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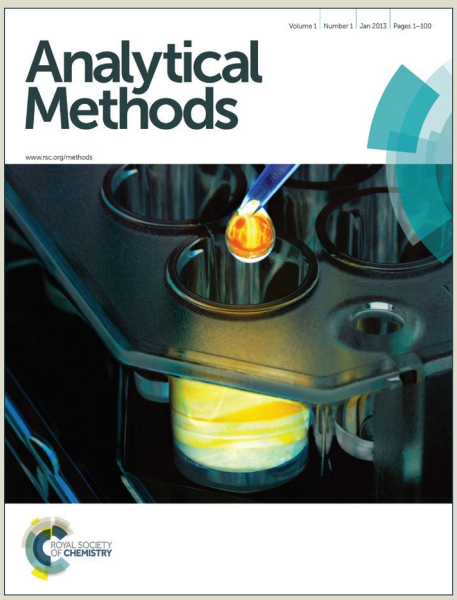

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Speciation of inorganic arsenic in rice using hydride generation atomic absorption spectrometry (HG-AAS)

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Inorganic arsenic (iAs) concentration in rice is a current concern worldwide. A method for iAs determination in rice using hydride generation atomic absorption spectrometry (HG-AAS) is proposed. Arsenic species were extracted from rice using 0.14 mol L^{-1} HNO_3 assisted by microwave radiation or 0.28 mol L^{-1} HNO_3 and heating in water bath prior to As determination by HG-AAS. Selective generation of arsine of As(III) was achieved with 0.1% (m/v) $NaBH_4$ and 10 mol L^{-1} HCl. The iAs was determined by pre-reduction of all inorganic arsenic and determination under the same conditions used for As(III). By difference, the concentration of As(V) was estimated. Ascorbic acid (1% m/v) and KI (0.2% m/v) in presence of 1.2 mol L^{-1} HCl were used for As pre-reduction. The limit of detection (LOD) of As(III) was 1.96 ng g^{-1} and that of iAs was 3.85 ng g^{-1}, which are feasible for As(III) and iAs speciation in rice. Accuracy was evaluated by analyte recovery tests and analysis of certified reference rice. Total arsenic (tAs) was quantified using alkaline extraction with $K_2S_2O_8$ and HNO_3, followed by As detection by HG-AAS. Thirteen samples of different types of rice (polished, brown, organic, and parboiled) grown in Southern Brazilian were analyzed. The iAs, As(III) and As(V) concentrations found were consistent with those already reported for rice.

1 Introduction

Arsenic, which is potentially toxic to humans, occurs naturally in many chemical forms. The toxicity of As varies widely, ranging from highly hazardous inorganic arsenicals (namely arsenite - (As(III)), and arsenate – As(V)) to relatively harmless organic species (monomethylarsonate - MMA and dimethylarsenate - DMA). Organic arsenic (oAs) such as arsenobetaine (AsB) and arsenocholine (AsC), usually found in animal tissues, are also effectively non-toxic towards living organisms. Therefore, determination of total arsenic (tAs) content in a sample does not reflect the level of hazard of the element actually present, and it is important to determine the various forms of As for providing a much clearer view of the risk associated with exposure to As. In the case of rice grains, inorganic arsenic (iAs) - comprising As(III) and As(V) - are commonly found, which is the major source of potentially harmful As compounds for most people. Exposure to iAs is linked to an increased risk of cancer.[1-4]

Arsenic in rice grains is present primarily as iAs and DMA.[2,5] The arsenic compounds are either in the soil or in the irrigation water - the As compounds in the soil may include those from agricultural inputs. It has been observed that rice accumulates high concentrations of As in its grain compared to other cereal crops.[6] The main reason would be the anaerobic conditions of As cultivation (usually in irrigated paddy fields) where As is more available.[7] Flooding of soil leads to a rapid mobilization of As, mainly as As(III), in the soil solution.[7] Low concentration of MMA has also been detected in rice. However, in a survey undertaken by the Food and Drug Administration (FDA)[8] where over 1300 samples of rice products were analyzed the MMA concentration was lower than 13 ng g^{-1} in 97% of the samples. Only in 1% of the samples the MMA concentration fell between 20 and 30 ng g^{-1}. Pétursdóttir *et al*.[5] analyzed 32 samples of rice from different countries and detected MMA in 3 of them; the maximum MMA concentration found was 7.2 ng g^{-1}.

