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Investigation of arsenic species stability by HPLC-ICP-MS in plants stored under different conditions for 12 months ☆

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ABSTRACT

In studies of speciation, the conservation of species in their original forms is a very challenging issue, and the reliability of the results deeply depends on it. Preliminary steps of sample preparation and storage temperature may influence the conservation of species and extraction efficiency. Plant sample (*Brachiaria brizantha* Stapf. cv. Marandu) grown in As-containing soil was harvested and divided into four portions. The first portion was frozen-dried, milled with liquid N_2 and stored at room temperature; the second portion was lyophilized and stored at 4 °C; and the remaining portions were kept at two storage temperatures (−18 and −80 °C) without any prior procedure. Aliquots of samples stored under different conditions were extracted every 2 months for a 12-month period. The total content of As was 2.30 ± 0.27 mg kg^{-1} of dried and ground sample and As species were determined by HPLC-ICP-MS. The extraction was more efficient in sample aliquots that were lyophilized and ground (87–90%) than those only stored under different temperatures (53–66%). Furthermore, only for lyophilized and ground samples dimethylarsenic acid (DMA) was extracted. Concerning the storage time under the two tested temperatures, no significant differences on species were found and they remained stable. Standard deviations for replicates of samples that were not frozen-dried and ground were higher, and this behavior can be explained by sample heterogeneity. For the As species here studied it can be concluded that lyophilization and cryogenic grinding strategies were the most suitable sample pretreatments for As speciation in plant tissue.

1. Introduction

Arsenic speciation in aquatic and terrestrial biota is an important topic, since plants are often the main form of potentially toxic element input into food chain, either directly by ingestion of edible plants or indirectly from animals (meat and milk) that eat leaves used for livestock fodder [1]. The determination of chemical species provides information about toxicity, availability and essentiality; however it requires a more complex procedure of analysis compared to total determination. In order to obtain reliable results, more rigorous analytical procedures taking into account all steps, such as sample preparation (drying, grinding, extraction) and storage (temperature, time, radiation), must be employed [2–4]. Additionally, to show that species conversion does not occur confers reliability to the results.

Usually, procedures employed in trace analysis involve drying and grinding of samples. The sample preparation for speciation is milder than for total content determination; thus, it is worth discussing whether the preliminary procedures also should be gentle. Some authors advised the direct analysis of fresh plant; however, this procedure is often difficult due to moisture content and sample heterogeneity [5]; others recommended the drying of the plant since it is believed that plant moisture can cause species conversion [6]. A paper has reported that milling is important to assess the species contained inside the cellular structure of the vegetable and also for providing greater homogeneity [7]. Furthermore, some studies focused on the storage temperature and divergences have been consistently found. Recent researches indicate that the plant should be kept at a low temperature, while others consider that the freezing/defrosting process of the sample led to species conversion and, therefore, they must be kept at room temperature [8,9].

Another important point is the time that the sample remains in its original state. Most works about speciation analysis do not assess the sample stability on long-term storage, which is critical to determine the maximum storage time that still preserves the species. Samples of algae *Cystoseira mediterranea Sauvageau* were stored under different conditions for 45 days [9]. Bluemlein et al. [10] analyzed samples stored

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at −80 °C and the concentration of arsenic–phytochelatin complexes, remained relatively constant during 21 days; on the other hand Raab et al. [11] noticed that long-term storage of plant at −20 °C is not appropriate for conserving As–phytochelatin complexes.

High performance liquid chromatography–inductively coupled plasma mass spectrometry, HPLC-ICP-MS, is one of the most widely employed hyphenation techniques for speciation studies due to the simple coupling, high separation power of HPLC and suitable detection power of ICP-MS [12,13].

The aim of this work was to evaluate the As species stability along 12 months with periodical determination of them (each two months), using HPLC-ICP-MS. Also, different storage temperatures and state of the sample, drying and grinding, were evaluated. The sample chosen *Brachiaria brizantha* Stapf cv. Marandu is a type of grass used in Brazilian cattle feed. In order to increase the As occurrence in plant leaves and thus ensuring species quantification, the plant was cultivated in a soil containing As(V) as $Na_2HAsO_4 \cdot 7H_2O$.

