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Journal of Chromatography A, 1097 (2005) 1–8
JOURNAL OF CHROMATOGRAPHY A
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# Evaluation of a focused sonication probe for arsenic speciation in environmental and biological samples

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Received 26 April 2005; received in revised form 29 July 2005; accepted 4 August 2005
Available online 24 August 2005

**Abstract**

Arsenic speciation analysis suffers in general from high sample handling time required by sample preparation. In a previous work, ultrasonic probe has been proved to reduce sample treatment time for arsenic extraction in rice to only a few minutes. Base upon the obtained results, here several extraction media for chicken, fish and soil samples (SEAS G6RD-CT2001-00473) have been studied and evaluated employing the same technique. Chicken sample needed an enzymatic treatment in order to liberate the species linked to the protein matrix. Extraction of the major species in fish, AsB, was quantitatively achieved in water in 1 min. Also 1 min was enough to leach about 85% of species present in soils and sediments, mainly the inorganic ones, using $H_3PO_4$. In all cases, no inter-conversion among As species was observed. The five species found in those samples were separated using an improved HPLC–ICP-MS method in only 11 min, with detection limits at the ng $l^{-1}$ level. The proposed methods were validated by analysing several Certified Reference Materials: SRM 1568a rice flour, CRM-627 tuna fish tissue, SOIL-7 soil and MURST-ISS-A1 Antarctic sediment.


*Keywords:* Rice; Fish tissue; Soil; Chicken; Arsenic speciation; Focused ultrasound probe; HPLC–ICP-MS

## 1. Introduction

Nowadays it has become clear that mobility, bioavailability, stability, retention and toxicity of trace elements depend on both the chemical form in which they enter the system and the final form in which they are present [1]. Thus, the assessment of environmental impact and risk to human health must be based on the identification and quantification of the different chemical forms of the elements [2]. In the case of arsenic, the inorganic forms arsenite and arsenate have been related to an increased risk of cancer as well as to cardiovascular diseases [3], whereas methylated forms of arsenic such as methylarsonic acid (MMA) and dimethyarsinic acid (DMA) are significantly less toxic. Other species such arsenobetaine (AsB), arsenocholine (AsC) and arsenosugars are essentially non-toxic [4].

Human uptake of arsenic mainly occurs via the food chain (dietary sources and drinking water) and occupational exposure [5,6]. The permissible level for total arsenic in drinking water is 10 μg $L^{-1}$ but for food products a definitive value has not yet been implemented. However, the Food and Agriculture Organisation/World Health Organisation (FAO/WHO) recommended a provisional tolerable weekly intake of not more than 15 μg inorganic As/kg body weight [7]. The major amount of dietary arsenic ingested comes from seafood and marine fish which, fortunately, have the ability to bio-accumulate the non-toxic AsB. In contrast, rice is a bio-accumulative plant for the more toxic arsenic species arsenate and arsenite [8]. Also, arsenic at high concentration levels is present in soils and sediments in places with intense mining and other anthropological activities, mainly production of insecticides, herbicides and fungicides [9]. Typically, methanol, water, their mixtures and sometimes chloroform or trifluoro acetic acid (TFA) have been the extracting solvents used for arsenic species extraction from marine fish [10–13] or rice samples [14–19]. Although these extraction systems provided quantitative arsenic recovery, the procedures are, in general, time-consuming due to long extraction times required and the several analytical steps involved. A large number of procedures have been developed to liberate metals from soils based in the different affinities for the matrix. The most widely applied has been the sequential extrac-

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doi:10.1016/j.chroma.2005.08.012

tion scheme proposed by Tessier et al. [20]. Those methods are usually affected by selectivity, re-adsorption problems, large treatment times and the great number of steps involved [21]. Their application to chemical speciation has shown low feasibility in the case of As because its occurrence at different oxidation states and binding behaviour depended on the chemical form. Arsenic demands for mild extractions so as to maintain the species integrity [22]. Several authors have demonstrated the ability of diluted phosphoric acid [23–25], hydroxylammonium hydrochloride [9,26] or ammonium oxalate [9,27] for this purpose. The main problems encountered were low recovery and oxidation of As(III) to As(V). Extraction in the mentioned media is usually aided by techniques such as shaking, sonication, microwave-assisted or accelerated solvent extraction [28].