The extent of the contamination of rice with iAs became apparent as more measurements were made and the results published.[2] China has legislation for maximum levels (MLs) of iAs in rice (200 ng g^{-1}),[9] whereas in Europe and America there are ongoing studies to establish a legislation for MLs of iAs in rice.[5,8] Analytical methods capable of distinguishing between inorganic and methylated As compounds are necessary to support such regulation because the toxicity of As varies widely. It has been difficult to establish the MLs for iAs in rice because of analytical concerns. This has occurred because methods for As speciation in rice has been seen as complex and difficult to be implemented in any laboratory.

High performance liquid chromatography (HPLC) has been used for the separation of As species, coupled with hydride generation atomic absorption spectrometry (HG-AAS) or atomic fluorescence spectrometry (HG-AFS).[10] In recent years, HPLC-ICP-MS (high performance liquid chromatography-inductively coupled plasma mass spectrometry) has mostly been employed for As speciation in rice, including iAs and oAs.[2,11-14] However, as only iAs is of toxicity concern, non chromatographic methods for iAs speciation in rice have been

proposed. In this case, HG-AFS[15,16] or HG-ICP-MS[5] have been employed. Methods using these techniques are considered simpler than those using HPLC-ICP-MS. For higher sample throughput, speciation using HG-AFS or HG-ICP-MS might be more suitable for non-specialized laboratories. However, the HG-AAS technique is still the most popular and preferred in terms of simplicity, sensitivity, precision, speed, and cost.

In hydride generation, sodium borohydride ($NaBH_4$) and hydrochloric acid (HCl) are usually employed to convert As species in aqueous solutions into volatile hydrides. All of the commonly encountered arsenic compounds in rice (arsenite, arsenate and dimethylarsinate) react with $NaBH_4$ in aqueous solution to form the corresponding volatile hydrides. The extent of the reaction depends on the conditions, but all of the iAs in a solution can be converted to arsine (AsH_3) and transferred to the vapour phase. By using the selectivity of hydride generation[17] in different reaction media, iAs speciation is possible. The selective iAs in rice can be quantified by pre-reduction of inorganic As(V) (ascorbic acid and potassium iodide have been used) [15,18] or oxidation of As(III) to As(V) (hydrogen peroxide has been used).[18] The pre-reduction or oxidation step is usually carried out because the HG efficiencies of As(III) and As(V) are different. The selective hydride generation approach has already been applied to As speciation in several kinds of samples (*e.g.* biological tissues, food and drinking water). Methods based on oxidation state of As species were developed using hydride generation-cryotrapping associated with AAS[19], ICP-MS[20] and AFS[21] for preconcentration and separation of arsines, with appropriate limits of detection (LODs).

Solid phase extraction (SPE) followed by (HG-AAS) detection was used for iAs speciation in rice. A SPE column was used for separation of arsenosugars whereas the SPE eluate containing iAs was collected off-line and pre-reduced prior to As determination by HG-AAS.[31] In the present study it is develop a method for iAs speciation in rice using selective hydride generation prior to As detection by AAS. Strong acidic condition (10 mol L^{-1} HCl) and low $NaBH_4$ concentration (0.1% m/v) are used for the selective generation of arsine from As(III).

2 Experimental Part

2.1 Instrumental

An atomic absorption spectrometer (PerkinElmer Analyst 200) equipped with a deuterium lamp for background correction and associated with a flow injection system (FIAS 100) was used for the determination of As. A peristaltic pump (Minipuls 3 from Gilson, Villiers Le Bel, France) was used to introduce air into the gas-liquid separator. Figure 1 schematizes the arrangement of the system. The sample is injected via valve **a**, carried by HCl, mixed with $NaBH_4$ in the confluence **b** and then conducted to the gas-liquid separator (GLS). The reacting mixture is removed from GLS to waste by gravity.

An electrode discharge lamp (EDL) from PerkinElmer, operated at 400 mA, was used as radiation source. The GLS was developed in the laboratory; it is a "U" tube type GLS (see Fig.1), made of borosilicate glass. The dimensions and geometry of this GLS made it difficult for water droplets to enter the transfer line. The absorbance was measured at 193.7 nm, whereas the slid width was 0.7 nm. All measurements were fulfilled using integrated absorbance. The quartz cell (16 cm long x 0.7 i.d. - from PerkinElmer) was kept at 900 °C during operation. The reagents (ascorbic acid + KI) used for As(V) reduction may change the internal surface of the quartz cell. Thus, two quartz cells were available; one was used solely for As(III) determination while the other was for iAs.

A microwave oven (Berghof, Speedwave 4) equipped with PTFE-PFA flasks was employed for rice acid digestion and As species extraction. A hot plate was employed for alkaline extraction for tAs determination by HG-AAS. A centrifuge (Fanem, Baby 206, São Paulo, SP, Brazil) was employed for the separation of phases of rice slurry.

