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Evaluation of extraction procedures for the ion chromatographic determination of arsenic species in plant materials

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Abstract

The determination of arsenic species in plants grown on contaminated sediments and soils is important in order to understand the uptake, transfer and accumulation processes of arsenic. For the separation and detection of arsenic species, hyphenated techniques can be applied successfully in many cases. A lack of investigations exists in the handling (e.g., sampling, pre-treatment and extraction) of redox- and chemically labile arsenic species prior to analysis. This paper presents an application of pressurized liquid extraction (PLE) using water as the solvent for the effective extraction of arsenic species from freshly harvested plants. The method was optimized with respect to extraction time, number of extraction steps and temperature. The thermal stability of the inorganic and organic arsenic species under PLE conditions (60–180°C) was tested. The adaptation of the proposed extraction method to freeze–dried, fine-grained material was limited because of the insufficient reproducibility in some cases.

Keywords: Extraction methods; Pressurized liquid extraction; Plant materials; Organoarsenic compounds; Arsenic

1. Introduction

The existence of arsenic compounds, like arsenite or arsenate, in the terrestrial and aquatic environment is linked with the high risk of toxicity of this element to living organisms [1–4]. Therefore, in several organisms metabolization reactions to form less dangerous arsenic compounds like arsenobetaine or arsenocholine take place, for example, in marine organisms. The identification and quantification of the arsenic species in biological samples can give a more detailed description of the uptake mechanisms, accumulation efficiencies and of course the assessment of the real risk of toxicity.

Normally, for the determination of the total amounts of metals and metalloids different digestion techniques (e.g., high-pressure, sonification or microwave-assisted) together with the application of strong acids or oxidizing agents can be used successfully [5,6]. In order to determine elements in their different valence states, more moderate procedures have to be applied. One of the most popular procedures is shaking of the ground material for hours with water [7], water–methanol [8] or water–methanol–chloroform mixtures [9]. The disadvantages of this technique are the long shaking times, additional operations (e.g., filtration) and low and varying

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efficiencies of extraction. Recently, a microwave-assisted extraction procedure was proposed for sample pretreatment for the determination of arsenic species in fish tissue [10], which allows an effective extraction at elevated temperature of 65°C in a drastically reduced analysis time.

Pressurized liquid extraction (PLE; also known under the trade name ASE, accelerated solvent extraction) should also be able to extract arsenic species effectively and automatically within a short time. However, PLE typically works at higher temperatures and pressures compared with the microwave digestion procedure described in Ref. [10], such that a degradation of the compounds of interest cannot be excluded completely. Therefore, the ability of PLE as a pretreatment for the analysis of inorganic and organic arsenic species in environmentally and biologically interesting samples like plants was studied. The aim of the work was focused on a high-efficiency extraction of arsenic species to allow their determination at ultratrace levels and on the thermal stability of the species to detect the real species distribution in the original sample.

Fresh plant material and freeze–dried standard reference materials of plants were investigated with respect to their extraction behavior with water and water–methanol solutions. Most investigations with PLE have been carried out using different organic solvents [11], whilst PLE of organometallic and redox species with water as solvent is a new direction.

For determining the arsenic species ion chromatography (IC) was coupled with inductively coupled plasma mass spectrometry (ICP-MS) as a sensitive and selective detector. The separation of eight arsenic species was performed with a gradient of diluted nitric acid using a high-capacity anion-exchange column [12].

2. Experimental

2.1. Equipment

The PLE was performed with an ASE 200 device (Dionex, Sunnyvale, CA, USA). The extraction cartridges used for all experiments had a volume of 11 ml. A constant pressure of ca. 10 MPa was held during extraction.

The IC system consists of a binary HPLC pump LC 250 (Perkin-Elmer, Norwalk, CT, USA), a pneumatic injection valve with a 200-μl sample loop (Dionex) and a high-capacity anion-exchange column IonPac AS7/AG7 (Dionex) [12,13].

An ELAN 5000 ICP-MS system (Perkin-Elmer Sciex, Thornhill, Canada) was used for the element-selective detection. The chromatographic device was coupled with the ICP-MS system via a cross-flow nebulizer. Instrumental conditions were as follows: radiofrequency (RF) power: 1060 W; plasma gas flow-rate: 15 l min^{-1}, auxiliary flow-rate: 0.85 l min^{-1}, nebulizer flow-rate: 0.9 l min^{-1}; dwell time: 1 s; isotope monitored: mass 75.