The field of arsenic speciation analysis has grown rapidly in recent years, especially with the implementation of high-performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS) [29]. However, the quality of arsenic speciation is limited by the long time of sample preparation step. In this context, sonochemistry has emerged as an interesting approach [30–33] that has been already employed in a previous paper [34], where this technique was applied to arsenic extraction from rice samples. The main responsible of sonochemistry success is the acoustic cavitation, provoked by the bubbles formed by a wave sound in a liquid that compresses and decompresses continuously. The results are extreme temperatures and pressures generated in the liquid at a micro level as well as solute thermolysis and formation of hydroxyl radicals and hydrogen peroxide [35]. As a consequence, when a solid is present in aqueous medium, the analyte present in the solid may be partially or totally extracted into the liquid medium [36] faster than by other classical methods.

The aim of the present study was to apply this technique to different matrices other than rice: biological (chicken, fish) and environmental (soils and sediments) in order to reduce the treatment time required for As speciation, to keep the maximum recovery and to avoid species inter-conversion and/or degradation. Therefore, various extractant media have been employed, depending on the matrix nature as well as As species present in the sample. A preceding optimization of the chromatographic method employed in previous papers [19,34] was necessary in order to separate all the species present in the samples, including rice, in the isocratic mode and to increase sensitivity. Discussion about how arsenic could be linked to the matrix has been included. Finally, the proposed method was validated by analysing several reference materials.

## 2. Experimental

### 2.1. Instrumentation

An ultrasonic homogenizer, model SONOPULS HD 2200 (Bandelin, Germany), equipped with a converter UW 2200, SH 213 G horn as amplifier and sonotrode MS 73 (3 mm titanium microtip) was used for sample treatment. A centrifuge model 5804 Eppendorf (Hamburg, Germany) was used for phase separation after the extraction step.

Table 1
Instrumental parameters for total As determination and As speciation analysis

| | |
|---|---|
| ICP-MS | |
| RF power | 1350 W |
| Ar flow rate | Plasma gas: 15.3 L $min^{-1}$<br>Nebulizer: 1 L $min^{-1}$<br>Auxiliary 0.9 L $min^{-1}$ |
| Isotope monitored | $^{75}As$ |
| Integration time | 0.1 s (spectrum) per point |
| Points per peak | 3 |
| HPLC | |
| Column | PRP-X100 anion exchange<br>Dimensions: 250 mm × 4.1 mm, particle size 10 μm |
| Guard column | PRP-X100 anion exchange<br>Dimensions: 4.6 mm |
| Mobile phase | 10 mM $HPO_4^{2-}/H_2PO_4^-$; 2% (v/v) MeOH; pH 8.5 |
| Injection volume | 100 μL |
| Flow rate | 1.5 mL $min^{-1}$ |
| Mode | Isocratic |

The ICP-MS used for As determination was an HP-4500 Plus (Agilent Technologies, Analytical System, Tokyo, Japan), equipped with a Babington nebulizer, Fassel torch and double pass Scott-type spray chamber cooled by a Peltier system. Single ion monitoring at *m*/*z* 75 was used for data collection. Monitoring of Cl (*m*/*z* 35) species was carried out in order to evaluate possible ArCl (*m*/*z* 75) polyatomic interference.

The chromatographic system consisted of a model PU-2080 Plus pump, (JASCO Corporation, Tokyo, Japan) and a PRP-X100 analytical and guard anion-exchange column (Hamilton, Reno, NV, USA). The column effluent was directly introduced into the nebulizer via a PTFE capillary tube (0.5 mm i.d.). The samples were injected through a six-port valve (Rheodyne 9125, USA). The ICP-MS operating conditions used for total As determination and arsenic speciation along with chromatographic parameters are summarised in Table 1.

### 2.2. Reagents and standards

High-purity deionized water (Milli-Q Element system, Millipore, USA) was used for sample and standard solutions preparation. Ten milligrams per litre stock solutions, expressed as metal, of MMA and DMA, were prepared in 4% $HNO_3$ by dissolving adequate amounts of $CH_3AsO_3Na_2$ (MMA) and $(CH_3)_2AsO_2Na{\cdot}3H_2O$ (DMA), both 98% purity from Merck (Darmstadt, Germany). One thousand milligrams per litre stock solutions of As(V) and As(III) were prepared from $AS_2O_5{\cdot}2H_2O$ (98.5%) from Merck (Darmstadt, Germany) and $AS_2O_3$ (99.5%) from J.T. Baker (Deventer, Holland), respectively. One thousand milligrams per litre stock solution of Arsenobetaine (99%) (AsB) was prepared from Tri Chemical Laboratory Inc., Japan. Germanium, purchased from High-Purity Standard (Charleston, USA), was used as internal standard. All these solutions were kept at 4 °C and stored in high density polyethylene (HDPE) bottles until use. Working solutions were prepared daily.