For As determination by ICP-MS, an ELAN DRC II instrument (from PerkinElmer/SCIEX), operated in standard mode was employed. Pneumatic nebulization was employed as sample introduction system.

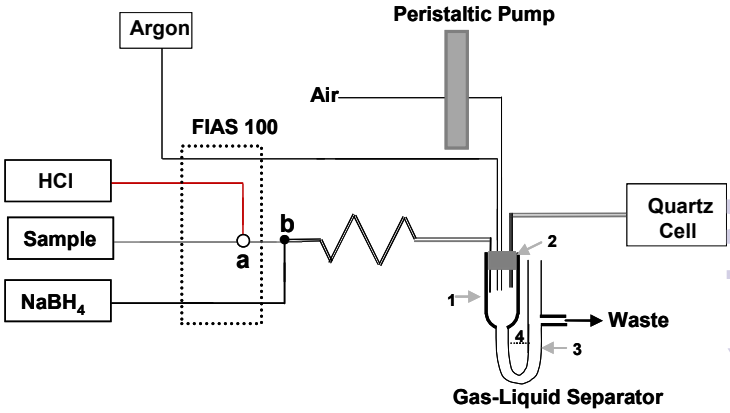

Fig.1 Schematic presentation of the instrumental arrangement of the HG-AAS for As determination; HCl and $NaBH_4$ solutions flow rate: 4 mL min^{-1}; argon flow rate: 50 mL min^{-1}; air flow rate: 1.65 mL min^{-1}; sampling loop: 250 µL; **a**: valve; **b**: confluence. Red-white tygon tubes are employed for the carrier (HCl) and $NaBH_4$ solutions while a black-black tygon tube is for air introduction into the gas-liquid separator. A PTFE (polytetrafluorethylene) tubing (100 cm long x 0.8 mm i.d) is employed to transport the solution from **b** to the gas-liquid separator. 1: 9 cm high x 1 cm i.d; 2: silicone stopper; 3: 11 cm high; 4: 1 cm.

2.2 Reagents, Solutions and Materials

Water (resistivity of 18.2 Ω) purified in a Milli-Q system (Milliprore, Billerica, MA, USA) was used for all solutions and samples. Nitric acid (HNO_3, 65% in mass, Merck), hydrogen peroxide (H_2O_2 30% v/v, Merck) were used for As species extraction in rice or acid digestion in microwave oven. Solutions containing 1 to 10 mol L^{-1} HCl were evaluated for As hydride generation, whereas a 0.14 mol L^{-1} HNO_3 solution was employed for As species extraction from rice. Hydrochloric acid (HCl, 37% in mass, Merck, Darmstadt, Germany) and sodium tetrahydroborate ($NaBH_4$, purity 99%, Acros Organics, Geel, Belgium) were used for As hydride generation.

Solutions of $NaBH_4$ ranging from 0.02 to 1.5% (m/v) were prepared in 0.01% (m/v) NaOH (Merck) in the day of their use. Potassium persulfate ($K_2S_2O_8$, Synth, Diadema, SP, Brazil) was used as oxidant for tAs extraction in rice. To this end, a 2% (m/v) $K_2S_2O_8$ solution was prepared in water and used. For tAs determination using HG-AAS, calibration solutions ranging from 1.5 to 12 µg L^{-1} were obtained by serial dilution of a 1000 mg L^{-1} As(V) stock solution (Merck). These calibration solutions were prepared in 1% (v/v) HNO_3 and 0.4% (m/v) $K_2S_2O_8$ in order to match the medium of the samples extract.

An As(III) stock solution containing 1000 mg L^{-1} As was prepared by dissolving As_2O_3 (Merck) in water previously sonicated for 30 minutes to remove the dissolved oxygen. Calibration solutions (0.5 to 8.0 µg L^{-1}) of As(III) were prepared in 0.14 mol L^{-1} HNO_3 or 0.28 mol L^{-1} HNO_3 by serial dilution of the As(III) stock solution. Reference solutions of As(V) were prepared in 0.14 mol L^{-1} HNO_3 by serial dilution of a 1000 mg L^{-1} As(V) solution (from Merck). Stock solutions containing 1000 mg L^{-1} of dimethylarsinic acid (DMA - $C_2H_6AsO_2Na$) from Sigma Aldrich (St. Louis, USA) or 1000 mg L^{-1} monomethylarsonic acid (MMA - $Na_2CH_3O_3.As.6H_2O$) from Chem Service (West Chester, USA) were also prepared. Reference solutions of DMA and MMA were prepared in water in the day of use. Ascorbic acid (Nuclear, Brazil) and potassium iodide (KI - Vetec, Brazil) were used for reduction of As(V).

