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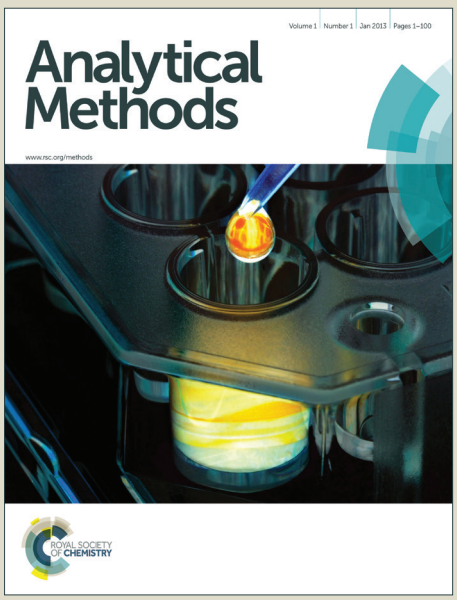

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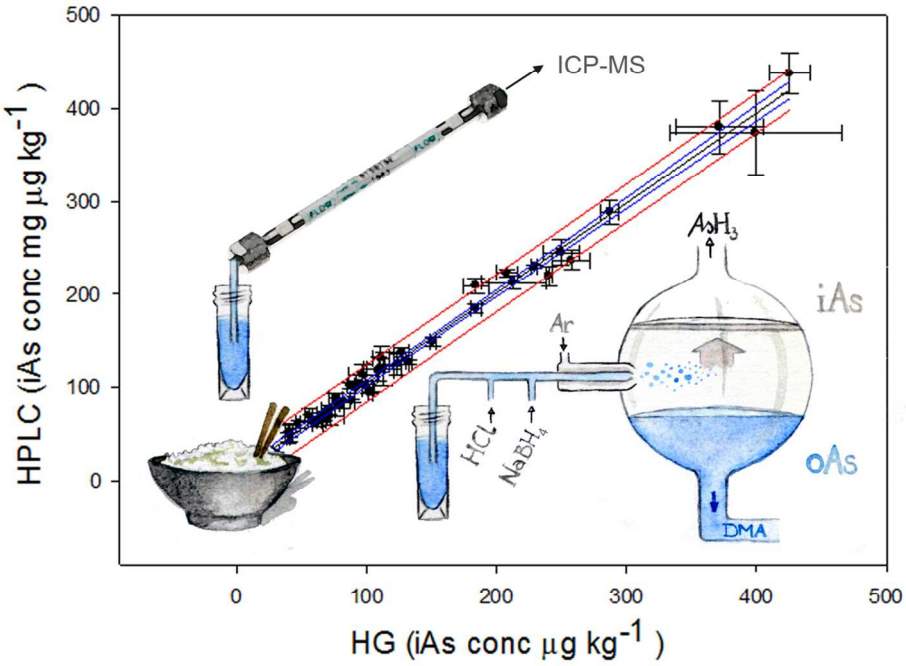

Using hydride generation for the determination of inorganic arsenic in rice gives the same result as HPLC
499x364mm (72 x 72 DPI)

Hydride generation ICP-MS as a simple method for determination of inorganic arsenic in rice for routine biomonitoring

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Abstract

Inorganic arsenic (iAs) concentration was measured in 44 rice product samples, covering a wide range of iAs concentrations, using both hydride generation (HG) ICP-MS and HPLC-ICP-MS. Linear regression showed good linearity (R^2 of 0.99) with a slope close to 1 (0.969 ± 0.015) and similar sensitivity showing that HPLC can robustly be replaced by a simple HG system, shortening the measurement time and resulting in easier data treatment as no manual integration of peaks is necessary. With upcoming regulations on iAs concentration in rice in the EU it is important that regulators do not prescribe only one standard method since it excludes new instrumental developments.