2. Experimental

2.1. Reagents and standards

All glassware was immersed into 10% $v\,v^{-1}$ HNO_3 solution overnight before using and further thoroughly rinsed with distilled–deionized water (18.2 MΩ cm, Milli-Q, Millipore, Bedford, MA, USA). The standards used in the speciation studies were $Na_2HAsO_4 \cdot 7H_2O$ (Vetec, Rio de Janeiro, RJ, Brazil), As_2O_3, $CH_4AsNa_3 \cdot 5H_2O$ and $C_2H_7AsO_2$ (Supelco, Bellefonte, PA, USA). Analytical grade methanol was also used for column cleaning (Tedia Company, Fair-field, OH, USA). The mobile phase, phosphate buffer, for liquid chromatography experiments was prepared from respective salts, K_2HPO_4/KH_2PO_4 (Labimpex, Diadema, SP, Brazil). Ultrapure HNO_3 was obtained from a sub boiling distillation system (Milestone, Sorisole, Italy) and H_2O_2 30% $v\,v^{-1}$ (Synth, Diadema, SP, Brazil) was used in microwave-assisted digestion for total As determination in samples. Solutions used to build up the analytical calibration curve for total As determination were prepared by diluting the corresponding 1000 mg L^{-1} standard stock solutions (Quemis, São Paulo, SP, Brazil) with distilled–deionized water.

2.2. Instrumentation

Total As concentration in digested plant samples were determined using a quadrupole-based ICP-MS (ICP-QMS, 820-MS, Varian, Mulgrave, Australia), equipped with a collision–reaction interface (CRI), which is composed of a modified skimmer and sampler cones through which H_2 or He can be introduced to correct for spectral interferences. Four different strategies were evaluated for total As determinations: H_2 at 60 or 80 mL min^{-1}, He at 80 mL min^{-1} (both gases introduced individually through the skimmer cone), and without using the CRI. Speciation analysis was carried out using a ProStar HPLC System, Varian composed of two pumps, a Rheodyne sample injector (model 9725i), a dual wavelength ultraviolet–visible, UV–Vis, detector and an anion exchange column (IonoSpher A 250 × 4.6 mm, Varian) was used to promote the separation of As species. Chromatograms were recorded using ICP-QMS software. HPLC software was only used to control the solvent elution and pressure. The mobile phase consisted of a phosphate buffer solution, pH 5.4, 12.5 mmol L^{-1} and flowing at 1.0 mL min^{-1} in isocratic mode. The chromatographic column outlet was directly connected to the ICP-MS nebulizer. Operating parameters for the HPLC-ICP-MS arrangement are shown in Table 1. For sample preparation, a cryogenic mill (CryoMill, Retsch,Hann, Germany), a freeze-drier (E-C, MicroModulyo, New York, NY, USA), a closed vessel microwave oven (Ethos 1, Milestone-MLS, Sorisole, Italy), a water bath with heating and agitation (Novatecnica, Piracicaba, SP, Brazil) and a centrifuge (Celm, Barueri, SP, Brazil) were used.

Table 1
Instrument configuration and operating conditions for ICP-QMS and HPLC.

ICP-QMS	
RF applied power	1.4 kW
Plasma gas flow rate	18.0 L min^{-1}
Nebulizer gas flow rate	1.02 L min^{-1}
Auxiliary gas flow rate	1.8 L min^{-1}
Sheath gas flow rate	0.13 L min^{-1}
Sample depth	5.5 mm
Points per peak	15
Scans per replicate	5
Replicates per sample	3
Nebulizer	Seaspray
Spray chamber	Scott-type
Spray chamber temperature	2 °C
Mass-to-charge ratios (m/z) monitored	75 and 77
Scan mode	Peak hopping
Acquisition mode	Time resolved
HPLC	
Column	IonoSpher A 250 × 4.6 mm
Mobile phase	Phosphate buffer 12.5 mmol L^{-1}, pH 5.6
Flow rate	1 mL min^{-1}
Injection volume	50 μL