Before use, the glassware and polypropylene flasks were left in contact with 50% (v/v) HNO_3 or 10% (v/v) HNO_3 for 48 hours followed by washing with purified water. The quartz cell surface was cleaned/activated by immersing it in HF + HNO_3 (7 + 3) solution for 10 minutes. Then, the quartz cell was thoroughly washed with water and left drying at room temperature before use.

2.3 Samples

Thirteen samples, one kg each, comprising different types and varieties of rice (brown, polished, parboiled, organic or conventionally cultivated in irrigated fields in Rio Grande do Sul, Brazil) were bought in fairs and supermarkets. About 50 g of each sample were ground in agate mortar and sieved to obtain particles with diameter < 0.08 mm. The certified rice flour NIST 1568a (from National Institute of Standards and Technology) and ERM-BC211 (from European Reference Materials) were analyzed as received (without grinding and sieving).

2.4 Procedures

2.4.1 Total Arsenic

Alkaline extraction was used for tAs that was determined by HG-AAS. To this end, 250 mg of rice were transferred to graduated glass vials to which 1 mL 10% (v/v) HNO_3 and 2 mL 2% (m/v) $K_2S_2O_8$ were added. The mixture was heated in water bath on hot plate at 80 - 90 °C for 3 hours. After this period, the mixture was left cooling to room temperature. Then, the volume was elevated to 10 ml by adding water and the mixture centrifuged at 1600 rpm for 5 minutes. tAs was determined in the supernatant. This procedure was employed for the determination of tAs by HG-AAS due to the absence of As signal in the solution of rice sample after acid digestion in microwave oven. The main reason would be the effect caused by nitrogen oxides present in the sample solution,[22,23] which must be reduced before As determination by HG-AAS. Thus, alkaline extraction instead of acid digestion was used to simplify the work. 0.75% (m/v) $NaBH_4$ and 6 mol L^{-1} HCl were used in the determination of tAs by HG-AAS.

Four samples of rice were acid digested in microwave oven and tAs measured by ICP-MS as an independent technique in order to validate the alkaline extraction method. In this case, 3 mL HNO_3 and 2 mL H_2O_2 were added to 250 mg of sample in the respective flask of the microwave oven employed for the rice samples digestion. The microwave oven program given in Table 1 was run. The obtained solution was transferred to graduated polyethylene vial and the volume completed to 15 mL.

Table 1 Microwave heating program for rice digestion.

Step	T (ºC)	P (bar)	Ramp (min)	Holding (min)	Power (%)
1	160	30	10	15	70
2	180	35	5	15	90
3	50	25	1	10	0

2.4.2 Arsenic Speciation

Following reported methods for As speciation in rice,[24] the As species were extracted using 0.14 mol L^{-1} HNO_3. Huang *et al.*[12] demonstrated that the integrity of As(III) and As(V) species were preserved in a narrow range of HNO_3 concentration. The released matrix led to significant As(V) reduction and As(III) oxidation by hot HNO_3 < 0.28 mol L^{-1} and > 0.70 mol L^{-1}, respectively. However, 0.14 mol L^{-1} HNO_3 was used in the present method for selective extraction of inorganic As species, as cited in reference 24. At the conditions employed in the present method, recovery of spiked As(III) and As(V) revealed no species oxidation/reduction. In the present method, 5 mL 0.14 mol L^{-1} HNO_3 were added to 500 mg of sample in the microwave oven flask and the mixture let standing for 12 h (overnight). Subsequently, microwave radiation was applied (5 min 50 °C, 5 min 75 °C, 30 min 95 °C). After reaching the room temperature, the slurry obtained was transferred to graduated polypropylene vial and the volume was elevated to 14 mL by adding 0.14 mol L^{-1} HNO_3. The final slurry was centrifuged at 1600 rpm for 5 minutes. Then, the iAs and As(III) present in the supernatant were determined by HG-AAS. The iAs extraction with 0.28 mol L^{-1} HNO_3, as proposed by Huang *et al*., was also evaluated. In this case, 5 mL of 0.28 mol L^{-1} HNO_3 were added to 250 mg of sample in a graduated polypropylene vial. The mixture was heated at 95 °C for 90 minutes in water bath on hot plate. Then, the mixture was left standing to achieve the room temperature, followed by centrifugation and supernatant analysis. Results obtained using either 0.14 mol L^{-1} HNO_3 or 0.28 mol L^{-1} HNO_3 for i(As) extraction in rice were in agreement and, therefore, both methods are suitable.