*Streptomyces griseus* (protease Type XIV) was obtained from Sigma (Sigma–Aldrich, Steinheim Germany) and *Bacillus subtilis* (α-amylase) was obtained from Fluka (Sigma–Aldrich, Steinheim, Germany). HPLC-grade methanol from SDS (Peypin, France), and $H_2O_2$ (30%, p/v) from Panreac (Barcelona, Spain) were used. $(NH_4)H_2PO_4$ from Merck (Darmstadt, Germany) was employed as mobile phase. Ammmonium oxalate (98%) from PANREAC (Barcelona, Spain) and orthophosphoric acid (85%) from Carlo Erba (Milano, Italy) were used as extractants for soil samples.

### 2.3. Samples

Arsenic was determined in rice, chicken, fish and soil samples. All of them are candidate reference materials (prepared within the framework of European project SEAS (ref. SEAS G6RD-CT2001-00473). Sample preparation of the lyophilised pool was carried out at the Institute for Reference Materials and Measurements (IRMM) in Geel (Belgium). The samples were kept frozen (−20 °C) for further analysis. Rice sample was previously grounded, sieved (<125 μm particle size) and γ-irradiated to preserve the sample from bacteriological activity. No instability of As species in the lyophilised samples was observed during the 6-month test period [37].

Also, four Certified Reference Materials (CRMs) were selected for method validation. The first was a rice reference material, SRM 1568a (NIST, USA). The second was a tuna fish tissue, CRM-627 (BCR, Belgium). In addition to certified total arsenic content, this material is certified for two arsenic species: AsB and DMA. The third was a soil sample, SOIL-7 (IAEA, Austria) and the last one was an Antarctic sediment reference material, MURST-ISS-A1 (BCR, Belgium).

### 2.4. Extraction procedure

The sample was introduced in a Teflon vial together with the extraction media. The relation sample mass/extraction media volume for each sample was the following: 0.1 g $ml^{-1}$ for rice; 0.05 g $ml^{-1}$ for chicken; 0.02 g $ml^{-1}$ for fish and 0.01 g $ml^{-1}$ for soil. The probe was then introduced into the solution and sonication was applied during different times at 30% amplitude (being 40% the maximum value recommended by the manufacturer). To avoid sample contamination, the probe tip was rinsed several times with deionized water and cotton tissue dried between sample runs. The extracts were centrifuged at 5000 rpm for 10 min and the supernatant was passed through a 0.22 μm nylon syringe filter before analysis. This procedure was applied in triplicate throughout the method optimisation. Aliquots and dilutions of the supernatant were taken for both, total arsenic measurement and speciation analysis.

## 3. Results and discussion

### 3.1. Improvement of the isocratic chromatographic separation

Initially, the chromatographic conditions previously developed [34] were chosen by employing a polymeric anion-exchange column, PRP-X100 and mobile phase of 10 mM $HPO_4^{2-}/H_2PO_4^-$ at pH 6.0. Under these conditions, As(III) and AsB co-elute at the void volume; optimization of the buffer concentration and pH to final values of 10 mM and 8.5, respectively, led to baseline resolution between the two species, keeping the isocratic mode. Co-elution of As(III) and DMA was obtained beyond that pH value. Although these conditions gave good separation for the five targeted species, the analysis time increased from 10 to 16 min. Then, flow rate was increased from 1 to 1.5 ml $min^{-1}$, causing no effect in terms of peaks resolution, but the sensitivity was affected because, in general, nebulization in ICP-MS is less effective at high flow rates. Some authors have proved the positive effect of organic solvents, in particular methanol, on the ICP-MS As and Se response [38,39], favouring the nebulization yield as well as the ionisation degree for elements with high ionisation potential. The optimal concentration, 2% of methanol was added to the 10 mM phosphate mobile phase obtaining a satisfactory separation in 11 min with threefold enhancement of the As signal. Fig. 1 shows a 10 μg $L^{-1}$ As species standard mixture. The detection limit (D.L.), calculated as three times the standard deviation of the blank divided by the calibration slope, was, under these conditions 13.6, 19.6, 12.7, 14.3 and 19.4 ng $L^{-1}$ for AsB, As(III), DMA, MMA and As(V), respectively. Quantification was based on peak area measurements. Precision of the developed method was determined at a concentration of 10 μg $L^{-1}$ and was not higher than 5% for all species ($n = 7$) under study. The lineal range was from D.L. to 1000 μg $L^{-1}$ for all species.

### 3.2. Determination of total and arsenic species

Rice, chicken, fish and soil samples from SEAS project were used all throughout this study. In all cases, the extracts were analysed both for total arsenic by direct ICP-MS nebulization and for As species, applying the above optimised HPLC–ICP-MS method.