A 4100 ZL atomic absorption spectrometer equipped with a FIAS-400 flow injection device (both Perkin-Elmer) was used for the determination of the total concentration of arsenic in the extracts after hydride generation and in situ preconcentration in a palladium-modified graphite furnace.

2.2. Chemicals and standards

The solvents for extraction were prepared from deionized water (Milli-Q Ultrapure water systems, Millipore) and HPLC-grade methanol (Merck, Darmstadt, Germany). Each of the stock solutions containing 1 g As l^{-1} were prepared from arsenic trioxide [As(III)] (Fluka, Buchs, Switzerland) dimethylarsinic acid (DMA), arsenic standard [As(V)] (both from Merck), methylarsonic acid (MMA), arsenobetaine (AsB), arsenocholine (AsC), tetramethylarsonium bromide (0.1 g As l^{-1}) (TMA) and trimethylarsine oxide (TMAO). The organoarsenic compounds MMA, AsB, AsC, TMA and TMAO were obtained from Institute of Analytical Chemistry, Karl-Franzens-University, Graz, Austria. These solutions were freshly mixed and diluted to the final concentrations daily.

2.3. Samples

Freshly harvested plant material (*Holcus lanatus*) grown on an arsenic-contaminated sediment was used for the method development and optimization

of the PLE conditions. The plant material was stored in a closed box at 4°C. The extraction (PLE and shaking) of fresh plant materials (cell structure intact) was compared with those of powder of freeze–dried white clover (standard reference material BCR 402) and sea lettuce (standard reference material BCR 279). In the reference materials the plant structure was completely destroyed. The total concentrations of arsenic in these certified reference materials (CRMs) are 0.093 and 3.09 mg kg^{-1}, respectively.

2.4. Extraction procedures

2.4.1. Extraction by shaking

In each case 4–5 g of the ground fresh plant material or 1–3 g of the freeze–dried standard reference materials filled in PTFE bottles (100 ml) were extracted for 2 h with 30 ml of water or water–methanol solution by a bottle rotator at 30 min^{-1} at ambient temperature. The supernatant was filtered with a cellulose acetate filter (pore width 0.45 μm), and aliquots of 200 μl were injected into the chromatographic device within 24 h. During this time the extracts were stored at 4°C.

2.4.2. Pressurized liquid extraction

The composition of the extraction solvent, the number of repetitive extraction steps, the time regime and the temperature program was controlled automatically by the device mentioned above. Deionized water and water–methanol solutions were used for the extraction. The volume of the supernatant ranged from 15 to 20 ml. It was diluted to a final volume of 50 ml. More detailed information is given in the text or in the captions to the figures.

2.5. Ion chromatographic separation

A gradient elution with diluted nitric acid and a flow-rate of 1.0 ml min^{-1} was chosen for the separation of the arsenic species. The composition of the eluent and the time regime for the gradient was as follows: eluent A (0.4 m*M* HNO_3), eluent B (50 m*M* HNO_3), both eluents contain 0.05 m*M* benzene-1,2-disulfonic acid (Fluka). The following time regime for the gradient was applied: (step 1) 1 min, 100% A; (step 2) 1 min, 0% A linear; (step 3) 5 min, 0% A; (step 4) 2 min, 50% A linear; (step 5) 50% A; (step 6) 0.5 min, 100% A linear; (step 7) 5 min, 100% A.

A typical ion chromatogram detected at m/z 75 (As) is shown in Fig. 1.

3. Results and discussion

Preliminary investigations of the extraction of arsenic species from freshly harvested green plant materials (leaves of *Holcus lanatus*) were performed using the conventional shaking procedure and the PLE method at 120°C and 100 bar with deionized water as the solvent and identical masses and dilutions. Ion chromatograms (Fig. 2) obtained from the first PLE extract after 5 min extraction time, and from bottle shaking, show a substantially enhanced efficiency for the extraction of arsenite and arsenate by PLE. A preliminary calibration result showed that the efficiency of extraction of both arsenite and arsenate is approximately three times higher working with PLE in comparison to the shaking procedure. This observation initialized a series of systematic investigations in order to optimize parameters for: (1) high extraction efficiency, (2) stability of the species and (3) saving of time and handling. The reproducibility of the PLE procedure was studied using freshly harvested leaves of *Holcus lanatus* (4).