The accuracy was evaluated through the analysis of certified reference material and analyte recovery tests. To evaluate the analyte recovery, before submitting the sample to the extraction procedure, 5 mL of solution containing 50 mg L^{-1}As(III), As(V) and DMA were added to a rice brown sample in order to obtain 42 ng g^{-1} of As(III), As(V) and DMA.

For pre-reduction of iAs, 0.1 g KI + 0.02 g ascorbic acid + 1 mL HCl were added to 5 mL of rice sample extract and left standing for 1 h. Then, the volume of the mixture was elevated to 10 mL by adding water. Thus, the final concentrations of KI and ascorbic acid were 1% (m/v) and 0.2 % (m/v), respectively. The calibration solutions were treated in the same manner that the extracts of samples.

3 Results and Discussion

3.1 Method Development

Preliminary tests revealed poor sensitivity and precision. Then, air was introduced into the gas-liquid separator in order to improve these parameters. As can be seen in Fig. 2, the As

absorbance increases more than 3-fold with the air addition. In this way the relative standard deviation (RSD) of 10 measurements was less than 5%.

Atomization of hydrides in a heated quartz cell occurs *via* collisions with free H radicals, which can be formed by decomposition of hydrogen molecules. The improvement of the hydrides atomization in presence of H radicals has been reported by Dedina in several papers.[25-27] Hydrogen radicals are formed by reactions among hydrogen and oxygen, from the $NaBH_4$ decomposition and contaminants in reagents and carrier gas, respectively. Oxygen present in the air may have enhanced the production of H free radicals and increased the As absorbance, as observed in Fig. 2.[17] The decrease of the absorbance observed for air flow rate higher than 1.65 mL min^{-1} was possibly caused by the consumption of hydrogen.[28] Two processes are related to decreasing of free analyte atoms inside atomization quartz cells. One is the mechanical process that is based on the flow of the carrier gas, which pushes free atoms to external parts of the quartz cell. The other is related to chemical reactions occurring along the atomizer (critically influenced by the atomizer inner surface). The free H radical mechanism satisfactorily explains the enhancement of the As absorbance as a function of the air flow rate. However, the decrease of free analyte atoms has not been fully elucidated yet.[27,29] In the present study it was ultimately concluded that additional investigations are necessary for better understanding the signal enhancement depicted in Fig.2

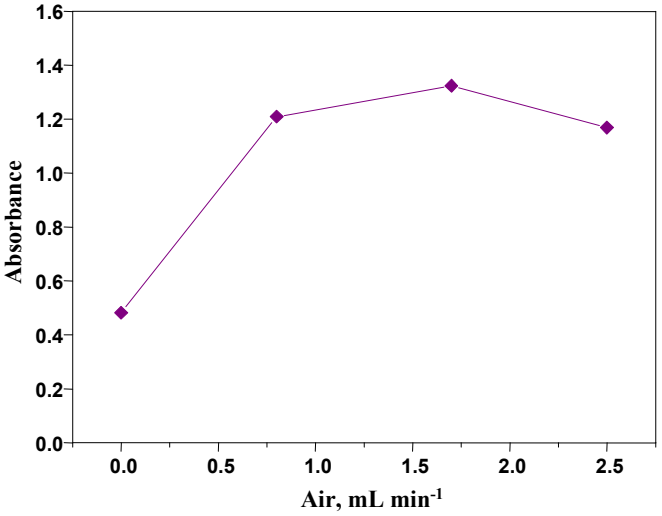

Fig. 2 Influence of air introduced into the gas-liquid separator on the absorbance of As (III) (10 µg L^{-1} in 1 moL L^{-1} HCl); $NaBH_4$ concentration: 0.5% m/v. See Fig. 1 for additional information.