#### 3.2.1. Rice

Parameters such as extraction time, extraction media, ultrasound probe amplitude, temperature, tip depth, were optimized and reported in a previous work [34]. Briefly, from the different extraction media tested, quantitative extraction for total arsenic (>95%) and 90% recovery as sum of the arsenic species was obtained by applying the enzymatic treatment, using an aqueous mixture of protease Type XIV and α-amylase. The amount of As(III) found was ten times higher compared to the other extraction media. The extraction recovery obtained for the other species present in rice (DMA, MMA and As(V)) was virtually the same for all media tested, indicating that they are not chemically associated to the rice matrix. The other parameter that revealed great influence in the total and species extraction recovery was the sonication time. Three minutes was enough to obtain quantitative recovery in the case of rice. The rest of variables appeared to have lower influence, thus being set for the matrices tested here as follows: 30% amplitude, room temperature, 1 cm immersion depth of the tip into the solution.

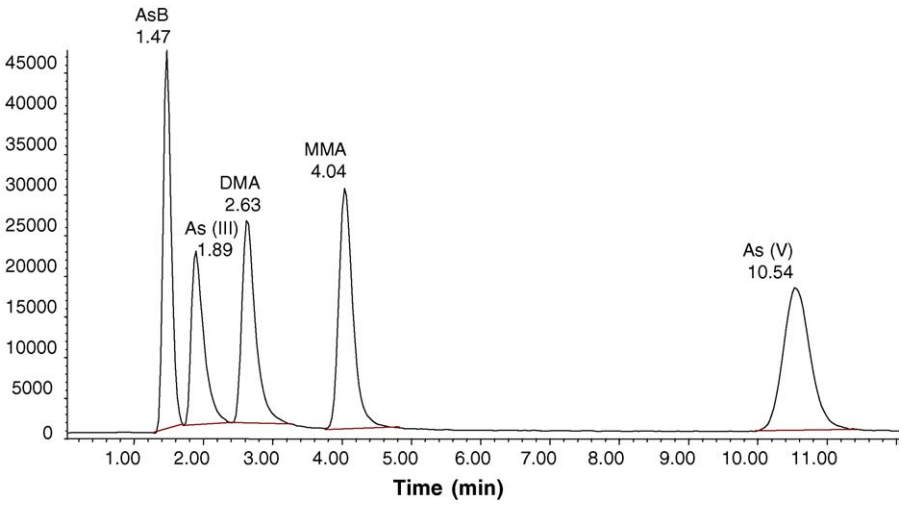


Fig. 1. Typical chromatogram obtained for a stock solution of As species at 10 μg $L^{-1}$ (expressed as As concentration).

### 3.2.2. Chicken muscle

The extraction media evaluated for this sample were water and MeOH, further enzymatic hydrolysis (employing protease type XIV). In a first attempt, the ultra-sonication time applied was 1 min, obtaining total As recoveries of 56.9 ± 9.1%, 43.2 ± 4.2% and 22.3 ± 2.7% for enzymatic, water and MeOH treatment, respectively. Again, the enzymatic treatment showed the best results. Also, differences in arsenic species were observed depending on the extraction media employed (Fig. 2a).

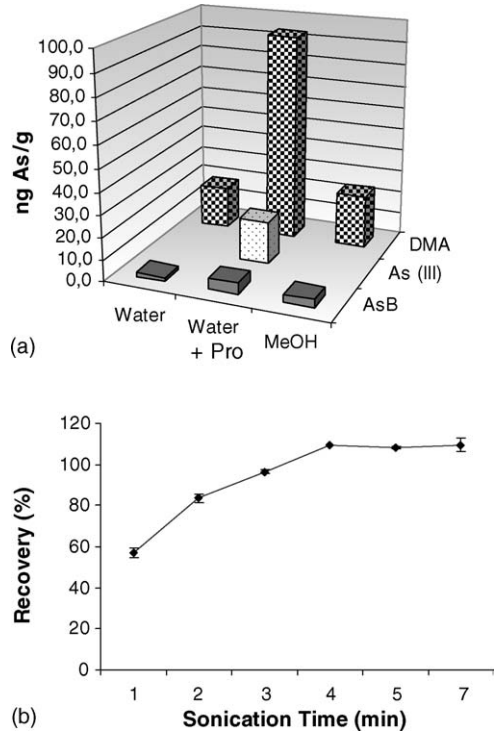


Fig. 2. Determination of total and speciated arsenic in chicken: (a) speciation arsenic vs. extraction media. One minute of sonication time and 30% amplitude (values expressed as relative species concentrations). (b) Total arsenic vs. sonication time. Extraction media: water + protease and 30% amplitude. Values expressed as percentage of recovery for total arsenic.