3.1. PLE procedure for fresh plant material

(1) The first studies were directed to estimate the extraction efficiency of leaves of *Holcus lanatus* in dependence of the extraction temperature (Fig. 3), the number of extraction steps and the solvent composition (both in Table 1).

The extraction efficiency can be improved by increasing the temperature from 60 to 180°C. The influence of temperature on extraction efficiency is more significant for arsenate than for arsenite which points again to a stronger linkage of arsenate to the matrix. For a efficient extraction of arsenorganic compounds, like AsC and DMA, enhanced temperatures are necessary.

However, both the organoarsenic compounds and the other soluble organic matter have comparable solubility characteristics, resulting in increasingly

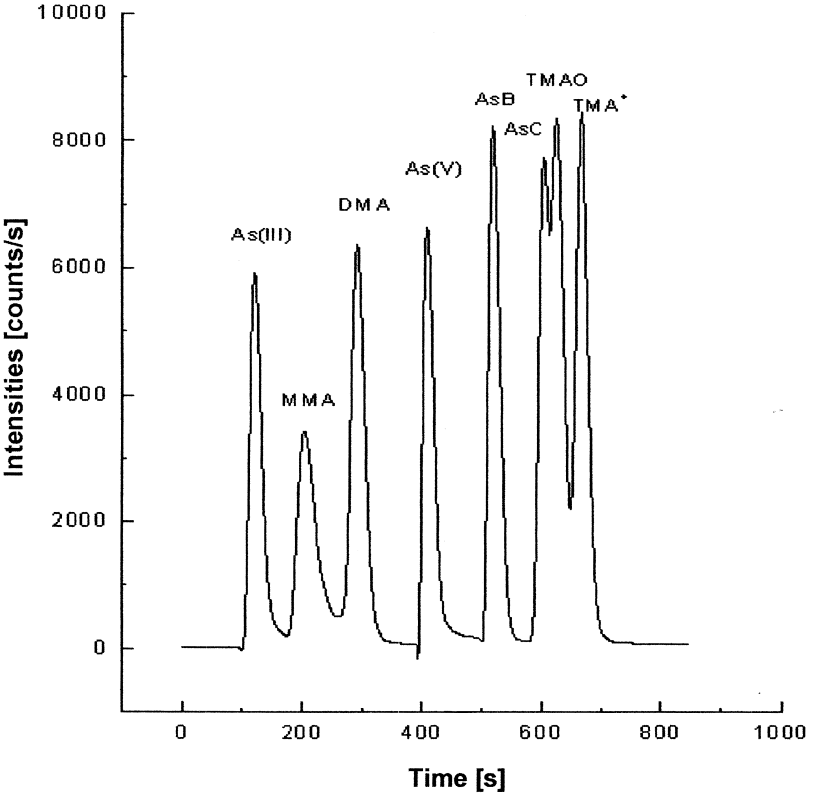

Fig. 1. IC–ICP-MS chromatogram of standards of arsenic species (20 μg As l^{-1} of each compound).

darker extracts (changing from transparent, light orange to thick, dark brown) with increasing temperature. This phenomenon was observed also using PLE with water for the extraction of chlorophenols from soil samples [14]. Consequently, the subsequent IC separation is influenced by relatively high amounts of dissolved organic residues. From the point of view of long-term stability of the analytical column and avoidance clean-up steps, the temperature should be limited to 150°C using a 5 min extraction time.

The extraction efficiency depends also on the number of steps used for the subsequent extraction of the same sample (Table 1). Within a 5-min extraction time (at 120°C), the extractable arsenite was quantitatively dissolved. However, remarkable amounts of arsenate were still extracted in the second and third extraction step, and this is presumably attributed to a stronger interaction of the doubly-charged arsenate with the plant matrix, in contrast to the probably more mobile, neutral arsenious acid.