2.3. Cultivation and sampling of B. brizantha cv. Marandu in As contaminated soil

Freshly harvested plant material (*B. brizantha* cv. Marandu) grown on an As contaminated soil was used for the As speciation study. Plant samples were cultivated in a greenhouse in pots containing 25 kg of a typical Brazilian soil (Dark-Red Latosoil) that was sampled from the surface layer of the soil down to a depth of 20 cm. In a preliminary study, As concentrations added to the soil were evaluated to prevent the death of the plants and at the same time to ensure enough absorption of As for performing speciation studies. The optimal concentration was established as 10 mmol of As, as $Na_2HAsO_4 \cdot 7H_2O$, per kg of soil. It was an experimental cultivation that is representative of field conditions adopted for cattle feed, in terms of soil acidity and nutrients content. Samples from eight pots were harvested 10 cm above the ground; therefore, only leaves were analyzed.

2.4. Pre-treatment of sample and storage conditions

The whole homogenized amount of sample was transported to the laboratory and divided into 5 portions. Portion A was immediately extracted (Day 0) without any processing; portion B was frozen-dried for 48 h and then ground in a freezer mill containing liquid N_2 and after that stored at room temperature; portion C was frozen-dried for 48 h and stored at 4 °C; portion D was stored at −18 °C and portion E was stored at −80 °C, portions D and E were stored without any previous treatment. All portions were stored for 1 year in each specific condition and were analyzed at 2-month basis intervals. All procedures were carried out in triplicate.

2.5. Total As determination and As speciation analysis

The portion that was frozen-dried and ground was digested (200 mg of samples, 6 mL of HNO_3 7 mol L^{-1} and 2 mL of H_2O_2 30% $v\,v^{-1}$). Sample digestion was carried out in a closed-vessel cavity microwave oven and the mixture was submitted to a three-step heating program: 15 min ramp to reach 120 °C, followed by 20 min to reach 200 °C, and a 20 min final step at 200 °C. The sample and a standard reference material of tomato leaves (NIST SRM 1573a, National Institute of Standards and Technology, Gaithersburg, MD, USA) were analyzed in triplicate with their corresponding digestion blanks. Samples and blank solutions were transferred to polypropylene flasks and the volumes were made up to 15.0 mL with distilled–deionized water. Further dilution was

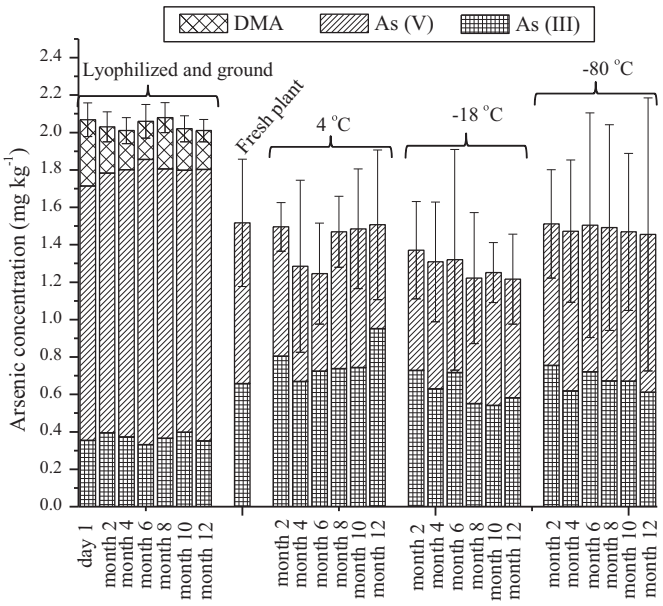

Fig. 1. Concentrations of As species in sample aliquots (mg kg^{-1}, mean and standard deviations, $n = 3$) under different storage conditions.

performed before analysis to ensure a maximum concentration of dissolved solids of 0.1% $m\ v^{-1}$.

Only the portion A was extracted once because it corresponds to the fresh plant; the remaining four portions were extracted each 2 months in a 1-year period. After extraction, four As species (As(III), As(V), MMA and DMA) were spiked into the extracting solutions previously mixed with a plant (lyophilized and ground) cultivated in the absence of As, as reported in a previous work [14]. All measurements were carried out in triplicate. The extraction procedure was performed by adding 10 mL of 2% $v\ v^{-1}$ HNO_3 to 200 mg of the sample. Extraction was carried out in a water bath at 37 °C with shaking (150 rpm) for 90 min. After extraction, the mixture was centrifuged and the supernatant was collected for analysis [14]. All sample extracts were filtered through a 0.45 μm filter paper (Millipore) and stored at −20 °C until analysis.