Two species are present in the three media tested: AsB, and DMA, only the enzymatic hydrolysis extract showed As(III), leading us to postulate the association of inorganic arsenic to chicken proteins, similar to the findings for rice. In addition, the increasing amount of DMA extracted by applying the enzymatic treatment in comparison to the others is noticeable: from 20 to 93 ng/g in water and enzyme medium, respectively. In order to enhance total As recovery, sonication time was enlarged from 1 to 7 min (plotted in Fig. 2b). The higher sonication time the smaller is expected to be the particle size of the treated sample, which in turn shall benefit the extraction efficiency. At $t = 4$ min, quantitative extraction is reached, being the species recovery 82.7 ± 1.3, calculated as sum of species.

### 3.2.3. Fish

The same extraction media, including enzymatic treatment, than for chicken samples were tested here at 1 min sonication time, yielding recoveries >90% for total As content in all cases. Water was selected as extraction media for this matrix. From this high amount easily extracted, 99% corresponded to AsB; the rest of about 1%, was an unknown species eluting at the void volume (presumably arsenocholine). A small peak corresponding to the retention time of As(III) could not be quantified (under detection limit). The found species distribution was expected, since AsB has been identified to be the major arsenical compound in a great variety of fish tissues [10].

### 3.2.4. Soil

Soft extractants usually employed like water, ammonium oxalate (0.2 mol $L^{-1}$) and $H_3PO_4$ (1 mol $L^{-1}$) were selected in order to extract arsenic species from soil. First, the extraction time was optimised for each medium until reaching complete recovery for total As. The results are plotted in Fig. 3, showing not much effect from 30 to 300 s, with best recoveries always obtained for $H_3PO_4$. Speciation analysis was then undertaken in the 30 s extract for the three media tested. Fig. 4 shows the presence of four arsenic species in all cases: As(III), DMA, MMA and As(V), being the last one the major species. Comparably to

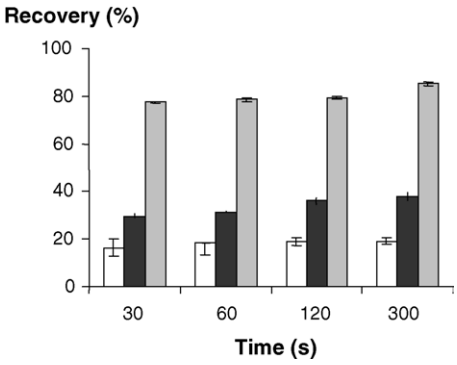


Fig. 3. Total As recovery for several soft extractions of soil sample at different sonication times: (□) water; (■) 0.2 M ammonium oxalate; (▩) 1 M $H_3PO_4$; 30% amplitude; room temperature.

the total As extraction, the optimal media for arsenic species was $H_3PO_4$, with significant differences for all species except DMA, which was extracted at the same level with the three extractants, which in turn indicates that it is the only species which does not seem to establish strong links with the solid matrix. The sum of species extracted in the acid medium represents around 80%, which fits with the total As content of the extract. In order to evaluate the combined effect of sonication time at acid conditions on the arsenic species, the $H_3PO_4$ extracts shown in Fig. 3 at different times were also analysed. Concentration of As(III) decreased at 60 s, disappeared when the sonication time raised to 300 s, and a slight but noticeable increase in As(V) concentration was observed. This oxidation has been attributed to the radiolysis of the acid induced by the focused ultrasound energy. As time increases, hydrogen peroxide and oxygen radicals are generated in the solution leading to redox transformations. Oxidation of As(III) has been checked by spiking a sample with a standard of this species; no oxidation was observed at 30 s, but at higher times (from 60 s onwards) the transformation was evident. In order to improve As recovery, two subsequent steps of 30 s were