The HCl and $NaBH_4$ concentrations that would lead to selective hydride generation of As(III) and iAs were evaluated and studied. As shown in Figs. 3(a) and 4(a), by using 10 mol L^{-1} HCl and 0.1% (m/v) $NaBH_4$ only As(III) and MMA are detected. However, it has been observed in previous studies[5,8] that MMA is commonly not detected in rice. Thus, by using 10 mol L^{-1} HCl and 0.1% (m/v) $NaBH_4$ it is possible to determine As(III) in rice, considering the absence of MMA. In this condition the contributions of DMA and As(V) are negligible.
It has been reported by Shraim *et al.*[30] that the generation of hydrides from DMA in solution is irrelevant in presence of $NaBH_4$ up to 2% (m/v) and 6 mol L^{-1} HCl. According to Pétursdóttir *et al.*,[5] in presence of 5 mol L^{-1} HCl and 2% (m/v) $NaBH_4$ the contribution of DMA as dimethylarsine is only 2 - 4% while MMA forms methylarsine at approximately 40% efficiency. Nevertheless, MMA is generally absent in rice or present in very low concentration and would not affect the quantification of iAs. In the present study it was observed that in presence of 6 mol L^{-1} HCl and increased $NaBH_4$ concentration (see Fig.4 (b)) there was arsine generation from DMA. Therefore, it was concluded that it would be necessary to reduce As(V) to As(III) and measure iAs at the same condition used for As(III). As can be seen in Figure 3 (b), with pre-reduction of As(V) both As(III) and As(V) can be measured under the same condition used for As(III) (10 mol L^{-1} HCl and 0.1% $NaBH_4$) without contribution of DMA.

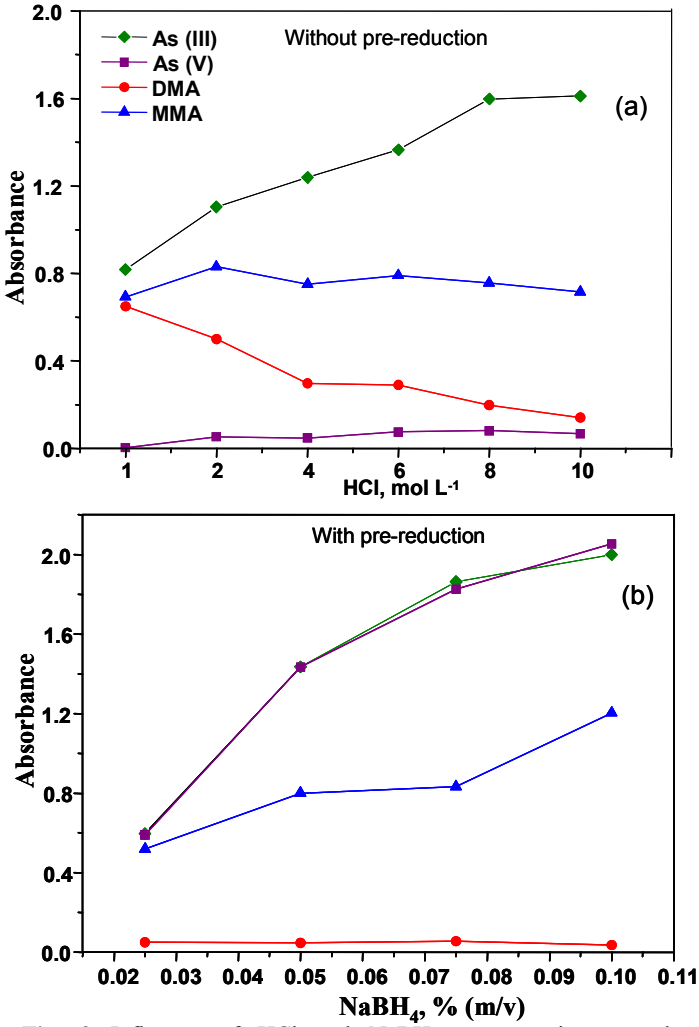

Fig. 3 Influence of HCl and $NaBH_4$ concentrations on the absorbance of As species (10 µg L^{-1} each); DMA: dimethylarsinic acid; MMA: monomethylarsonic acid. The $NaBH_4$ and HCl concentrations were 0.1% (a) and 10 mol L^{-1} (b), respectively. The absorbances in (b) correspond to As species submitted to pre-reduction using KI (5% m/v) + ascorbic acid (1% m/v).

3.2 Accuracy

Initially, a reference solution containing 50 μg L^{-1} of As(III) and As(V) in 0.14 mol L^{-1} (v/v) HNO_3 underwent the same treatment of samples. The recovery of As(III) and As(V) was close to 100%, demonstrating the species preservation. In a subsequent step, a sample of brown rice was spiked with As(III), As(V) and DMA. As can be seen in Table 2, the recoveries of As(III) and As(V) were close to 100%. Hence, the As species were conserved or the interconversion among them was very low. The results given in Table 2 also demonstrate that hydride generation from DMA is not detected at the conditions used for selective hydride generation, allowing the speciation of iAs and As(III).

Table 2 Recovery of As spiked to brown rice. Concentrations are the mean and standard deviation of three determinations (n = 3).