Introduction

Inorganic arsenic (iAs) is a known carcinogen and considered to be one of the most toxic arsenic species whereas organoarsenic species are generally considered less toxic.[1] Rice has been under scrutiny in recent years because of its relatively high concentrations of iAs and given that rice is a staple food around the world it is one of the main dietary sources of iAs.[2] Today, China has legislation

on maximum levels (MLs) of iAs in rice (0.15 mg kg^{-1}), [3] however no such legislation exists on iAs in rice, nor any foodstuff, in the EU. iAs is also the focus of attention in the US, where the Food and Drug Administration (FDA) has just published the results of iAs in more than 1300 samples of rice and rice products [4] with plans of conducting a comprehensive risk assessment. A recent proficiency testing (IMEP-107) organised by the European Union Reference Laboratory for Heavy metals in Feed and Food and the International Measurement Evaluation Program concluded that "[…]the concentration of inorganic As determined in rice does not depend on the analytical method applied and that introduction of maximum level for inorganic As in rice should not be postponed because of analytical concerns". [5] Following this a draft maximum level of iAs in rice was proposed in 2012 by the Joint FAO/WHO committee on contaminants in foods.[6] However, these proposed draft MLs (0.3 and 0.2 mg kg^{-1} in raw and polished rice, respectively) have not yet been set in legislation, because of lack of detailed information on rice. This is the case because arsenic speciation in rice is seen as complicated and it needs sophisticated methods often not available in laboratories which aims at large sample throughput for biomonitoring purpose.

The dominant arsenic species found in rice are iAs and dimethylarsinic acid (DMA) with only trace amounts of methylarsonic acid (MA) and/or tetramethylarsonium (TETRA) if present at all. [4, 7] Hydride generation (HG) has been established as a powerful sample introduction technique and beneficial alternative to nebulization techniques in analytical atomic spectrometry.[8] It usually uses sodium borohydride ($NaBH_4$) and hydrochloric acid (HCl) to convert species in aqueous solutions into volatile hydrides.[9] HG was popular for the determination of arsenic in the 70s and 80s,[10] however, since not all arsenic species form hydrides that posed a challenge for the determination of total arsenic with HG. This was used as an advantage for speciation of arsenic, e.g. by using the selectivity of hydride generation from different reaction media.[11] Today the most common method of arsenic speciation is applying HPLC for the separation of arsenic species hyphenated to an arsenic detector; HG-AAS, HG-AFS or in more recent years most often to ICP-MS.[1, 12, 13] When HG is coupled with ICP-MS for arsenic determination today it is mainlyemployed to increase sensitivity and eliminate

matrix interferences,[14, 15] and when HG-ICPMS is used for speciation in most cases HPLC is coupled in as well.[13, 16-19]

The European Committee for Standardization (CEN) has initiated projects aimed at establishing standard methods for the determination of iAs in both food and feed. Previously a CEN method on the determination of iAs in seaweed was published in 2008 (EN 15517:2008) based on acid extraction followed by hydride generation atomic absorption spectrometry (HG-AAS), [20] however, the method states it is not suitable for iAs concentrations below 1 mg kg^{-1} whereas the majority of seaweed samples would fall below this limit.[21] Recently a CEN method (EN 16278:2012) for the determination of iAs in animal feed using (HG-AAS) after microwave extraction and an offline seperation of species by solid phase extraction (SPE) was published.[22] This method has further been applied to both seafood and rice samples [23, 24] with good agreement between results for iAs using SPE-HG-AAS and HPLC-ICP-MS. Currently a CEN project is on-going with the aim of a standard method for the determination of iAs in food of plant and marine origin using anion exchange HPLC-ICP-MS after water bath extraction.[25] Therefore the CEN has recently prescribed a new standard method for the determination of iAs in feed utilizing HG-AAS, a method which additionally has been shown to be applicable for the determination of iAs in rice, whereas the future focus of the method of choice for food samples of plant and marine origin appears to be based on HPLC-ICP-MS. Therefore, laboratories wanting to measure both iAs in feed and food by using standard methods would have to have both an HG-AAS system to measure feed as well as an HPLC-ICP-MS system for food.

HPLC-ICP-MS is a well-established robust method for the determination of iAs, however, it has been shown that other – often cheaper - methods can give the same results. [24, 26, 27] For large throughput of samples other methods of speciation might be quicker, cheaper and more convenient and therefore more urgently needed data can be generated by non-specialised laboratories.

The aim of this paper is to apply a recently published method[27] of hydride generation for the determination of iAs for rice samples to illustrate that iAs can be determined without chromatography. 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. MA forms

methylarsine at approximately 40% efficiency with the method, however, since MA is generally absent from rice – or only present in trace amounts – this should not affect the quantification of iAs. The previously optimised method will be applied to a range of rice samples and compared to HPLC-ICP-MS data of the same extracts for further validation of the method.