3. Results and discussion

The total As content was determined using the introduction of H_2 gas through the skimmer cone at a flow rate of 80 mL min^{-1} to minimize polyatomic interferences. Therefore, total As content in *B. brizantha* adopted in all calculations of extraction efficiency was 2.30 ± 0.27 mg per kg of lyophilized and frozen-dried sample. The certified value for As in tomato leaves NIST 1573a is 0.112 ± 0.004 mg kg^{-1} and a concentration of 0.118 ± 0.008 mg kg^{-1} was determined, therefore evidencing the accuracy of the analytical method. As we previously reported, small interconversion of spiked As(III) and As(V) was observed and recoveries for As(III), DMA, MMA and As(V) were 68, 105, 93 and 126% [14].

Fig. 1 shows an overview of the As species extracted in samples stored under different conditions; the standard deviation (errors bars) is relative to the sum of the species. First of all, it is noted that extraction was more efficient in samples that were lyophilized and ground (87–90%) compared to those only stored under different temperatures (53–66%) (Fig. 1 and Table 2). Also, the As(III)/As(V) ratio changed when comparing a sample of fresh plant with a plant sample aliquot lyophilized and ground. The sample lyophilized and ground contained higher concentration of As(V); on the other hand, the lyophilized sample stored at 4 °C contained higher concentration of As(III). The portion stored at −18 °C diverges slightly; months 2 and 6 presented more As(III) and the remaining months, more As(V). The sample stored at −80 °C again contained more As(V), although lower concentration than the sample lyophilized and ground. The portion conditioned at −80 °C is the most similar to the fresh plant. It is difficult to infer whether there are species interconversions during the lyophilization and grinding process or during freezing/defrosting of sample. Although the fresh sample was also analyzed, low extraction efficiency of species did not guarantee complete reliability in the results. Such behavior is difficult to explain because we cannot assert which procedure is generating artifacts.

Table 2
Extracted As(III)/As(V) ratio and total extraction efficiency (%), $n = 3$.

Portion	Processing and storage temperature	Storage period	As(III)/As(V)	Extraction efficiency (%)
A	Fresh plant	0	0.77	66.0 ± 14.8
B	Lyophilized and ground[a]	1 day	0.26	89.9 ± 3.91
B	Lyophilized and ground[a]	2 months	0.29	88.3 ± 3.48
B	Lyophilized and ground[a]	4 months	0.26	87.4 ± 3.04
B	Lyophilized and ground[a]	6 months	0.22	89.6 ± 3.91
B	Lyophilized and ground[a]	8 months	0.25	90.4 ± 3.48
B	Lyophilized and ground[a]	10 months	0.28	87.8 ± 3.04
B	Lyophilized and ground[a]	12 months	0.24	87.4 ± 2.61
C	Lyophylized and chopped[b]	2 months	1.16	65.0 ± 2.65
C	Lyophylized and chopped[b]	4 months	1.09	55.9 ± 20.0
C	Lyophylized and chopped[b]	6 months	1.39	54.2 ± 11.7
C	Lyophylized and Chopped[b]	8 months	1.01	63.9 ± 8.26
C	Lyophylized and chopped[b]	10 months	1.00	64.6 ± 13.9
C	Lyophylized and chopped[b]	12 months	1.71	65.5 ± 17.4
D	Chopped[c]	2 months	1.13	59.6 ± 11.3
D	Chopped[c]	4 months	0.93	56.9 ± 13.9
D	Chopped[c]	6 months	1.19	57.4 ± 25.6
D	Chopped[c]	8 months	0.82	53.1 ± 15.2
D	Chopped[c]	10 months	0.76	54.4 ± 6.96
D	Chopped[c]	12 months	0.92	52.9 ± 10.4
E	Chopped[d]	2 months	1.00	65.7 ± 12.6
E	Chopped[d]	4 months	0.72	64.0 ± 16.5
E	Chopped[d]	6 months	0.92	65.4 ± 26.1
E	Chopped[d]	8 months	0.82	64.8 ± 23.9
E	Chopped[d]	10 months	0.84	63.9 ± 18.3
E	Chopped[d]	12 months	0.73	63.3 ± 31.7

[a] Room temperature.
[b] 4 °C.
[c] −18 °C.
[d] −80 °C.