To estimate the influence of organic solvents on the extraction efficiency, solutions of water–methanol (90:10 and 80:20, v/v) were used for PLE at 120°C. As shown in Table 1, methanol decreases the extraction efficiency for arsenite, as well as for arsenate. Therefore, the following investigations with fresh plant material were performed only with bidistilled water as solvent.

(2) The stability of the arsenic species during PLE were investigated as a function of the temperature ranging from 60 to 180°C, as shown in Table 2. To simulate the thermal behavior of arsenic species in biological samples, non-contaminated ground plant material was spiked with a certain amount of standard solutions of the arsenicals. The dependence of the relative recoveries, related to the mean value of peak area of each component at 60°C, on tempera-

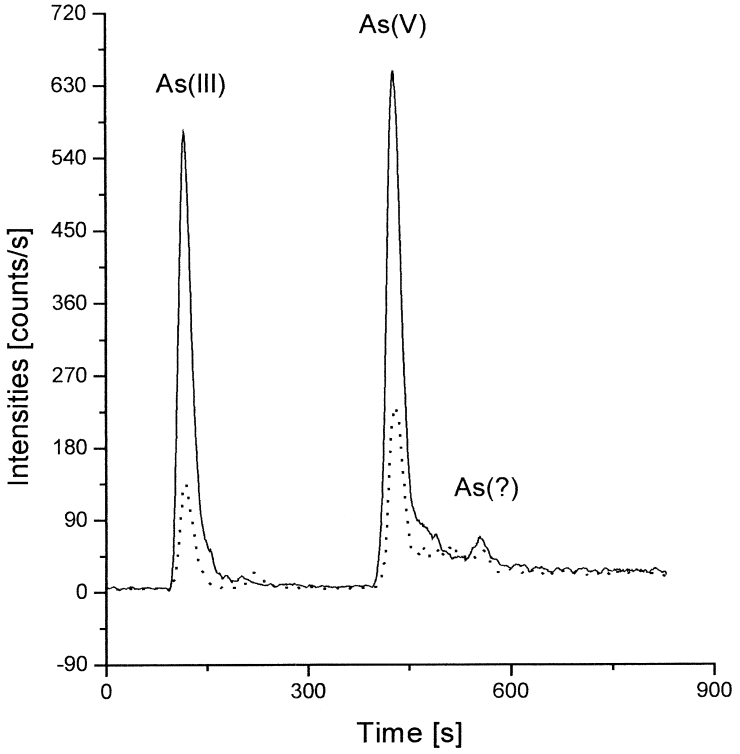

Fig. 2. IC–ICP-MS chromatograms of arsenic species after extraction of ground fresh plant material (*Holcus lanatus*) by shaking (dotted line) and PLE (solid line). Shaking procedure: 2 h, water, ambient temperature; PLE: 5 min, 120°C, water.

ture shows that for the most arsenic species, except for arsenite, a sufficient stability was observed over the entire temperature range. A direct correlation between arsenite diminution and the formation of oxidation products (e.g., arsenate) could not be found.

(3) An important advantage of PLE is its ability for automation. However, a time-consuming step still remains in the entire extraction procedure: the preparation (grinding) of the plant material under investigation for extraction. If it would be possible to save the time for homogenization of the plants then the extraction would be still more effective. A second aspect of the use of non-cut plants is that the influence of oxygen on the stability of arsenic species can be further reduced. Therefore, the extraction yield of fresh harvested and only coarse cut-up leaves of *Holcus lanatus* was compared with that obtained for fine-ground material of the same plant. The concentration of extractable arsenate and DMA seems to be independent of the degree of grinding, as shown in Table 3. An opposite effect was observed in the case of arsenite. Fine-grinding of the fresh plants led to a higher concentration of arsenite in the extract. As expected, the standard deviations analyzing non-ground material are somewhat higher compared to those for fine-ground ones.