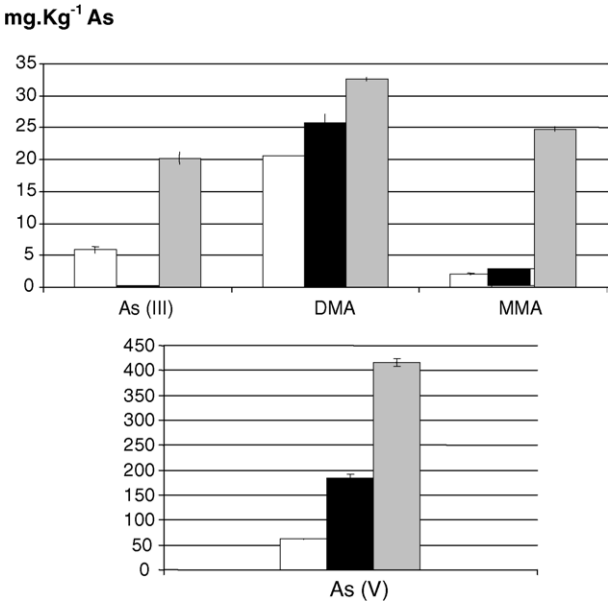


Fig. 4. Concentration of species arsenic (μg As/g) in soil sample by soft extractions. $T$ = 30s; 30% amplitude; room temperature; (□) water; (■) 0.2 M ammonium oxalate; (▩) 1 M $H_3PO_4$.

applied, reaching an optimal value of 85.56 ± 1.40%. No further improvement by applying additional steps was obtained.

Table 2 summarised the experimental set-up for all extractions and the optimal conditions selected for each type of sample.

### 3.3. Validation

Analysis of several Certified Reference Materials was performed in order to validate the optimized extraction methods in rice, chicken, fish and soil. Table 3 summarises the total and species content in arsenic for SEAS and CRM samples, showing in most cases good agreement between the reference values and results obtained by applying the proposed method.

Table 2
Experimental set-up for the extractions and optimal parameters of the extraction methods selected

| Sample | Mass sample (mg)<br>EM[a] volume (ml) | Volume vial (ml) | EM tested | ST[b] tested (min) | Optimal conditions ST (s)–EM |
|---|---|---|---|---|---|
| Rice | 300<br>3 | 10 | See ref. [34] | See ref. [34] | 180–aqueous solution (1 mg mL$^{-1}$ α-amylase + 10 mg mL$^{-1}$ protease) |
| Chicken | 150<br>3 | 10 | Water<br>MeOH<br>Enzymatic hydrolysis | 1 for all media<br>1–7 for enzymatic hydrolysis | 240–aqueous solution (5 mg mL$^{-1}$ protease) |
| Fish | 100<br>5 | 25 | Water<br>MeOH<br>Enzymatic hydrolysis | 1 for all media | 60–water |
| Soil | 100<br>10 | 25 | Water<br>0.2M ammonium oxalate<br>1 M orthophosphoric acid | 0.5–5 for all media | 30 (two steps)–1 M orthophosphoric acid |

Fixed parameters: 30% amplitude, room temperature; tip depth 1 cm.
[a] EM: extraction media.
[b] ST: sonication time.

Table 3
Total arsenic and arsenic species content in SEAS samples and certified materials

| Sample | Total As | Recovery (%) | AsB | As(III) | DMA | MMA | As(V) | Sum of species (%) |
|---|---|---|---|---|---|---|---|---|
| Rice | 193.2 ± 13.1 (195.8 ± 13.3)[a] | 98.4 ± 6.7[b] | nd[c] | 129.2 ± 3.1 | 31.5 ± 1.6 | 1.9 ± 0.7 | 15.4 ± 3.8 | 90.2 ± 1.3[d] |
| SRM 1568a | 286.4 ± 6.1 (290.0 ± 30.0)[e] | 99.1 ± 2.1 | nd[c] | 68.3 ± 3.7 | 135.4 ± 4.1 | 8.1 ± 1.3 | 20.5 ± 2.3 | 82.3 ± 4.2[f] |
| Chicken | 184.3 ± 2.3 (168.6 ± 6.8)[a] | 109.3 ± 1.4 | 4.7 ± 0.8 | 2.3 ± 0.3 | 133.0 ± 2.7 | nd[c] | nd[c] | 82.7 ± 1.6[d] |
| Fish | 42.20 ± 0.09 (43.30 ± 3.91)[a] | 97.5 ± 0.2 | 38.9 ± 0.2 | <LOD | nd[c] | nd[c] | nd[c] (0.95 ± 0.01)[a] | 91.0 ± 0.5[d] |
| CRM-627 | 3.8 ± 0.1 (4.8 ± 0.3)[e] | 79.2 ± 0.5 | 3.6 ± 0.1 (3.8 ± 0.2)[c] | nd[c] | 0.14 ± 0.01 (0.15 ± 0.02)[c] | nd[c] | 0.01 ± 0.00 | 79.9 ± 0.5[f] |
| Soil | 518.62 ± 6.46 (612.21 ± 23.12)[a] | 84.71 ± 1.31 | nd[c] | 26.3 ± 0.3 (18.2 ± 4.4)[a] | 32.6 ± 0.2 (29.2 ± 3.6)[a] | 21.5 ± 1.1 (33.4 ± 12.5)[a] | 443.4 ± 7.5 (513.6 ± 20.1)[a] | 85.6 ± 1.4[d] |
| Soil-7 | 9.5 ± 0.2 (13.4 ± 0.1)[e] | 70.93 ± 1.9 | nd[c] | 0.7 ± 0.1 | nd[c] | <LOD | 8.0 ± 0.1 | 65.1 ± 0.1[f] |
| MURST-ISS-A1 | 3.4 ± 0.1 (4.4 ± 0.1)[e] | 78.07 ± 1.23 | nd[c] | 0.3 ± 0.1 | nd[c] | 0.4 ± 0.1 | 2.1 ± 0.1 | 62.8 ± 1.8[f] |