As reported by Huang et al. [15] interconversions between As(III) and As(V) are not only related to the extractant media, but also to the resulting matrix from the extraction process. Huang and Ilgen [16] recommended As speciation directly in fresh plants samples, because they believe that the storage and drying steps cause modifications in the forms of original species. These authors also noticed a decrease in extracted As when samples were not dried. Additionally, the As(III)/As(V) ratio has changed, similarly as observed in present study.

In contrast, Pell et al. [9] reported that non-ground plants (only chopped) showed low recovery of species, probably due to the lack of access to the retained species in the plant cellular structure. These authors also noted that frozen plants had an average recovery 60% lower than plants that were not stored under low temperatures. They attributed this effect to As losses during freeze/defrosting process, and for this reason they recommended the use of frozen-dried samples, but not frozen storage.

It can be seen in Table 3 that, only in lyophilized and ground samples the DMA (As methylated form) species was extracted. This is probably due to the fact that some species are linked to plant cellular structures and since the plant has not been ground, the access to such species becomes more difficult. Schmidt et al. [17] reported no difference between fresh and ground plants considering extracted amounts of As(V) and DMA; however, the amount of As(III) extracted from the ground plant was almost twice compared to the fresh ones. These authors also pointed out the importance of sample homogeneity because standard deviations were higher in fresh plants, as expected.

Taking into account the storage time under different temperatures, no significant differences on species were found and they remained relatively stable (Table 3). However, it is possible to observe longer time stability for the lyophilized and ground sample, when compared to the samples just stored at low temperatures.

Standard deviations between the replicates for the non-dried samples were higher and this behavior probably is related to sample heterogeneity (Table 3). It may be inferred that particle size distribution has exerted a great influence on extraction efficiency and selectivity, since only on lyophilized and ground sample was possible to extract DMA. Alava et al. [18] highlighted that particle size has crucial importance in As speciation regarding extraction efficiency. But unlike the data obtained in the present work, these authors noticed that the extraction efficiency for each species is similarly affected by particle size, except for As(III), which was extracted in smaller quantities in whole grains.

The best strategy to keep As species depends on the type of sample investigated and the species of interest. According to Bluemlein et al. [10] in studies of As species linked to peptides, lyophilization is not a good alternative once it was observed the occurrence of glutathione and phytochelatins losses and compound disintegration.

It can be seen that there is some controversy in the literature about the best procedure for sample storage; therefore, the type of plant, investigated species, the time between extraction and analysis and equipment available must be carefully considered. While some authors recommended grinding and drying of samples to achieve greater homogeneity and access to species, others believe that such procedures can lead to losses or species conversion [19,20].

4. Conclusions

It can be concluded that for As species evaluated in the present work, lyophilization and cryogenic grinding were the best sample preparation strategies. On the other hand, sample storage at low temperatures resulted in poor extraction efficiency. Furthermore, for each condition As species have remained relatively stable along 1-year period. The ratio As(III)/As(V) changed when comparing no pretreated (fresh plant) and pretreated sample (lyophilized and ground), indicating possible interconversion during drying and grinding process; however, as the extraction efficiency of fresh sample was low nothing can be asserted about it. The sample particle size was the critical factor for choosing the best processing and storage procedure of sample.

Table 3
Arsenic species concentration (mg kg^{-1}, mean and standard deviations, $n = 3$) found in plants (*Brachiaria brizantha*) stored under different conditions.