Experimental section

Chemicals and Standards

Ultrapure water (>18 MΩ cm) was used for all analytical purposes. For calibration of total As and measurements with HG, a 1,002 mg As L^{-1} certified As stock solution (as H_3AsO_4 in 0.5 M HNO_3) was supplied by Merck (UK). Quantification for speciation using HPLC-ICP-MS was performed with dimethylarsinic acid sodium salt (DMA, 98 %; ChemService, USA). As internal standard rhodium (Specpure, Alfa Aesar, Germany), 1,000 mg L^{-1} solution, was diluted to 1 or 25 µg L^{-1} for HG measurement or total arsenic/speciation respectively. Nitric acid (HNO_3, 69 %) was obtained by Fluka (UK). Ammonium solution (28 %) and ammonium carbonate were obtained from BDH (UK). Hydrogen peroxide (H_2O_2, >30 % w/v) sodium hydroxide (NaOH, laboratory reagent grade (LR)) and hydrochloric acid (HCl, 32 %, LR grade - used for the hydride generation reaction), was supplied by Fisher Scientific (UK). Sodium borohydride ($NaBH_4$, 99 %) was from Acros Organics (UK). Antifoam B emulsion (aqueous – silicone emulsion) was purchased from Sigma-Aldrich (USA). All chemicals used were at least of analytical grade unless otherwise stated.

Samples

A variety of rice products were purchased from local stores in Aberdeen, Scotland (N=32) and additionally the grain of different rice varieties grown under arsenic exposure in the greenhouse was measured (N=12). Subsamples (30 g of the commercially available rice products, 15 g of the exposed rice grain) of the rice were taken and ground to a fine homogeneous powder using a coffee grinder.

For quality control two rice samples of well-established iAs concentration were included: IMEP-107 rice (Institute for Reference Materials and Measurements, Geel, Belgium)[5] and rice CRM NIST 1568a.[2]

Sample preparation

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

Rice samples (0.1 g) were extracted for determination of iAs in 10 mL of 1% HNO_3 and 1% H_2O_2 (5 min 50 °C, 5 min 75 °C, 10 min 95 °C). Preparing the calibration standards in 1% H_2O_2, same as the samples, is of significant importance. The samples were centrifuged at 13,000 rpm for 10 min prior to analysis with HPLC-ICP-MS.

Instrumental setup

The Agilent Hydride Generation (HG) Accessory for ICP-MS was used. This set up has been described in detail elsewhere. [27] Briefly, the samples were injected via an autosampler and transported to the hydride generator (0.5 mL min^{-1}) where the sample mixed with HCl (5 M, 2.5 mL min^{-1}) and $NaBH_4$ (2% (w/v) in antifoam, 0.5 mL min^{-1}) in a mixing coil before entering the gas liquid separator (GLS). The gaseous sample was then transported to the ICP-MS with an argon gas flow (0.3 L min^{-1}) using the make-up gas line of the ICP-MS, separating online the iAs from the DMA. To this an argon flow (0.85-0.95 L min^{-1}) carrying a nebulized solution of the IS using the peristaltic pump of the ICP-MS was added creating wet plasma conditions.

The Agilent triple quadrupole ICP-MS 8800 (ICP-QQQ) was used for arsenic detection. Measurements were carried out in two gas modes (no gas and O_2) in the reaction/collision cell. In O_2 mode, arsenic was measured indirectly as $^{75}As^{16}O^+$ on m/z 91.

Speciation was carried out on an Agilent 1100 HPLC system connected directly to the ICP-MS. A PRP X-100 Hamilton anion exchange column (10 µm, 4.6×250 mm) was used with a flow rate of 1 mL min^{-1} of the mobile phase (20 mM ammonium carbonate (pH 8.5)).