Concentrations: rice and chicken in ng As/g; fish and soils μg As/g.
[a] Reference value from SEAS project report.
[b] This recovery is calculated as: (As total/As reference value from project SEAS) × 100.
[c] Not detected.
[d] This recovery is calculated as: (As total (sum of species)/As reference value from project SEAS) × 100.
[e] Certified value.
[f] This recovery is calculated as: (As total (sum of species)/As certified value) × 100.

Recovery of total As and species in tuna fish tissue (CRM 627) was lower compared to that obtained for fish from the SEAS project. This material has been analysed by other authors [40], reporting a 95% recovery of total arsenic content by applying acid digestion with $HNO_3 + H_2SO_4$. However, the quantification of arsenic species led the authors to over-estimate the AsB content (10% more) since this species concentration was calculated as difference (total digested arsenic minus DMA + As(V)). This fact could be an indication of a strongly linked arsenic fraction, only measurable in more aggressive conditions with the consequential species transformations. The total As recovery in Soil-7 and Sediment MURST-ISS-A1 also were only about 75%, slightly lower than in SEAS soil, most probably due to the low total arsenic concentration of these samples and the high variability of soils, due to the different matrix components.

### *3.4. Relationship of extraction media and nature of the sample*

Fig. 5 shows typical chromatograms obtained for each sample analysed under the optimal conditions. The strong inter-relation between nature of the sample and the optimum extractant for maximum species recovery can be clearly deduced from this table. Taking rice as first example, As(III) is extracted only by applying an enzymatic leaching procedure. This fact implies accumulation of this chemical form associated to rice proteins. Possibly As(III)-proteins bonding mechanism involves proteins containing thiol groups (cysteine residues), such as the so-called "prolamins rich in sulphur", about 8% of total rice protein content. Protease, which breaks up peptide bonds, mixed with amylase, which liberates starch–protein bound, is then the most efficient extraction media. Similarly, the results found in chicken showed association of As(III) and DMA to chicken proteins. Eventually the rupture of peptide by the enzyme better solubilizes chicken proteins, which in turn alleviates arsenic species extraction. It is known that inorganic arsenic is the chemical form bound to proteins, but there is also evidence that organic forms can bind to proteins [41]. At this point, it is interesting to mention that the chicken breast muscle analysed came from 70-day-old white cockerels, subjected to a diet enriched with As(III) [37]. The inter-comparison exercise organised in the frame of SEAS project revealed DMA as the main species present in chicken breast muscle accounting for more than 50% of the total content. This may indicate a methylation as result of an As(III) detoxification mechanism during metabolism of the cockerels. Our results agree with those reported in the mentioned project and besides, our study observed that in the biomethylation of inorganic arsenic, the formed species are binding to chicken protein. On the contrary, fish has appeared to be the easiest extractable sample, with quantitative recovery after only 1 min of sonication in aqueous medium. This soft treatment was efficient due to, first the high amount of AsB, non-associated to any matrix macromolecule and second, to acoustic cavitation, which produces intense local heating and high pressures [42] increasing the analyte solubilization.

Finally, the As fraction extracted from a highly complex matrix such as soil evidenced a strong dependence on the extrac-