Processing and storage temperature	Storage period	As(III)	As(V)	DMA	Sum of species
Fresh plant	0	0.658 ± 0.051	0.859 ± 0.049	–	1.52 ± 0.34
Lyophilized and ground[a]	1 day	0.354 ± 0.005	1.36 ± 0.08	0.354 ± 0.007	2.07 ± 0.09
Lyophilized and ground[a]	2 months	0.396 ± 0.007	1.39 ± 0.05	0.247 ± 0.004	2.03 ± 0.08
Lyophilized and ground[a]	4 months	0.373 ± 0.004	1.43 ± 0.03	0.209 ± 0.002	2.01 ± 0.07
Lyophilized and ground[a]	6 months	0.332 ± 0.007	1.52 ± 0.06	0.204 ± 0.006	2.06 ± 0.09
Lyophilized and ground[a]	8 months	0.366 ± 0.003	1.44 ± 0.08	0.275 ± 0.007	2.08 ± 0.08
Lyophilized and ground[a]	10 months	0.397 ± 0.009	1.40 ± 0.07	0.221 ± 0.003	2.02 ± 0.07
Lyophilized and ground[a]	12 months	0.351 ± 0.005	1.45 ± 0.04	0.207 ± 005	2.01 ± 0.06
Lyophylized and chopped[b]	2 months	0.804 ± 0.064	0.692 ± 0.017	–	1.50 ± 0.13
Lyophylized and chopped[b]	4 months	0.670 ± 0.085	0.616 ± 0.074	–	1.29 ± 0.46
Lyophylized and chopped[b]	6 months	0.724 ± 0.025	0.521 ± 0.035	–	1.25 ± 0.27
Lyophylized and chopped[b]	8 months	0.739 ± 0.019	0.731 ± 0.029	–	1.47 ± 0.19
Lyophylized and chopped[b]	10 months	0.744 ± 064	0.741 ± 0.070	–	1.48 ± 0.32
Lyophylized and chopped[b]	12 months	0.952 ± 0.050	0.555 ± 0.055	–	1.51 ± 0.40
Chopped[c]	2 months	0.729 ± 0.076	0.642 ± 0.029	–	1.37 ± 0.26
Chopped[c]	4 months	0.630 ± 0.081	0.679 ± 0.024	–	1.31 ± 0.32
Chopped[c]	6 months	0.717 ± 0.043	0.602 ± 0.010	–	1.32 ± 0.59
Chopped[c]	8 months	0.552 ± 0.053	0.670 ± 0.085	–	1.22 ± 0.35
Chopped[c]	10 months	0.542 ± 0.019	0.710 ± 0.030	–	1.25 ± 0.16
Chopped[c]	12 months	0.582 ± 0.012	0.634 ± 0.081	–	1.22 ± 0.24
Chopped[d]	2 months	0.755 ± 0.044	0.757 ± 0.039	–	1.512 ± 0.29
Chopped[d]	4 months	0.619 ± 0.029	0.854 ± 0.015	–	1.473 ± 0.38
Chopped[d]	6 months	0.721 ± 0.023	0.783 ± 0.012	–	1.504 ± 0.60
Chopped[d]	8 months	0.672 ± 0.014	0.819 ± 0.043	–	1.492 ± 0.55
Chopped[d]	10 months	0.671 ± 0.012	0.798 ± 0.056	–	1.469 ± 0.42
Chopped[d]	12 months	0.612 ± 0.033	0.843 ± 0.017	–	1.455 ± 0.73

[a] Room temperature.
[b] 4 °C.
[c] −18 °C.
[d] −80 °C.