Apecies	Found in the Sample, ng g^{-1}	Spiked, ng g^{-1}	Found in the Spiked Sample, ng g^{-1}	Recovery, %
As(III)	40 ±3	42	84 ± 2	102
As(V)	89 ± 3	42	126 ± 9	96
DMA	nd	42	41 ± 2	nd

nd: not detected

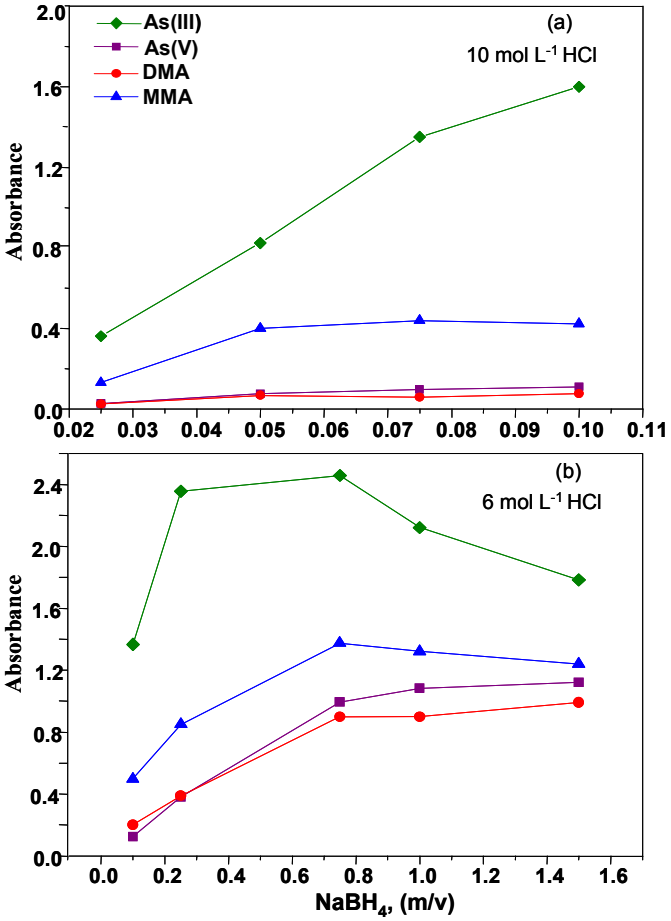

Fig. 4. Influence of $NaBH_4$ concentration on the absorbance of As species (10 μg L^{-1} each); DMA: dimethylarsinic acid; MMA: monomethylarsonic acid.

3.2 Limits of Detection

The limits of detection (LODs) of As (III) and As(V) are given in Table 3, together with the calibration curves parameters and conditions used for the selective hydride generation. The LODs were calculated following the 3s criterion - s is the standard deviation of 10 consecutive runs of the sample blank. The LODs were calculated by considering 500 mg of sample in a final volume of 14 mL of slurry. In the case of alkaline extraction (using $K_2S_2O_8$) the LOD was 14 ng g^{-1}, by considering 250 mg of sample in 10 mL of slurry. The LOD of tAs is the highest due to the oxidizing medium (0.4% m/v $K_2S_2O_8$ + 1% v/v HNO_3) of samples and calibration solutions. The LOD of the proposed method is feasible for iAs speciation in rice and has the same order of magnitude of that reported for HG AFS, that was 1 ng g^{-1}.[15] It is worth citing that the sensitivity of AFS is remarkably better than that of AAS for As. The low LOD of the present method can be attributed to the use of EDL as radiation source, low dilution of the sample and high sampling volume (250 μL). The GLS employed also played an important role because a greater amount of rice could be processed without excessive foaming, which would force the introduction of water into the transfer line (from GLS to quartz cell) and increase the noise.

Samples Analysis

The developed method was applied to the analysis of different types of rice. The As species concentrations found are given in Table 4. The results obtained are consistent with those reported for Brazilian rice[12] and others.[5,12,16] The results given in Table 4 also reveal the importance of pre-reduction of As(V) and measuring it under the same condition used for As(III). There was contribution of DMA if iAs was measured using 1% $NaBH_4$ and 6 mol L^{-1} HCl; as can be seen in Table 4 - the iAs and tAs concentrations found in four rice samples were similar. Thus, the DMA species may not be extracted by 0.14 mol L^{-1} HNO_3 in a given type of rice but may be in other.