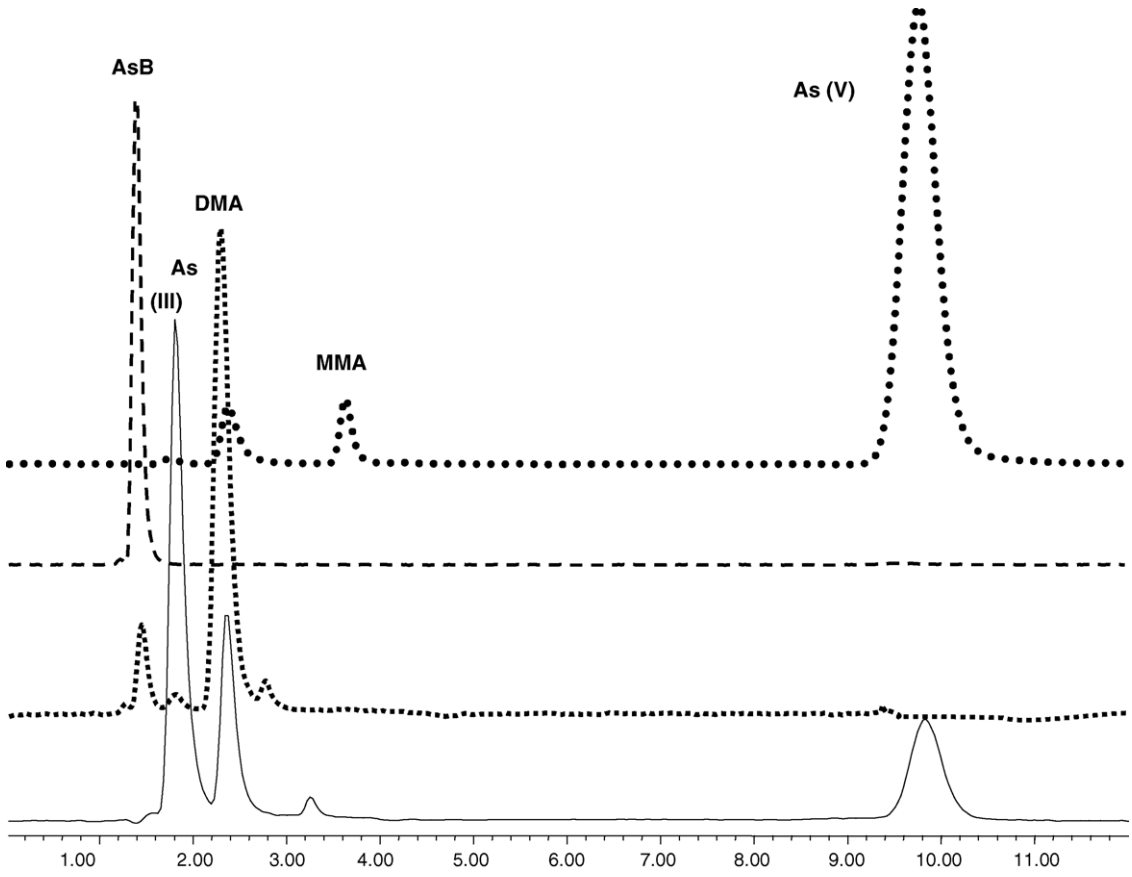


Fig. 5. Chromatograms obtained for SEAS samples: (—) rice, (▪▪▪▪▪) chicken, (- - -) fish and (• • • • •) soil. Chromatographic conditions from Table 1. Extraction conditions from Table 2.

tant selected. Thus, the low values obtained with ammonium oxalate led us to suggest that the arsenic present in this matrix is not integrated to crystalline iron and manganese oxy-hydroxides fraction. The ability of this reagent to complex with $Fe^{III}$ breaking the oxide crystals is well known. On the other hand, the high recovery of total As and As species (85%) obtained corresponds to the labile fraction, which is the most immediately dangerous to the environment. The 15% left are presumably bound to sulphides and/or organic matter and/or silicate fraction and would need stronger acid conditions to break these bounds with high probability of species transformation via oxidation.

## 4. Conclusions

A simple, fast and reliable method for As species extraction and detection in different matrices has been developed. The time required for both sample treatment and isocratic chromatographic analysis is about 40 min. Excellent detection limits between (13 and 20 ng $L^{-1}$) have been achieved. Ultrasound probe sonication has shown to be a powerful tool for sample treatment allowing, in most cases, quantitative extraction in very short time (from hours to a few minutes) compared to other methods, simplifying also sample handling. The species preservation under optimized conditions led us to postulate the binding of some As species to the matrix components. So, different extraction media for rice, chicken, fish and soil yielded recoveries in the range 70–109% for total arsenic and 86–91% for arsenic species. Future investigation will be focused on partial automation of these processes and also to the immobilisation of the enzymes in order to reduce the relatively high analysis cost, due high enzyme consumption.

## Acknowledgements

The authors gratefully acknowledge the Spanish Ministry of Science and Technology for the Ramón y Cajal contract of Riansares Muñoz-Olivas. This work has been carried out with the economical support of the CICYT project ref. BQU-2002-01348.

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