Results and Discussion

Quality control

The totAs concentration in NIST 1568a was determined as 295 ± 6 µg kg^{-1} in good agreement with certified value of 290 ± 30 µg kg^{-1} (n=3), and IMEP-107 was found to contain 173 ± 1 µg kg^{-1} totAs (n=3) also in good agreement with 172 ± 18 µg kg^{-1} reported in the proficiency testing. For speciation the iAs concentration was in good agreement with reported values for IMEP-107 rice (HG: 100 ± 11 µg kg^{-1} and HPLC 110 ± 12 µg kg^{-1} (n=15), reported 107 ± 14 µg kg^{-1}) [5] and NIST 1568a (HG: 94 ± 8 µg kg^{-1} and HPLC 105 ± 4 µg kg^{-1} (n=3), reported 94 ± 12 µg kg^{-1}). [2] The column recovery was good for both QC materials; IMEP-107 rice and NIST 1568a; 98 ± 9% (n=12) and 101 ± 4% (n=3), respectively. IMEP-107 rice was analysed with every batch of samples and from these multiple measurements the RSD within-day and the RSD between measurement days was calculated. The reproducibility and repeatability of the two methods were very similar. The RSD within analysis day were on average 3% for both HG and HPLC and the RSD between analysis days (RSD of all replicas for all measurement days) was 11% for both HG and HPLC. A blank sample was analysed with every batch of samples.

Comparison HG-ICP-MS and HPLC-ICP-MS

The extraction efficiency for the various types of rice grains was generally good, 91 ± 10 % (ranging from 73-111%). The rice flour showed 55% extraction efficiency, the rice paper 87% and the rice noodles 72%. Even for the few samples where the extraction is not complete the results can be used for comparison between HG and HPLC since the same sample extract was used for both measurements. With regard to MLs, despite the low extraction of rice flour it was under 150 µg kg^{-1} in totAs concentration and therefore under both the Chinese ML for iAs as well as proposed EU MLs.

The column recovery of the HPLC was quantitative (94 ± 10%) for all samples. An overview of the iAs, DMA and totAs concentration is given in Table 1. MA was detected in a few samples, however, the concentration was below LOQ for almost all samples and it did not exceed 7 μg kg^{-1}. This is in accordance with an extensive survey undertaken by the US FDA where over 1300 samples of rice products were measured. [4] In this survey 97% or more of the rice products fell below LOD or LOQ (under 13 μg kg^{-1}) for MA. Only 1% of the samples fell between 20 and 30 μg kg^{-1}, with the highest reported concentration of 25 μg kg^{-1}. MA is therefore not a determining factor in the totAs concentration of rice and at these low concentrations MA does not influence the determination of iAs with this HG-ICP-MS method.

Table 1 reveals that all of the commercial rice products fall below the proposed EU MLs for iAs in rice (200 μg kg^{-1}) as well as the MLs for iAs in China (150 μg kg^{-1}). However, if only the totAs had been measured 29% (10 out of 34) of the samples would have exceeded the EU proposed draft MLs and 38% (13 out of 34) the current Chinese ML. The iAs conc. ranged from 26% - 84% of the totAs concentration for the commercial rice samples.

Table 1. Overview of the determination of iAs in 32 rice products and 12 rice grain samples from rice grown under arsenic exposure. Data given for iAs determined by HG and HPLC (coupled with ICP-MS), for DMA and MA as well as the totAs concentration. All data given ± SD, with n=3 for speciation and n=2 or 3 for totAs.