4 Conclusions

A method of iAs speciation in rice by HG-AAS was developed. The iAs species can be extracted with 0.14 mol L^{-1} HNO_3 under microwave radiation or 0.28 mol L^{-1} HNO_3 and heating in water bath on hot plate. Selective hydride generation is achieved using 0.1% $NaBH_4$ and 10 mol L^{-1} HCl. In such condition hydrides of As(III) and MMA are generated only. As(V) submitted to pre-reduction with KI + ascorbic acid is determined as As(III). The type of gas-liquid separator used must ensure that there is no excessive effervescence, avoiding transport of water droplets into the capillary between the gas-liquid separator and quartz cell.

Accurate determination of tAs in rice by HG-AAS was possible by using 2% (m/v) $K_2S_2O_8$ + 10% (v/v) HNO_3 and heating in water bath on hot plate for As extraction. Accuracy

was assured by matching the calibration solutions and sample with respect to the $K_2S_2O_8$ and HNO_3 concentrations.

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Table 3 Limit of detection (LOD), parameters of calibration curves, and conditions used for selective hydride generation for As species in rice, extracted with 0.14 mol L^{-1} HNO_3.

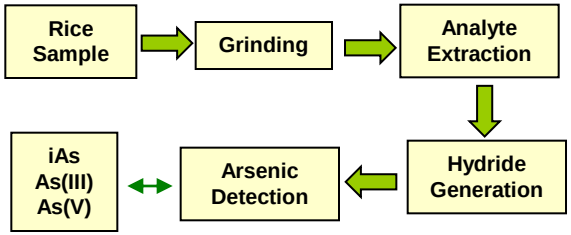
Specie	LOD		Calibration Curve		Hydride Generation	
	µg L^{-1}	ng g^{-1}	r	Linear Regression Equation	HCl, mol L^{-1}	$NaBH_4$, % (m/v)
As(III)	0.07	1.96	0.9965	y = 0.065x + 0.006	10	0.1
As(i)[a]	0.14	3.85	0.9996	y = 0.074x - 0.023	10	0.1

[a]: pre-reduction using 5% KI + 1% ascorbic acid + 1. 2 mol L^{-1} HCl

Table 4 Concentration of As species found in rice using HG-AAS. The As(V) concentration was obtained by difference (iAs - As(III)). Concentrations are the mean and standard deviation of three determinations (n = 3). iAs species were extracted with 0.14 moL^{-1} HNO_3.

Sample	Concentration, ng g^{-1}			
	iAs	As(III)	As(V)*a*	tAs
Brown/Organic and Byodinamic	145 ± 4	113 ± 15	32 ± 15	241 ± 9
Brown	88 ± 5	51 ± 1	37 ± 5	133 ± 9
"Agulhinha"/Polished/Organic	143 ± 5 (222 ± 3)b	105 ± 1	39 ± 5	223 ± 9 (229 ± 18)c
"Agulhinha"/Polished	68 ± 2 (175 ± 7)b	62 ± 2	5 ± 3	163 ± 3 (153 ± 18)c
"Cateto"/Polished/Organic	56 ± 3 (132 ± 7)b	47 ± 3	8 ± 5	133 ± 6 (121 ± 8)c
"Cateto"/Brown/Organic	128 ± 6 (200 ± 10)b	98 ± 5	30 ± 8	203 ± 5 (207 ± 11)c
"Cateto"/Brown	163 ± 2 (162 ± 1)e	116 ± 3 (112 ± 2)e	46 ± 4 (50.0 ± 1.9)e	258 ± 10
"Cateto"/Polished	87 ± 1	58 ± 1	29 ± 1	136 ± 6
Parboiled White	150 ± 4	75 ±4	76 ± 5	168 ± 5
Parboiled/Organic	102 ± 7	70 ± 1	32 ± 7	116 ± 2
Red/Integral	169 ± 5	158 ± 2	11 ± 6	222 ± 1
"Arbóreo"/Polished	125 ± 1	97 ± 4	28 ± 4	150 ± 7
Polished	54 ± 4	30 ± 8	24 ± 8	83 ± 9
NIST 1668a	-	-	-	270 ± 15 (290 ± 30)d
ERM – BC211	116 ± 3 (124 ± 11)d	104 ± 4	12 ± 5	257 ± 15 (260 ± 13)d

a: estimated by difference; *b*: without pre-reduction of iAs (using 1% $NaBH_4$ and 6 mol L^{-1} in hydride generation); *c*: determined using ICP-MS; *d*: certified value; e: 0.28 mol L^{-1} was used for As species extraction



Inorganic arsenic (iAs) is very toxic. iAs in rice was determined using selective hydride generation conditions and atomic absorption spectrometry.