(4) The reproducibility of the PLE procedure had been studied using green leaves harvested in May and September 1999. The results are given in Table 3 using four samples prepared from each of a pool of fine-ground or crushed fresh plant material. The standard deviations of the arsenic species concentrations were calculated from the ion chromatographic data (peak areas were integrated with Microcal Origin 5.0, peak fitting module) of four PLE runs carried out on 1 day. The standard deviation values of approximately 20% or lower indicate a sufficient reproducibility for these trace concentrations of arsenic species determined. To estimate the accuracy

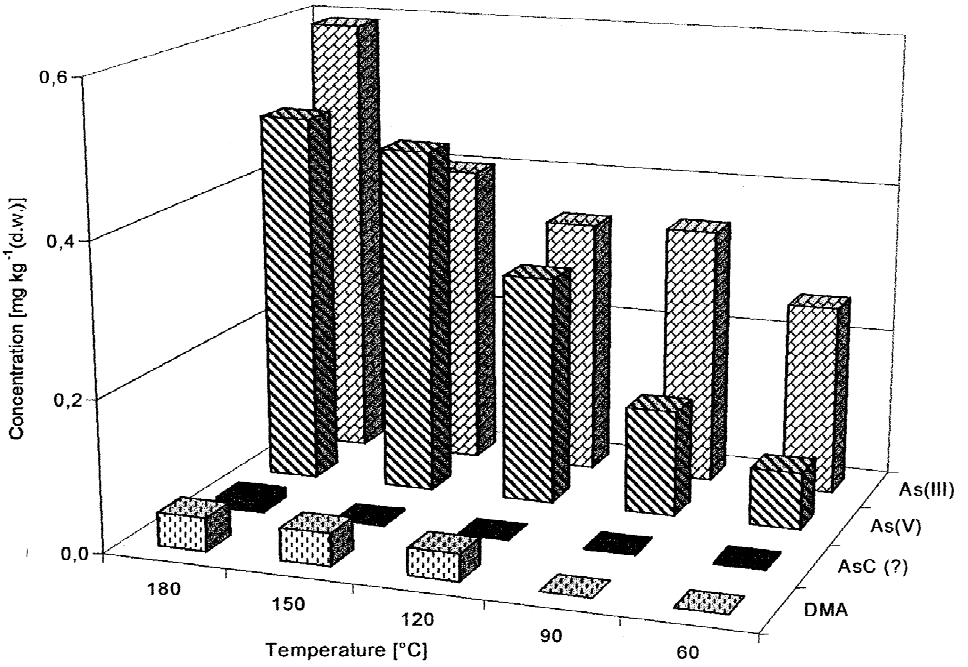

Fig. 3. Dependence of extraction efficiency on temperature: extraction of fresh, ground plant material (*Holcus lanatus*). PLE conditions: extraction time: 5 min, solvent: water.

of the results obtained by IC–ICP-MS the total concentration of arsenic in PLE extracts was determined by atomic absorption spectrometry, as described in Section 2.1. The total concentration of arsenic in fresh plants harvested in September 1999 was 9.90 ± 1.85 mg kg^{-1}. This value is in good agreement with the sum of the arsenic species in the PLE extracts of 10.3 ± 0.8 mg kg^{-1} calculated from the chromatographic data.

Table 1
Dependence of signal intensity (peak areas) on subsequent extraction steps and the methanol content of the solvent[a]

Solvent	Extraction step	As(III)	As(V)
Water	First	14 300	19 500
	Second	2700	15 500
	Third	720	10 800
Water–methanol (90:10, v/v)	First	5330	13 700
	Second	2230	12 200
Water–methanol (80:20, v/v)	First	6130	10 800
	Second	2370	7450

[a] PLE conditions: extraction time at each step 5 min, temperature 120°C, ground fresh plant material.

To calculate the extraction efficiency of the PLE procedure the total amount of arsenic obtained by a microwave-assisted digestion with nitric acid was compared with the sum of the arsenic species in PLE extracts. The total amount of arsenic in the fine-ground material harvested in May 1999 was determined to be 2.67 ± 0.16 mg As kg^{-1}. Compared to this value approximately 30% of the arsenic species (0.78 ± 0.04 mg As kg^{-1}) are extracted without loss of the original state of arsenic in plants using PLE. From these results it can be derived that other arsenic compounds exist which are more strongly bound to the matrix and therefore not extractable by PLE using water and/or which are not detectable (e.g., arsenosugars) with the chromatographic procedure applied in this work.