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References

[1] J. Bundschuha, B. Nath, P. Bhattacharya, C.-W. Liu, M.A. Armienta, M.V.M. López, D.L. Lopez, J.-S. Jean, L. Cornejo, L.F.L. Macedo, A. Tenuta-Filho, Arsenic in the human food chain: the Latin American perspective, Sci. Total Environ. 429 (2012) 92–106.
[2] J.L.G. Ariza, E. Morales, D. Sánchez-Rodas, I. Giráldez, Stability of chemical species in environmental matrices, TrAC Trends Anal. Chem. 19 (2000) 200–209.
[3] C.D.B. Amaral, J.A. Nóbrega, A.R.A. Nogueira, Sample preparation for arsenic speciation in terrestrial plants—a review, Talanta 115 (2013) 291–299.
[4] C. B'Hymer, J.A. Caruso, Arsenic and its speciation analysis using high performance liquid chromatography and inductively coupled plasma mass spectrometry, J. Chromatogr. A 1045 (2004) 1–13.
[5] A.C. Schmidt, K. Kutschera, J. Mattusch, M. Otto, Analysis of accumulation, extractability, and metabolization of five different phenylarsenic compounds in plants by ion chromatography with mass spectrometric detection and by atomic emission spectroscopy, Chemosphere 73 (2008) 1781–1787.
[6] L. Jedynak, J. Kowalska, M. Kossykowska, J. Golimowski, Studies on the uptake of different arsenic forms and the influence of sample pretreatment on arsenic speciation in White mustard (*Sinapis alba*), Microchem. J. 94 (2010) 125–129.
[7] W. Zhang, Y. Cai, K.R. Downum, L.Q. Ma, Arsenic complexes in the arsenic hyperaccumulator *Pteris vittata* (Chinese brake fern), J. Chromatogr. A 1043 (2004) 249–254.
[8] K.A. Mir, A. Rutter, I. Koch, P. Smith, K.J. Reimer, J.S. Poland, Extraction and speciation of arsenic in plants grown on arsenic contaminated soils, Talanta 72 (2007) 1507–1518.
[9] A. Pell, A. Márquez, R. Rubio, J.F. López-Sánchez, Effects of sample processing on arsenic speciation in marine macroalgae, Anal. Methods 5 (2013) 2543–2550.
[10] K. Bluemlein, A. Raab, J. Feldmann, Stability of arsenic peptides in plant extracts: off-line versus on-line parallel elemental and molecular mass spectrometric detection for liquid chromatographic separation, Anal. Bioanal. Chem. 393 (2009) 357–366.
[11] A. Raab, A.A. Meharg, M. Jaspars, D.R. Genney, J. Feldmann, Arsenic–glutathione complexes—their stability in solution and during separation by different HPLC modes, J. Anal. At. Spectrom. 19 (2004) 183–190.
[12] S. Husted, D.P. Persson, K.H. Laursen, T.H. Hansen, P. Pedas, M. Schiller, J.N. Hegelund, J.K. Schjoerring, Review: the role of atomic spectrometry in plant science, J. Anal. At. Spectrom. 26 (2011) 52–79.
[13] W. Maher, F. Krikowa, M. Ellwood, S. Foster, R. Jagtap, G. Raber, Overview of hyphenated techniques using an ICP-MS detector with an emphasis on extraction techniques for measurement of metalloids by HPLC–ICPMS, Microchem. J. 105 (2012) 15–31.
[14] C.D.B. Amaral, A.G.G. Dionísio, M.C. Santos, G.L. Donati, J.A. Nóbrega, A.R.A. Nogueira, Evaluation of sample preparation procedures and krypton as an interference standard probe for arsenic speciation by HPLC-ICP-QMS, J. Anal. At. Spectrom. 28 (2013) 1303–1310.
[15] J.H. Huang, G. Ilgen, P. Fecher, Quantitative chemical extraction for arsenic speciation in rice grains, J. Anal. At. Spectrom. 25 (2010) 800–802.
[16] J.H. Huang, G. Ilgen, Factors affecting arsenic speciation in environmental samples: sample drying and storage, Int. J. Environ. Anal. Chem. 20 (2006) 347–358.
[17] A.C. Schmidt, W. Reisser, J. Mattusch, P. Popp, R. Wennrich, Evaluation of extraction procedures for the ion chromatographic determination of arsenic species in plant materials, J. Chromatogr. A 889 (2000) 83–91.
[18] P. Alava, T.V. Wiele, F. Tack, G.D. Laing, Extensive grinding and pressurized extraction with water are key points for effective and species preserving extraction of arsenic from rice, Anal. Methods 4 (2012) 1237–1243.
[19] M. Leermakers, W. Baeyens, M. De Gieter, B. Smedts, C. Meert, H.C. De Bisschop, R. Morabito, Ph. Quevauviller, Toxic arsenic compounds in environmental samples: speciation and validation, TrAC Trends Anal. Chem. 25 (2006) 1–10.
[20] R. Rubio, M.J. Ruiz-Chancho, J.F. Lopez-Sanchez, Sample pretreatment and extraction methods that are crucial to arsenic speciation in algae and aquatic plants, TrAC Trends Anal. Chem. 29 (2010) 53–69.