	20	1.2	57
Different whole grain varieties				
SM, Basmati	unknown	18	1.0	128
SM	unknown	78	3.7	231
SM	unknown	100	3.3	315
SM	unknown	201	8.3	184
SM	unknown	64	1.5	158

Conclusion

Combining the resolving capability of IC with the detection power of ICP-MS allows fast, easy and reliable analysis of trace elemental species, with control of the fully integrated IC-ICP-MS system enabled using a single platform with a common software package. The metal free ICS-5000 IC and the powerful interference removal capabilities of the iCAP Q ICP-MS offer a platform that enables contamination and interference free analysis of arsenic species in complex samples. The developed IC-ICP-MS method is well suited for the determination of inorganic arsenic in rice and rice products with excellent detection limits and stability of retention time. Determination of iAs in a range of rice samples available in European supermarkets or from local markets showed that the amount of iAs in rice is highly variable. The samples of whole grain rice contain unsurprisingly relatively high amounts of iAs. This is well known, since the majority of As is stored in the bran and kernel of the grain, which are not removed in whole grain rice, but removed during the production of white rice. Of most concern should be the high iAs content in rice products specifically marketed for baby food. The problem of arsenic in rice has been known for several years and it is somewhat worrying that the producers of these products seem not to have taken steps to reduce the arsenic burden in these products.

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