A comparison of the fine-ground samples harvested in the beginning of the growth (May) and on the end (September) showed that during this time the arsenic concentrations in the plant, *Holcus lanatus*, increased by a factor of 50 for As(V) and 4 for

Table 2
Thermal stability of arsenic species: dependence of recovery on extraction temperature (recoveries normalized to the values obtained at 60°C)[a]

Temperature (°C)	Recovery (%)				
	As(III)	MMA	DMA	As(V)	AsC
60	100	100	100	100	100
90	104.6	96.1	87.1	97.9	99.5
120	77.6	80.5	83.6	97.8	92.0
150	67.0	87.7	81.8	106.0	93.7
180	89.6	91.8	78.5	87.8	76.2

[a] PLE conditions: extraction time 5 min, solvent water, ca. 5 g of fresh plant material was spiked with 10 μg As (absolute) of each arsenic species.

As(III). More detailed investigations about seasoning of transfer and accumulation behavior of arsenic species in plants are in progress and are carried out on the basis of the proposed extraction procedure.

Summarizing the results of optimization, it can be remarked that PLE using water as the solvent, extraction times between 5 and 15 min and extraction temperatures of 120 to 150°C (10 MPa) can be successfully applied to arsenic species extraction from fresh plant material and their subsequent IC–ICP-MS analysis. However, more investigations are necessary to explain the differences in the behavior of arsenite and arsenate.

3.2. Application to standard reference materials

In a second series of experiments standard reference materials of freeze–dried plants (certified values for the total concentration of arsenic) were tested for an effective extraction under the same PLE conditions (120°C, one step) used for the fresh plants. Chromatograms of the extracts of white clover and sea lettuce are shown in Fig. 4 for both PLE and shaking procedures.

The PLE of white clover with deionized water, as well as with water–methanol (90:10, v/v) as the extracting agent supplied a much larger quantity of both arsenite and arsenate. However, in the case of sea lettuce the PLE efficiency, in comparison to the shaking procedure, was improved only for arsenate and three (unknown) arsenic compounds (not identified) with water as extracting agent.

The extraction efficiency of arsenite from sea lettuce was similar for PLE and for shaking. Shaking seems to be more efficient for arsenate using a water–methanol solution as solvent. The increased peak height of arsenite (Fig. 4B and D) in contrast to water extraction is attributed to an enhanced ionization of arsenic in the plasma (ICP-MS) caused by methanol co-eluting with arsenite. The first investigations of the extraction of homogenized fine-ground, freeze–dried biological material suffers from

Table 3
Comparison of analytical data (mean values and standard deviations): determination of arsenic species in fresh plant material (*Holcus lanatus*) using PLE procedure for extraction ($n=4$); concentration given in mg kg^{-1} (dry mass); PLE conditions: extraction time 5 min, extraction temperature 120°C, solvent water

Time of harvest	Pre-treatment	Concentration [mg kg^{-1} (dry mass)][a]			
		As(III)	As(V)	DMA	Σ (all As species)[b]
May 1999	Non-ground	0.27±0.03	0.21±0.046	0.031±0.007	0.50±0.09
May 1999	Fine-ground	0.50±0.03	0.21±0.04	0.037±0.007	0.78±0.04
September 1999	Fine-ground	1.5±0.25	8.5±1.0	0.16±0.02	10.3±0.8

[a] Mean value and standard deviation.
[b] Sum of all As species (including non-identified peaks).