Type	HG iAs (µg kg^{-1})	HPLC iAs (µg kg^{-1})	HPLC DMA (µg kg^{-1})	HPLC MA (µg kg^{-1})	totAs (µg kg^{-1})
Basmati	41 ± 4	53 ± 7	8 ± 1	<LOD	100 ± 12
White Rice	71 ± 5	76 ± 5	14 ± 4	<LOQ	124 ± 1
Pudding Rice	124 ± 9	125 ± 11	44 ± 5	<LOD	202 ± 4
Brown Rice	127 ± 6	137 ± 5	35 ± 2	<LOD	205 ± 2
Arborio Risotto	113 ± 13	120 ± 18	63 ± 7	<LOQ	236 ± 15
Paella Rice	66 ± 4	70 ± 3	17 ± 1	<LOD	121 ± 6
Long Grain Rice	103 ± 2	94 ± 1	218 ± 9	<LOQ	392 ± 23
Thai Jasmine	61 ± 4	64 ± 3	49 ± 5	<LOD	143 ± 3
Japanese Rice	101 ± 5	99 ± 5	123 ± 1	<LOQ	252 ± 10
Rice Noodles	27 ± 1	29 ± 1	9 ± 1	<LOD	73 ± 2
Rice Flour	40 ± 1	46 ± 5	19 ± 2	<LOD	102 ± 6
Vietnamese Rice Paper	21 ± 2	28 ± 1	<LOQ	<LOD	58 ± 10
Long Grain Rice	40 ± 2	52 ± 10	39 ± 3	<LOQ	111 ± 8
Spanish Paella	67 ± 2	67 **± 3**	13 ± 1	<LOD	109 ± 7
Basmati (white)	72 ± 11	69 ± 9	24 ± 1	<LOD	240 ± 5
Organic Long Grain Brown	111 ± 7	131 ± 14	54 ± 7	<LOQ	207 ± 15
Thai Jasmine (white)	62 ± 4	62 ± 3	49 ± 2	<LOD	171 ± 5
Risotto	97 ± 11	114 ± 10	72 ± 9	<LOQ	221 ± 17
Long Grain white	47 ± 2	61 ± 4	19 ± 4	<LOD	102 ± 9
FLG Thai (white)	88 ± 3	102 ± 3	52 ± 5	<LOD	197 ± 9
Organic (white)	65 ± 4	65 ± 2	11 ± 1	<LOD	92 ± 4
Long grain (white)	89 ± 2	85 ± 1	16 ± 1	<LOD	121 ± 11
Carnaroli Risotto Rice	81 ± 2	82 ± 4	84 ± 2	<LOD	210 ± 15
Whole Grain	133 ± 2	127 ± 2	151 ± 12	7.2 ± 0.3	370 ± 19
Paella	60 ± 5	65 ± 2	38 ± 1	1.2 ± 0.1	136 ± 1
Organic Basmati (white)	95 ± 3	104 ± 3	21 ± 2	<LOD	117 ± 13
Org ArbRis	109 ± 12	119 ± 13	60 ± 8	<LOD	150 ± 7
Basmati	76 ± 6	88 ± 6	28 ± 4	<LOD	91 ± 8
Organic Basmati (white)	30 ± 2	29 ± 2	27 ± 1	<LOD	84 ± 2
Organic Long Grain (white)	151 ± 2	149 ± 5	50 ± 1	1.6 ± 0.2	123 ± 13
Whole grain	55 ± 2	70 ± 7	21 ± 2	<LOD	91 ± 11
Long Grain white	77 ± 2	84 ± 5	29 ± 8	<LOQ	103 ± 1
Lemont (Low As exposure)	229 ± 2	230 ± 2	66 ± 1	<LOD	323 ± 19
Kitrana (Low As exp.)	184 ± 4	209 ± 8	275 ± 10	<LOD	484 ± 5
Dom Solid (Low As exp.)	213 ± 25	213 ± 8	168 ± 7	<LOQ	384 ± 21
YRL-1 (Low As exp)	287 ± 7	288 ± 13	42 ± 4	<LOQ	350 ± 15
IC Italian Card. (Low As exp.)	372 ± 34	380 ± 29	101 ± 2	<LOQ	489 ± 20
9524 (Low As exp.)	184 ± 4	184 ± 5	79 ± 1	<LOD	292 ± 11
Lemont (High As exp.)	208 ± 8	223 ± 4	91 ± 4	<LOD	332 ± 7
Kitrana (High As exp.)	240 ± 2	220 ± 11	790 ± 5	7 ± 1	1015 ± 59
Dom Solid (High As exp.)	258 ± 14	237 ± 11	660 ± 25	5 ± 1	923 ± 6
YRL-1 (High As exp.)	426 ± 16	438 ± 22	214 ± 6	1.2 ± 0.4	630 ± 100
IC Italian Card. (High As exp.)	399 ± 55	374 ± 45	798 ± 61	1.7 ± 0.4	1259 ± 48
9524 (High As exp.)	249 ± 14	245 ± 14	494 ± 8	<LOD	746 ± 35