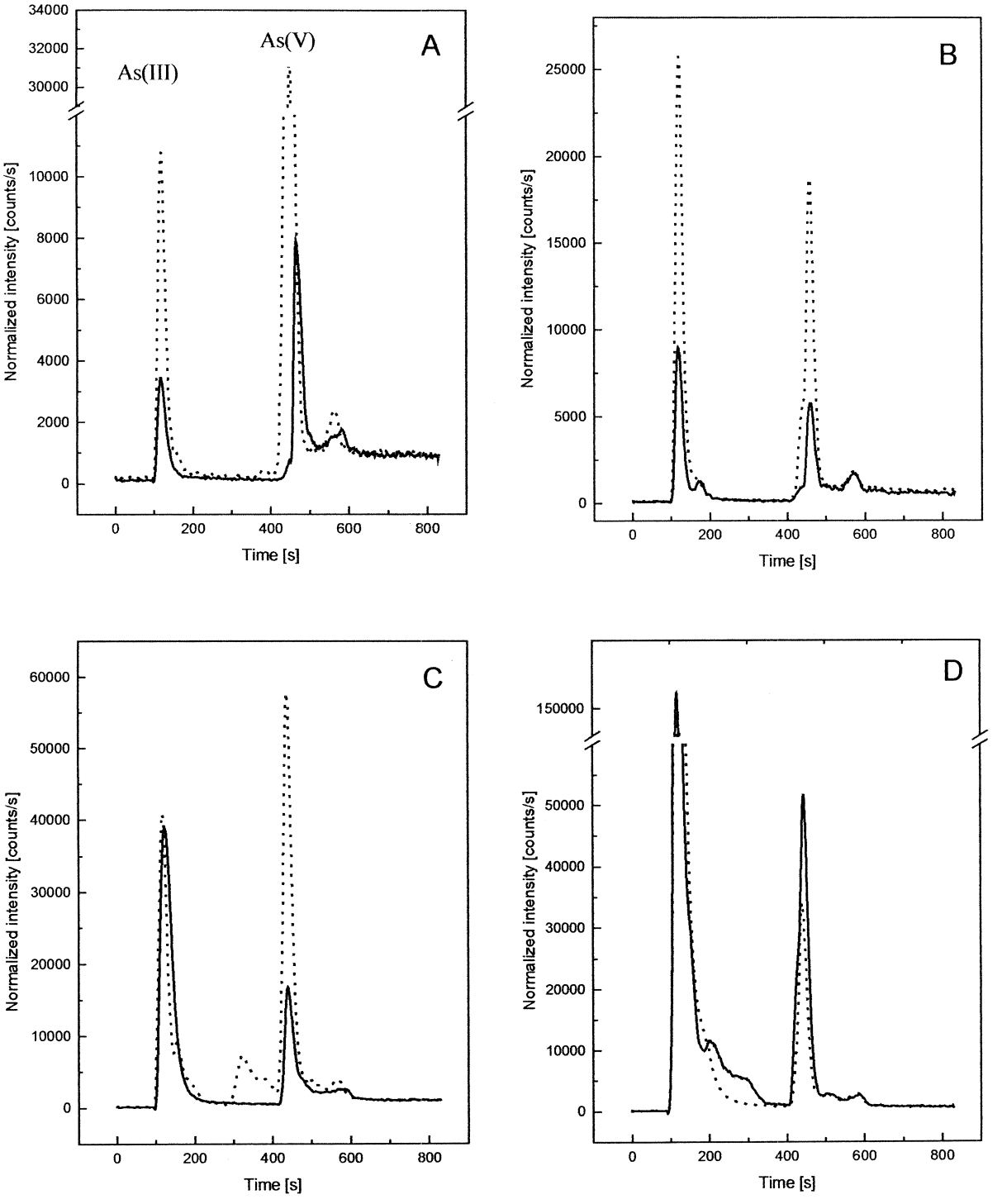

Fig. 4. IC–ICP-MS chromatograms of PLE extracts (dotted line) of freeze–dried, fine-ground standard reference material in comparison to extraction by shaking (solid line) (A, B): BCR 402, white clover; (C, D) BCR 279, sea lettuce; (A, C) extraction with water; (B, D) extraction with water–methanol (90:10, v/v).

an insufficient reproducibility and a lack of interpretable results. The attempt to improve the homogeneous penetration (as one possible reason) of the sample by the solvent during the extraction by mixing inert auxiliary materials with the standard reference materials was also not successful.

4. Conclusions

PLE is a suitable and effective technique for water extraction of arsenic species from fresh plant material. The extracts can directly injected into the chromatographic system. The elevated temperatures used by this method have an insignificant influence on the stability of the species studied. More investigations are necessary to explain the different behavior of arsenite and arsenate in subsequent extraction steps, and depending on the preparation of the plants. The PLE of freeze–dried, fine-ground biological material, e.g., standard reference material of plants seems to be more complicated. Further investigations are necessary to improve the reproducibility and efficiency of PLE for species analysis in this kind of standard reference material.

Generally, the validation of analytical results for species analysis in fresh plant materials cannot be solved satisfying; standard reference materials of plants certified for elemental species do not exist and the extraction behavior of freeze–dried standard reference materials is quite different to that of fresh samples.

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