LOQ HG: 5 µg kg^{-1}, HPLC: 1.1 µg kg^{-1}

When considering the whole dataset of all tested rice samples the iAs concentration found with HG vs HPLC, Figure 1a, showed good linearity (R^2 of 0.99) and a slope close to 1 (0.969 ± 0.015). A linear regression assumes that the x values are considered error-free, however, when both the x and y data have error a Deming regression, which allows for errors in both x and y variables, can be used. A Deming regression of the data showed that the intercept value is 2.96 with a 95% confidence interval including 0 (-0.19 – 6.12) and the slope coefficient is 0.976 with a 95% confidence interval including 1 (0.942 – 1.009), therefore there are no systematic or proportional differences between the two methods. The effect of DMA is minimal where even at high concentrations of DMA in the exposed rice samples the concentration of iAs is within SD for almost all samples when comparing HG and HPLC. This can be seen when looking at the arsenic exposed rice which has a diverse DMA profile where the proportion of DMA to totAs ranges from 10 - 80%. Figure 1b shows the iAs conc. found with HG and HPLC as well as the DMA present in the samples. It is evident that no significant difference is found despite the DMA concentration ranging up to 80% of the totAs conc.

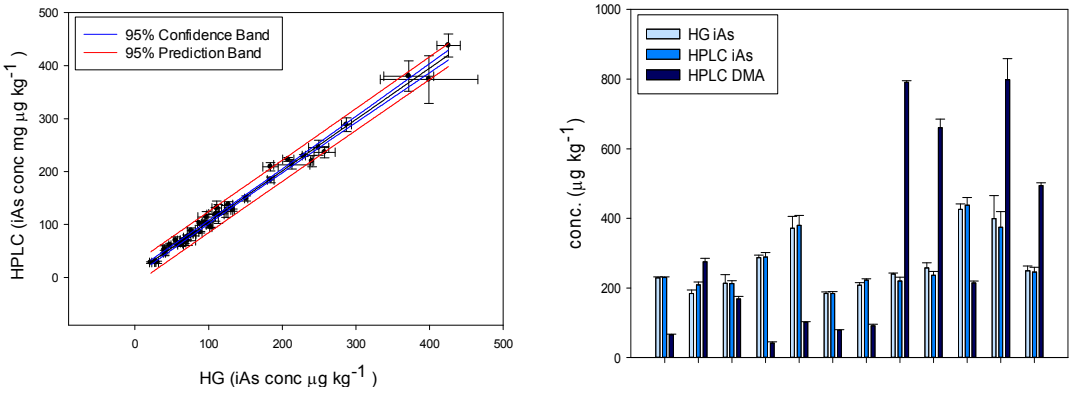

Figure 1. a) Regression of the iAs concentration found with HG vs HPLC. b) Arsenic samples grown at arsenic exposure, determined with HG and HPLC – influence of the diverse DMA profile of the samples on the iAs concentration.

HG coupled to ICP-MS is a sensitive method that can quantify low ppb levels in the sample extract, and down to 10 µg kg^{-1} iAs in the sample – which is a factor of 15 lower than the iAs MLs in China. Using similar experimental parameters, such as acid and $NaBH_4$ concentrations, this method could be adapted to other cheaper instruments such as atomic fluorescence detection (HG-AFS). The change of the detection system might influence the LOQ, but the principle of iAs detection from a solution

containing DMA and iAs using HG remains the same. The LOQ could be remedied by using higher sample to extraction solution ratio or use higher sample and reagents uptake rate while holding the same HCl ratio and LOQs relevant to proposed MLs of iAs could easily be obtained.

Our data show that a robust simple method for the determination of iAs can be used which does not rely on a combination of HPLC and ICP-MS, but the HPLC can be replaced by a simple HG system and possibly the ICP-MS can be replaced by AFS. This illustrates that regulators should not prescribe one standard method for the analyte iAs in rice because it excludes new instrumental and method developments to make the analytical method more affordable and therefore more available for non-specialised laboratories.

Conclusions

Applying HG-ICP-MS and HPLC-ICP-MS on 44 rice products, using the same sample extracts for both measurements, show a y=x relationship over a wide linear range where the influence of DMA on the iAs concentration in the samples is insignificant. The sample preparation is a straight forward microwave extraction and the separation of iAs and DMA is performed online, with only about 4 minute sample run time followed by a convenient data treatment as no integration of peaks is necessary. With increasing demand and interest in the reliable determination of iAs in food, especially rice, this method can be a valuable tool with a quick turnover time.

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